METABOLIC STUDIES IN DISORDERS OF ACID-BASE EQUILIBRIUM OF INFANCY AND CHILDHOOD.

BY

NOAH MORRIS, M.D., B.Sc.

PREFACE.

The thesis which is here presented is devoted to a study of some metabolic problems of disturbances of acid-base equilibrium in infancy and childhood. I have refrained from including a consideration of blood chemistry except in so far as it throws light on the underlying metabolism.

The work on which this thesis is based was carried out in the biochemical laboratory and wards of the Royal Hospital for Sick children, Glasgow during the years 1926-1933. Some of the results have not yet been published but much of it has already appeared in print in The Archives of Disease in Childhood, Lancet, Acta Paediatrica (Stockholm), British Journal of Experimental Pathology, Journal of Physiology, and Biochemical Journal. These papers with others are bound in a separate volume which is sent herewith.

I personally am entirely responsible for the composition of the thesis and the conclusions contained therein and also for the biochemical work except for assistance received from Dr. O. Macrae in the performance of some of the calcium and phosphorus analyses. I am deeply indebted to Dr. Leonard Findlay/

Findlay, formerly Professor of Paediatrics in the University of Glasgow, Professor G. B. Fleming and Dr. Stanley Graham at present visiting physicians at the Royal Hospital for Sick Children, Glasgow for affording me the fullest possible facilities for investigation in their wards. To Dr. Graham I would like specially to tender my thanks for his encouragement throughout. We have collaborated in the writing of a book on Acidosis and Alkalosis which was published last year and much of the work reported here was initiated as a result of our discussions on the problems of acid-base equilibrium. I also wish to acknowledge my indebtedness to the Directors of the Hospital for their ready help in providing laboratory equipment and to the Medical Research Council for a personal grant which I received during the performance of this work.

CONTENTS.

CONTENTS(continued).

---0---

Chapter One

Introduction

THE SIGNIFICANCE AND DETERMINATION OF DISTURBANCES OF ACID-BASE EQUILIBRIUM.

Chapter One

Introduction

THE SIGNIFICANCE AND DETERMINATION OF DISTURBANCES OF ACID-BASE EQUILIBRIUM.

There are three stages in the evolution of any scientific advance. The first is a period in which it is either entirely neglected or regarded with too rigorous a scepticism. The second stage is one in which the over-sceptical attitude is followed by an equally exaggerated credulity. Finally comes the period in which it is estimated at its proper value. At the present time the clinical conception of acidosis is in the second stage with the result that many conditions are cheerfully labelled acidosis. No state from debility to rheumatism is safe from this diagnosis and this is especially true for infancy and childhood. So much is this the case that the scientific foundation on which is based the conception of acidosis is likely to be lost sight of and ignored. This would be a loss both from the practical and scientific aspects of medicine, since the elucidation of the problems of acidosis has thrown considerable light on many aspects of clinical medicine/

medicine and has led to the adoption of valuable therapeutic measures. The treatment of chronic nephritis and diabetic coma has been considerably aided by making use of the knowledge revealed by a study of acidosis, while the basis of the treatment of tetany in children is derived from physiological studies in acid-base equilibrium.

Normal life cannot be sustained unless the reaction of the body and its fluids is kept practically constant. Metabolic processes, however, lead to the formation of acids such as carbonic and lactic and the diet contains varying amounts of acid and alkali. The digestive juices dispose of both acid and alkali. The net result of all these processes is the production in the body of a great excess of acid over base. It is said that in the course of each twenty-four hours the lungs get rid of the equivalent of 20-40 litres of normal acid (carbonic), and the kidneys of 50-150 c.c. (chloride sulphate, phosphate). It is clear that even in health adjustments must always be taking place. So delicate are these compensatory measures that changes during health can hardly be detected by the methods at our disposal. Haldane has stressed the point that this regulation of reaction is not merely the maintenance of equilibrium of acid and base: compensatory/

compensatory measures are themselves the result of physiological activity. The manifestations of disturbed acid-base equilibrium should therefore be thought of not merely as evidence of morbid functioning, but as physiological adjustments to alterations in the tissue fluids. Consequently, the proper interpretation of signs and symptoms associated with disturbances in acid-base equilibrium may become a matter of extreme importance. These disturbances are of special importance in the growing child. Not only are they more frequently encountered in infancy and childhood, but the ability to overcome the upset and re-establish normal equilibrium is less perfectly developed. It is as if the young organism lacked practice and experience.

Maintenance of a constant reaction of the blood, however, is much more complicated than the mere regulation of excretion of acids and bases. Respiration and urinary excretion, by which means practically all the acid is got rid of, are influenced by many factors other than the presence of acid substances in the blood. It is well, therefore, to remember that a study of acid-base equilibrium throws light only on one aspect of the problem of disease. Not only are other factors of importance but they generally act and are reacted on by the state of acid-base equilibrium.

There are two possible deviations of acid-base equilibrium from normal viz., acidosis and alkalosis. Neither is a disease entity any more than fever is a diagnosis. Unfortunately many clinicians are satisfied with the labels of acidosis and alkalosis, apparently oblivious to the fact that only the first step has been taken in the consideration of the condition. Actually the alteration in acid-base equilibrium is generally a reaction of the body as a whole to some pathological process. The signs and symptoms by which acidosis and alkalosis are detected mean little unless there is an understanding of their mode of production and a realisation that they form part of the body's adaptation to disease. As L.J.Henderson has remarked - "Nothing is more wonderful or instructive than the condition of an individual who has long been suffering from a progressive disease like chronic nephritis, who is approaching his end but who remains adapted in every part and in every activity to the changed and almost impossible conditions of life". Even restricting one's outlook severely to the realm of practical therapeutics it is advisable if at all possible to have a knowledge of the underlying causes and take efficient counter measures and not merely bring back the reaction of the blood to normal limits. To take an example frequently met with in paediatric practice there/

there is the condition of acidosis encountered in severe gastro-enteritis. The mere neutralisation of the acidosis by alkali is per se of comparatively little value unless it is recognised that the cause of the acidosis is a three-fold one viz. loss of fluid, loss of salts, and increased production of ketones. With this knowledge of the pathogenesis of acidosis in gastro-enteritis it is a simple matter to lay down the practical measures to be adopted in such a condition. These are the administration of abundant fluid, salts and glucose. Such treatment by dealing with the factors producing acidosis is usually sufficient to restore acid-base equilibrium to normal just as the removal of bacterial toxins is the best antipyretic. This is not to deny the value of simple ant-acidotic measures in those conditions about the etiology of which we know nothing. In this connection it is necessary to remember that acidosis may quickly become an emergency, that has to be dealt with immediately in order to prevent a fatal issue. This is due to the fact that a rapidly developing acidosis may cause such a strain on the cellular processes that irreparable damage may be produced if the strain is not quickly relieved.

There are, therefore, two aspects of acid-base disturbance which have to be considered. In the first place disturbances/

disturbances of metabolism may lead to relative excess of acid or alkali causing acidosis or alkalosis. In the second place acidosis and alkalosis lead to attempts on the part of the body to deal with, and compensate for the disturbance. Not infrequently these compensatory measures if pushed to excess lead to further disturbances of metabolism which may be of very serious import. There is a further advantage to be derived from a study of acid-base equilibrium. From a knowledge of the compensatory measures carried through by the body it has been possible to use artificial inducement of acidosis to obtain similar effects. Thus the fact that acidosis leads to dehydration has been made use of in the treatment of oedema.

CLASSIFICATION OF DISTURBANCES OF ACID-BASE BALANCE.

In 1921 Van Slyke showed that there are nine possible variations in the relationship of the three variables, cH, H_2CO_3, and $BHCO_3$, of which only one is absolutely normal. These have been classified in the following groups:-

(1) Uncompensated Alkali Excess. - The $BHCO_3$ is increased, the H_2CO_3 increased to a less extent, and the pH increased.

(2) and (3) Uncompensated Carbon Dioxide Deficit. - The H_2CO_3 is decreased, $BHCO_3$ decreased to a less extent, and the pH increased.

(4) Compensated Alkali Excess. - The $BHCO_3$ is increased, the H_2CO_3 is increased to the same extent, and the pH is normal.

(5) Normal Acid-Base Equilibrium. - All three variables are normal.

(6) Compensated Alkali Deficit. - The $BHCO_3$ is diminished, the H_2CO_3 diminished to the same extent, and the pH is normal.

(7) and (8) Uncompensated H_2CO_3 Excess. - The H_2CO_3 is increased, the $BHCO_3$ is increased to a less degree, and the pH is diminished.

(9) Uncompensated Alkali Deficit. - The $BHCO_3$ is diminished, the H_2CO_3 diminished to a less degree, and the pH is diminished.

From this classification it becomes apparent that by the term compensated is meant an alteration in the combined carbon dioxide without a change in the pH. For example, one form of compensated acidosis (Group 6) is that in which the bicarbonate is lowered but the pH remains unaltered, while in a further stage of this type of acidosis (Group 9), the pH is lowered and the condition becomes uncompensated. Actually, however, in a compensated acidosis there must be a change in the pH, but it is of a degree which is not detected because the methods of estimation are not delicate enough./

enough. Any fall in the $BHCO_3$ is certainly followed by a corresponding fall in the H_2CO_3 in an effort to maintain or re-establish acid-base equilibrium. The fall in H_2CO_3, however, lags behind the fall in $BHCO_3$, and this must produce a slight but certainly definite alteration in the ratio whether or not it can be detected by present-day chemical methods. If this lag in the fall of the free carbon dioxide did not occur, i.e. if the ratio were immediately restored exactly to normal but at a lower level of bicarbonate there would be no stimulus for the bicarbonate to return to its previous level. It is the alteration in the ratio which affords a stimulus to the body to expel more acid and to call more alkali into the blood and thus to restore the bicarbonate to its normal level. It has been shown that a rise of 0.2, volume per cent. in the H_2CO_3 without change in the $BHCO_3$ is sufficient to double the resting alveolar ventilation. Yet, this acidosis, though sufficient to stimulate the respiratory mechanism to increase its activity by 100 per cent., represents a difference in the pH of only 0.028 (say pH 7.401 to pH 7.373), a change which can just be detected by accurate physico-chemical measurements. For this reason it would appear that the use of the terms compensated and uncompensated

is/

is of no particular advantage: the term compensated is so called merely because of the lack of delicacy in the methods employed for the determination of the pH.

The following classification suggested by J.B.S. Haldane seems much more helpful as it gives in each case an indication of the particular procedure employed by the body in the production of the change.

(1) Non-Gaseous Acidosis. - In this there occurs a primary fall in the combined dioxide with a secondary fall, but less marked, in the free carbon dioxide. This is the classical acidosis, the acid poisoning type which occurs in diabetic coma and gastro-enteritis. It is characterised by a fall in pH and a diminished alkaline reserve. The clinical manifestation of increased respiration is the result of the necessity of getting rid of carbon-dioxide.

(2) Gaseous Acidosis. - Here the primary factor is an increase in the free carbon dioxide with a consequent rise, but to a less degree, in the combined carbon dioxide in an attempt to restore the normal ratio. Here the pH is lowered and the alkaline reserve or total carbon dioxide is also increased. A disturbance of this nature occurs in morphine poisoning where, owing to diminished sensitiveness of the respiratory centre, the subject breathes more slowly and thus/

thus conserves free carbon dioxide which provides the necessary increase in stimulation required by the respiratory centre. In other words, the depressed respiratory centre requires a bigger "head" of carbon dioxide to stimulate it.

(3) Non-Gaseous Alkalosis. - There is a rise in the amount of available combined carbon dioxide, followed by a less marked rise in the free carbon dioxide. The pH is increased and the total carbon dioxide is increased. The breathing is slow and shallow, in an effort to conserve the free carbon dioxide and thus to restore the normal ratio.

(4) Gaseous Alkalosis. - Here the primary factor is a diminution in the free carbon dioxide followed by a relatively smaller diminution in the combined carbon dioxide. This gaseous alkalosis is seen in conditions where there is "washing out" of the carbon dioxide as in cases of forced over-ventilation or hyperpnoea, which has been observed as a sequela of encephalitis lethargica and occurs at high altitudes and in other severe anoxaemic conditions. The pH is increased and the total carbon dioxide diminished.

It is apparent that a non-gaseous acidosis and a gaseous alkalosis are both associated with a low total carbon dioxide/

dioxide content and likewise a low alkaline reserve. Similarly a gaseous acidosis and a non-gaseous alkalosis produce a high total carbon dioxide and a high alkaline reserve. This serves to illustrate the fallacy of using the alkaline reserve or the total carbon dioxide figure alone as an indication of the state of the acid-base balance.

There is no royal road to the diagnosis of acidosis or alkalosis. There is no single clinical sign, symptom or chamical test which is pathognomonic of either condition, Alteration in respiration, reduction or increase in alkaline reserve and physico-chemical changes in the urine are individually of little value. The only thing that will clinch matters is finding an actual change in the reaction of the blood. This, however, is likely to prove a help in but few cases. Apart altogether from technical difficulties which prevent it being used as a routine clinical test it has to be remembered that although variations in pH are usually kept in the one person within limits that are practically within experimental error, there is a fairly wide variation of pH in different healthy subjects: thus, a value which is quite normal for one person may be a definite shift to the acid or alkaline side for another. In many cases/

cases, therefore, it would be necessary to know the value of the pH prior to the onset of the illness before one could diagnose acidosis or alkalosis. It is therefore only by a consideration of all the evidence clinical and laboratory that one can come to any definite conclusion. In many diseases unfortunately, the knowledge of the nature of the acid-base disturbance is still so scanty, that such a procedure is likely to lead to fallacious conclusions. Before the various tests can be used with any degree of safety it is necessary that a thorough investigation be made of the various manifestations clinical and biochemical of the acid-base disturbance in these conditions. In a number of diseased conditions, however, the clinical and biochemical findings have been correlated so that it is possible now to label them acidosis or alkalosis. In such cases single findings may be of considerable value in determining the severity of the condition and providing an index to rational and successful therapy. Here, however, it is wise to utter a word of caution that a return of the pH of the blood to normal does not necessarily mean a return of health. Thus, by the administration of sufficient alkali it may be possible to restore the pH while the distribution of ions and the

water/

water metabolism may be very far from normal. This shows the danger of considering the disturbance in acid-base equilibrium as the sole abnormality and forgetting the changes that have been brought into being by the acidosis or alkalosis.

---0---

Chapter Two

Chapter Two

METABOLIC REACTIONS TO ACIDOSIS PRODUCED BY AMMONIUM CHLORIDE.

Acidosis is so frequently put forward as the underlying pathological condition in such a variety of disorders that it is important to appreciate what actually are the metabolic manifestations of the acidotic state. Clinically one seldom, if ever, has an opportunity of studying acidosis uncomplicated by some other factor such as inanition or toxaemia. It is therefore advisable in an investigation of the metabolic reactions to a disturbance in acid-base equilibrium to have these secondary factors as far as possible excluded. This can only be done when the acidosis is induced in a healthy individual with the minimal amount of upset especially as regards food-intake, and such a condition is most nearly attained in the acidosis produced by the ingestion of ammonium chloride.

Haldane was the first to show that ammonium chloride taken in large amounts led to a marked acidosis. Gamble and Ross, Baird, Douglas, Haldane and Priestley, and Gollwitzer-Meier/

Gollwitzer-Meier confirmed these results, indicating that after administration of ammonium chloride the bicarbonate content of the plasma was reduced to a greater extent than was the tension of carbon dioxide, producing therefore an increase in hydrogen-ion concentration. Similar results have been obtained with calcium chloride (Haldane, Hill and Luck) and magnesium chloride (Gollwitzer-Meier). Ammonium chloride is however, the most suitable for determination of the effects of mild acidosis on the metabolic processes of a healthy individual. Both calcium and magnesium ions have a sedative effect whereas the ammonium radicle is very rapidly changed by the liver into the inert substance urea which is rapidly excreted (Stewart and Haldane: Mainzer). Furthermore, the acidotic effect of calcium and magnesium salts depend upon the retention of a large proportion of the cations in the intestine which probably leads to alteration in the absorption of phosphorus.

Most workers are agreed that there is an increased output of lime in the urine during acidosis. The effect on the faecal excretion of lime and the influence of the acidotic state on phosphorus metabolism have not, however, been clearly determined. Steenbock, Nelson and Hart pointed out the detrimental effect of acid-forming diets on calcium/

calcium retention and calcification in animals. Sawyer, Baumann and Stevens found an increased urinary output of calcium and phosphorus in two children during a period of high fat intake: in only one, however, was there an increase in the faecal amounts of these substances, while in the other there was a decrease. In a study of acid and base-forming diets in adult women Bogert and Kirkpatrick did not obtain a constant change in the amount of faecal calcium during the period of acid-forming diet although the urinary lime was always increased. In infants Flood found that administration of deci-normal hydrochloric acid led to no alteration in the retention of calcium, although this substance always appeared in slightly increased amount in the urine. Ross and Scriver reported that an increase in faecal calcium occasionally occurred after administration of ammonium chloride to children.

It has been shown by Haldane and Gamble, Ross and Tisdall that salts such as calcium and ammonium chloride produce their diuretic effect in virtue of their acid-producing powers. This relationship between water-loss and acidosis has frequently been commented upon in states of dehydration accompanying gastro-enteritis. Gamble, Blackfan and/

and Hamilton, and Rölling have shown the close relationship between the extra loss of water by the kidney and the excess excretion of fixed base. In this research it is hoped to bring forward evidence as to the part played by calcium and phosphorus and in general to attempt a correlation between the various metabolic changes following on the production of an acidosis by ammonium chloride administration.

PRESENT INVESTIGATIONS.

The subjects of the study were four apparently normal children - N.G., female aged 11 years; W.C., male aged 9 years; N.M., female aged 10 years; and J.F., male aged 9¼ years. Each had recovered from a mild attack of rheumatism and appeared quite well. The diet throughout the period of the investigation was constant, consisting of cow's milk with sugar sufficient to satisfy the caloric requirements of the child. After at least three days on the arranged diet the urine and faeces were collected with the usual precautions for periods of seven or six days as stated. Thereafter one gram of ammonium chloride was given five times daily in capsule form. The excreta were again collected for a period of seven days. In the case of N.M. and J.F. the ammonium chloride/

chloride was continued so as to include a third period of five and six days respectively. In the case of N.M. 4 cc. of cod-liver oil was given thrice daily during this last period.

CLINICAL FEATURES.

No apparent change was produced in the appearance of any of the children during or following the ingestion of the ammonium chloride. In two cases the administration continued for a period of eighteen days without any sign of respiratory or other disturbance although the daily intake varied from 0.166 to 0.247 g.per kilo.of body weight. In several other subjects the dosage was as high as 0.40 g. per kilo. daily for ten days without the appearance of any symptoms. Haldane produced in himself marked respiratory distress by taking one dose of ammonium chloride equivalent to 0.25 g. per kilo.body weight. Koehler found that administration of 10 - 15 g. of ammonium chloride daily to well-developed adults produced definite symptoms of listlessness, thirst, diuresis and muscular aches: these subjects, however, were all patients recovering or recovered from lead-poisoning. Three explanations may be offered for the absence of symptoms in the subjects of this investigation. First, children may not be as susceptible as adults to the action of ammonium chloride./

chloride. It is well known that children tolerate a relatively much larger dose of such a drug as salvarsan than do adults. It seems strange, however, that this should be the case with an acid-producing substance when the peculiar susceptibility of the young to disturbances of acid-base equilibrium is remembered. Secondly, the milk diet used in this study may be of importance since it contains an excess of fixed base over mineral acid. It is possible that this excess base enabled acidosis to be more effectively withstood than would otherwise have been the case. Thirdly, the division of the daily dose into five portions may have allowed the compensating reactions of the body to come into play before there was any necessity for visible extra effort on the part of the respiratory or other system. The last seems the most likely explanation; but whatever the cause may have been, the absence of clinical manifestations of acidosis in no way invalidates this study for, as will be shown later, the blood analyses were indicative of a disturbance of the acid-base equilibrium towards the acid side. The principal object was to study the changes over a period of at least several days, and it would have been manifestly impossible to have accomplished this in the presence of respiratory/

TABLE 1.

Changes in Chemical Composition of Blood following Administration of Ammonium Chloride.

Name	Stage	(1)	(2)	(3)	(4)	(5)	(6)
		CO_2 Vol.%	Cl' Mg.%	Fixed base c.cm. N/10%	N.P.N. mg.%	Calcium mg.%	Phosphorus mg.%
N.G.	Normal	55.1	300	158	36.5	-	-
	7 days NH_4Cl	38.7	350	154	46.1	-	-
W.C.	Normal	68.2	340	148	37	10.6	5.2
	4 days NH_4Cl	41.4	360	157	-	-	-
	8 " "	40.3	340	157	44	10.1	6.5
J.F.	Normal	66.7	240	154	35.5	9.1	-
	9 days NH_4Cl	49.1	360	159	48.5	9.1	-
	19 " "	45.8	320	154	40.1	8.80	-
N.M.	Normal	60.6	285	147	35	9.25	4.2
	3 days NH_4Cl	45.1	290	-	-	-	-
	6 " "	41.8	-	-	-	-	-
	9 " "	43.4	280	132	46	9.8	4.1
	13 " "	41.5	320	137	32	-	-

respiratory distress or other evidence of acute acidosis.

CHANGES IN CHEMICAL COMPOSITION OF THE BLOOD.

The changes in the chemical composition of the blood found during the ammonium chloride period are in close agreement with those reported by Haldane, and Gamble, Blackfan and Hamilton. The changes in the individual constituents which are detailed in Table 1 will be discussed in turn.

A. Carbon Dioxide. The total carbon dioxide of the blood was reduced in every case. Keith and Whelan found that the plasma carbon dioxide dropped about the fourth or fifth day of the administration of ammonium chloride. In three of the subjects of the present investigation the carbon dioxide content was estimated two or more times during the ammonium chloride period. From these results it is evident that the reduction in the carbon dioxide content reached what was practically its maximum, comparatively early in the reaction to ammonium chloride. Continued administration of the acid-producing substance had but little further effect on the carbon dioxide content of the blood. This is probably due to the fact that the other regulating mechanisms came into play, and thus protected the carbon dioxide content and almost certainly the pH, from further reduction.

B. Chlorine. The chlorine was moderately increased. The increase in chlorine when calculated in milli-equivalents (Table 2)/

TABLE 2.

Showing Compensatory Decrease and Increase of Chlorine and Bicarbonate Ions Respectively.

Name	Stage	HCO'_3 c.cm. N/10%	Cl' c.cm. N/10%	HCO'_3 Cl' c.cm. N/10%	Change of (HCO'_3 + Cl') from normal	Change in fixed base
N.G.	Normal	24.6	84.5	109.1	-	-
	7 days NH_4Cl	17.3	98.6	115.9	+ 6.8	-4
W.C.	Normal	30.4	95.7	126.1	-	-
	4 days NH_4Cl	18.5	101.4	119.9	-6.2	+9
	8 " "	18.0	95.7	113.7	-12.4	+9
J.F.	Normal	29.8	70.0	99.8	-	-
	9days NH_4Cl	21.9	101.4	123.3	+ 23.5	+5
	19 " "	20.4	90.1	110.5	+ 10.7	0
N.M.	Normal	27.1	80.3	107.4	-	-
	3days NH_4Cl	20.1	81.7	101.8	-5.6	-
	9 " "	19.4	80.3	99.7	-7.7	-15
	13 " "	18.5	90.1	108.6	+ 1.2	-10

(Table 2) did not compensate for the decrease in milli-equivalents of bicarbonate. Baird, Douglas, Haldane and Priestley found that the carbonate ion of the plasma and tissues is partly replaced by the chlorine ion in conditions of acidosis. Gamble, Blackfan and Hamilton also reported similar results with both ammonium and calcium chloride. Mainzer and Joffe showed that the change in the carbon dioxide content of the blood was always less than what would be expected if only fixed base, chlorine and carbonic acid took part in the equilibration. In the present series, however, this replacement was by no means exact even when allowance was made for the change in fixed base. In W.C. there was an actual increase in fixed base accompanied by a deficit in the sum of bicarbonate and chloride. In J.F., on the other hand, the increase in chloride over-compensated the loss in bicarbonate: in this case the control period was characterised by a very low chlorine content. An objection may be raised that these analyses were performed on whole blood and not on plasma, but it should be remembered that the cell walls are equally permeable to chlorine and carbonic acid. It would seem, therefore, that all the acid ions take part in mutual compensation. As will be indicated later, there/

there was but little change in the inorganic phosphorus of the blood. Indeed, the possible limits of variation of the amounts of this substance and sulphate render a change in either almost negligible as a compensatory factor. It is probable that the organic acids of the blood play an important part in balancing excess or deficit of the other acids, as has been suggested by Gamble and his co-workers in alkalosis resulting from experimental obstruction of the pylorus. No data are presented here on the change in base-combining power of the plasma proteins. The findings of Keith and Whelan, and Fólling suggest that such change is trifling. Mainzer and Joffe, however, on the strength of calculations made by Van Slyke on horse's blood suggest that concentration and dilution of blood by altering the base-combining capacity of the protein is sufficient to explain the difference between the expected and actual amounts of carbon dioxide.

C. Fixed Base. Gamble, Blackfan and Hamilton state that there is a very slight reduction in the fixed base of the serum, but Fólling reports a fall of 15.4 milli-equivalents per litre in calcium and ammonium chloride acidosis. In two of the cases there was a decrease in the fixed base content of the serum: in one of these (N.M.) the reduction amounted to/

Fig. I.

Changes in Calcium and Phosphorus Content of Serum.

Case E.C.

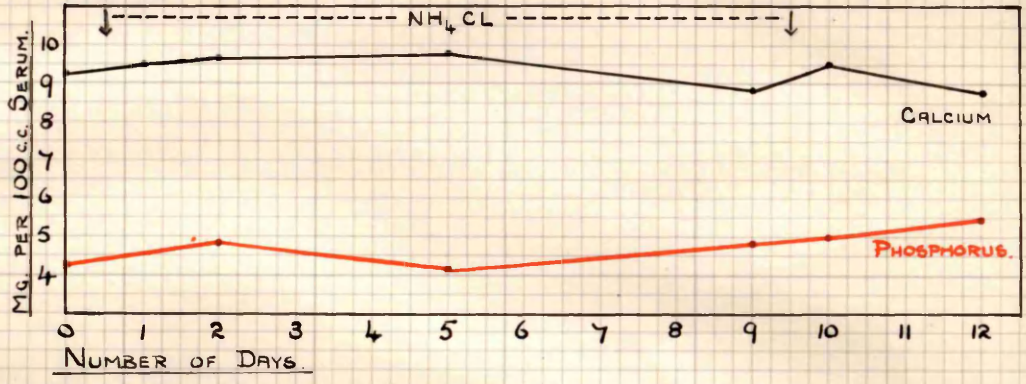
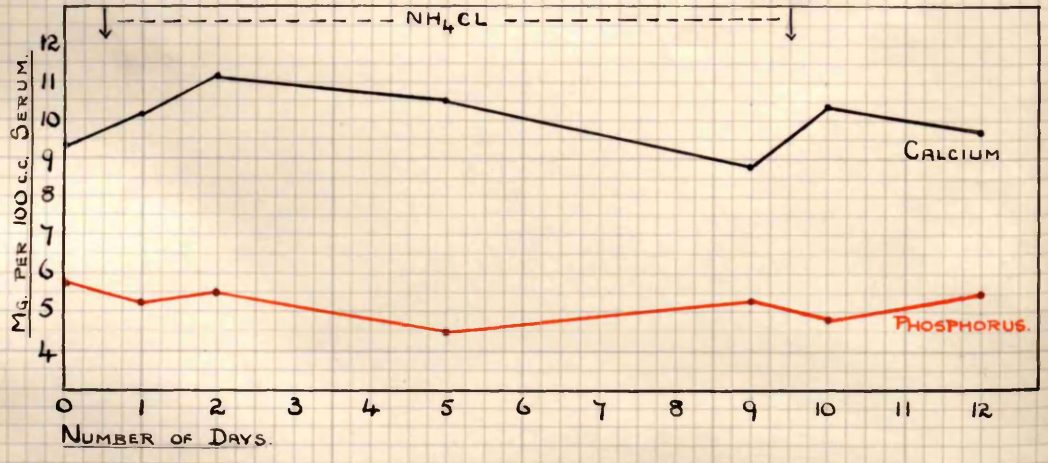


Fig. II.

Changes in Calcium and Phosphorus Content of Serum.

Case A.M.



to 15 milli-equivalents per litre, resulting in a value outside the normal limits. In the other two cases there was an increase in fixed base but the raised values fell within normal limits.

D. Calcium and Phosphorus. In 1924 Stewart and Haldane noted a 10 per cent. rise in the serum calcium of a healthy adult, following the administration of 25 grm. ammonium chloride. Haldane, Wigglesworth and Woodrow found no significant change in the inorganic phosphorus of the serum during acidosis but observed a slight fall as the acidosis was passing off. In the results recorded in Table 1, where the estimations were made after a variable period from the commencement of the acidosis, no constant change in either calcium or phosphorus was observed. In another two subjects more frequent analyses of the serum calcium and phosphorus were made during the administration of ammonium chloride (figs. 1 & 2). In both, the serum calcium showed an initial rise which persisted till the fifth day, thereafter falling below the normal level: on the cessation of ammonium chloride administration the calcium immediately rose somewhat above the "control" level and then returned to normal. The serum phosphorus moved in the inverse direction to calcium.

E. Non-protein Nitrogen. In every case this was found

TABLE 3.

Blood Analyses in Cases of Clinical Acidosis.

Name	Condition	CO_2 Vol.%	N.P.N.Mg.%
J.H.	Acidosis;cyclical vomiting Recovered 2 days	36.5 48.8	60.0 40.0
H.P.	Gastro-enteritis Recovered	37.6 57.0	76.4 38.0
H.F.	Acidosis; ?gastro-enteritis Recovered	28.7 89.0	78.0 47.3
Mc.P.	Acidosis;gastro-enteritis	26.8	52.1
E.J.	Acidosis	36.1	109.1
E.F.	Gastro-enteritis	28.7	78.1
C.K.	Ileo-colitis	32.7	53.1
J.McG.	Gastro-enteritis	27.5	86.1

to be increased. In the two cases in which it was determined twice during the administration of ammonium chloride, the value had fallen at the second estimation. Keith and Whelan have shown that there is no increase in the ammonia content of the blood after ingestion of ammonium chloride. In the present series if the increase in non-protein nitrogen had been due to ammonia there would have been a rise of 8.5 to 15.8 mgrm. per cent. in the ammonia value. Such an amount of circulating ammonia would almost certainly have produced marked symptoms of poisoning, as ammonia is a very toxic substance. It is, therefore, highly improbable that the increase in non-protein nitrogen is due to ammonia nitrogen. A more reasonable explanation is that the rise is the result of increased breakdown of tissue proteins consequent on the disturbance of acid-base equilibrium. This view is supported by blood analyses in some cases of frank clinical acidosis (Table 3.) It will be seen that in three cases during the stage of acidosis the non-protein nitrogen was increased, to fall to normal in the post-acidotic period. In cases of alkalosis where it also rises the suggestion has been put forward that the cause is renal inefficiency. This can hardly have been the case here since, as will be seen/

TABLE 4

Showing Intake, Faecal and Urinary Outputs, and Retention of Calcium and Phosphorus . (grams).

Name	Period		Total intake		Faecal Output		Urinary output		% of total output in urine		Total Retention		Retention per kg. per day.	
	Intake	Days	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5
N.G.	Normal	7	20.16	27.72	9.508	6.818	1.406	11.55	12.8	62.8	9.246	9.352	.05	.05
	NH_4Cl	7	20.16	27.72	12.445	8.984	2.949	12.222	19.0	57.6	4.766	6.514	.025	.034
W.C.	Normal	7	20.16	27.72	14.983	14.390	1.521	9.464	9.2	39.7	3.66	3.87	.018	.019
	NH_4Cl	7	20.16	27.72	15.938	14.564	2.60	12.600	14.0	46.4	1.62	0.56	.008	.002
J.F.	Normal	6	13.44	18.48	8.621	7.494	0.683	7.686	7.3	50.6	4.136	3.30	.034	.027
	NH_4Cl	6	13.44	18.48	11.158	9.564	2.133	7.812	16.0	44.9	0.149	1.104	.001	.009
	NH_4Cl	6	13.44	18.48	8.823	7.728	2.099	7.392	19.0	48.9	2.518	3.36	.021	.028
N.M.	Normal	7	19.60	26.95	10.959	9.627	1.784	11.102	14.0	53.5	6.857	6.121	.033	.029
	NH_4Cl	7	19.60	26.95	19.649	17.445	3.192	11.928	13.7	40.7	-3.241	-2.759	-.015	-.013
	NH_4Cl & cod-liver oil	5	14.00	19.25	17.799	16.425	2.829	10.212	13.7	38.3	-6.608	-7.387	-.044	-.050

TABLE 5

Showing Ash, Calcium, Phosphorus and Fat

Content of Faeces.

Daily Figures (g.)

Name		Period 1 (normal		Period 2 (NH_4Cl)	
		Total quantity in faeces grm.	% in faeces	Total quantity in faeces grm.	% in faeces
N.G.	Faec.wt.(Dried)	8.94	-	9.51	-
	Ash	2.843	31.8	3.443	36.2
	CaO	1.358	15.0	1.778	18.0
	P_2O_5	0.974	10.9	1.283	13.0
	Total fat	3.105	34.75	2.784	29.28
	Comb.fatty acids	2.542	28.45	2.237	23.53
	Free fatty acids	0.170	1.9	0.234	2.46
	Neutral fat	0.393	4.4	0.313	3.29
W.C.	Faec.wt.(Dried)	12.24	-	12.63	-
	Ash	4.993	40.8	5.178	41.0
	CaO	2.140	15.0	2.277	18.0
	P_2O_5	2.056	14.0	2.081	14.0
	Total fat	4.218	33.7	3.528	27.94
	Comb.fatty acids	2.491	20.6	2.000	15.84
	Free fatty acids	0.976	8.19	1.041	8.24
	Neutral fat	0.594	4.91	0.487	3.86

TABLE 5 (continued)

Name		Period 1 (normal)		2 (NH_4Cl)		3 (NH_4Cl)	
		Total quantity in faeces grms.	% in faeces	Total quantity in faeces grms.	% in faeces	Total quantity in faeces grms.	% in faeces
.F.	Faec.wt.(Dried)	9.39	-	13.29	-	10.14	-
	Ash	3.117	33.2	4.186	31.5	3.326	32.8
	CaO	1.437	15.3	1.860	14.0	1.471	14.5
	P_2O_5	1.249	13.3	1.594	12.0	1.288	12.7
	Total fat	3.45	36.79	5.564	41.89	3.333	32.92
	Comb.fatty acids	2.335	24.95	3.03	22.81	2.107	20.81
	Free fatty acids	0.815	8.652	2.174	16.37	0.928	9.18
	Neutral fat	0.300	3.188	0.360	2.71	0.297	2.93
.M.	Faec.wt.(Dried)	8.65	-	13.69	-	18. 05	-
	Ash	3.529	40.8	6.119	44.7	7.834	43.4
	CaO	1.565	18.0	2.807	20.0	3.556	19.0
	P_2O_5	1.375	16.0	2.492	18.0	3.285	18.0
	Total fat	2.85	33.03	3.936	28.75	6.58	36.45
	Comb.fatty acids	2.204	25.48	2.909	21.25	5.008	27.75
	Free fatty acids	0.236	3.43	0.602	4.40	0.826	4.58
	Neutral fat	0.376	4.12	0.424	3.10	0.743	4.12

seen later, there was a definite increase in the urinary output of ammonia, a good indication of unimpaired renal function.

METABOLISM OF CALCIUM AND PHOSPHORUS.

The intake and output of calcium and phosphorus in each case is detailed in Table 4 which also shows the retention and the partition of these substances between urine and faeces. In Table 5 are given the results of the faecal analyses for calcium, phosphorus and fat. It will be convenient to give a brief account of the results in each case, and thereafter to summarise and discuss the bearing of these findings on the general problem of mineral metabolism.

N.G. The retentions of CaO and P_2O_5 were reduced. The output of lime in the faeces was increased as the result of an increased percentage of CaO in the faeces and also a rise in the weight of the dried faeces. The urinary excretion of lime was also increased. The output of P_2O_5 by the urine was likewise raised, but the decreased retention of P_2O_5 was mainly the result of an increased faecal content of this substance. As with lime, the rise in faecal phosphorus was consequent on an increased percentage output together with a rise in the faecal weight. The percentage of ash was also increased/

increased, while the percentage and absolute amounts of total fat and combined fatty acids were decreased.

W.C. The results here were practically identical with those recorded in N.G. One point of difference may be noted, namely, the fact that there was but little increase in the faecal output of P_2O_5, the lowering of the retention value being due to a fairly marked rise in the urinary content.

J.F. In this case there were two successive periods on ammonium chloride. The retentions of CaO and P_2O_5 were reduced during the first of the two periods but returned practically to normal in the second. There was a rise in the urinary CaO in both periods, and the urinary P_2O_5 in each was practically unchanged. The reduced retantions of lime and phosphorus were due entirely to the increased faecal output resulting from a rise in the faecal weight; the percentages of ash, calcium and phosphorus were all reduced. The percentage of total fat in the faeces was increased during the first period because of the marked increase in free fatty acids, while in the second period the value for total fat was slightly below that of the normal. The percentage of combined fatty acid was reduced during

both periods.

N.M. With this subject there were two periods on ammonium chloride, the first for 7 days and the second for 5 days during which time 4 c.cm. of cod-liver oil was given three times daily in addition. A negative balance of both lime and phosphorus was found during each ammonium chloride period, being much more marked on the second. The urinary output of lime and phosphorus was increased on both occasions, the increase in urinary phosphorus being much more marked in the second of the two periods. The percentages of lime, phosphorus and ash in the faeces were increased, but the marked rise in the total faecal output of CaO and P_2O_5 was chiefly the result of the striking increase in the weight of the dried faeces. In the first period the percentages of total fat and combined fatty acids were reduced but in the second, during which cod-liver oil was also administered, these percentages were increased slightly above those in the normal period.

A survey of these results indicates that there was a reduction in the retention of lime and phosphorus in all four cases. Increased excretion of calcium was apparent in both urine and faeces, in two instances being more marked in/

in the latter and in one in the former. The increased output of phosphorus was more marked in the faeces in three cases.

In spite of the large amount of work done in connection with calcium metabolism there is as yet no definite information as to the extent of absorption of this element, and it is still a matter for conjecture how much of the faecal calcium has been absorbed and excreted through the bowel wall, and how much has passed through the gut unabsorbed. Grosser found that subcutaneous injection of calcium salts led to an increased excretion by the bowel, and Salvesen showed that in parathyroidectomised dogs, of calcium chloride injected intravenously, nine-tenths was excreted in the faeces and one-tenth in the urine. Percival and Stewart isolated the large intestine in cats and found that the intravenous administration of calcium chloride was followed by a marked increase in the excretion of calcium by the large intestine, but no change in the urinary output. Mainzer showed that intravenous administration of calcium chloride led to acidosis presumably because the bulk of the calcium was excreted by the gut, Bauer, Albright and Aub have published the results of an investigation of the calcium metabolism on a very low calcium intake in 13 normal adults,

on/

on whom there were 46 three-day periods of investigation. With the exception of a single period in one case, they found in all a negative balance of calcium, and with the exception of three periods there occurred in the faeces a greater amount of lime than had been ingested. In one case (N.M.) of the present series during each of two periods on ammonium chloride there was a greater amount of calcium in the faeces than had been ingested. From these results it is justifiable to conclude that in ammonium-chloride acidosis, excretion of calcium through the bowel wall can occur.

In one period at least (N.M., 2nd period) there was unequivocal evidence of calcium excretion by the bowel wall. As there was no alteration in the lumen of the gut other than the temporary presence of ammonium chloride which will be shown was practically completely absorbed, it seems reasonable to assume that absorption of lime was unaltered during the ammonium chloride period, so that the excess of faecal calcium must have been the result of excretion by the bowel wall. On similar grounds it would appear that the excess of faecal phosphorus in the ammonium chloride period was the result, not of decreased absorption, but of increased excretion through the wall of the intestine.

Goto/

Goto reported an increased excretion of calcium by the urine in acidotic conditions. Indeed, the investigations of Nelson on the mineral metabolism of diabetic patients and of epileptic subjects fed on ketogenic diets have shown that the kidneys are capable of excreting large amounts of lime, so much so that more than half of the total output of calcium may take place through the urinary system. Keith and Whelan, however, observed but little change in the urinary calcium during administration of ammonium chloride. In the present series the percentage of total output of calcium appearing in the urine was increased in three cases while the actual amount of lime excreted in the urine was greater in the ammonium chloride period in all four cases. Albright, Bauer and Ropes and Bernhardt, who also found an increased renal excretion of calcium concluded that this increase was due to diversion from the faeces but attributed this effect to a systemic acidosis rather than to any change in the reaction of the intestinal contents. In all four cases recorded here, the actual amount of calcium found in the faeces was increased during the ammonium chloride period so that diversion from the faeces could not have played a part in the increased renal excretion. In Nelson's cases also the rise in/

TABLE 6.

Showing Intake, Output and Retention of Chlorine (c.cm.N/10 Cl).

Name	Period	Intake	Output		Retention	
			Urine	Faeces	Total	Per kg. per day
N.G.	Normal	4133	3585	36	+512	+2.8
	NH_4Cl	10675	9518	40	+1117	+6.1
W.C.	Normal	4133	3815	15	+303	+1.6
	NH_4Cl	10675	9853	17	+805	+4.3
J.F.	Normal	2755	2829	-	-74	-0.6
	NH_4Cl	8365	7299	-	+1066	+8.8
	NH_4Cl	8365	7656	-	+709	+5.8
N.M.	Normal	4018	3706	-	+312	+1.5
	NH_4Cl	10560	10040	-	+635	+3.0
	NH_4Cl (5 days)	7545	7678	-	-133	-0.9

in urinary output was accompanied by slight increase in the faecal calcium. These results indicate, therefore, that the presence of acidosis induces an increased excretion of lime either by urine or faeces or both.

Fitz, Alsberg and L.J. Henderson and J.B.S. Haldane (1925) have shown that administration of ammonium chloride leads to an increase of phosphate in the urine. This has been confirmed by the present results which show that as with lime the excretion of phosphorus by the faeces is also increased. It would appear therefore that when there is a state of acidosis without alteration of the reaction of the intestinal contents both the urine and faeces may act as vehicles for the transport of excess minerals.

CHLORINE METABOLISM.

The excretion of chloride normally takes place through the urine and the sweat; the faecal output is practically negligible. The latter was estimated in two cases, and as will be seen from the results in Table 6 the faecal excretion of chloride was relatively minute both during the control and ammonium chloride periods.

During the control period there was a small retention of chlorine except in the case of J.F. where there was a very slight/

TABLE 7.

Percentage Excretion of Chlorine in First 24 hours following Ingestion of Sodium Chloride.

Amt.of NaCl given (g.)	Form in which NaCl given	Diet	% Excretion of extra salt during lst 24 hrs.
4.5	Saline	Salt poor	53
4.5	Saline	Salt rich	62
10.0	Solid in capsule	Salt poor	50
10.0	Solid in capsule	Salt rich	67

Fig. III.

Graph Showing Daily Urinary Output of Water, Chlorine, Fixed Base, Ammonia and Titratable Acid.

Case N.G.

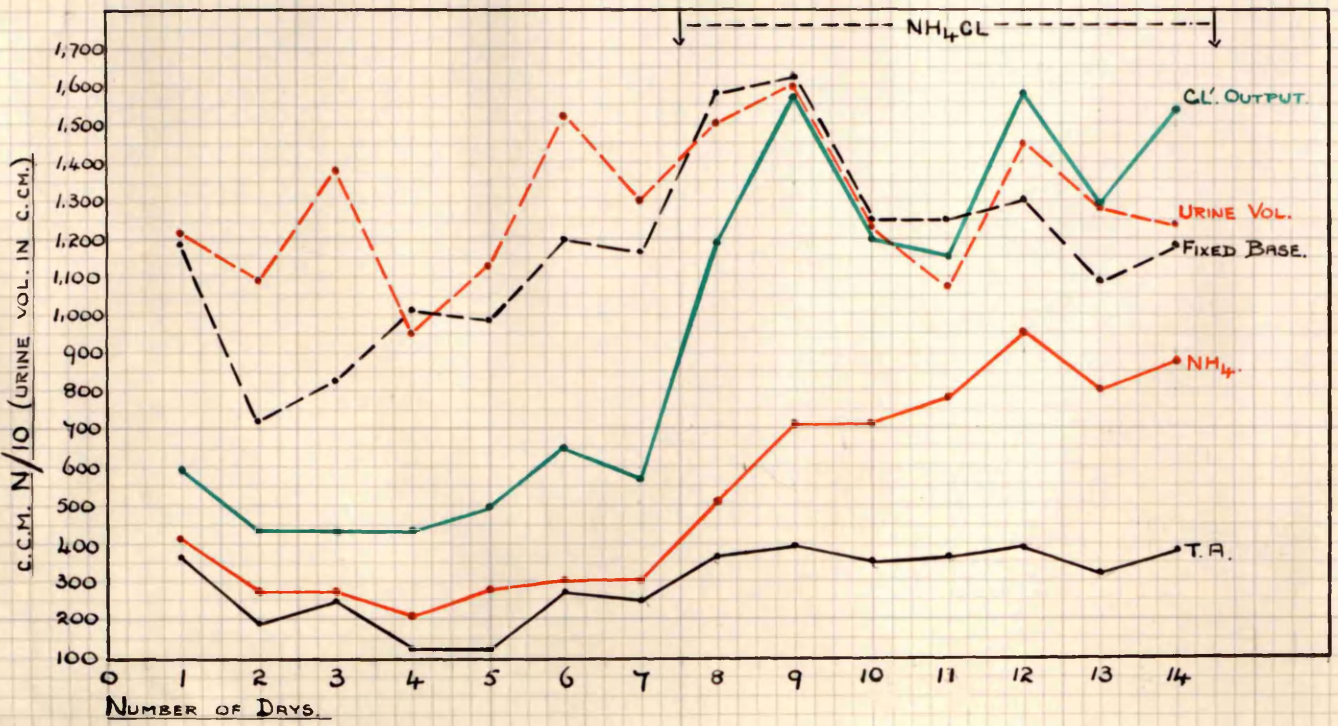


Fig. IV.

Graph Showing Daily Urinary Output of Water, Chlorine, Fixed Base, Ammonia and Titratable Acid.

Case W.C.

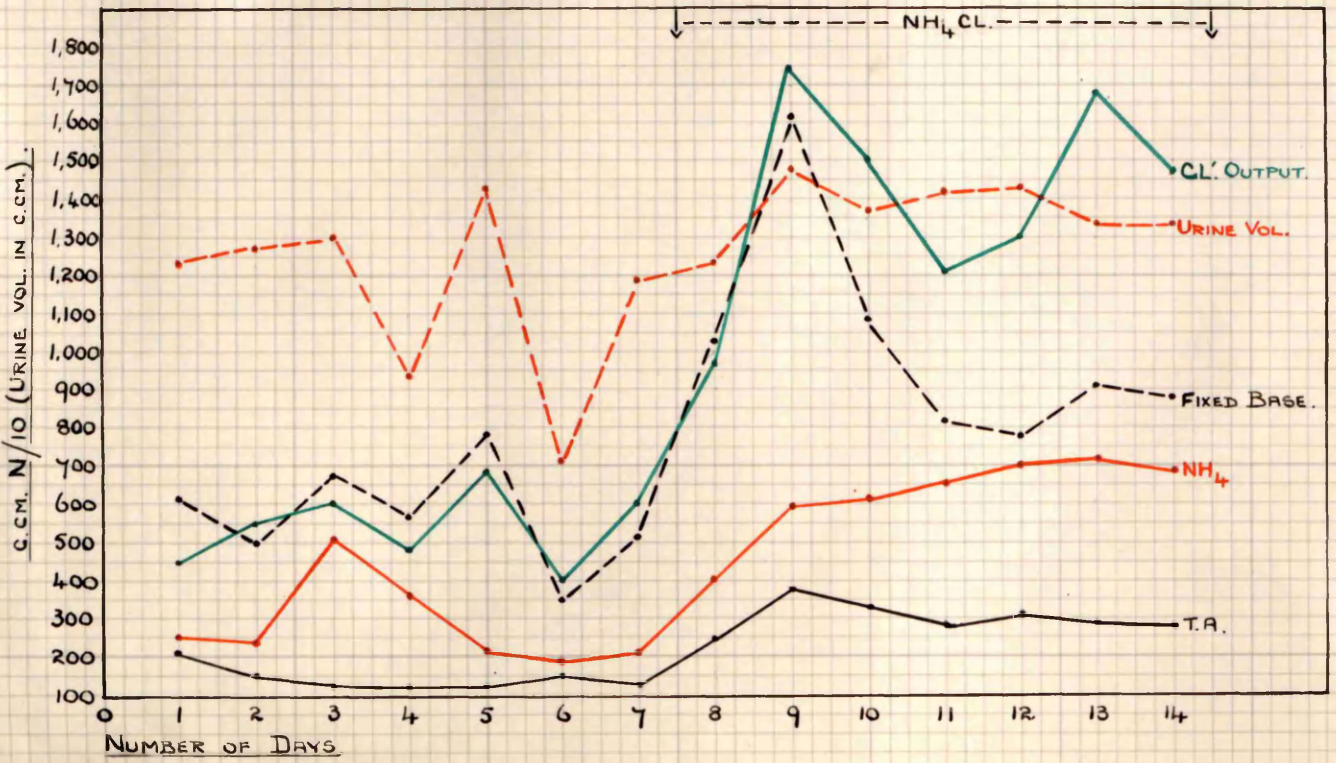


Fig. V.

Graph Showing Daily Urinary Output of Water, Chlorine, Ammonia and Titratable Acid.

Case J.F.

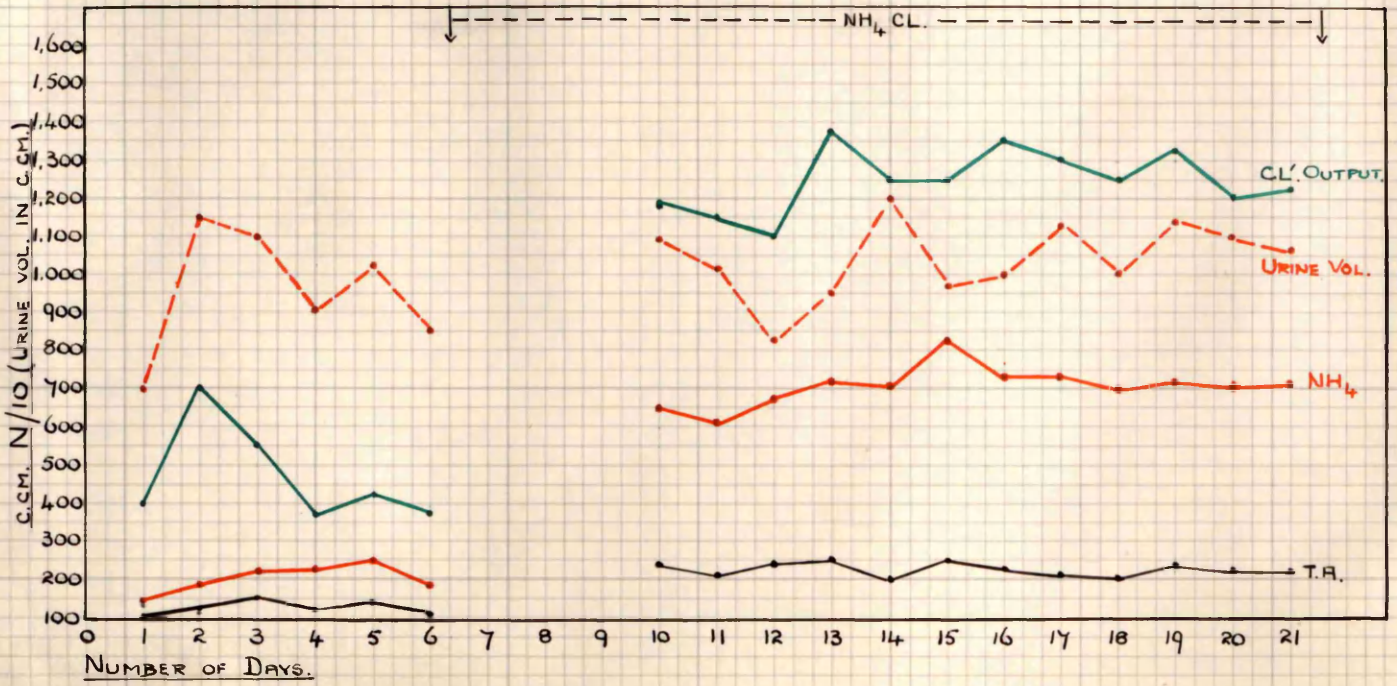
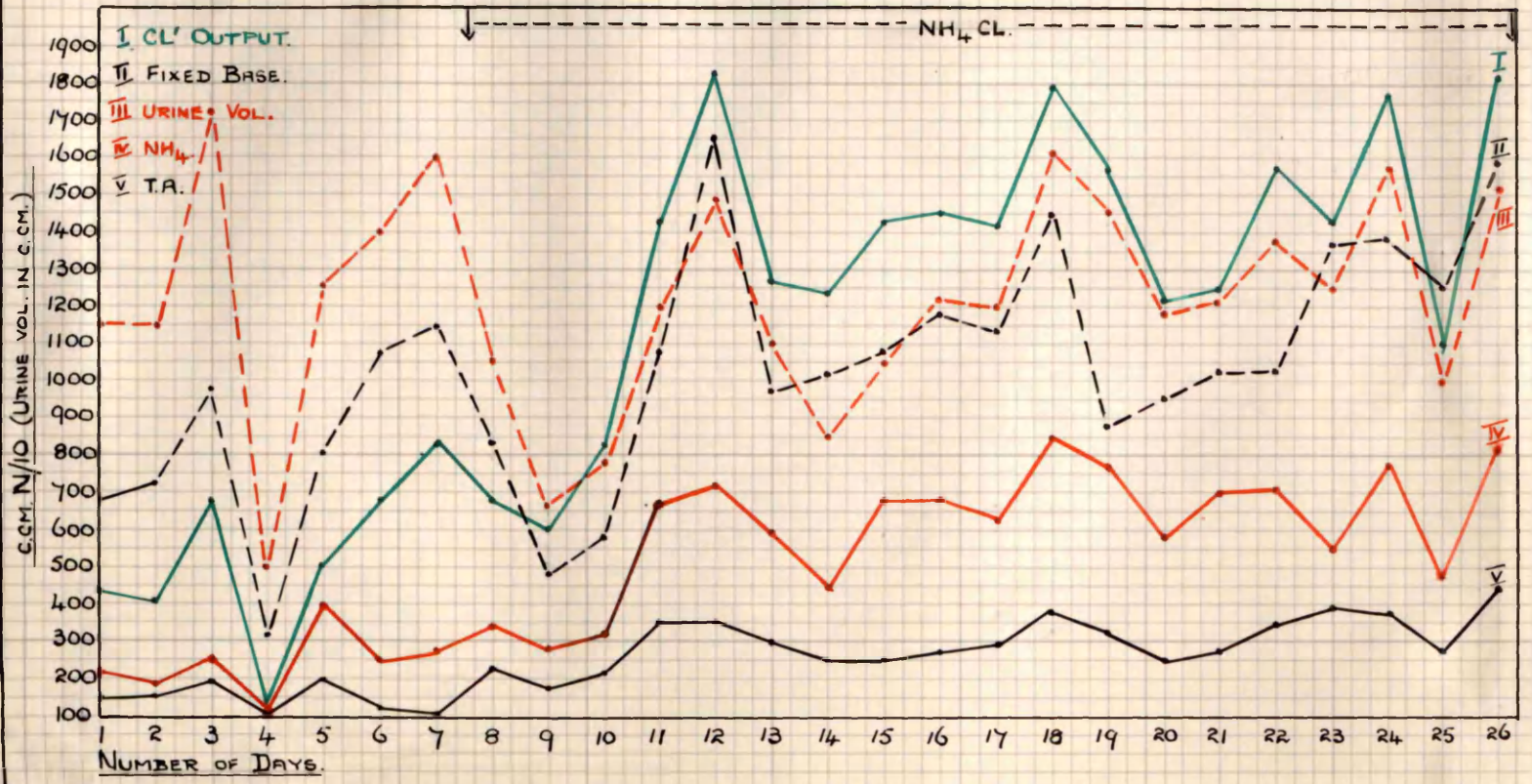


Fig. VI.

Graph Showing Daily Output of Water, Chlorine, Fixed Base, Ammonia and Titratable Acid.

Case N.M.



slight negative balance. While ammonium chloride was being administered the retention was increased in every case except during the second period of N.M., which was characterised by a slight negative balance.

When one comes to examine the daily figures it is plain that all the subjects reacted immediately to the extra chlorine by the excretion of a greatly increased amount of this substance in the urine. In N.G. the output was doubled on the first day, so that only about 30 per cent. of the extra chlorine had been retained. This corresponds to what happens when sodium chloride is given. Table 7 indicates the percentage excretion of chlorine in the first 24 hours following ingestion of sodium chloride: the subject was a healthy boy aged 11 years.

With the exception of N.M. the daily curve of NaCl output (Fig. 3-6) during administration of ammonium chloride shows that the peak of chloride excretion occurred on the second or third day. This was followed by a drop lasting two or three days, which was succeeded on the fifth or sixth day by a peak reaching almost to the level of the first. In the case of N.M. there was a complete absence of the first peak but the second was quite marked. It will be noted in every/

every case that this second peak corresponded with the maximum rise in the output of ammonia. It is therefore fair to conclude that this secondary rise in the excretion of chlorine was associated with the increased ability of the kidney to supply ammonia. Indeed, a glance at the chlorine and ammonia curves following this second peak shows in every case some parallelism, indicating a correlation between these two substances. This parallelism is not noticeable during the first five days of ammonium chloride administration. There is also a fairly marked correlation between the amount of urinary chloride and fixed base both during the control and the ammonium chloride periods, with the exception of the control period of N.G. The urinary volume and chlorides also show some parallelism especially during the ammonium chloride periods.

URINARY VOLUME,

Gamble, Ross and Tisdall reported an increase of urinary volume in children during the administration of either calcium or ammonium chloride. Keith and Whelan, however, found no change in the volume of urine excreted by a normal individual during ingestion of ammonium chloride. During such administration there was in all the cases except the first/

Table 8.

Showing Intake, Urinary and Faecal Outputs and Retention of Fixed Base (c.cm.N/10 Monovalent Base).

Name	Period	Intake	Output			Retention	
			Urine	Faeces	Total	Total	Per kg.per day
N.G.	Normal	16254	7172	4561	11733	4521	24.5
	NH_4Cl	16254	9105	5397	14502	1752	9.4
W.C.	Normal	16254	5442	6579	12021	4233	22.4
	NH_4Cl	16254	8434	6695	15129	1125	6.0
J.F.	Normal	10836	4494	4135	8629	2207	18.1
	NH_4Cl	10836	5465	5036	9501	1335	11.0
	NH_4Cl	10836	5841	4069	9910	926	7.5
N.M.	Normal	15803	5726	5563	11289	4514	21.5
	NH_4Cl	15803	8229	8039	16268	-465	-2.2
	NH_4Cl	11290	6616	7617	14233	-2943	-19.6

first period of N.M. an increase in urinary volume, not, however, as marked as might have been expected. The daily output of urine varied greatly, frequently falling much below the maximum observed in the control period (Figs. 3-6).

AMMONIA AND TITRATABLE ACIDITY OF THE URINE.

The output of ammonia and titratable acid was increased in every case during the administration of ammonium chloride. (Figs. 3-6). The maximum output of titratable acid was reached by the second day, following which there was usually a very gradual decline in the output. The ammonia content of the urine did not attain its greatest value till the 5th or 6th day, and in the case of J.F. the 9th day. Thereafter the output of ammonia remained at a constant level, except in the case of N.M. where considerable variations were observed from day to day. The ammonia output was not estimated in the days following the ammonium chloride period, but Gamble and others have shown that the output remains definitely above normal for some days following the administration of acid salt.

FIXED BASE.

The output of fixed base (Table 8) was with the exception of the control period of W.C., and the second ammonium chloride period of N.M., somewhat greater by the urine than/

TABLE 9 .

Showing Relationship of Outputs of Calcium and Total Fixed Base.

Name	Period	% of calcium to output of total fixed base	
		Urine	Faeces
N.G.	Normal	7.0	74.3
	NH_4Cl	11.5	82.4
W.C.	Normal	10.0	89.8
	NH_4Cl	11.0	99.0
J.F.	Normal	5.4	74.1
	NH_4Cl	14.0	79.0
	NH_4Cl	12.8	77.5
N.M.	Normal	11.1	70.1
	NH_4Cl	13.8	87.6
	NH_4Cl	15.0	83.3

than by the faeces. The faecal output of base was chiefly composed of calcium which constituted from 70 to 90 per cent. in the control periods and 77 to 99 per cent. in the ammonium chloride periods (Table 9). Thus, not only did ammonium chloride increase the output of fixed base by the faeces, but it also raised the proportion of calcium to other base. In the urine the calcium formed 5.4 to 11.1 per cent. of the fixed base in the control periods and 11 to 15 per cent. in the test periods. The urine, therefore, showed during administration of ammonium chloride an increase in total fixed base, and a slight rise in the relative proportion of calcium to other base. The urinary fixed base reached its maximum within three days of the commencement of administration, thereafter falling to slightly above the average level of the control period (Figs. 3-6). This is in agreement with the findings of Gamble, Blackfan and Hamilton with several acid-producing salts. The retention of fixed base varied from 18.1 to 24.5 c.cm. N/10 per kilo of body weight per day during the control periods. It was always diminished during ammonium chloride administration, and was negative during both the periods of N.M., the loss being entirely accounted for by calcium.

METABOLIC REACTIONS TO ACIDOSIS.

Against the production of a non-gaseous acidosis such as is produced by ammonium chloride the organism has the following general defences:- (1) an increase in the available base of the blood; (2) an increased excretion of volatile acid by the lungs; and (3) an increased supply of base for neutralizing acids that are to be excreted.

(1) INCREASE IN AVAILABLE BASE OF THE BLOOD. - The fixed base is maintained at a fairly constant level. By a reduction in the carbon dioxide content of the blood a certain amount of base is freed and rendered available for the neutralization of other acid radicles. It has been shown that the base-combining powers of protein and inorganic phosphorus are reduced with a fall in the pH of the blood, but the amount of base released by these changes in such a condition as prevails in the experiments detailed here is practically negligible. The fall in the blood carbon dioxide, therefore, undoubtedly constitutes the chief immediate response to acidosis of the non-gaseous variety. In the subjects of this study the increase in available base produced/

produced by the reduction in carbon dioxide could not have amounted to more than 230 c.cm. N/10 (on the assumption that the blood-volume was one-thirteenth of the body weight). One gram of ammonium chloride (i.e. one-fifth of the daily intake) contains 187 c.cm. N/10 acid so that two such doses were much more than sufficient to use up all the base made available by the reduction in carbon dioxide. The relief afforded by this means could only have been temporary, since prolonged administration of ammonium chloride did not to any extent further reduce the carbon dioxide content of the blood.

In clinical acidosis figures for total carbon dioxide have been noted much lower than the lowest in the present series. Such low figures supply definite evidence of an inability of the other compensatory mechanisms to deal with the situation either because of the suddenness of the demands (as in Haldane's case with an avalanche of 25 grams of ammonium chloride), or because of the functional inefficiency, relative or absolute, of the other defensive reactions, as in diabetic or uraemic coma. One point of practical interest arises from a consideration of the results of this investigation. It will be remembered that the reduction in the carbon dioxide content of the blood reached its maximum very shortly after

ammonium chloride administration was commenced, due in all probability to efficiency of the regulating mechanism outside the blood. Accordingly if in any case of clinical acidosis the carbon dioxide content of the blood falls persistently from day to day, it may be taken as an indication that the other regulating reactions are unable to cope with the amount of acid and that it is essential to supply fixed alkali in the form of sodium or potassium salts to neutralise and transport the acid.

(2) INCREASED EXCRETION OF VOLATILE ACIDS BY THE LUNGS. - Respiratory changes leading to an increased output of carbon dioxide must naturally follow its displacement from the union with base. Otherwise the tension of carbon dioxide in the blood would increase and lead to the lowering of the pH of the blood. In the present series there were no marked respiratory alterations so that the excretion of the extra carbon dioxide must have been of such relatively small amount that no apparent strain was put on the respiratory system.

(3) SUPPLY OF BASE FOR THE EXCRETION OF ACID. - The kidney is undoubtedly the principal organ for the excretion of the non-volatile acid radicles. The sweat glands may play an/

an important part in the metabolism of chlorine when large amounts of sweat are produced, although Schwenkenbecker and Spitta conclude that not more than one gram of sodium chloride is excreted daily even during profuse sweating. In the absence of hyperidrosis, at any rate, it is justifiable to assume that the amount of electrolyte lost in this way is practically negligible. The bowel certainly plays a part in mineral metabolism, but as far as the actual excretion of chlorine is concerned the intestinal output is negligible. Katzenstein has shown that administration of either acid or alkali has no effect on the pH of the faeces although the urinary reaction is immediately affected while Scheer concludes that the acidity of the faeces in infants is not due to excess of acids over bases in the diet but to processes such as bacterial action in the intestine. Accordingly one may conclude that the extra acid supplied in these experiments must have been excreted by the kidneys.

At the lowest possible value of the urinary pH chlorine cannot be excreted as a free acid, so that it requires a full equivalence of base. This base can be obtained in three ways. (a) Base may be released from weak acids which can be excreted either free or with only a partial complement of base. (b) Extra ammonia may be formed. (c) Fixed base may/

may be supplied from the tissues and tissue-fluids.

(A) Release of Base from weak Acids. - This is, of course, an accompaniment, if not the result, of increased acidity of the urine, which decreases the base-combining powers of the weaker acids. Change of phosphate from the mono-hydrogen to the di-hydrogen variety forms the best example of the saving of base effected in this way.

If it is assumed that during the control period the pH of the urine was 6.81, and during the NH_4Cl period 5.91, the amount of base saved by change of phosphate from Na_2HPO_4 to NaH_2PO_4 may be calculated as follows:-

at pH 6.81 - 50 per cent. of phosphorus is in the form of NaH_2PO_4

" " 5.91 90 " " " " " " " " " "

Of 1000 mgrm. phosphorus,

at pH 6.81 500 mgrm. are present as NaH_2PO_4

" " 5.91 900 " " " " "

In changing, therefore, from pH 6.81 to 5.91, 400 mgrm. are converted from the mono - to the di-hydrogen variety. Since one H-ion is involved in the change of each phosphate molecule it would require 1 litre of normal acid to change 1 litre of normal phosphorus (i.e., 31 grm. phosphorus) from the mono - to the di-hydrogen phosphate.

To/

To change 400 mgrm. P. would require $\frac{0.400 \times 1000}{31}$ c.cm. N/1 acid, (i.e., 13 c.cm. N/1 acid).

By change of urinary pH from 6.81 to 5.91 there will be a saving of 130 c.cm. N/10 base for every gram of phosphorus excreted.

This saving is indicated by the increase in titratable acidity. The response of the urinary system in this direction reaches its maximum within a very short time of the commencement of ammonium chloride administration. The base so released amounts, however, to only a small part of that likely to be required in any but the very mildest forms of increased acid excretion, and it certainly would be hopelessly inadequate to meet the requirements of even the smallest degree of acidosis that could be recognised clinically.

(B) Increase in Ammonia Formation. - The work of Benedict and Nash has shown that ammonia is formed in the kidney. In cases of marked renal inefficiency the ammonia output is low: this must play an important part in the production of renal acidosis. In the subject with normal renal function the supply of ammonia forms a most important bulwark against acidosis. The increase in ammonia formation takes some time to reach its maximum. Some

Fig. VII A.

Showing Daily Output of Ammonia Before, During and After Administration of Ammonium Chloride. (g. V daily).

Case J.McF.

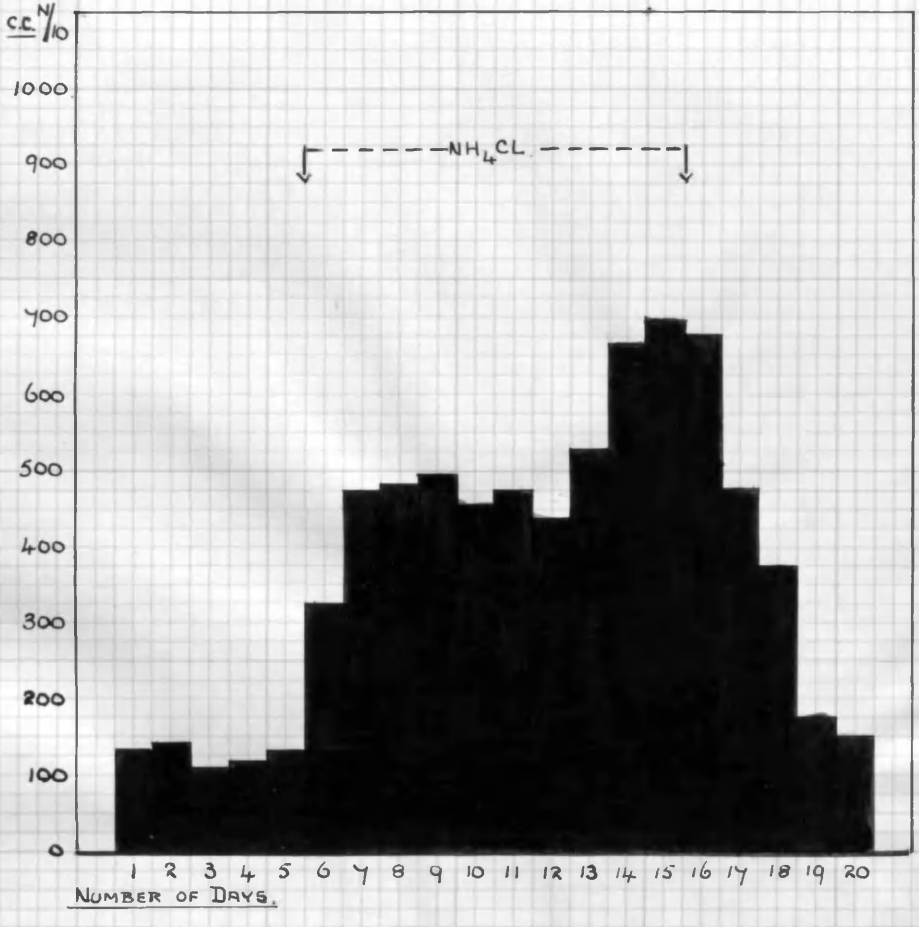
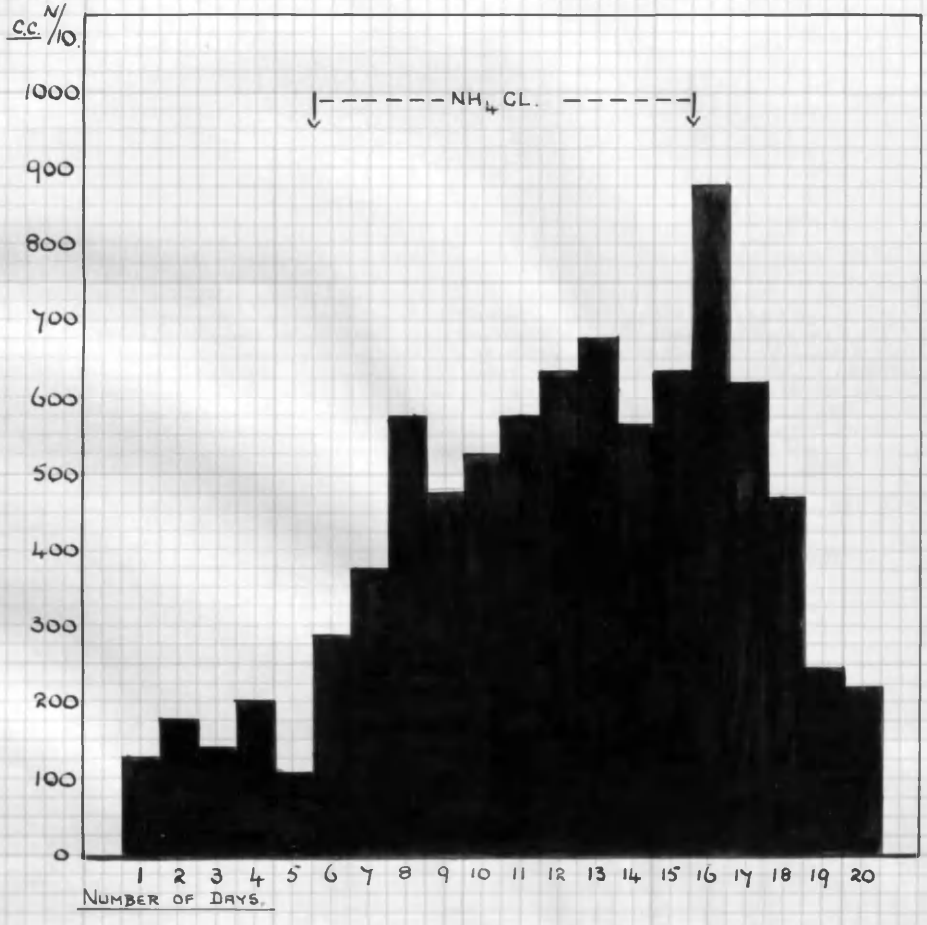


Fig. VIIB.

Showing Daily Output of Ammonia Before, During and After Administration of Ammonium Chloride (g. V daily).

Case E.Cr.



mechanism is therefore required to tide over the needs of the excretory system for more base until the supply of ammonia is sufficient to meet the demands. This mechanism will be discussed in the next section, but before leaving the question of increased ammonia production it is well to remember that the increase in ammonia output is continued after the need for increased acid excretion has ceased. This is well shown in the accompanying graphs (Figs. 7 A and B) where the output of ammonia before, during and after ammonium chloride administration is plotted. It is evident that the fall of urinary ammonia to the normal level does not take place till the fifth day after the cessation of ammonium chloride ingestion. As Gamble and his co-workers have pointed out this continued formation of ammonia is of vital importance in restoring the depleted stores of body to normal.

(C) Supply of Fixed Base from Tissues and Tissue-Fluids. - This method provides the chief immediate means whereby excess anions are excreted. The base may be derived from the bones or from the other tissues in which latter case it must be accompanied by fluid in order to prevent disturbances of osmotic equilibrium.

In the bones calcium is found as phosphate with a small amount/

amount of carbonate. Accordingly the release of calcium entails the freeing of phosphorus which must also be excreted. In this transaction, however, there is a distinct saving of base. In bone two molecules of phosphorus neutralize three molecules of calcium (i.e., six equivalents of monovalent base). As excreted, however, the phosphorus in the urine is monovalent while in the faeces it has probably about the same valency as in the plasma, namely, 1.8. Accordingly for every equivalent of bone phosphorus excreted in the urine we have the saving of two equivalents of base, while for every equivalent in the faeces the saving effected is 1.2.

If it is assumed that the excess of calcium excreted is derived in proportionately equal amounts from the carbonate and phosphate of the bone, then one-fifth of this excess comes from the carbonate. Therefore the amount of base rendered available by the release of calcium carbonate may be calculated as the equivalents of monovalent base contained in one-fifth of the total excess calcium found in the excreta. The amount of base obtained from the phosphate may be calculated from the excess phosphorus as follows:-

(Excess urinary P. in c.cm.N/10X2.0)+(Excess faecal P.in c.cm. N/10 X 1.2)

It may be objected that not all of the extra calcium and phosphorus/

phosphorus come from bone. As the calcium content of the non-osseous tissues is relatively minute, bone must form the chief source of calcium. Phosphorus, however, plays an important part quantitatively in practically all metabolic processes. In three of our cases there was more extra phosphorus than extra calcium found in the excreta, thus showing that bone is not the source of all the extra phosphorus. The amount of extra phosphorus excreted from the extra-osseous source probably forms, however, only a small fraction of the total, and can only modify slightly the saving of base as calculated from the above formula.

The fixed base from the non-osseous tissues consists almost entirely of sodium and potassium, the former derived chiefly from extra-cellular, the latter from intra-cellular constituents. Both, however, are associated with an equivalent amount of acid. If this base is excreted, some means must be found for dealing with its anions. Of these carbon dioxide is excreted by the lungs, while protein which holds about ten equivalents of base is probably catabolised. With the exception possibly of some of the organic acid, the remaining anions demand their full quota of base for neutralising purposes. The water carrying the base/

TABLE 10

Showing The Means Employed in Neutralising The Excess Acid.

Name	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
	Incr. in Cl'	Incr. in T.A.	Incr. in NH_4	Incr. base derived from			Tissue fluid base	Incr. in urinary volume	Calcula c.cm.N/ B.Cl pe 100 c.c excess H_2O
				$CaCO_3$	Faecal P'	Urinary P'			
N.G.	5933	917	3298	320	370	192	837	850	98.4
W.C.	6038	1172	2452	145	30	896	1343	1475	91.1
J.F.	4470 4827	527 503	3051 3670	285 116	355 40	36 nil	216 498	231 526	93.5 94.7
N.M.	6334	1150	2768	721	1340	236	121*	nil	-

*This amount of fixed base was probably supplied without the accompaniment of fluid by the reduction of the fixed base content of the blood by 15 c.cm. N/10 per 100 c,cm.

base must also be got rid of in order to prevent an upset of osmotic equilibrium. Accordingly for every 150 c.cm. N/10 base there will be rendered available only about 40 c.cm. N/10 for neutralising extra acid. This 40 c.cm. is made up of 24 c.cm. from B. HCO_3 and the remainder from B. protein and organic salts. The efficiency in supplying base is thus only 26 per cent. even when blood plasma is the fluid called upon, and must be less in the case of the tissue-juices where the protein content is lower. Accompanying this 150 c.cm. N/10 base will be 100 c.cm. water which will contain its normal quota of fixed acid (Cl', SO"4). The amount of tissue fluid excreted during the ammonium chloride period is indicated by the increase in urinary volume. The chlorine content of this excess in urinary volume should, if the hypothesis put forward be correct, approximate to that of the plasma.

In Table 10 are given the figures indicating the methods whereby the excess of excreted acid (chlorine) has been neutralised. Column 1 gives the values for the excess excretion of chlorine. Columns 2 to 7 indicate the amount of base derived from the sources indicated for the neutralisation of this excess chlorine. The figures in

columns/

columns 2 to 6 are calculated from the results of the analyses, but those in column 7 have been obtained as follows:- Cl - (T.A. + NH_4 + Base derived from bone). The results do not contain values for sulphates and organic acids which, as has been shown by Gamble, are also excreted in excess during acidosis. These acid radicles probably come from tissue juices and carry down with them their quota of base from the tissue-juices. Thus the calculation of the figures in column 7 in the manner stated obviates any error due to the presence of excess sulphates. The figures for base given in column 7 indicate the amount of tissue-fluid base combined with chlorine, in other words the amounts of B.Cl. Accordingly if these figures are divided by the corresponding increase in urinary volume (Column 8) the results obtained should give the value for the percentage of B.Cl in the tissue fluid (Column 9). It is evident that these values lie between 90 and 100, therefore within the normal limits of chlorine in plasma and presumably in tissue juice. Despite the fact that the method of calculation is, and must be, one of comparatively rough averages, the consistency of the values so obtained is sufficiently striking to afford strong support to the thesis which has been advanced.

Several/

TABLE 11.

Showing Output of CaO and P_2O_5 in g. per day before , during and after Administration of Ammonium Chloride.

Name	Period	Urinary Output		Faecal Output		Total Output	
		CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5
J.McF.	(1)7 days	0.247	1.884	0.899	1.082	1.146	2.966
	(2)3 "	0.299	1.950	-	-	-	-
	(3)7 "	0.284	1.517	1.506	1.759	1.790	3.276
	(4)3 "	0.152	1.560	-	-	-	-
E.Cr.	(1)7 days	0.216	1.243	0.188	0.265	0.404	1.508
	(2)3 "	0.247	1.368	-	-	-	-
	(3)7 "	0.282	1.364	0.838	1.043	1.120	2.407
	(4)3 "	0.140	1.133	-	-	-	-

Period (1) Diet only.
" (2) Diet + NH_4Cl gm.v 1st 3 days.
" (3) Diet + NH_4Cl gm.v 4th - 10th days incl.
" (4) Diet only, 1st 3 days after cessation of NH_4Cl administr.

Several further points may be noted. If the view proposed is correct, one would have expected that the bulk of the excess phosphorus would have been excreted in the urine, since output by the kidneys effects a saving of two equivalents of base per equivalent of phosphorus, whereas for faecal excretion the economy achieved is only 1. 2. The fact that the bulk of the excess phosphorus is not excreted in the urine is probably related to the fact that there is a close association between the calcium and phosphorus in the faeces.

Another point of interest is the different extent to which the various mechanisms are brought into play in reacting to excess acid. For the immediate supply of base both bone and tissue-juices are called upon. The one exception to this is the ammonium chloride period of N.M., where practically all the necessary fixed base was derived from bone; less urine was passed than in the control period indicating a lack of response on the part of the tissue fluids. As to the rapidity with which the osseous tissue responds to the stimulus of an acidosis, the urinary calcium and phosphorus output was determined in two children on constant diet before and during the first three days of ammonium chloride administration. The results (Table 11) indicate that extra calcium and phosphorus/

phosphorus are found in the urine during the first three days of ammonium chloride ingestion. They also show the fall in the urinary output of calcium and phosphorus immediately after the administration of ammonium chloride had ceased. H.L.White obtained an increased urinary output of phosphorus within four hours of the administration of acid, but considered this merely a temporary phenomenon. Haldane, Hill and Luck, however, found a definite increase in the urinary output of phosphorus for 24 hours following the ingestion of large amounts of calcium chloride. On the other hand Gamble, Blackfan and Hamilton observed no significant change in phosphorus excretion after the intake of moderately large amounts of acid. These divergent results would appear to be due to the fact that in some instances a large proportion of the extra phosphorus is excreted through the urine while in others it appears almost intirely in the faeces. Occasionally, as in Case J.McF., during the height of the acidosis produced by ammonium chloride the urinary output of phosphorus is decreased below the normal level although the total output is greatly increased.

A consideration of the evidence adduced shows the onset of acidosis is resisted by three main reactions, which may be termed (a) Haemato-respiratory, (b) Call on fixed base of the tissues, (c) Increased production of ammonia.

The haemato-respiratory defence meets the first onslaught of excess acid. It consists of various buffering mechanisms in the blood itself, resulting in the neutralisation of the strong acid and its replacement by one which is very poorly dissociated, carbon dioxide. The excess of free carbon dioxide is dealt with by the respiratory system. It is clear that the store of available carbonate is limited so that this defence must be very speedily reinforced to prevent severe acid-poisoning.

Very rapidly the kidneys respond with an increased output of un-neutralised acid but owing to the limits of hydrogen-ion concentration within which the kidneys work, such an increase can only account for relatively small amounts of acid. Accordingly the two other defensive processes must be called into play. While the two are probably brought into action almost immediately, ammonia production takes some days before it reaches its maximum. Accordingly the fixed base provides a temporary means of dealing with the excess acid. That it is only temporary is demonstrated by the fact that when renal efficiency is seriously impaired as in chronic interstitial nephritis, acidosis frequently ensues indicating that the amount of acid produced, although not excessive, has depleted the/

the store of fixed base.

The call for base is met by withdrawal of lime from the bones and potassium and sodium from the soft tissues and tissue fluids. In conditions associated with long continued excess acid production there often ensues decalcification and in the growing child dwarfism. Of more immediate urgency is the fact that withdrawal of sodium from the tissue fluids entails the removal of large amounts of water which generally leads to dehydration. This constitutes one of the chief dangers of acidosis and must be remembered when therapeutic measures are undertaken, especially when associated with the acidosis there is loss of fluid by the bowel. An attempt to make good the loss of tissue fluid is possibly the cause of the increased tissue catabolism which results in increased production of acid.

Last of all to reach its maximum is the increased production of ammonia. In a sense this defence is the most important and powerful of all since not only can it deal with relatively enormous quantities of acid but it can and does permit of the strengthening of the other defences. It has been pointed out that excess production of ammonia obviates the necessity of excreting large amounts of fixed base the store/

store of which can therefore be replenished. The importance of ammonia as a defensive agent against acidosis emphasises the importance of a plentiful supply of fluid. The beneficial effect of ammonia production can only be realised when it is excreted and the amount excreted depends in great part on the volume of urine.

SUMMARY.

The effect of prolonged administration of ammonium chloride on the metabolism was studied in four apparently healthy children. In none was any clinical manifestation of acidosis produced.

The changes in the blood consisted of:-

(a) Reduction in carbon dioxide content occurring early;

(b) Moderate increase in chlorine content which did not exactly compensate the deficiency in carbon dioxide.

(c) Slight increase in serum calcium and reduction in serum phosphorus.

The changes in metabolism consisted of:-

(a) Increased urinary output of titratable acid and ammonia. The latter did not reach its maximum till the sixth day/

day of ammonium chloride administration or later.

(b) Increased output of chlorine. Two peaks were apparent one on the second and one about the sixth day, the latter usually coinciding with the first maximum output of ammonia.

(c) Increased output of calcium and phosphorus by urine and faeces and decreased retention. Short term experiments on other subjects indicated that the increase in output of calcium and phosphorus probably occurred within three days of the commencement of ammonium chloride administration.

(d) Increased excretion of fixed base by urine and faeces.

---0---

Chapter Two.

Appendix A.

A NOTE ON THE EFFECT OF INGESTION OF HYDROCHLORIC ACID ON THE EXCRETION AND RETENTION OF MINERALS.

It has long been stated that diets in which mineral acid elements exceed fixed base tend to produce decalcification (Berg: Ferrier). Givens and Mendel, Goto, Stehle and McCarty, Fiske, Goodall, Hathaway and West, and Aub, Fairhall, Minot and Reznikoff have all shown that ingestion of mineral acids such as HCl leads to increase in urinary output of calcium and phosphorus. This has been attributed to the more acid reaction of the intestinal contents which as Telfer has shown causes a better absorption of minerals. The statement has been made by Bernhardt, Givens and Mendel and Irving that hydrochloric acid increases urinary calcium at the expense of the faecal content. Stehle and Shohl and Sato, however, found an increased faecal output of lime after the ingestion of hydrochloric acid.

Moreover/

TABLE 12

Showing Intake, Faecal and Urinary Outputs and Retention of Calcium and Phosphorus (g).

Name	Period		Total Intake		Faecal Output		Urinary Output		% of total output in urine		Total Retention	
	Intake	Days	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5
McI	Milk	7	16.80	24.64	13.754	13.869	0.608	8.512	4.2	38.0	2.438	2.259
	Milk HCl	7	16.58	24.08	13.350	11.92	1.766	11.408	11.6	48.9	1.460	0.752
W.	Milk	7	15.75	23.10	12.378	13.268	0.924	7.238	6.9	35.3	2.447	2.594
	Milk HCl	7	15.44	22.58	9.448	8.851	4.165	15.47	30.6	63.7	1.827	1.746

TABLE 13.

Showing Ash, Calcium, Phosphorus and Fat

Content of Faeces.

		Period 1. (normal)		Period 11. (HCl)	
		Total in g.	% in Faeces	Total in g.	% in Faeces.
R.McL	Faecal wt. (Dried)	57.55	-	59.6	-
	Ash	-	55.1	-	48.4
	CaO	13.754	23.9	13.350	22.4
	P_2O_5	13.869	24.1	11.92	20.0
	Total Fat	10.958	19.04	11.795	19.79
	Combined Fatty Acids	5.013	8.71	7.432	12.47
	Free Fatty Acids	3.403	5.74	2.736	4.59
	Neutral Fat	2.642	4.59	1.627	2.73
D.W.	Faecal wt.(Dried)	80.9	-	54.3	-
	Ash	-	37.8	-	40.0
	CaO	12.378	15.3	9.448	17.6
	P_2O_5	13.268	16.4	8.851	16.3
	Total Fat	15.848	19.59	10.061	18.53
	Combined Fatty Acids	8.882	10.98	5.560	10.24
	Free Fatty Acids	5.242	6.48	2.053	3.78
	Neutral Fat	1.723	2.13	2.450	4.51.

TABLE 14

Showing Titratable Acidity, Ammonia and Sodium Chloride Output in Urine.

Name	Period	Volume cc.	Titratable Acidity cc.N/10	Ammonia cc. N/10	Sodium Chloride cc.N/10.
R.McI	1(Normal)	7440	1067.6	1762.4	2342
	2 (HCl)	8155	2691.6	9596.0	13551.
D.W.	1(Normal)	7295	841.6	1334.4	2428
	2. (HCl)	8880	2501.0	8989.0	13636

Moreover the results obtained in ammonium chloride acidosis indicate that even without apparent alteration in the reaction of the intestinal contents the presence of increased amounts of acid in the body will lead to an increased excretion of minerals by the kidney. The following investigation was undertaken to determine whether the increased renal output of calcium and phosphorus could be entirely attributed to better absorption in the gut or whether the action of the increased amount of acid on the metabolic processes of the body also played an important part.

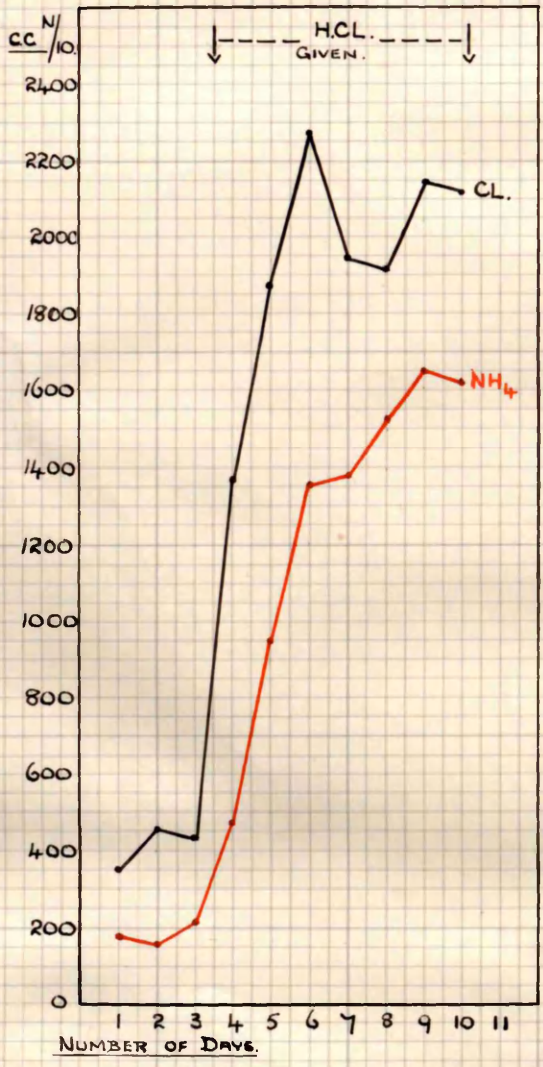
The effect of ingestion of hydrochloric acid was determined on two children who were kept on a constant milk-diet throughout the investigation. During the last seven days they were given 20 cc. of N/1 hydrochloric acid five times daily.

The results detailed in Tables 12, 13 and 14 correspond well with those obtained after ammonium chloride ingestion except that the faecal excretion of both phosphorus and calcium is reduced. The mineral retention is markedly diminished due to the very greatly increased output in the urine. Figure 8 which shows the daily excretion of ammonia and chloride indicates that as in ammonium chloride acidosis it/

Fig. VIII.

Showing Ammonia and Chlorine Output Before and During Administration of Hydrochloric Acid.

Case R. McI.



Case D. W.

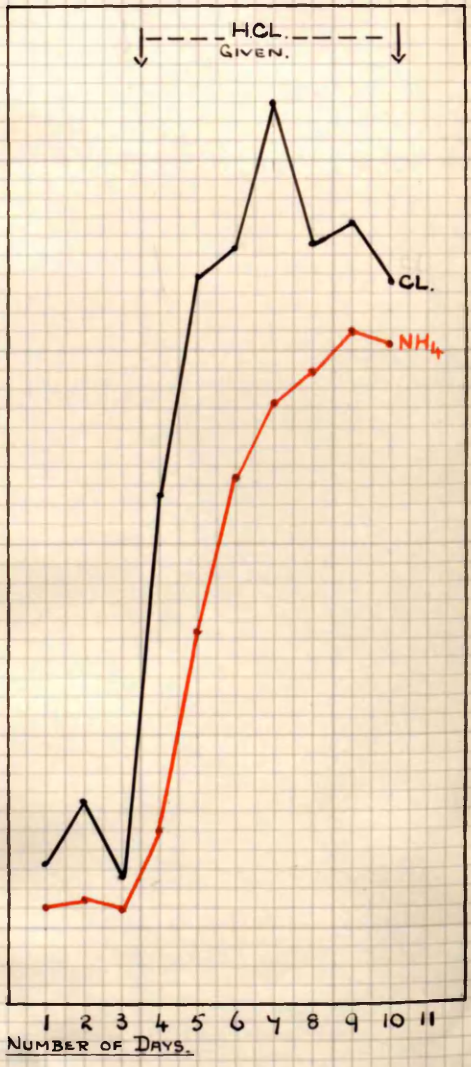


TABLE 15.

Showing the Means Employed in Neutralising the Excess Acid Excreted by the Urine.

Name	Increase in Cl.	Base released by incr. in T.A.	Base derived from $CaCO_3$	Base derived from Phosphate	Increase in Ammonia	Tissue fluid Base	Incr. in Urinary Volume	Calcula cc.N/10 BCl per 100 cc. Excess Urinary Water
R.McI.	11208	1559	54	271	7655	1569	1585	99.0
D.W.	11209	1552	22	1090	7834	711	715	99.0

All figures indicate cc.N/10 except those for urinary volume which are in cc.

it requires about six days for the ammonia output to reach its maximum. The first peak of chloride excretion occurs on the third or fourth day, later than was observed with ammonium chloride; but on the day of maximum ammonia excretion there is a tendency for a second chloride peak. Furthermore, when the source of supply of the base required for the transport of the extra acid is investigated in the manner previously suggested similar results are obtained. It is apparent from Table 15 that the calculated molar concentration of BCl in the excess urinary water excreted during the H Cl period falls within the normal limits of the chlorine concentration of the plasma.

Undoubtedly the diversion of much of the mineral excretion from the intestinal to the renal route is due to the increased acid reaction in the gut which renders the minerals more readily absorbed but in the light of the results recorded here it seems reasonable to suggest that the faecal and urinary outputs of minerals must be considered together and the increased total excretion referred to the call on the osseous tissues for more base. It is clear that the mechanisms involved in the transport of excess acid are similar whether the acid is given in the form of free acid or a neutral salt.

---0---

TABLE 16.

Showing Effect of Acidotic Conditions on

Urinary Excretion of Calcium.

Name	Nature of Acidosis	Urinary Output of CaO. (mg.per day)	
		Normal	Acidosis
N.G.	NH_4Cl	201	421
W.C.	NH_4Cl	217	371
J.F.	NH_4Cl	114	356
N.M.	NH_4Cl	283	456
McF.	NH_4Cl	247	284
Cr.	NH_4Cl	216	282
R.McI	HCl	87	252
D.W.	H Cl	132	595
J.G.	Diabetic + Ketosis	103	700
J.McK.	Diabetic + Ketosis	386	520

+ The normal period in these two subjects was obtained by administration of sufficient insulin to prevent glycosuria and acetomuria. I am indebted to Dr. F.J.Ford for these figures.

Chapter Two.

Appendix B.

A NOTE ON THE URINARY EXCRETION OF CALCIUM AND ACID-BASE EQUILIBRIUM.

Effect of Administration of Alkali on Mineral Metabolism.

A survey of the effects of ammonium chloride or hydrochloric acid ingestion shows that a constant feature is the increase in the output of calcium by the urine. The accompanying table (16) summarises the results already detailed in the previous sections. In addition it contains figures for the urinary calcium of output of two diabetic children on constant diet during two periods in one of which no insulin was given, thus causing a fairly well-marked ketosis. It is obvious that in acidosis produced by excess ketone-production there is also an increase in the excretion of calcium by the urine. These figures suggest that the amount of calcium eliminated by the kidneys is dependent on the amount of acid substance to be got rid of by the body. If this hypothesis has any basis in fact the administration of/

of alkali by lessening the amount of excess acid to be excreted ought to produce a definite decrease in the urinary output of calcium.

Dagois and Stolte attributed the favourable effect of malt soup on the calcium retention by infants to the alkali in the soup but Sato showed that any such effect was not the result of contained alkali and indeed that addition of alkali led to poorer retention. Shohl and Sato found that the addition of sodium bicarbonate to the diet decreased the urinary excretion of lime and phosphorus and Bogert and Kirkpatrick obtained similar results by the use of a diet containing excess of base. Zucker found that the addition of 15 g. of sodium bicarbonate to a diet containing 1.5 g. calcium led to the appearance of 22 per cent. of the excreted calcium in the urine whereas on the same diet without the extra alkali the urine contained 29 per cent. of the calcium output. Farquharson, Salter, Tibbets and Aub observed but little change in the elimination of lime after the administration of large amounts of alkali. The problem, however, is complicated by the fact that alkali in large amount causes a rise in the pH of the intestinal contents which will impair the absorption of minerals. Thus Irving has shown

TABLE 17

Showing the Effect of Intake of Alkali on the Excretion and Retention of Calcium and Phosphorus.(g.)

Name	Period	Total Intake CaO	P_2O_5	Urinary Output CaO	P_2O_5	Faecal Output CaO	P_2O_5	Total Reten CaO	P_2O_5
N.M.	Normal	17.91	24.64	1.174	8.649	16.913	17.140	-0.177	-1.149
	Bicarb.	17.91	24.64	1.934	9.300	17.061	15.917	-1.085	-0.577
J.H.	Normal	22.40	30.80	0.818	12.775	11.945	10.816	+9.637	+7.209
	Bicarb.	22.40	30.80	1.404	11.880	26.157	21.867	-5.161	-2.947

TABLE 18.

Showing Effect of Intake of Alkali on Titratable Acidity, Ammonia, Urea and Sodium Chloride in Urine.

Name	Period	Volume	Titr.Acidity cc.N/10	Ammonia cc.N/10	Urea g.	Sodium Chloride g.
N.M.	Normal	7595	921.8	1339.8	103.72	17.72
	Bicarb.	8950	33.6	120.8	115.83	17.60
J.H.	Normal	8295	1371.0	1442.6	133.50	19.01
	Bicarb.	8635	31.2	121.8	141.73	15.87

that the absorption of calcium chloride is enhanced by the addition of acidifying acetate solutions and impaired by citrate solutions, and Hjort noted that less calcium was absorbed when it was administered as the carbonate than as the lactate. Hamilton and Moriarty from investigations on infants concluded that the amount of lime in the faeces depended upon the fixed base and buffer content of the milk. Albright and Ellsworth who found that on administration of alkali there was an increased faecal output of phosphorus attributed this to impaired absorption.

The following experiments were carried out to determine the effect of alkali administration on calcium and phosphorus retention and mode of excretion. Each subject received a constant diet, whole milk and sugar throughout the investigation. During the last seven days each was given 10 g. of sodium bicarbonate daily. The results are given in tables 17 and 18.

In both children the urinary and faecal outputs of calcium were increased and the retention impaired. In one a negative balance of phosphorus was slightly reduced owing to diminution in the faecal output, although the amount in the urine was increased. In the other a positive was converted/

converted into a negative retention of phosphorus because of the great increase in the faecal excretion which overshadowed the diminished urinary output. It is interesting to note that the excretion of water and urea by the urine was slightly increased while the chloride output was slightly decreased. The diminished excretion of the latter was due to the sudden fall in output on the first two days of alkali administration: in the last few days the amount of chloride reached the level of the normal period.

It is clear, however, that these results lend no support to the view that excretion of calcium by the kidney is increased only in acidotic conditions. Apparently any disturbance of acid-base equilibrium leads to increased loss of calcium, part of which excess is lost by the urine. The cause of the loss in acidotic conditions has been shown to be due to the action of acid substances on the bone, the liberated calcium serving the useful purpose of neutralising the acid. There is no obvious explanation for the loss of calcium in states of alkalosis. It is possible that the presence of excess base has an action on the bone-phosphatase whereby calcium is not so efficiently laid down in the bone but with the insufficient data available it is idle to theorise.

---0---

Chapter Three

THE ACIDOSIS OF SALICYLATE POISONING.

Chapter Three

THE ACIDOSIS OF SALICYLATE POISONING.

Ever since the introduction of salicylic acid compounds by Maclagan in 1874 it has been noticed that signs and symptoms of intolerance were not uncommon especially where larger doses were given unaccompanied by alkali. There has been much discussion as to the fundamental nature of these disturbances. While the majority of workers have considered the condition of salicylate poisoning to be associated with, if not caused by acidosis, no unequivocal evidence has been adduced in support of this view. The administration of alkali with salicylate was strongly recommended in 1908 by Lees, who stated that the tolerance of the patient was greatly enhanced and modern workers have justified the combined salicylate-alkali therapy as a method of preventing acidosis. Thus Bernard and Merklen state that acidosis is occasionally met with during salicylate administration and that there is lowering of the alkaline/

alkaline reserve with ketonuria. They further state that these manifestations disappear when sodium bicarbonate is given. Certain observers have, however, within recent years denied the necessity of alkali administration. Hanzlik in a comprehensive monograph on the salicylates written in 1924, asserts that "it is hardly conceivable that the true reaction of the blood could be sufficiently altered so as to cause real acidity, and the results of old and recent observations confirm this opinion". If by real acidity is meant a pH of the blood less than 7.0 then this statement is almost certainly correct. By acidosis, however, is generally understood not an actual acid reaction of the blood but a reaction less alkaline or more acid than what is found in healthy subjects. Hanzlik further goes on to state that alkalis do not prevent or modify the appearance of toxic symptoms. He quotes Meara as stating that the use of alkali "is directed more by tradition than rationale", but Meara was discussing the alkali treatment of rheumatism rather than the beneficial effect of the addition of alkali to sodium salicylate.

At the Royal Hospital for Sick Children, Glasgow, it has been the custom to give sodium salicylate combined with twice/

twice the amount of sodium bicarbonate to every rheumatic patient during residence in hospital. The dosage of salicylate usually employed is 15 grains four-hourly, or 90 grains daily; occasionally 20 grains four-hourly (120 grains daily) are given. With these doses signs of intolerance are seldom observed. Vomiting is the only one which may be said to occur with any frequency and if Langmead's advice is remembered and the bowels kept freely open, it is rare that any intermission of the drug therapy is necessary. There are, of course, cases in which there does seem to be a special idiosyncrasy to salicylate just as occurs with many other drugs, e.g., arsenic and morphine. It is therefore advisable to commence with smaller doses which can be gradually increased till the usual amounts are reached. Aspirin is thought by some to be tolerated better than salicylate of sodium, but actually the tolerance would appear to be related to dosage rather than to the particular form of salicylate used.

CLINICAL OBSERVATIONS OF THE VALUE OF THE ADDED ALKALI.

A logical method of testing the value of the added sodium bicarbonate seemed to be to give certain patients salicylate alone and observe the ill-effects, if any, produced and/

TABLE 19.

Showing Tolerance of Children to Sodium Salicylate without Sodium Bicarbon

Name	Age (Years)	Daily dose of Sod.Salicyl. (Grains)	Duration of treatment (Days)	Vomiting	Other signs of intolerance	Acetonuria
B.G.	7	50	40	nil	nil	nil
M.N.	12	50	33	nil	nil	nil
F.K.	10	60	35	1	nil	nil
I.J.	12	60	14	9*	nil	nil
J.McL.	9	60	48	1	nil	± When vomiting occurred.
J.W.	8	60	30	4	nil	on 2 ± occasions.
K.C.	9	60	16	5	nil	± occasionally
A.S.	6	90	3	3	Tremor, nervousness acidosis.	± last day
J.S.	7	90	4	2	Nervousness, headache, depression,	+ last day
W.P.	7	90	2	2	Tremor, Nervousness, headache.	nil

*This patient vomited twice before commencement of sodium salicylate administration.

and then to add bicarbonate and note any diminution or alleviation of the toxic manifestations. It was soon found that 60 grains daily was the maximum amount which, without the addition of alkali, could be tolerated by the average child without toxic signs appearing. 50 grains daily in no case produced any ill effects, but 60 grains not infrequently produced slight vomiting which was not always attributable to constipation (Table 19).

If the daily amount was increased to 90 grains, signs of intolerance were invariably produced in 3 to 5 days, and in some cases these were of a severe and alarming nature. Vomiting was a constant and early sign and usually preceded all other manifestations of salicylism. Drowsiness and confused mental states or mental torpor occurred in some. Many became apathetic and disinterested, and in one instance the speech was thick and slurring. Air-hunger of greater or less severity, but not necessarily in proportion to the other signs, occurred in all cases and sometimes to a marked degree; this was the typical acyanotic dyspnoea which is known to be associated with an acidosis of the acid-poisoning type. Acetonuria was present in the majority of the cases, but was never extreme and in a few cases was entirely/

TABLE 20.

Showing Beneficial Influence of Previous Administration of Sodium Bicarbon on Tolerance of Children to Sodium Salicylate

Name	Age: (Years.)	Duration of administration of Sod.Sal. gr.90 Sod. Bic.gr.180. (Days.)	Vomiting	Duration of administration of Sod. Sal. gr.90. (Days)	Vomiting	Other signs of intolerance
A.McG.	11	80	nil	28	nil	nil
J.W.	9	78	nil	18	nil	nil
M.J.	11	68	nil	33	Once on 6th day	nil
M.F.	9	67	nil	20	nil	nil
M.J.	8	34	nil	11	nil	nil
R.McI.	9	28	nil	12	Once on 2nd.day.	nil
D.W.	8	27	nil	15	Once on 8th day	nil
R.S.	8	24	nil	23	Twice	nil
A.McC.	11	7	nil	6	Twice on 5th day. Twice on 6th day	Headache, depressio

entirely absent. Tinnitus, strangely enough, was not troublesome and in the present investigation was not complained of by any of the patients. This manifestation, however, has occasionally proved troublesome in some patients receiving both sodium salicylate and sodium bicarbonate in the routine way. All the above signs and symptoms disappeared rapidly on discontinuing the salicylate, or on the addition of twice the amount of bicarbonate.

It is an interesting and practical observation that in those patients to whom salicylate and bicarbonate had been previously given, the signs of poisoning on omitting the alkali either did not develop, or if they did, were not as severe as in those to whom no alkali had previously been given. From Table 20 it will be seen that of the ten patients to whom sodium salicylate and sodium bicarbonate had previously been given, four showed no toxic symptoms, while vomiting occurred in five, only one of whom suffered with headache and depression. The last child, it is interesting to note, had only had the alkali combined with the salicylate for a short period of seven days.

It seems indisputable, then, that whatever else the alkali may do, it is very valuable in preventing the development

of the signs and symptoms of salicylate poisoning. So striking is this that it seems unjustifiable to prescribe large doses of salicylate without giving in addition sodium bicarbonate.

THE NATURE OF THE TOXIC MANIFESTATIONS.-

There is considerable difference of opinion regarding the nature of the toxic manifestations caused by the salicylates. Walter in 1877 found that the CO_2 content of the blood of an animal poisoned with sodium salicylate was reduced to 11.2 volumes per cent. and therefore argued that death could not be due to an acidotic condition, a conclusion that would not now be drawn from this evidence. Meyer stated that sodium salicylate inhibited oxidation but did not influence the alkalinity of the blood. Scott, Thorburn and Hanzlik using colorimetric dialysis methods did not detect any change in blood reaction or decrease of alkaline reserve in men, dogs or cats receiving full doses of salicylate. Hanzlik came to the conclusion that salicylate poisoning was not of the nature of an acidosis but none of his objections are incompatible with such a disturbance. He drew a comparison between salicylate poisoning and the condition present in diabetic acidosis which/

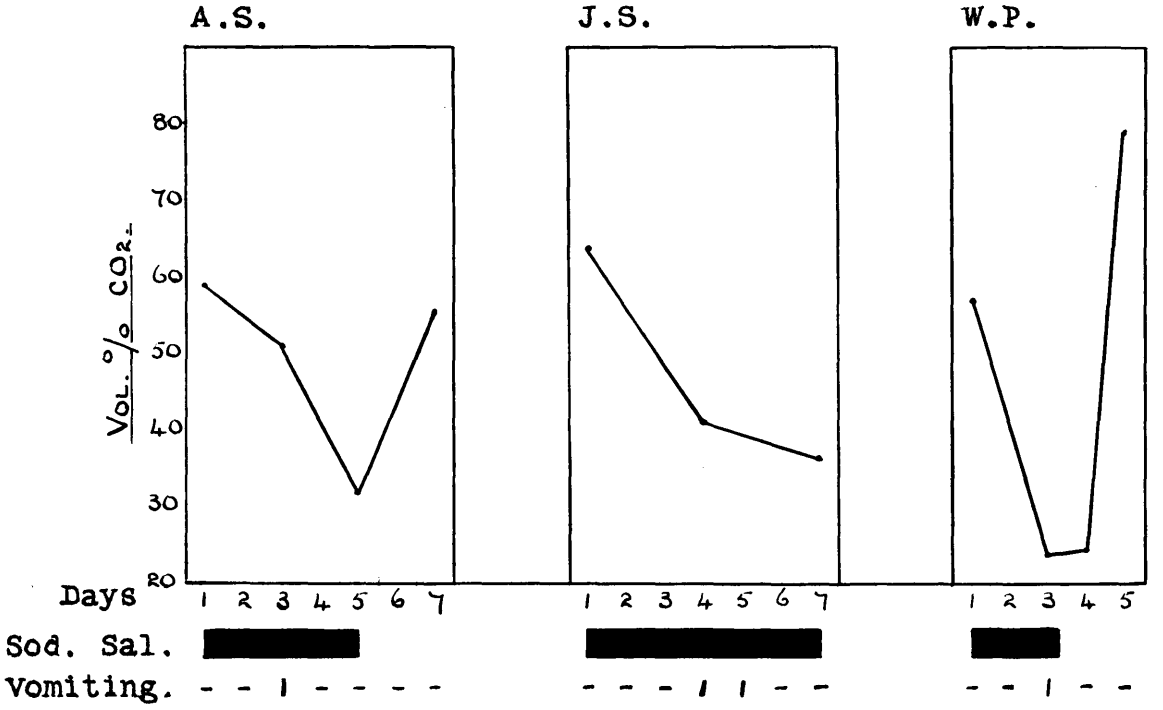
TABLE 21.

Showing Changes in CO_2 Content of Blood after Administration of Sodium Salicylate without Bicarbonate.

Name	CO_2 Content of Blood - vol.. per 100 cc.		Daily Dose of Sod. Sal. (grains.	No.of Days on Sodium Salicylate.
	Before Salicylate	During Salicylate		
J.S.	61.8	48.8	15	4
W.P.	62.6	47.0	30	4
M.N.	63.1 "	40.9 37.5	30 30	3 6
J.S.	58.2	33.5	60	3
J.A.	59.8	33.0	60	4
J.K.	61.3	25.0	90	5
J.McL	64.8	18.4	90	4
M.D.	57.5	23.8	90	3
J.G.	58.9	23.2	90	2
A.S.	53.0	35.6	9 0	3.
P.P.	47.1	21.7	90	2
J.S.	57.0	36.8	90	1

Fig. IX.

Effect of Sodium Salicylate on CO_2 Content of Blood.



which he put down to the action of certain enol acids but because he could find no shift in the hydrogen ion concentration of the blood he concluded that there was no acidosis.

Changes in Carbon Dioxide Content of the Blood.

In the present investigation are recorded the results of administration of sodium salicylate without alkali. In every case changes were noted in the carbon dioxide content of the blood which showed a marked reduction (Table 21 and Fig. 9). When the dose of sodium salicylate amounted to 90 grains daily the carbon dioxide fell to a figure below 42 volumes percent. With values between this and 35 volumes per cent. symptoms such as vomiting and air-hunger began to appear. At 30 volumes per cent. symptoms were invariably present and frequently severe. On discontinuing the drug the blood returned to normal fairly quickly but an even more rapid recovery was noted when sodium bicarbonate was given although in some cases the salicylate was continued.

A fall in the carbon dioxide content of the extent recorded here must mean the development of a non-gaseous acidosis or of a gaseous alkalosis. The clinical picture of a moderately severe case was that of a non-gaseous acidosis. The/

Fig. X.

Theoretical Carbon Dioxide Dissociation Curve of Blood in Salicylate Poisoning (Case R.H.).

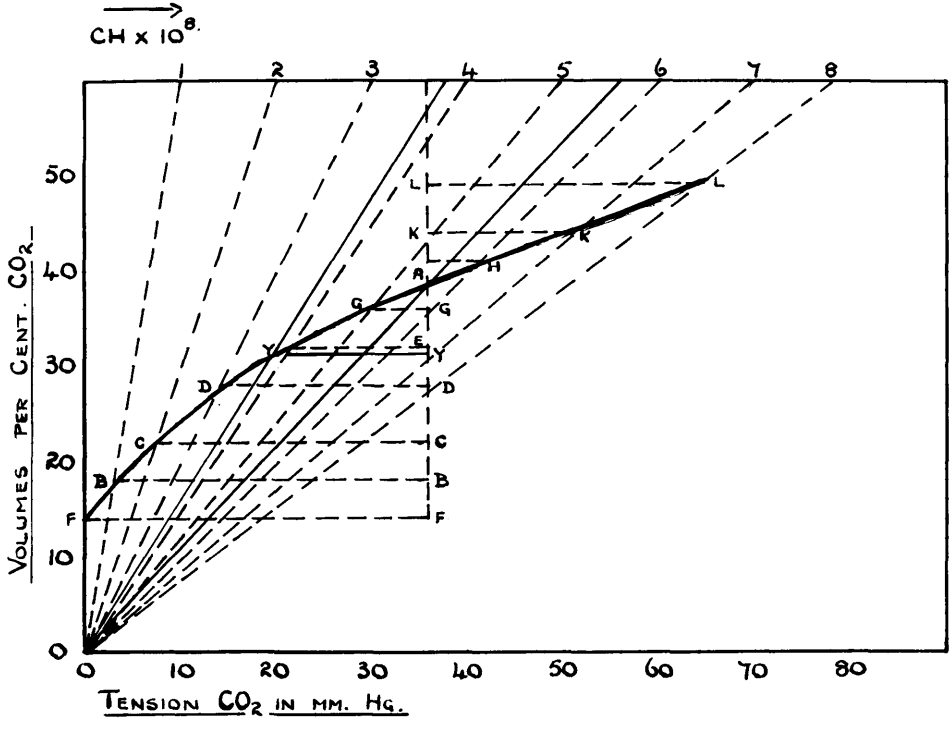
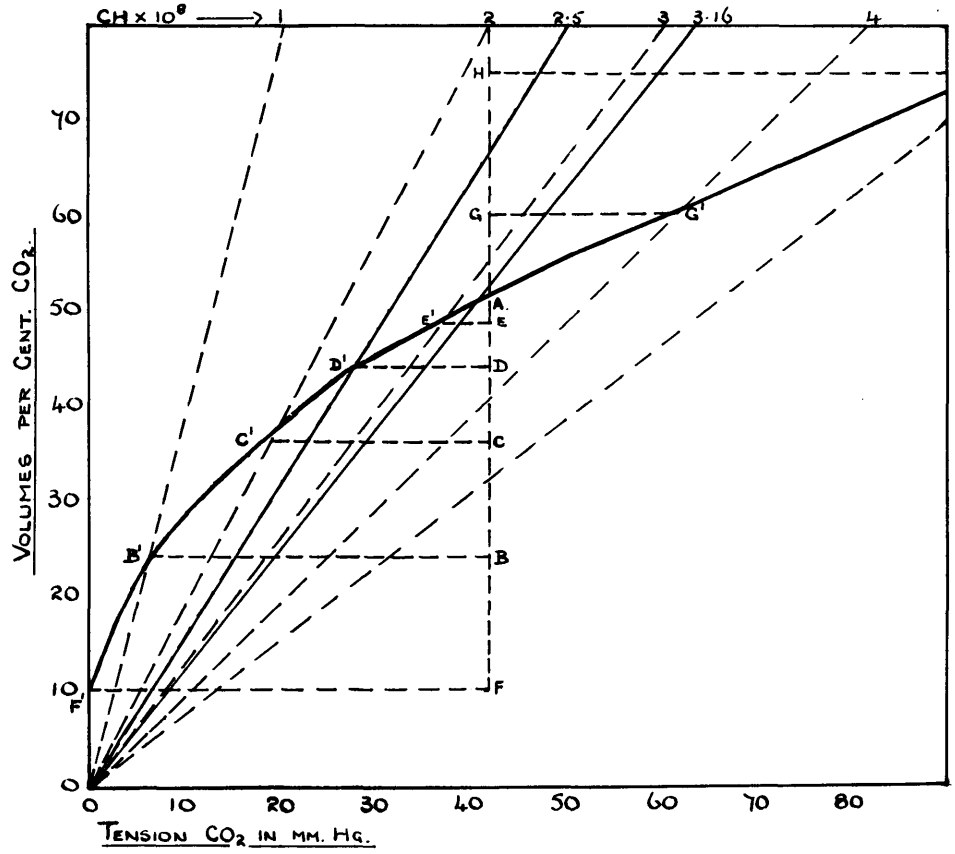


Fig. XI.

Theoretical Carbon Dioxide Dissociation Curve of Blood in Healthy Boy.



The air-hunger so characteristic of acidosis was sometimes very marked, the deep and rather frequent respirations being readily heard when one entered the ward. Furthermore, the fact that administration of alkali caused a return to normal clinical and biochemical conditions is definite proof that salicylate poisoning is not a state of alkalosis.

Theoretical Carbon Dioxide Dissociation Curve of the Blood.

Opportunity was taken in one case of salicylate poisoning to construct a theoretical carbon dioxide dissociation curve of the blood according to the method described by Barcroft, Dryerre, Meakins, Parsons, T.R., and Parsons, W. The child, R.H., aged 5 years convalescing from a mild attack of rheumatism, had been receiving sodium salicylate (10 grains) with sodium bicarbonate (20 grains) six times daily for four days when he suddenly became dyspnoeic. The carbon dioxide content of the venous blood was found to be 32.4 volumes per cent. A theoretical curve (Fig. 10) was constructed from the following data:

CO_2 tension in mm.Hg.	CO_2 Content in vol.%	C H X 10^8
36.3	38.4	5.6
20.0	31.0	-

A/

Fig. XII

Showing Relationship between Hydrogen-ion Concentration and Carbon Dioxide Content of Blood in Salicylate Poisoning (Case R.H).

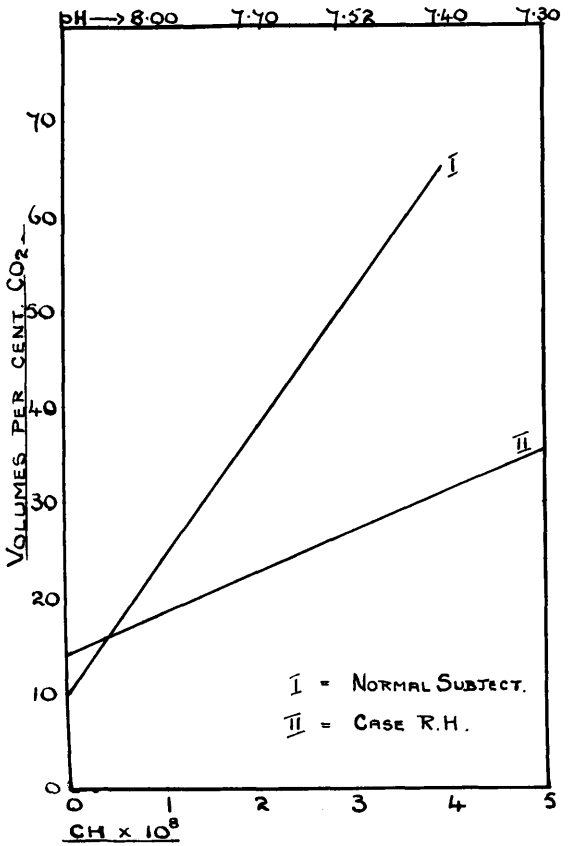
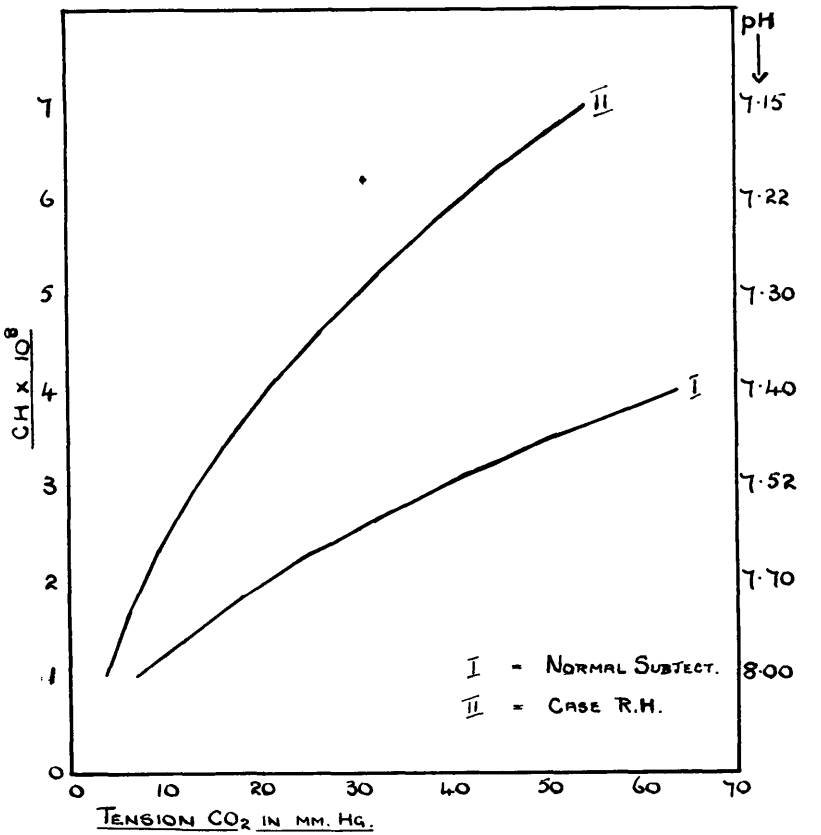


Fig. XIII.

Showing Relationship between Carbon Dioxide Tension and Hydrogen-ion Concentration of Blood in Salicylate Poisoning (Case R.H.).



A comparison of this curve with one obtained (Fig. 11) from the blood of a healthy child shows that it is definitely lower. At a tension of 40 mm.Hg. the blood contains only 40.1 volumes per cent. instead of 50 volumes per cent., i.e. about 20 per cent. below the standard. The relationships between cH and carbon dioxide content and carbon dioxide tension are graphed in figs. 12 and 13 respectively. From these it is evident that the same increase in carbon dioxide content or pressure produces a greater rise in cH in the salicylate than in the normal blood. The relationship between the volume of carbon dioxide and CH is expressed by the equation

$$V\ CO_2 = c + b\ (cH \times 10^8)$$

where V CO_2 = Carbon dioxide content in volumes per cent.

c = theoretical amount of available alkali united with carbon dioxide at zero tension of carbon dioxide.

b = degree of buffering represented by the increase in the volume per cent. of carbon dioxide per unit increase of cH.

This equation is easily deduced from the theoretical dissociation curve. The figures obtained were c - 14.0 and b - 4.4. Barcroft and his co-workers give the limits of normality as c 27.0 to 9.0 and b 6.5 to 10.1. It is clear that/

that the amount of alkali (c 14.0) is well within the limits of normality while the degree of buffering (b 4.4) is diminished. The faulty buffering is possibly due to the toxic action of the salicylic acid on the biological processes constituting the so-called buffering mechanism.

The question of the production of an acidosis in the poisoning by salicylate can be answered in the affirmative. The clinical picture, the behaviour of the carbon dioxide content and the response to alkali point very convincingly to this. The problem that now presents itself is the nature of the excess acid. There are two possibilities either that it is exogenous or that it results from an accumulation of normal or abnormal acid metabolism due to insufficient elimination.

IS SALICYLIC ACID THE EXCESS ACID?

Although in this investigation the salicylate was always given as a neutral salt one naturally turns to the salicylic radicle as a possible cause. The concentration of salicylate in the blood after prolonged administration of salicylate is reported by Hanzlik to vary between 18 and 35 mgm. in the rheumatic patient. The amount of salicylate in the blood of each of three patients was estimated after six days' administration of sodium salicylate first without and then with/

TABLE 22.

Influence of Sodium Salicylate with and without Sodium Bicarbonate on the Blood Content of Salicylate and CO_2

NAME	J.B.			M.M.			I.S.		
Period	1.	2.	3.	1.	2.	3.	1.	2.	3.
Salicylate content mg. per 100 cc.	-	9.3	29.0	-	6,9	13.0	-	13.0	94.0
milli-mol.per litre	-	0.67	2.09	-	0.50	0.94	-	0.94	6.80
CO_2 content vol. per 100 cent	50.6	29.5	59.7	49.8	29.8	52.3	53.9	17.1	47.1
milli-mol.per litre	22.6	13.1	26.7	22.3	13.2	23.4	24.0	7.3	21.0

Period 1. - Control

" 2. - Sod. Salicylate alone.

" 3. - Sod. Salicylate and Sod. Bicarb.

with sodium bicarbonate. The blood was taken early in the morning prior to any food or drug. The results are noted in Table 22.

Free Salicylic acid was never found except in traces, the drug always being present as the salt. The salicylate content of the blood in the periods without alkali varied between 6.9 and 13.0 mg. per 100 cc. while in the alkali periods these figures were increased two to fourfold.

The fact that salicylate was present in the blood in greater abundance in the alkali period in spite of the absence of symptoms is itself evidence against salicylic acid being the excess acid responsible for the locking up of available base. Furthermore, the amount found to be present during the low carbon dioxide period is quite insufficient to compensate as a base-holding substance for the deficit in carbon dioxide. The maximum amount of salicylic acid found in the blood during the presence of toxic symptoms amounted to 13 mg. per 100 cc. which is equivalent to 0.94 milli-mol per litre. This would only account for a diminution in the carbon dioxide of 2.1 vol. per cent., whereas the fall in carbon dioxide amounted to 36.8 vol. per cent. It is clear therefore, that the fall in carbon dioxide is not merely the result/

TABLE 23.

Changes observed in the Urine as a result of Administration of Sodium Salicylate with and without Sodium Bicarbonate

Name	Period	Volume in c.cm.	Titratable acidity in c.cm.N/10	Ammonia in g..	Total Nitrogen g..	$\frac{NH_3 N \times 100}{\text{Total N}}$	Urea g..	NcCl g.
J.B.	1	1,171	167.2	0.378	9.31	4.0	18.81	2.780
	2	954	255.3	0.311	8.22	3.8	13.24	1.906
	3	1,257	32.9	0.100	8.081	1.2	15.66	2.425
M.M.	1	1,126	155.2	0.291	8.49	3.4	15.85	2.093
	2	1,125	222.3	0.231	7.68	3.0	15.43	1.545
	3	1,299	34.2	0.062	8.18	0.77	15.97	2.398
J.S.	1	970	132.5	0.344	7.92	4.34	15.99	1.727
	2	721	176.9	0.189	7,41	2.70	13.11	1.061
	3	1,103	11.7	0.052	8.03	0.65	16.59	1.776

Period 1 - No medication

" 2 - Sod. salicylate.

" 3 - Sod. salicylate + sod. bicarbonate.

result of its replacement by salicylic acid. The other alternative is the presence of an excess of acid produced as a result of disordered metabolism.

URINARY CHANGES PRODUCED BY ADMINISTRATION OF SALICYLATE.-

It has long been known that albuminuria occasionally appears during salicylate administration. This naturally leads to the assumption that salicylates damage the renal cells. Hanzlik and his co-workers have published results which indicate a diminution in renal functional efficiency.

In three cases a study was made of the changes which occurred in the urine as the result of salicylate administration with and without the addition of alkali. The urine was collected from each patient for three periods of 6 days each. The first was a control period, during which time no drugs were given. During period two sodium salicylate (six doses daily of 15 grains in one, and 10 grains in the other two cases) was given; and during period three, the same amount of salicylate with twice the amount of sodium bicarbonate. During all three periods the diet, consisting of measured amounts of milk and sugar, was kept constant. In no case was there any loss by vomiting . These results are tabulated in Table 23.

From/

From the results some interesting facts emerge, the most striking being the fall in the ammonia coefficient of the urine during period 2. As is well known a rise in this figure would be expected if the condition were an acidosis of the acid-poisoning type, and the fact that in each of the three cases there was a slight but distinct fall would appear to point to impaired renal function. During period 3 as expected, the fall was pronounced due, of course, to the extra amount of fixed alkali available. Further investigation strengthens the suspicion of impaired renal efficiency during the administration of salicylate without alkali. In two of the subjects there was roughly a 20 per cent. reduction in the volume of urine excreted during period 2; in the third case there was no change. It has been noted by Hanzlik that full therapeutic doses lead to a decreased urinary volume and it is worthy of note that in all three instances the addition of the alkali resulted in a marked increase of diuresis. The urea output was also diminished in the second period and increased by the addition of the alkali. The total nitrogen of the urine was similarly affected, while evidence of chloride retention on salicylate alone is very definite. It may well be that the fall in carbon dioxide of the blood and tissues is responsible for this retention of chlorine in order/

TABLE 24.

Showing Effect of Administration of Sodium Salicylate with and without Sodium Bicarbonate on Urea-Concentrating Powers of Kidney.

(Urea Concentration Test).

Name	J. B.		M. M.		J. S.	
Period	2	3	2	3	2	
% of urinary urea before administration of urea	1.56	1.08	1.86	0.90	1.86	1
% of urinary urea one hour after administration of urea 15 grm.	1.74	1.74	1.44	1.86	1.26	2
% of urinary urea two hours after administration of urea 15 grm.	1.44	2.86	1.22	2.58	1.24	3

Period 2 - Sod. Salicylate.

Period 3 - Sod. Salicylate + Sod. Bicarbonate.

order to compensate for deficiency in acid radicle, but the other observations lend more support to diminished renal activity being the causal factor. The urea-concentration test (15 grm. urea given) also gave corroborative evidence (Table 24). Here again the alkali was found to restore the urea-concentrating power of the kidney to a normal level. Blood examination did not reveal any positive indication of kidney damage. The non-protein nitrogen and chlorine content were found to be within normal limits. Hanzlik states that while the non-protein nitrogen of the blood is somewhat diminished the urea nitrogen is increased. He also maintains that the impairment of kidney function occurs equally with and without the use of alkalis. The results with the urea-concentration test (Table 24), as well as the improved urinary output of those patients receiving sodium bicarbonate as shown in Table 23 would appear to warrant the statement that sodium bicarbonate protects renal function. A corroborative finding was the more frequent occurrence of albuminuria when the patients were on salicylate alone.

EVIDENCE AS TO THE PRESENCE OF EXCESS ENDOGENOUS ACID.-

It has been stated by Hanzlik that the symptoms of salicyl poisoning resemble those occurring in diabetic acidosis./

TABLE 25.

Changes in Fasting Blood-Sugar during Administration of Sodium Salicylate without Bicarbonate.

Name	Fasting Blood Sugar - mg. per 100 cc.		Daily Dose of Sod.Salic. (grains)	No.of Days on Sod. Salic.
	Before Salicylate	During Salicylate		
M.N.	94 63 " "	104 77 87 100	90 " " "	4 1 3 4
J.McL.	84	109	"	2
M.D.	79 63 " "	82 85 84 90	75 90 " "	1 1 3 4
J.K.	63	104	90	1
J.D.	77	77	90	3
M.A.	60	79	90	1
J.A.	69 "	81 109	90 "	1 3
A.S.	40 "	72 92	90 "	1 3
P.P.	77 "	89 95	90 "	1 3

Figures underlined were obtained during a period of Ketogenic diet.

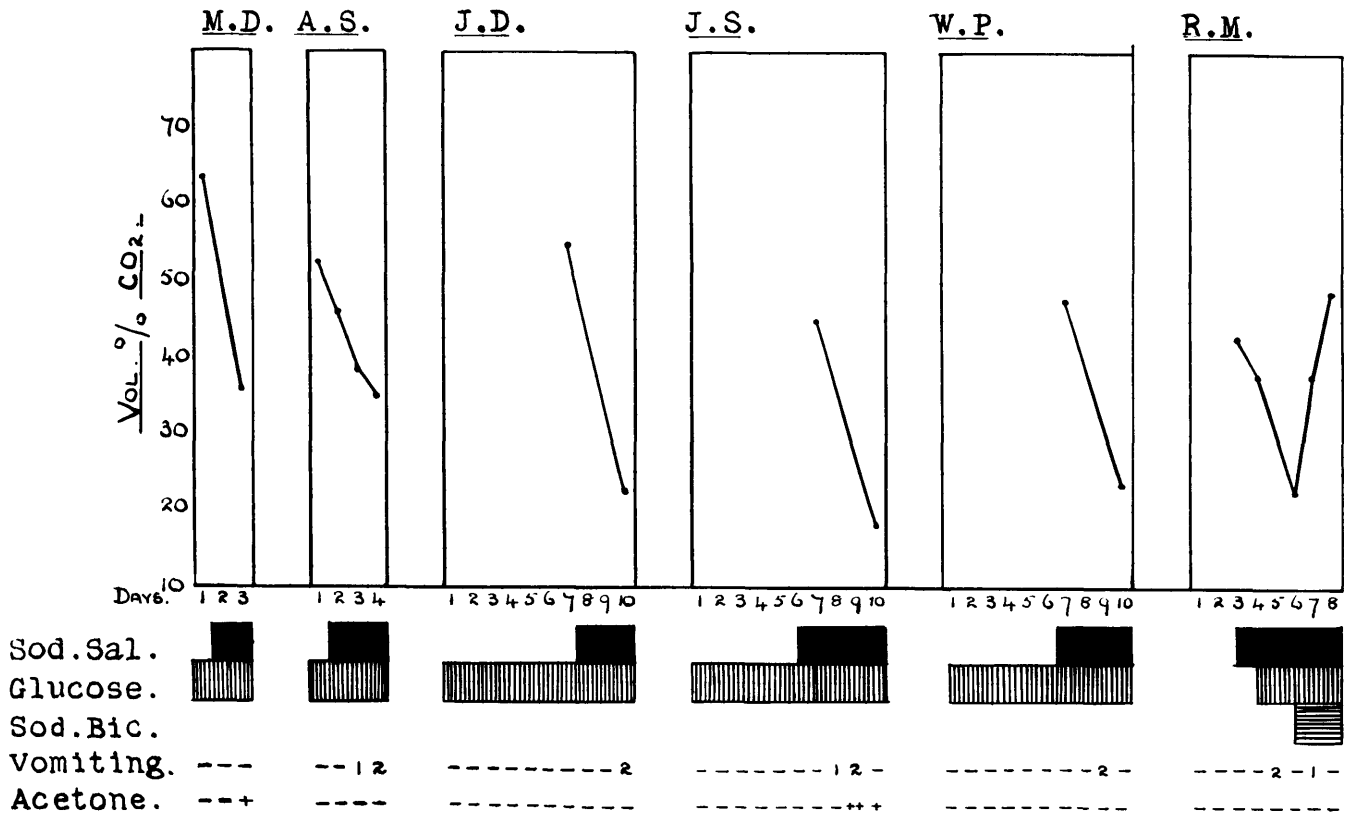
acidosis. He quotes references to states of ketosis occurring in poisoning from methyl salicylate and mentions the observation of Hurtley and Trevan on the similarity of the symptoms produced by intravenous administration of salicylate and sodium aceto-acetate. It is true that occasionally acetone appears in the urine during administration of salicylate, but even in severe cases of poisoning it is by no means a constant constituent of the urine, and when present, it is rarely in greater amount than is indicated by a mild Rothera reaction. It may be urged that impaired renal function is the cause of its non-appearance in quantity in the urine, but the work of Myers and Ferguson indicates quite clearly that both in rabbits and man administration of sodium salicylate leads to little change in the precentage content of acetone bodies in the blood, although some of the rabbits received fatal doses of salicylate. The fact that acetone was said to play a part in the production of symptoms suggested the advisability of investigating the effect of salicylate administration on the blood sugar as well as the influence of glucose ingestion in the prevention and amelioration of symptoms. The results contained in Table 25 show that the fasting blood sugar was higher/

higher during the period of salicylate ingestion, by 4 - 18 per cent. In only one case was the blood sugar unaltered after three days of salicylate administration. It would seem, therefore, that there is available in the blood-stream a sufficiency of glucose. It is possible that owing to the action of salicylate the tissues are unable to make use of this glucose. This, however, is improbable and a much more likely explanation would appear to be a toxic action on the liver, by which that organ is rendered less capable of storing food material. This would explain not only the rise of fasting blood sugar but also the slight excretion of acetone bodies, since the liver also acts as a reservoir of fatty acids. It is also interesting in this connection to draw attention to the diminution in the formation of urea.

If there is any basis for the suggestion that ketone bodies play a part in the production of the symptoms of salicyl intoxication one would expect that the administration of glucose would have an inhibitory effect. In order to test the protective action of glucose six patients were given in addition to the usual ward diet 50 grammes of glucose daily for periods of seven to one days prior to administration of/

Fig. XIV.

Effect of Glucose and $NaHCO_3$ in Prevention of Fall in CO_2 Content of Blood.



of salicylate. Thereafter sodium salicylate was administered in 15 grain doses six times daily while the glucose was continued. In each instance symptoms developed and the total carbon dioxide content of the blood fell just as quickly in those cases where no glucose or bicarbonate had been given previously or simultaneously. Fig. 14 shows the effect of glucose on the blood carbon dioxide content in salicylate administration and also illustrates the variability of the acetonuria which was absent in four of the six cases.

In contradistinction to the inefficiency of glucose in preventing a fall of the carbon dioxide content of the blood is the rapid recovery which occurs on administration of bicarbonate. This is well seen in Fig. 14 (Case R.M.) where the addition of sodium bicarbonate in 30 grain doses to the salicylate on the fourth day resulted in a rapid return of the blood carbon dioxide to normal and a disappearance of symptoms.

Still further evidence against the view that ketosis is an important factor in producing the symptoms of salicylism is obtained from a consideration of the results of the use of ketogenic diet prior to administration of salicylate. The ketogenic diet itself occasionally reduced the blood carbon/

carbon dioxide to below 40 volumes per cent., but the symptoms after salicylate administration were not aggravated nor did the administration of large amounts of glucose have any ameliorating effect either on the severity of the symptoms or on the level of the blood carbon dioxide.

Since the presence of a marked degree of ketosis did not aggravate the condition and the administration of glucose did not exert the slightest beneficial effect, it seems justifiable to conclude that the symptoms are not necessarily associated with incomplete fat oxidation.

Moderate increase of lactic acid has been observed in the blood of cats and rabbits after intravenous administration of salicylate preparations. Johnston who noted this could not correlate the lactate increase with the change in the alkaline reserve. There remains therefore the possibility of an excess production of the so-called "undetermined acid" which is mainly dependent on protein catabolism, together with a defect in the rate of elimination.

EXCRETION OF SALICYLATE.

In searching for the reason why sodium bicarbonate is effectual in preventing the signs and symptoms of salicylate poisoning there naturally arises the question of the rate of excretion/

TABLE 26.

Urinary Excretion of Salicylate during Administration o

Sodium Salicylate with and without Bicarbonate.

ıe	J.B.		M.M.		J.S.	
·iod	2	3	2	3	2	3
·al amount of sod.sal. given in grm.	23.33	23.33	23.33	23.33	34.99	34.99
;al amount of sod.bic. given in grm.	-	46.66	-	46.66	-	69.98
;al amount of sod.sal. ıcreted in grm.	6.069	20.25	6.57	21.33	13.17	27.91
of sod. sal. excreted	26.0	86.8	28.1	91.4	37.6	80.0

excretion of the drug with and without administration of alkali. Hanzlik states that about eighty per cent. of the amount ingested is excreted by healthy subjects but only sixty per cent. when there is rheumatic or other fever present. He was unable to demonstrate any difference in the duration or rate of excretion following the administration of alkali. Fleischer, however, stated that sodium bicarbonate shortens the period of salicylate elimination from 36 to 14 hours and Ehrmann obtained similar results.

The urine from periods two and three of the three children, (J.B., M.M., and I.S.) was analysed quantitatively for salicylic acid. The faeces were examined in one case only but only a negligible amount of salicylate was detected.

The results recorded in Table 26 show that in patients convalescing from acute rheumatism the administration of sodium salicylate without bicarbonate is followed by the excretion of only 26 - 37.6% of the intake. The giving of alkali, however, increased the output of salicylate two to fourfold so that 80 - 91.4% of the amount ingested was found in the urine.

It is probable therefore that one method whereby alkali prevents salicylate poisoning is the increased rate of excretion/

excretion of the latter drug. As has already been mentioned the concentration of salicylate in the blood is much higher during the periods of alkali administration and it is possible that this higher concentration enhances its therapeutic value so that there may be some foundation to the view of Lees that simultaneous administration of sodium bicarbonate and salicylate is beneficial apart from the prevention of salicylate poisoning.

CONCLUSIONS.

From the results obtained during this investigation it is permissible to draw the following conclusions:-

1. The oral administration of sodium salicylate alone in doses of over 60 grains daily to children results in the production of a non-gaseous acidosis which is not due to the replacement of carbonate by salicylate.

2. The development of the acidosis can be prevented by the administration of sodium bicarbonate but not of glucose. One effect of the alkali is to accelerate the rate of salicylate excretion.

3. The acidosis is accompanied by impairment of renal function associated with nitrogen and chlorine retention.

4. It is suggested that the cause of the acidosis is the excess accumulation of undetermined organic acid possibly nitrogenous in nature.

---0---

Chapter Three

Appendix.

A NOTE ON THE METABOLIC CHANGES FOLLOWING ADMINISTRATION OF SODIUM SALICYLATE TO CATS.

In view of the findings obtained in children following the administration of sodium salicylate it seemed worth while to investigate the effect of this drug on animals. Hanzlik and Karsner have found that cats receiving about 0.23 gm. of sodium salicylate per kilo. body weight whether per os or subcutaneously showed renal lesions varying from simple cloudy swelling of tubular epithelium to an acute tubular nephritis associated with acute glomerulitis. In view of these findings it was deemed worth while to investigate the effect of salicylate. The experiments detailed here were carried out with the view of throwing further light on nitrogen and chlorine metabolism which seemed to be definitely affected in children who had received salicylate without alkali.

Methods.

After a control period of seven days a sterile solution of/

of sodium salicylate was given intramuscularly daily. The cats received weighed amounts of oatmeal and milk; aliquot portions of each article of diet were analysed. The unused residues of the diet were kept, thoroughly mixed and analysed so that intake during each period of the experiment was accurately known. Urine and faeces were collected separately. The urine was analysed daily while the faeces for each period were collected, dried and analysed.

Results.

Of the three animals receiving 0.20 gm. sodium salicylate per kilo. body weight one died after the third injection and one after the fourth. In each case after the second injection the breathing became very heavy and the animals looked ill, remaining in a corner of the cage and taking no interest in their food. Post-mortem examination revealed no abnormality except marked bile-staining of the liver. Two cats received injections of 0.06 gm. sodium salicylate per kilo. body weight. Throughout the first seven days of salicylate administration cat 5 appeared quite normal and took its food while cat 4 seemed "off colour" and left some of its food. Cat 5 received salicylate injections for three more days during which time it gradually became more and more depressed./

TABLE 27.

Daily Output in Urine.

Cat No.	Period	Volume c.c.	Titr. Acidity c.c.N/10	Ammonia c.c.N/10	Urea g.	Purine Nitrogen mg.	Creatinine mg.	Creatine mg.
1	1	199	19.5	32.3	1.729	5.8	87.3	0
	2	143	35.1	40.5	1.737	7.6	86.3	28.3
2	1	154	16.7	26.8	1.480	-	86.0	0
	2	128	12.1	55.7	1.971	-	72.0	23.5
3	1	136	6.0	60.1	1.190	3.4	59.0	1.4
	2	124	14.5	76.5	2.285	10.0	79.5	16.0
4	1	147	15.2	66.9	1.770	2.6	98.3	1.9
	2	98	33.0	71.1	2.305	5.6	98.6	1.3
5	1	149	0	68.5	1.941	3.2	105.7	0
	2	143	0	143.0	0.769	4.2	103.7	0

TABLE 28.

Daily Intake, Output and Retention of Nitrogen in g..

Cat No.	Period	Intake	Output	Retention.
1	1	1.201	1.017	+0.184
	2	0.817	1.737	-0.920
2	1	0.921	0.857	-0.064
	2	0.409	0.937	-0.528
3	1	0.984	0.677	0.307
	2	0.490	1.432	-0.942
4	1	1.013	1.104	-0.091
	2	0.991	1.613	-0.622
5	1	0.970	1.080	-0.110
	2	0.998	1.251	-0.253

TABLE 29.

Daily Intake, Output and Retention of Sodium Chloride in mg.

Cat No.	Period	Intake	Output	Retention
1	1	184	166	+18
	2	119	278	-159
2	1	142	158	-16
	2	68	211	-143
3	1	137	119	+18
	2	74	424	-350
4	1	188	167	+21
	2	165	159	+ 6
5	1	187	167	+20
	2	188	158	+30

depressed.

Tables 27, 28 and 29 show the effect of salicylate on the composition of the urine. The findings may be summarised as follows:-

The volume was markedly reduced in all instances except in cat 5 where the reduction was trifling while the titratable acidity was increased except in cats 2 and 5.

Total nitrogen was definitely increased in all cases producing a very marked negative balance. The ammonia output was slightly raised except in cat 5 where the increase was very marked. The excretion of urea was also raised except in cat 5 where it was diminished while the purine nitrogen was increased in all cases, the increase being least marked in cat 5.

In cats 4 and 5 there was no change in the amounts of creatinine excreted during the salicylate period; practically no creatine appeared in the urine. In cats 1, 2 and 3, however, relatively large amounts of creatine were excreted. Ketonuria was not observed.

The chloride output was greatly increased in cats 1, 2, and 3 while cats 4 and 5 which received the smallest doses of salicylate showed reduced output. Cat 5, the sole animal not/

TABLE 30.

Showing Chlorine Content of Tissues in m.-eq. per kilo.

	Muscle	Liver	Kidney	Heart	Lung
Normal Limits	13 - 16	26 - 32	35 - 42	25 - 33	45 - 61
Cat 1	16.9	21.4	20.5	28.1	36.8
" 2	15.9	25.3	34.2	35.0	40.5
" 3	14.2	25.2	38.6	23.9	39.2
" 4	12.3	25.1	23.7	26.7	51.9
" 5	21.3	28.1	27.3	23.6	50.3
" A	23.9	37.9	45.5	31.6	44.3
" B	22.3	30.0	47.0	40.9	51.3

not to show symptoms of poisoning was the only one with an increased retention of chloride during the salicylate period while in cat 4 in which the symptoms were relatively slight, the retention, although less than in the control period, was still positive. Estimation of the chlorine content of various organs (Table 30) showed that the values fell within normal limits except in the muscle of cat 5 where there was an excess. The tissue chlorine was also estimated in two other cats (A and B) which had received sodium bicarbonate by stomach tube during the administration of large doses of sodium salicylate; in these the tissue chlorine was well above normal in all the organs except the lungs.

Discussion.

Cats are much more susceptible to salicylate than is the human subject since a daily dose of 0.26 gm. per kilo. body weight per day is relatively non-toxic to the child while it causes death of the cat within four days.

The metabolic results differ in some respects from those obtained in children in whom the urinary nitrogen, urea, ammonia and sodium chloride were diminished. In adults, however, Denis and Means and Grabfield and Knapp obtained an increase in the urinary excretion of total nitrogen and uric acid/

acid although in several patients with arthritis Denis found a diminished output of nitrogen. Histological examination revealed no pathological change in the kidneys. Albuminuria appeared in only one instance (cat 2). These results raise the question whether the kidney in the human subject and more especially the child is more susceptible to salicylate than in cats.

The increased output of total nitrogen and the appearance of creatinuria indicate that under the influence of salicylate there is an increase of tissue break-down. The fact that purines appeared in excess suggests that the nucleo-proteins are involved. It is worth noting that in cat 5 in which no symptoms were apparent, these changes were least marked.

The occurrence of dyspnoea associated with an increased output of free and ammonia-combined acid would indicate that in cats the administration of salicylate leads to the development of a non-gaseous acidosis not associated with ketosis. The accumulation of acid cannot be attributed to renal inefficiency as in the child but is probably due to the presence of acid substances resulting from tissue breakdown. There is no evidence as to the tissue particularly affected. The part that chloride plays is not clear but a hypothesis worth/

worth considering is that in the earlier stages of salicylate poisoning the retention of chlorine may lessen tissue breakdown but that when the salicylate action reaches a certain level sodium chloride is swept out along with the products of tissue breakdown.

GENERAL CONCLUSIONS.

An examination of the evidence derived from the metabolic investigations on the children and from the animal experiments permit the conclusion that salicylate poisoning is accompanied by a non-gaseous acidosis. In both the data are strongly suggestive of excess tissue breakdown with the resultant production of acid substances, and in the child there is indication of impaired renal function which would certainly aggravate any tendency to acidosis. Thus the chief cause of the acidosis is probably the excess production of acid metabolites. Furthermore, chlorine would appear to play an important part in the early stages of salicylate poisoning either by limiting the production of the acid metabolites or by reducing their toxic action.

It is more than likely that all the toxic symptoms and signs are not due to the presence of an acidosis. Thus Bernard and Merklen found a normal alkaline reserve in a patient/

patient with marked salicylate delirium. The delirium, speech disturbances, apathy and lassitude might quite well be the direct result of the salicylate itself or some of the compounds formed with it. Indeed, it is probable that salicylate disturbs the metabolism of all the tissues so that the nervous phenomena described can be more justifiably attributed to a direct action of salicylate on nervous tissue than to the effects of acidosis. The acidosis and the manifestations of nervous involvement are concomitant phenomena, and it would appear that the extent to which the carbon dioxide is lowered is generally a good index of the degree of general disturbance of the tissues.

Whatever may be the immediate cause of the toxic symptoms it is beyond dispute that administration of alkali effectively prevents in the vast majority of individuals the onset of clinical symptoms or chemical changes of poisoning. The alkali undoubtedly accelerates the excretion of salicylate and so prevents its accumulation in the tissues. This would to a great extent, explain its action in preventing signs of toxaemia. It would appear from the results shown that the functional activity of the kidney in children is restored to normal by the addition of sodium bicarbonate/

bicarbonate to the salicylate. Whether this is the sole action or not, one cannot say. It may in some way protect the tissues but conclusive evidence of this is lacking. At any rate the administration of sodium bicarbonate along with the salicylate is essential. Toxic manifestations are avoided and large doses of salicylate can be given with safety. Further, the alkali permits of a much greater concentration of salicylate in the blood, which presumably is of advantage in promoting the therapeutic effect.

---0---

Chapter Four

THE ALKALOSIS OF CONGENITAL HYPERTROPHIC PYLORIC STENOSIS.

Chapter Four

THE ALKALOSIS OF CONGENITAL HYPERTROPHIC PYLORIC STENOSIS.

There is a tendency for attention to be focussed on the mechanical factors in pyloric stenosis, the importance of which one cannot gainsay. There are, however, certain metabolic disturbances which also require consideration as they are of great significance in diagnosis and treatment. In congenital hypertrophic pyloric stenosis and indeed, in any obstruction of the upper part of the small intestine there is always present a well marked non-gaseous alkalosis. The problem of the pathogenesis of the latter condition presents points of considerable interest.

The earliest appreciation of the disturbances of acid base equilibrium in pyloric obstruction came from experimental work. In 1918, McCann showed that the carbon dioxide combining/

combining power of the blood plasma in dogs was greatly increased after the pylorus was ligated. Two years later, McCallum and his co-workers demonstrated the same phenomenon, and associated the rise in carbon dioxide with a fall in the chlorine content of the blood. The explanation they offered was that the chlorine deficiency was primary and due to a loss of chlorine by vomiting of gastric juice; this, they suggested led to a deficiency in the blood and tissue fluids of one acid radicle (chlorine) which necessitated the retention of another acid radicle (carbon dioxide) as a compensatory measure. These same investigators also found that the onset of tetany, which invariably occurred in experimental pyloric obstruction, could be prevented by intravenous administration of sodium chloride. Hastings, Murray and Murray, Haden and Orr, Gamble and Ross, Ingvaldsen, Whipple, Baumann and Smith, Dragstedt and White and Bridge have since 1920 amplified these observations on animals. Without exception these workers found that following ligation of the pylorus there was marked depletion of blood chloride with rise in the alkaline reserve and that administration of sodium chloride solution parenterally led to a temporary improvement with disappearance of most of the toxaemic/

toxaemic symptoms.

Brown, Eusterman, Hartmann and Rowntree, and later, Atchley and Benedict described similar biochemical findings in duodenal obstruction in the adult human subject and Ellis demonstrated the presence of alkalaemia in two cases. An identical picture was shown by Hardt and Rivers and Wildman to be present in patients with duodenal ulcer after intensive alkali treatment especially when there was some degree of renal inefficiency. Hartmann and Smyth, Maizels, and Maizels and McArthur have found that alkalosis is also not uncommon in infants who suffer from vomiting due to various causes. In the present communication are recorded clinical and biochemical observations of the disturbances in acid-base equilibrium in congenital hypertrophic pyloric stenosis.

CLINICAL OBSERVATIONS.

A most striking and probably the only important clinical manifestation of the presence of alkalosis in pyloric stenosis is depressed breathing. This is present in some form or another in practically every patient with pyloric stenosis of any duration. The depressed breathing is evidenced in one of three ways. First, there may be a shallow type of respiration, so shallow, indeed, that even with the bell/

bell of the stethoscope placed in front of the patient's nose and mouth it is often extremely difficult to hear the breathing. Secondly, the rate may be diminished, occasionally to eight or ten respirations per minute. Thirdly, there may be well marked and often alarmingly long periods of apnoea followed by three or four shallow respirations producing the typical Biot type of breathing. Most frequently all three manifestations are present together. It is not uncommon, however, for the respiration to be almost normal in rate over a period of a minute but shallow in depth with apnoeic periods occasionally appearing at longer intervals. Respiratory depression although an important diagnostic sign (Case J.L.), cannot be considered as pathognomonic of pyloric stenosis in infancy since a diminished respiratory rate may be due to encephalitis, cerebral haemorrhage and other conditions.

Case J.L. male, aged eight weeks. Normal labour; apparently healthy infant. Breast-fed; throve well, no vomiting; nothing unusual noted about the motions. For the past few weeks mother thought the infant was unduly quiet, "as if he were doped". She brought him to hospital because of this.

On/

On admission he was seen by Dr. Findlay, who observed that the respiratory rate was very depressed (twelve per minute), and the breathing shallow with apnoeic periods. Despite the absence of any history of vomiting he was led to suspect pyloric stenosis, and on examination, typical gastric peristalsis was evident and a pyloric tumour easily palpated. The head was small and the infant appeared mentally backward. The blood carbon dioxide was found to be 102.0 volumes per cent. The child was in hospital for four weeks, during which time the condition remained unchanged, and he vomited only once. Death occurred following a sudden bout of fever. Post-mortem examination revealed defective development of the cerebral hemispheres and the presence of a well-marked hypertrophy of the pylorus.

Accompanying the diminished pulmonary ventilation there is general lethargy, giving one the impression that the infant is under the influence of some hypnotic drug. It is interesting to note that in adults with marked pyloric obstruction there are symptoms such as lassitude and mental changes which have been ascribed to the presence of an alkalosis. This has been shown by Wildman to occur most frequently/

TABLE 31.

Biochemical Findings in the Blood in Pyloric Stenosis.

Name	Age in Weeks	CO_2 Vol. per cent.	Cl mg.per cent.	Non-protein Nitrogen mg.per cent.	Fixed Base mM. per litre.
R.C.	8	140.5	140	46	-
N.R.	4	140.0	181	80	-
A.M.	8	83.1	214	32	-
W.G.	6	85.0	190	35	-
M.M.	12	62.5	330	60	-
M.A.	8	125.6	112	145	-
A.W.	7	110.2	171	63	-
E.M.	8	100.5	169	86	-
J.K.	6	91.8	160	50	-
J.McK.	8	112.0	225	32	144
A.C.	3	113.0	240	34	-
G.H.	7	95.8	191	75	-
W.A.	3	103.7	305	38	-
"	4	148.0	134	183	-
W.D.	3	97.8	230	56	-
C.C.	4	66.5	282	38	-
J.R.	3	53.6	304	47	-
J.D.	3	104.1	220	51	-
"	4	98.1	210	44	-
"	5	106.8	223	56	-
"	6	101.9	265	40	-
R.M.	4	114.8	192	58	-
"	5	121.6	236	48	-
"	6	69.6	270	32	-
"	7	71.3	305	50	-
J.C.	4	97.6	263	48	-
J.B.	3	83.5	225	68	-
W.F.	5	93.4	187	100	-
P.T.	5	95.4	228	68	-
T.McK.	4	70.4	129	32	-
"	5	102.7	212	-	-
R.S.	3	107.0	271	39	-
"	4	102.4	305	65	-
J.T.	6	92.4	150	-	-
D.C.	5	81.2	220	-	-
"	6	70.6	291	-	-
A.L.	4	91.4	202	-	-
"	5	85.3	249	-	-
T.C.	5	100.8	259	-	-
J.J.	4	98.8	263	-	-

TABLE 31 (continued).

Name	Age in Weeks	CO_2 Vol. per cent	Cl mg. per cent.	Non-protein Nitrogen mg. per cent.	Fixed Base mM. per litre.
J.P.	5	118.7	192	-	151
T.S.	4	125.2	-	-	150
G.C.	7	47.0	249	-	160
W.B.	5	112.0	-	-	159
A.B.	3	117.0	121	-	152
M.McP.	5	62.7	295	-	154
D.F.	4	109.8	-	-	153
S.R.	4	112.0	-	-	143
E.F.	4	103.0	213	-	161.

frequently when the patient who has some form of peptic ulcer is receiving massive doses of alkali. Gatewood, Gaebler, Muntwyler and Myers observed that in patients with peptic ulcer, there was definite correlation between the drowsiness and mental depression and the alkalaemia which occurred during pyloric obstruction and disappeared when surgical measures afforded relief.

GENERAL BIOCHEMICAL FINDINGS.

The three outstanding changes observed in the blood were rise in the carbon dioxide content, fall in the chlorine content and, rather less frequently, an increase in the non-protein nitrogen value. As a rule these three findings were present together (Table 31) but the variations from the normal were not necessarily of the same degree. The fixed base of the serum was usually within normal limits but on two occasions it was slightly reduced (Table 31). A fall of ten per cent. below the normal minimum of serum base has also been found by Gamble and Ross in experimental pyloric obstruction just prior to the death of the animal. In two instances the plasma protein was determined and it was found to be 5.95 and 5.20 g. per 100 cc. respectively, while the minimum observed in apparently healthy infants of the same age/

age was 6.02 g. per cent. Determination of the relative volumes of plasma and corpuscles in two cases showed that the plasma volume was reduced to 51 per cent. compared to the normal 58 per cent. (Table 41). (vide page 109) Such a decrease in plasma volume which has been reported by McIver and Gamble to occur in experimental pyloric obstruction is probably due to water loss and in part conceals a fall in serum protein, which results from inanition. Maizels has reported an increase of haemoglobin content in the blood of infants with pyloric stenosis: this he also attributed to loss of fluid from the body.

The urinary reaction is in most cases alkaline but as Hartmann and Smyth have pointed out, an acid reaction is by no means uncommon. Myers and Booher have also recorded instances of alkalosis where the reaction of the urine was acid. These authors rightly emphasize the fact that the reaction of the urine is not a safe guide to the direction of the disturbance in acid-base equilibrium. Not infrequently acetonuria is present due to the insufficient supply of carbohydrate. The most important urinary finding, however, is the very great diminution or absence of chloride: rarely does the silver nitrate test yield more than a very faint/

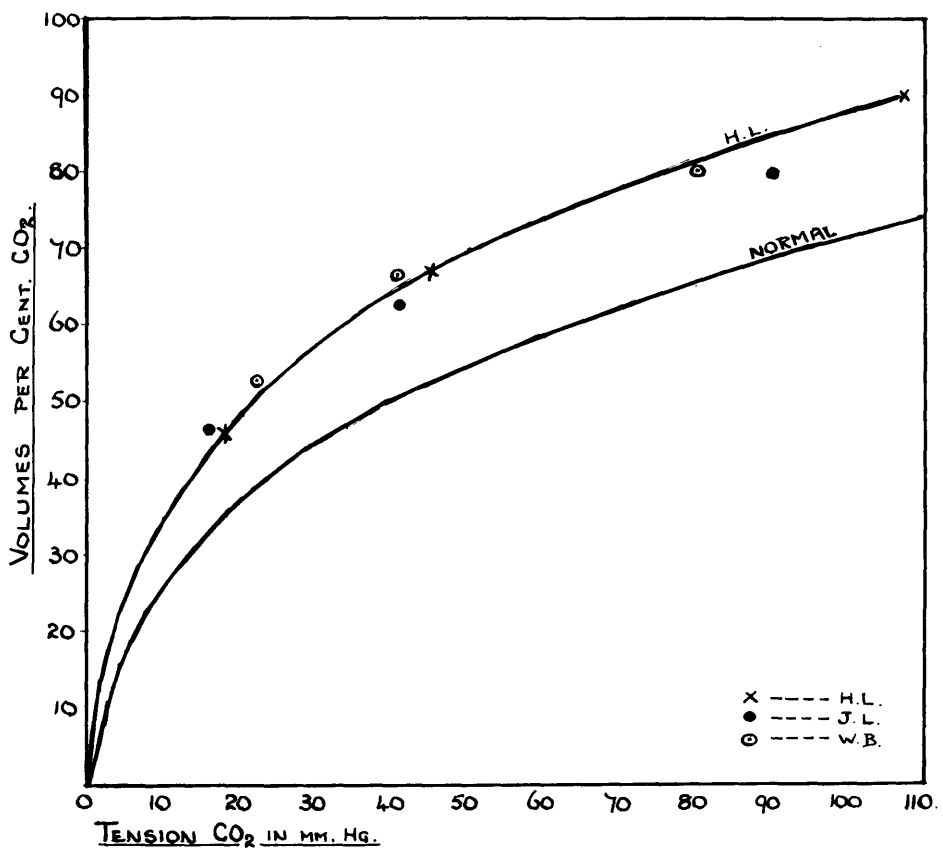
TABLE 32.

Data used for Construction of Carbon Dioxide Dissociation Curves in Pyloric Stenosis.

Name	CO_2 tension in mm.Hg.	CO_2 Vol. %	Percentage Difference from Standard Curve at a tension of 40 mm. Hg.
H.L.	44.8 105.5. 17.8	67.8 89.0 46.1	+ 25.5
W.B.	42.7 79.8 23.5	67.6 80.9 53.2	+ 30.0
J.L.	15.3 42.1 90.8	46.8 64.2 81.1	+ 23.5

Fig. XV.

The Carbon-Dioxide Dissociation Curve of the Blood in Congenital Hypertrophic Pyloric Stenosis.



faint haze. During the past seven years chloride has never been found to exceed a faint trace in the urine of infants with pyloric stenosis who have not received saline therapy. Conversely, however, pyloric stenosis is not necessarily present when urinary chlorine is diminished as this has been found in association with other conditions.

THE CARBON DIOXIDE CONTENT OF THE BLOOD .

A rise of the carbon dioxide content of the blood may occur either in non-gaseous alkalosis or gaseous acidosis. The carbon dioxide dissociation curve of the blood in each of three cases showed a shift to the left, (Fig.15 and Table 32) indicating an increased capacity of the blood to hold carbon dioxide, a finding equally characteristic of either of the above two disturbances of acid-base equilibrium. In a series of ten children Morris and Graham found that at a tension of 40 mm. Hg. the percentage content of carbon dioxide varied from 47 to 51.6 volumes with an average of 51.5 which falls almost exactly on the curve for normal blood as determined by Christiansen, Douglas and Haldane. It is convenient in describing carbon dioxide dissociation curves to indicate the position of each with reference to that of Haldane's blood which contained 51 volumes per cent. at a tension of 40 mm.Hg./

TABLE 33 .

pH Findings and CO_2 Content of Blood

Name	Diagnosis	CO_2 content Vol. %	pH
J.C.	Pyloric Stenosis	140.0	7.76
R.R.	" "	110.0	7.33
W.B.	" "	73.2	7.35
J.McL.	" "	53.4	7.39
M.R.	" "	110.9	7.52
J.L.	Encephalitis lethargica (Respirations 8/min.)	67.0	7.25

The pH was estimated by Dale and Evans' Colorimetric method.

40 mm. Hg. The limits for the normal children in Morris and Graham's series were - 8.9 and +9.8 per cent. while Peters, Barr and Rule found in a series of normal adults (3 of their own and 15 collected from the literature), a variation of - 15.6 to +9.8 per cent. In the three cases of pyloric stenosis the variations were +23.5, +25.5 and +30.0 per cent.

In a few instances the pH of the blood was determined (Table 33); any deviation from normal limits that did occur was toward the alkaline side. This is in accord with what has been found by Ellis in two cases of high intestinal obstruction where there was definite alkalaemia. In contrast with this it is interesting to note the pH value in the case of J.L. suffering from encephalitis. Here, although the respiratory rate was only eight per minute and the carbon dioxide content 67.0 volumes per cent., the pH was 7.25 indicating a gaseous acidosis caused by diminished respiratory activity. One can therefore conclude that in pyloric stenosis the disturbance of the acid-base balance is towards the alkaline side and that the change in the respiratory volume is attributable and therefore secondary, to alkalosis.

The Theoretical Carbon Dioxide Dissociation Curve in Congenital Pyloric Stenosis.

The capacity of the blood for holding carbon dioxide depends upon two factors which may be briefly described as the available alkali and the degree of buffering. The relative importance of these two factors in maintaining acid-base equilibrium is revealed by the theoretical dissociation curve desbribed by Barcroft, Dryerre, Meakins, Parsons and Parsons. Such a curve may be constructed if the carbon-dioxide content of a sample of blood be known for each of two tensions of carbon dioxide together with the pH of the blood at one of these tensions. The theoretical curve affords information (1) as to the amount of base left over after the affinities of all the non-gaseous acid radicles are satisfied, i.e. the minimal amount of base available for combination with carbon dioxide and (2) as to the extra amount of carbon dioxide which can be taken up by the blood as a result of unit increase of cH ($cH \times 10^8$) i. e. degree of buffering. In an attempt to throw more light on these factors in pyloric stenosis a theoretical dissociation curve was constructed from the blood of a patient showing very marked symptoms. The infant W.R. aged 7 weeks had the usual manifestations/

Fig. XVIA.

Theoretical Carbon-Dioxide Dissociation Curve of Blood of a Patient (W.R.) with Pyloric Stenosis.

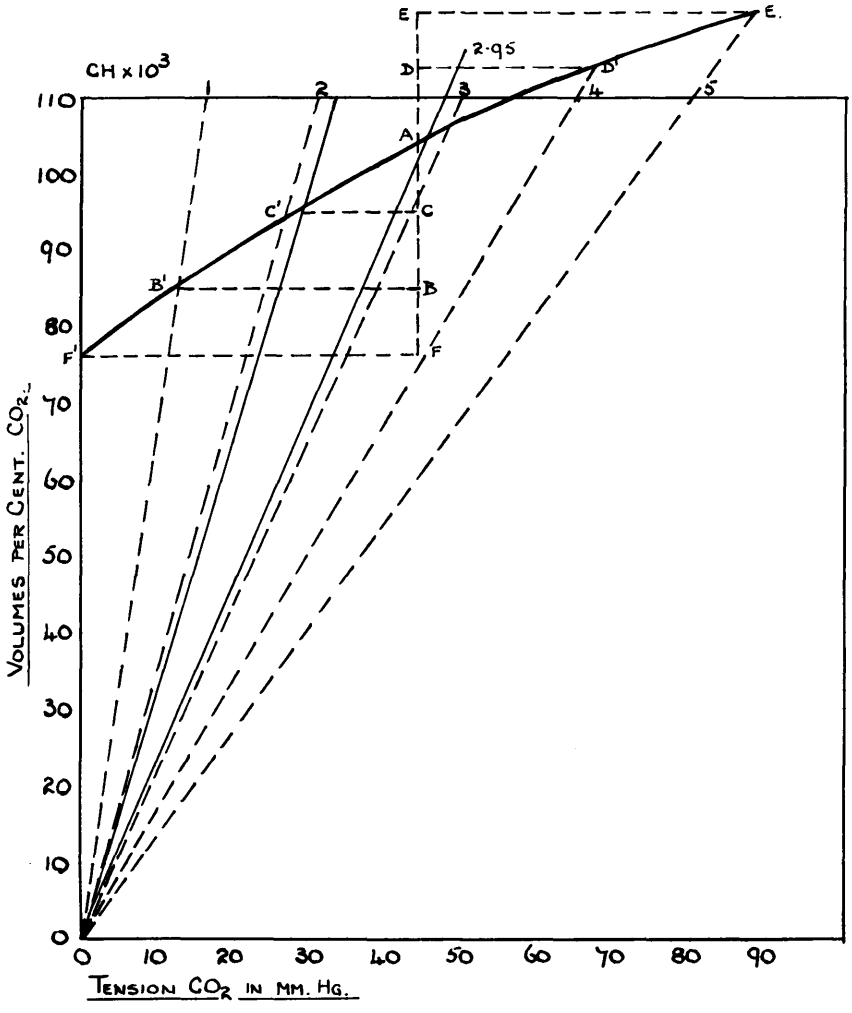
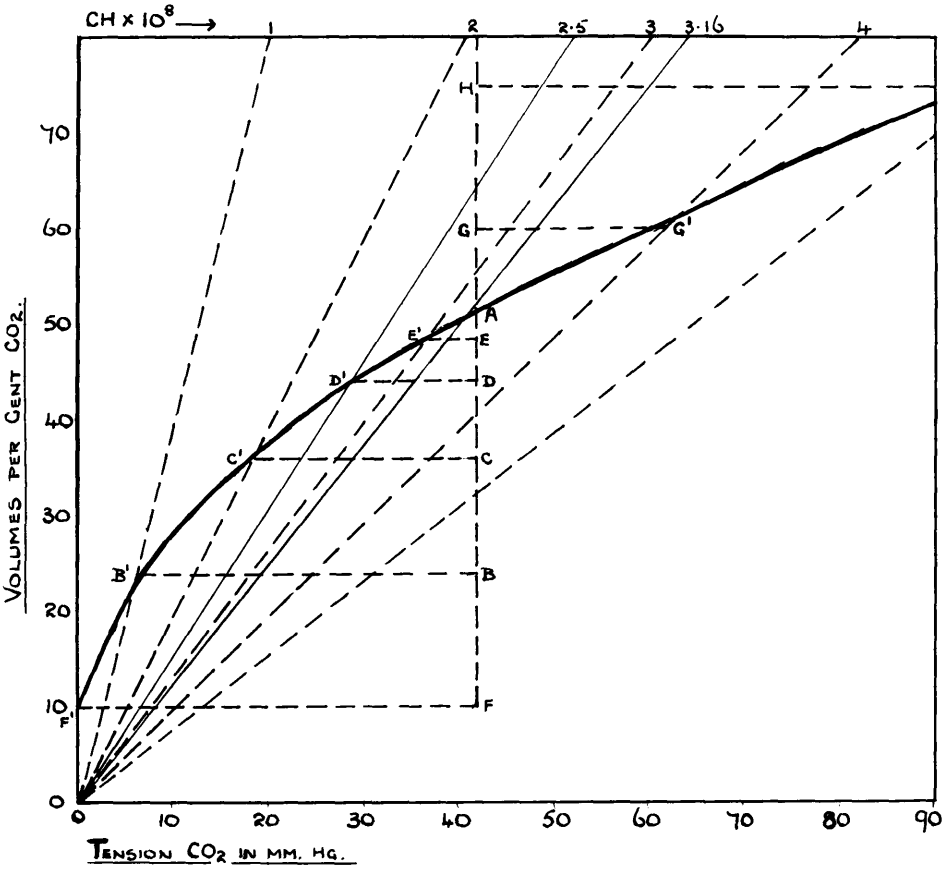


Fig. XVIB.

Theoretical Carbon-Dioxide Dissociation Curve of Blood of Healthy Child (B.H.).

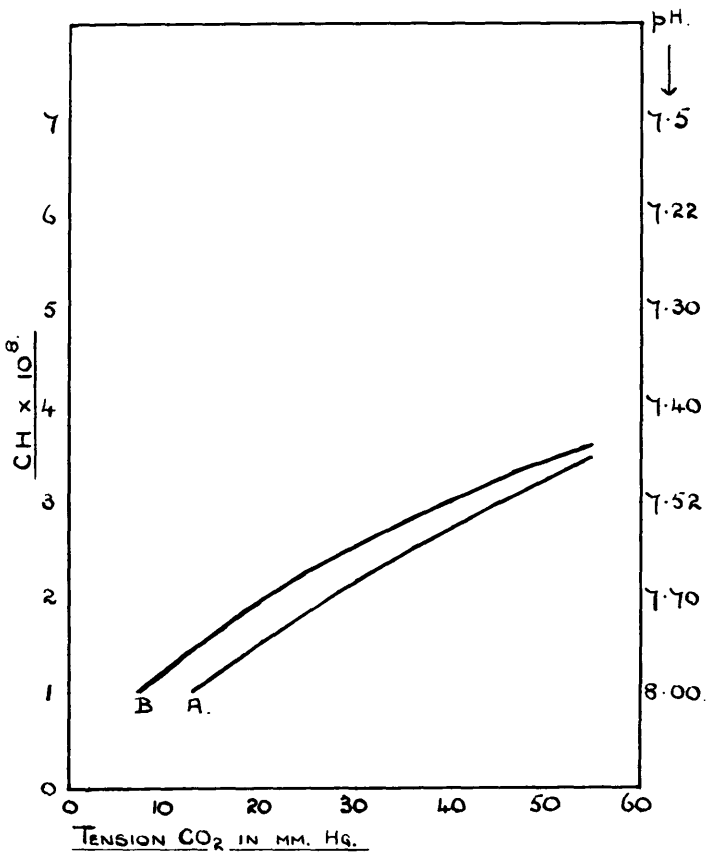


Constructed from the following data:-

CO_2 tension.	CO_2 in Vol.%	CH x 10^8
41.3	52.3	3.16
27.0	43.3	----

Fig. XVII.

Showing Relationship between CO_2 Tension and cH of Blood in Health and Pyloric Stenosis.



A = Pyloric Stenosis
B = Health.

manifestations - vomiting, visible gastric peristalsis, palpable pyloric tumour, very slow, shallow breathing and a blood carbon dioxide of 110 volumes per cent.

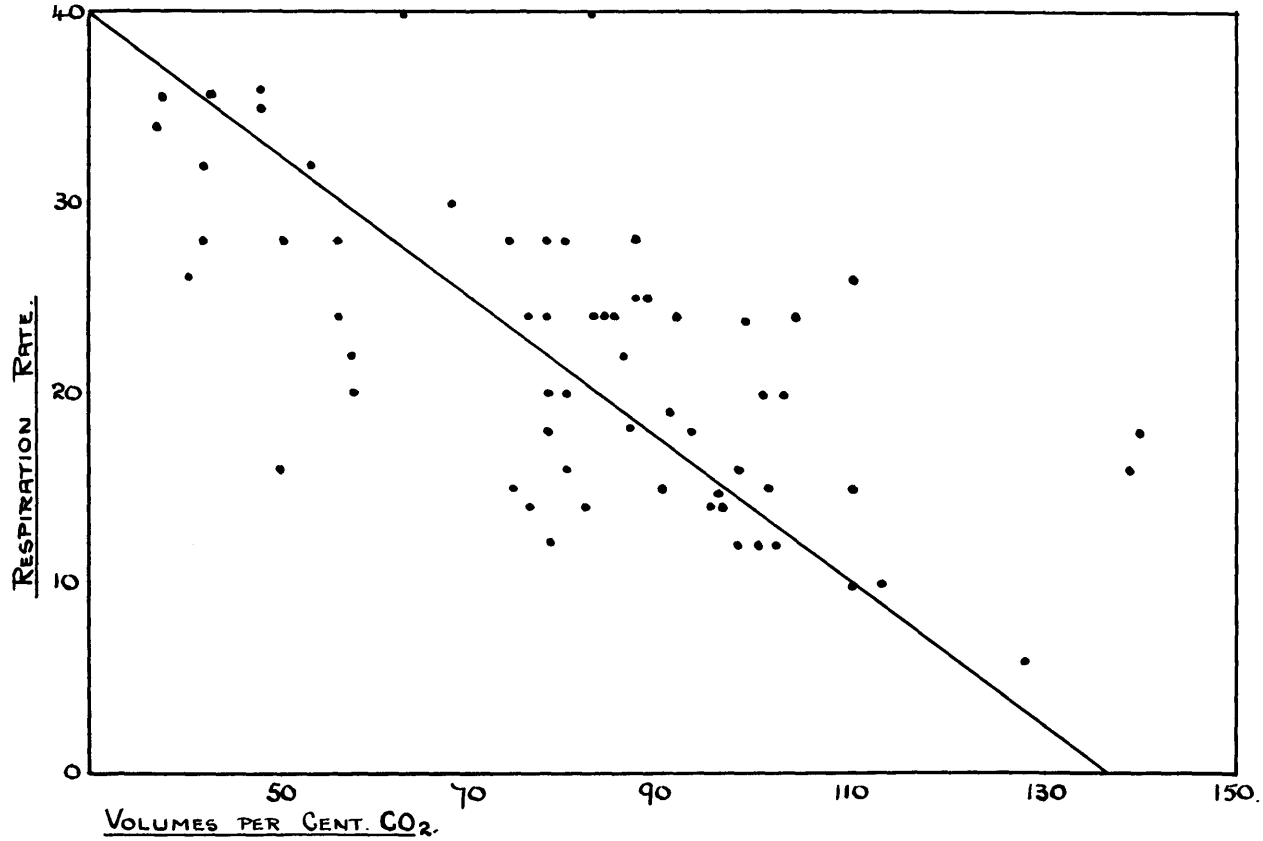
The points used in the construction of the curve were as follows:-

CO_2 tension	CO_2 in vol.%	cH X 10^8
28.5	96.0	-
43.4	103.8	2.95

It will be seen from Fig. 16A that the curve in pyloric stenosis is much higher than in health (Fig. 16B). At a tension of 40 mm. Hg. the volume was 102 vol. %, i.e. 100 per cent. above the standard. The height of the curve was the result of the large amount of available alkali. In this case the degree of buffering (b) was 9.1, quite within the normal limits (b = 6.5 to 10.1) given by Barcroft and his co-workers. The available alkali (c) had a very high value, 76.8 compared with the normal range of 27 to 9: this was almost certainly due to the impoverishment of the chlorine content of the blood. The relationship between tension of carbon dioxide and cH is shown in Figure 17 which indicates that for the maintenance of any given cH in pyloric stenosis the blood must have a higher tension of carbon dioxide than the/

Fig. XVIII.

Showing Relationship between Respiratory Rate and Total CO_2 Content of Blood.



the normal. This finding is clearly associated with the slow shallow breathing.

Relationship of the Carbon Dioxide Content of the Blood and Respiration.

One of the most striking correlations is that between the carbon dioxide content of the blood and the respiratory rate. This is all the more noteworthy when one remembers firstly that it is the total respiratory exchange and not merely the rate of breathing which is of significance and secondly that it is not the total carbon dioxide, but the ratio of the free to combined carbon dioxide which is the regulating factor in respiration. In figure 18 are plotted individual observations of the total carbon dioxide content of the blood against the respiratory rate at the time of withdrawal of the blood. The slower the respiratory rate, the greater is the increase in carbon dioxide. The correlation coefficient (r) of the carbon dioxide content of the blood and the respiratory rate is -0.609, with a probable error of 0.053 so that one is justified in stating that there is a significant relationship between the carbon dioxide content of the blood and the respiratory rate.

TABLE34.

Carbon Dioxide Content of Blood in Non-Obstructive Cases with Reduced Respiratory Rate.

Name	Diagnosis	Respiration-Rate per min.	Carbon-Dioxide Vol.per cent.
W.D.	Congenital Heart Disease	4	52.7
J.L.	Encephalitis Lethargica	8	62.7
"		8	67.0
"		14	56.5
M.M.	Cerebral Haemorrhage	10	57.5
R.M.	Prematurity	10	55.0
J.R.	Prematurity	12	60.6
J.G.	Meningitis	12	57.1
M.C.	Pyuria	18	55.4

(The use of Fisher's t test for small numbers also shows the significance of the correlation $t = \frac{r\sqrt{n'-2}}{\sqrt{1-r^2}}$ n' being the number of pairs of observations. The value of t was found to be 9.8 and n'-2 was 63 so that P, (the probability that the correlation was accidental) is less than one in a hundred).

This relationship between the carbon dioxide content and slow breathing only holds good in patients suffering from pyloric stenosis. In Table 34 are the findings obtained in a series of non-obstructive cases with reduced respiratory rate. It is clear that in these the carbon dioxide content was not high. Of value in the clinical differentiation of these two types of slow breathing is the degree of cyanosis, which is fairly well marked in the non-obstructive series but little if at all in pyloric obstruction.

The Carbon Dioxide Content of the Blood and Vomiting.

There is probably some relationship between the carbon dioxide content of the blood and the severity of the vomiting. It is admittedly difficult to gauge the amount of vomitus and, what is even more important, the amount of chlorine lost, but when the records of individual patients are examined it is found that, occasionally, the carbon dioxide content of the/

TABLE 35.

Showing High Carbon Dioxide Content of Blood in some Cases of Pyloric Stenosis despite the Mild Nature of Vomiting attacks.

Name	Severity of Vomiting	Blood	
		CO_2 content vol.%	Cl' content mg.%
J.L.	Nil	107.9	-
J.D.	Nil	104.1	220
"	Nil	98.1	210
R.S.	Nil	107.0	259
"	Slight	102.4	305
R.M.	Slight	121.6	236
J.J.	Slight	98.8	263

TABLE 36.

Blood-Chemistry in Non-Obstructive Cases associated with severe Vomiting.

Name	Condition	Blood		N.P.N.mg.%
		CO_2 vol.%	Cl mg.%	
P.McD.	Ileo-colitis	48.7	-	-
J.S.	Olitis media	54.7	280	33
R.D.	Ileo-colitis	59.7	315	78
P.O'D.	Pylorospasm	69.0	-	-
A.H.	Olitis media	60.1	285	39
W.B.	Inanition	59.5	-	-
J.H.	Gastritis	63.5	341	52
J.P.	Gastro-enteritis	43.6	328	-
K.M.	Meningitis	50.3	322	73
P.S.	Gastro-enteritis	59.5	336	44

the blood may have remained high although there had been no vomiting for several days prior to the blood analysis (Table 35). On the other hand, in ten patients suffering from vomiting, usually very severe, due to causes other than pyloric stenosis, the carbon dioxide content was never above 70 vol. per cent., and only three times exceeded 60 vol. per cent. (Table 36). Phélizot also found that vomiting in infancy was associated with a high alkaline reserve and diminished plasma chloride only when it was associated with pyloric stenosis or high intestinal obstruction. The evidence is therefore against the view that vomiting is the only cause of the alkalosis. This conclusion is supported by the fact that in a large series of cases free hydrochloric acid was never found in the vomitus and by the absence of any relationship between the carbon dioxide level and the length of time the vomiting has existed.

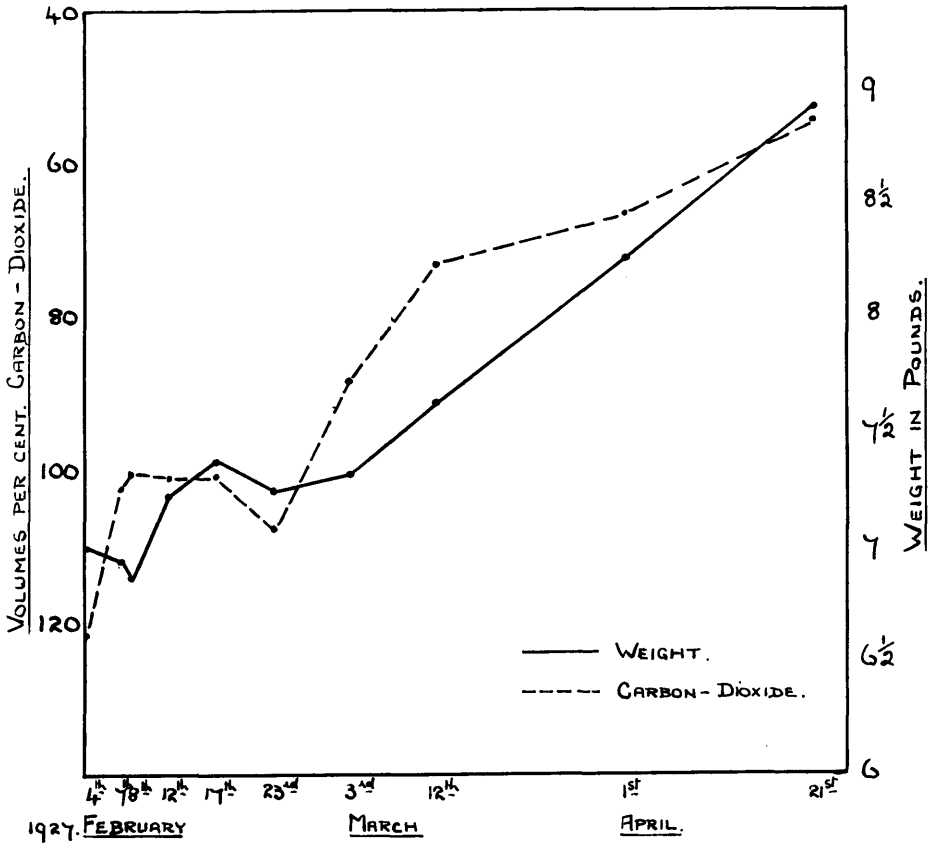
Relationship of Carbon Dioxide Content of the Blood to General Nutrition.

In the individual patient there is an undoubted association between the fall of the carbon dioxide content to a normal level and the increase in the weight of the infant. This is illustrated in Figure 19. An isolated observation

on/

Fig. XIX.

Showing Increase in Weight and Fall in Carbon-Dioxide Content of Blood (Case J.B.)



on the carbon dioxide content of the blood is, however, of no value in prognosis. A high value is not necessarily of graver significance than one moderately increased, although a further rise during the course of the illness does indicate an aggravation of the metabolic disturbance.

The fall in carbon dioxide and the improvement in general nutrition are concomitant events; the latter cannot be said to be caused by the former. In some instances the high carbon dioxide value was reduced by various measures without improvement in the general condition. In one case, by means of blood transfusion, the carbon dioxide content was reduced from 98.0 vol. per cent. before, to 70.2 vol. per cent. immediately after, transfusion. The donor's blood of which 110 cc. were given along with 40 c.c 0.9% saline, contained 62.1 vol. per cent. of carbon dioxide. Assuming the volume of the infant's blood to have been one fifteenth of the body weight (3.1 Kg.) i. e., 200 c.c. then 75.5 vol. per cent. represented the value one would have calculated from a mixture of the two bloods and the saline. Despite the fall in carbon dioxide content there was no apparent change in the clinical condition of the infant and four days later the value had returned to 97.8 vol. per cent.

In/

In another case the administration of calcium chloride caused a reduction of the carbon dioxide content from 110 vol. to 62.3 vol. per cent. without any clinical improvement.

These findings are of general importance since there is a tendency by some to consider that in all forms of acid-base disturbance a return of the carbon dioxide content of the blood to a normal value is the final proof of successful treatment. It cannot, however, be too strongly emphasized that the important factor is the underlying metabolic disturbance. It is comparatively easy to raise or lower the carbon dioxide content by free administration of alkali or acid, but this is of little value unless the underlying metabolic disturbance is also favourably affected.

RISE IN THE NON-PROTEIN NITROGEN CONTENT OF THE BLOOD.

This was a common but not an invariable finding (Table 31). (page 93) It was first reported in high intestinal obstruction by Tileston and Comfort in 1914 and Cooke, Rodenbaugh, and Whipple in 1916. Later workers (Felty and Murray: Foster and Hausler) have confirmed this observation. Haden and Orr state that the increase in non-protein nitrogen does not occur until there is marked depletion of chlorine. This was the general finding in the present series/

series but on three occasions the blood chlorine was above 280 mg. per cent. when the non-protein nitrogen exceeded 50 mg. per cent. Admittedly, however, a great increase was not observed, the highest value being 65 mg. per cent., when the chlorine content was within normal limits. Wildman found that during the alkaline treatment of patients with peptic ulcer the blood urea rose only when there was marked depletion or alkalosis. Haden and Orr attribute the rise in non-protein nitrogen to concentration of plasma from fluid loss and to increase in tissue destruction. This view is supported by the fact that in severe gastro-enteritis with and without vomiting the non-protein nitrogen of the blood is frequently increased. Some examples of this are given in Table 36. (page 101)

Brown, Eusterman, Hartmann and Rowntree suggested that the increase in non-protein nitrogen is the result of impaired renal activity and concluded that the tetany-like symptoms so frequently observed in animals with pyloric obstruction are really uraemic in nature. These authors have given details of the microscopic and macroscopic appearances of toxic nephritis occurring in six cases of fatal pyloro-duodenal obstruction. Ellis supported this view/

TABLE37.

Showing Percentage of Urea in Urine in Cases of Pyloric Stenosis and Non-Obstructive Conditions in Infancy.

Name	Condition	Carbon Dioxide Content of Blood vol. %	Urine (24 Hours) Volume cc.	Urine (24 Hours) Urea g.%
J.D.	Pyloric Stenosis	104.1	170	1.05
R.M.	" "	114.8	280	0.96
J.B.	" "	83.5	100	1.28
C.C.	" "	66.5	350	1.00
A.G.	Inanition	48.1	265	0.56
J.S.	Olitis Media	54.7	39	1.23
P.McC	Empyema	52.3	125	1.08
K.T.	Inanition	45.6	75	1.25
R.M.	Olitis Media	45.6	140	1.14
R.D.	Ileo-Colitis	59.7	85	1.15
J.McK	Gastro-enteritis	58.4	300	0.32
P.S.	Gastro-enteritis	59.5	84	0.50

view by his finding of a low percentage excretion of urea with a high blood urea and by the fact that an alkalosis can be produced by administration of alkali only when the renal function is impaired. In one of the two cases reported by him there was pyaemia involving the kidney and in both there was noted the excretion of an acid urine when there was definite alkalaemia. Venables stated that the alkalosis which may follow alkali treatment is associated with disturbance of renal function. These observations in so far as they relate to the human subject have been made on adults, in whom the presence of renal imapirment is not uncommon and may be merely incidental and not caused by pyloric obstruction. In the congenital hypertrophic pyloric stenosis of infancy, however, there is no evidence of disturbance of renal function apart from the increase in the non-protein nitrogen of the blood. On several occasions the percentage excretion of urea was estimated and found to be of the same order as that found in non-obstructive cases without renal damage (Table 37). This is in accord with the findings of Haden and Orr who showed that in experimental obstruction non-protein nitrogen is often present in the urine in very high concentration. It seems justifiable therefore/

TABLE 38

Showing Part played by Excess Non-Protein

Nitrogen in the Maintenance of Normal Molecular Content of Blood.

Name	CO_2 mM./litre	Cl mM/Litre	Excess N.P.N. mM./litre	CO_2 + Cl mM./litre	CO_2 + Cl + Excess N.P.N. mM/ litre
J.McK.	50	63	0	113	113
W.A.	46	86	0	132	132
"	66	37	27	103	130
R.M.	51	54	4	105	109
"	54	66	3	120	123
"	31	76	0	107	107
"	32	86	3	118	121
J.C.	44	74	3	118	121
J.B.	37	63	6	100	106
W.F.	41	52	11	93	104
P.T.	42	64	6	106	112
R.S.	48	76	1	124	125
"	45	86	5	131	136
E.C.	63	40	2	103	105
N.R.	63	51	8	114	122
A.M.	37	60	0	97	97
W.G.	39	54	0	93	93
M.M.	28	94	5	122	127
M.A.	56	31	20	87	107
E.M.	45	46	9	91	100
J.K.	41	46	3	87	90
A.C.	50	69	0	119	119
G.H.	43	54	7	97	104
W.D.	44	65	4	109	113
C.C.	30	79	1	109	110
J.R.	24	85	2	109	111
J.D.	46	63	3	109	112
"	44	60	2	104	106
"	48	63	4	111	115
"	45	75	2	120	122

therefore, to conclude that in infancy, at any rate, the increase of non-protein nitrogen cannot be attributed to renal damage.

Another view, put forward by Hartmann and Smyth, is that the increase in non-protein nitrogen is a compensatory measure for maintaining the normal concentration of the plasma. These authors calculated the molecular value of the excess non-protein nitrogen on the assumption that it is all urea and that two molecules of urea constitute one milli-equivalent. Similar calculations were made in those cases of the present series with the available data. Some of the findings, which are recorded in Table 38 lend support to their view. Especially is this the case in the patient W.A. whose blood was analysed on two separate occasions. The second analysis revealed a more marked diminution in chlorine with an increase in the carbon dioxide which did not compensate for the extra chlorine deficiency. On calculating the value for the excess non-protein nitrogen it is apparent that the deficiency is almost exactly balanced by the rise in non-protein nitrogen. Many of the other results, however, fail to support this view. Thus in the results of A.M., or W.G. the decrease in the combined carbon dioxide and chlorine value is in neither instance associated with any increase

TABLE 39.

Showing Normal Values for Blood Chlorine in some Cases of Pyloric Stenosis despite Presence of Alkalosis.

(The respiratory volume was reduced in all)

Name	Blood		Urinary Chloride
	Cl Content mg. %	CO_2 Content Vol.%	
J.D.	265	101.9	Faint Trace
R.M.	305	71.3	" "
J.C.	263	97.6	" "
R.S.	305	102.4	Nil
D.C.	291	70.6	Faint Trace
T.C.	259	100.8	Nil
J.J.	263	98.8	Trace.

TABLE 40.

Showing effect of Intravenous Administration of Saline on the Chlorine and Carbon Dioxide Contents of the Blood. (Figures are in milli-Mols.per litre to permit comparison).

Name	Carbon Dioxide		Chlorine	
	Before Saline	After Saline	Before Saline	After Saline
A.B.	47	51	34	60
J.K.	47	49	60	65
R.S.	48	45	73	90

Blood Chlorine. - The fall in the chlorine content of the blood is generally related to the rise in the carbon dioxide value and the degree of alkalosis. Essen, Kauder and Porges maintained that increase and decrease of plasma chlorine was associated with a corresponding movement of bicarbonate in the opposite direction. Straub and Atchley, Palmer. Loeb and Benedict could not, however, confirm this. In the present series instances have been encountered in which the typical picture of alkalosis, raised carbon dioxide content and depressed breathing, was present in spite of a comparatively normal value for blood chlorine (Table 39). In three cases intravenous administration of sodium chloride solution was followed by a rise in the blood chlorine but not by a commensurate fall of carbon dioxide (Table 40). These findings are sufficiently definite to make one hesitate to assume that the deductions drawn from the findings in experimental obstruction hold good in their entirety for pyloric stenosis in infancy.

Wildman reported a reduction of the ratio of cell to plasma chlorine in patients with peptic ulcer and hypochloraemia. Maizels found the same to occur in infants with pyloric stenosis. In two patients of the present series/

TABLE 41.

Showing Relative Volumes of Plasma and Cells

and Distribution of Blood Chlorine in Pyloric Stenosis.

Name	% Content of		Cl content mg.%			Cl cells / Cl Plasma
	Plasma	Cells	Whole Blood	Plasma	Cells	
J.D.	51.1	48.9	220	280	160	0.57
T.McK	51.3	48.7	129	162	94	0.58

series the ratio was determined and found to be 0.57 and 0.58 (Table 41), which values are within the limits (0.52 - 0.58) noted by Gram for normal individuals.

Urinary Chlorine. - It has already been remarked that in pyloric stenosis chlorine, if present in the urine, is only found in very minute amounts. This has been attributed to the impoverishment of the blood chlorine. Such cannot, however, always be the cause since in several instances there has been very little if any urinary chlorine, although the value for blood chlorine was within normal limits (Table 39). After operation chlorine does not begin to appear in the urine in significant amounts for at least six days. In two patients every sample of urine was examined for chlorine. It first made its appearance in one on the sixth, and in the other on the seventh, day after operation and then only in very small amounts: anly on the twelfth day did the chlorine concentration reach a normal value.

The absence of urinary chlorine is of some diagnostic value especially if this finding is obtained on several occasions. When, in addition, there is depressed respiration the diagnosis of congenital pyloric stenosis becomes/

TABLE 42.

Showing Percentage Retention of Chlorine in Pyloric Stenosis When Vomiting was slight or absent.

Name	Date	Amt. of NaCl injected (g)	Volume OF Urine	Na Cl %	excreted Total(g)	% Reten. of NaCl	Vomiting
J.D.1.	24.5.29		220	0.059	0.130		Once in 48 hours.
	25,5.29	1.08	170	0.105	0.179	95.4	
" 2.	3.6.29		800	0.012	0.096		Nil
	4.6.29	0.99	536	0.023	0.123	97.3	
J.R.	27.4.29		265	0.048	0.128		Once in
	28.4.29	0.54	335	0.070	0.232	80.7	24 hours
C.C.1.	17.3.29		232	0.000	0.000		Twice in
	18.3.29	0.99	350	0.047	0.164	83.4	48 hours.
2 after operation	1.4.29		784	0.483	3.787		Nil
	2.4.29	0.90	920	0.508	4.670	2.0	
R.N.	10.10.29		67	0.006	0.004		Once in
	11.10.29	0.90	129	0.059	0.076	92.0	24 Hours

TABLE 43.

Showing Percentage Retention of Chlorine in Non-Obstructive Conditions.

Name	Condition	Date	Amt. of NaCl injected (g)	Volume of Urine	NaCl %	Excreted Total (g)	% Retent of NaC
W.B.	Inanition	1.5.29.		91	0.585	0.532	
	(Vomiting)	2.5.29.	0.90	205	0.647	1.326	11.8
R.W.	Meningitis	5.8.29		370	0.303	1.120	
	(Vomiting)	6.8.29	0.72	450	0.420	1.790	7.0
P.McC	Empyema	10.8.29		95	0.573	0.544	
		11.8.29	0.81	125	0.855	1.069	35.2
J.S.	Otitis Media	23.9.29		239	0.245	0.586	
	(Vomiting)	24.9.29	1.35	285	0.491	1.359	42.7
K.T.	Inanition	2.9.29		80	0.292	0.234	
	(Vomiting)	3.9.29	0.72	75	0.621	0.471	67.0
A.G.	Inanition	12.8.29		265	0.363	0.961	
		13.8.29	0.90	500	0.398	1.990	0.0
J.B.	Gastro-	28.8.29		627	0.198	1.241	
	enteritis	29.8.29	1.08	825	0.269	2.219	9.5

becomes practically certain even when gastric peristalsis and a palpable pyloric tumour are not detected. The finding of abundant urinary chlorine in the absence of parenteral administration of saline is definite evidence that the condition causing the vomiting is not pyloric stenosis.

Retention of Chlorine after Parenteral Administration. - If saline is administered intravenously to an infant with pyloric stenosis, a very marked retention of chlorine takes place greatly exceeding that which occurs both in the normal infant and in most of those suffering from non-obstructive vomiting. In pyloric stenosis over 80 per cent. of the amount of chlorine injected was retained (Table 42) whereas of non-pyloric patients in only one did the retention exceed 60 per cent. (Table 43).

Wendt estimated the normal retention of chlorine in infancy to be 0.102 to 0.647 g. Na Cl per day. In the infants with pyloric stenosis the extra retentions after the administration of saline were 1.031, 0.963, 0.436, 0.826 and 0.828 g. Na Cl whereas in the non-obstructive conditions the corresponding figures were 0.106, 0.050, 0.285, 0.577, 0.483, 0.00, 0.102. It is clear that not only the percentage but also the absolute retention of chlorine is increased/

increased in pyloric stenosis. In the pyloric series only one retention figure fell within normal limits while the others were much higher: in non-obstructive conditions, on the other hand none of the retention values were high. In the second observation of J.D. the blood chlorine amounted to 265 mg. per cent. before the saline was injected.

The excess retention occurs even when there has been no vomiting for several days prior to the injection of saline and the blood has practically its full complement of chlorine. It is also to be noted in the series of four cases recorded in Table 42 that the greatest retention occurred when there was no vomiting during the observation period. In one patient (C.C.) the retention was only 20 per cent. ten days after operation. It may be concluded, therefore, that the body retains chlorine in amounts exceeding the normal whether as a result of fixation in and about the tissues or because of inability to excrete chlorine in the normal way. Hartmann and Smyth have shown that severe vomiting may lead to a depletion of blood chlorine but the fact that excess retention of chlorine does occur in pyloric stenosis even when the blood has its full complement, indicates that the metabolism of chlorine in

pyloric/

TABLE 44.

Comparing Retention of Chlorine and Fixed Base

in cases of Pyloric Stenosis and of Simple Inanition.

(Values given in milli-Mols. per litre to permit comparison.)

Name	Condition	NaCl injected	NaCl actual	retained % of extra intake	Fixed Base actual	retained % of extra intake
J.R.	Pyloric Stenosis	9.23	7.43	80.7	4.56	49.4
C.C.	Pyl.Sten.before operation	16.90	14.10	83.4	6.70	40.0
"	after operation	15.40	0.30	2.0	2.89	18.7
B.W.	Inanition	15.40	2.20	13.3	5.07	33.0

pyloric obstruction presents a different picture from that in non-obstructive conditions. This view is further strengthened by the observation that repeated administration of saline to an infant with pyloric stenosis may lead to a state of oedema much more marked than is found in non-obstructive cases after repeated injections of saline.

The retention of chlorine after the administration of saline is not accompanied by an equal retention of fixed base, as can be seen from Table 44. In the pyloric cases before operation there was a much lower retention of the extra base than of the extra chlorine whereas after operation and in the non-obstructive case the reverse occurred. It would seem, therefore, that in the infant with pyloric stenosis there is a preferential retention of chlorine over fixed base, whereas in the normal infant as Rominger and Meyer, Shohl, Shohl, Wakeman and Shorr, and Boldt, Brahms and Andresen have shown, more sodium than chlorine is retained after the administration of sodium chloride. Gamble and Ross have demonstrated in dogs with experimental occlusion of the pylorus a deficiency both of fixed base and chlorine, the deficit of the latter being the more marked because of the presence in the vomitus of HCl in addition/

addition to B Cl. This might explain the preferential retention of chlorine in pyloric stenosis or infancy were it not for the fact that in the cases examined vomiting was not a pronounced feature and the vomitus contained no free H Cl although neutral chlorine was always present.

Tissue Chlorine. - These observations on the retention of chlorine led naturally to the investigation of the existence of a partial chlorine vacuum in the tissues in the fatal cases. Haden and Orr have already demonstrated that in animals with experimental pyloric obstruction there is a chlorine deficiency in the tissues as well as in the blood. White and Bridge found that the tissue chlorine ran parallel with that of the blood.

Considering the importance of chlorine to the body economy, remarkably few investigations appear in the literature as to the variation in the content of the tissues. Von Noorden gives 0.188 per cent. Cl (52.9 c.cm. N/10) as the average of the bodies of new-born infants, the extreme values being 0.138 per cent. and 0.194 per cent. Analysis of individual tissues in the human subject are rare. Observations have, however, been made on adult tissues by Katz, Moraczewski and Hutchison who report figures varying from/

TABLE 45.

Showing the Chlorine Content of the Various Tissues of the Infant, Expressed in C.CM. N/10.

Group	A			B			C		
Tissue	Normal Cases (six)			Pylorics to whom no saline has been given. (4 cases)			Pylorics to whom saline had been given. (4 cases)		
	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Muscle	54.5	38.2	43.2	31.5	20.0	24.6	69,8	46.0	60.9
Liver	45.9	25.6	36.5	30.3	17.8	23.0	67.6	32.5	50.6
Lung	62.5	47.5	52.4	38.6	19.1	30.7	84.8	37.6	56.6
Heart	41.9	32.7	37.7	16.8	16.5	16.7	48.6	22.2	32.2
Kidney	44.8	39.8	42.1	29.2	15.9	23.0	73.2	29.0	49.2
Brain	58.5	35.9	48.9	41.5	8.2	24.9	62.8	39.7	48.9
Skin	47.7	32.5	39.2	25.2	19.1	24.5	65.4	39.4	56.3

from 0.070 per cent. (19.7 c.cm. N/10) in muscle to 0.219 per cent. (61.7 c.cm. N/10) chlorine in lung. Since no figures pertaining to individual tissues of infants could be found, it was considered advisable to obtain normal standards for the chlorine content of the various tissues as well as to determine the chlorine content of the tissues in the fatal cases of pyloric stenosis. One appreciates, of course, that actually no truly normal tissues can be had, but from the post-mortem material of those cases in which there was no reason to suspect any such change as has been observed in pyloric stenosis, samples of various tissues were obtained.

The method used in estimating the chlorine content of the tissues was the one elaborated by Van Slyke. Portions of the various organs were obtained at the time of the post-mortem examination, and in each case were minced, mixed and weighed as soon as possible. All determinations were done in duplicate, and duplicates were consistent in all cases, the error never exceeding 10 per cent. and usually being much less. In Table 45, the results of the analysis of six such normal cases are shown (Group A) as well as the figures for the analysis of the tissues of eight fatal cases of/

TABLE 47

Showing the Chlorine Content (NaCl c.cm.N/10%) of the Tissues of Four Fatal Cases of Pyloric Stenosis to whom Saline had been given.

Name	Muscle	Liver	Lung	Heart	Kidney	Brain	Skin
T.C.	-	32.5	37.6	22.2	29.0	39.7	39.4
J.M.	46.0	42.2	47.5	25.7	40.5	42.4	50.2
J.B.	67.1	60.2	-	48.6	54.1	50.7	65.4
R.S.	69.8	67.6	84.8	-	73.2	62.8	60.0

of pyloric stenosis in four of whom saline had been given (Group C), and four where no such treatment had been adopted (Group B). The individual values of the various tissues in the cases of Groups B and C are given in Tables 36 and 37.

It will be noted that in those cases not receiving saline the chlorine content is very greatly diminished, the maximal values of this group being usually below the minimal in the normals. These results point very convincingly to the existence of a diminished chlorine content of the tissues in cases of pyloric stenosis. Of the four cases of the last group, high values were obtained, the average for this series exceeding the averages obtained for the control group. It must therefore be concluded that the administration of saline is capable of raising the diminished tissue chlorine content to normal or even to values above normal. Furthermore, it is of interest to note that the samples of urine obtained from the patients in group C for several days before death contained only minimal amounts of chlorine.

THE OCCURRENCE OF OEDEMA IN PYLORIC STENOSIS.

In those infants with congenital pyloric stenosis who are/

are treated with continued intravenous or intraperitoneal injections of saline the occurrence of oedema is not an uncommon event. In the present section details are given of such a condition in three patients: in two the oedema was definitely related to the administration of saline but in the third no saline had been given. Parenteral administration of saline to healthy infants or to those suffering from gastro-enteritis rarely leads to the production of oedema, although a sudden rise in weight, presumably due to a retention of water, may occur. The anomaly of the presence in excess of a fluid rich in chlorine in the pericellular spaces during a condition characterised by chlorine impoverishment is certainly worthy of comment.

Case 1. J.B. Male infant: aged six weeks: vomiting for past five weeks.

13.XI.29. On admission, small emaciated infant weighing 2.2 kg. Visible gastric peristalsis: pyloric tumour palpable: respiration shallow with definite apnoeic periods, rate being 25 per minute.

Blood Analysis -	Carbon dioxide	83.0 vols. per cent.
	Chlorine	225 mg. per cent.
	Non-protein Nitrogen	68.0 mg. per cent.

Urine/

Urine - No chlorine found.

Normal saline (100 cc.) was injected intravenously.

14.Xl.29. Feet puffy.

Normal saline (100 cc.) injected intravenously.

15.Xl.29. Definite generalised oedema.

Normal saline (100 cc.) injected intravenously.

16.Xl.29. Oedema more marked. Breathing still shallow, 20 per minute.

Blood Analysis - Chlorine 320 mg. per cent.

Non-protein Nitrogen 52mg. per cent.

18.Xl.29. Infant looking better. Oedema of feet and ankles still present but less marked. Respiratory rate increased (25 - 30 per minute) but breathing shallow with apnoeic periods.

19.Xl.29. Oedema very much less. Vomiting less.

20.Xl.29. No oedema: infant looks "pinched".

Blood Analysis - Chlorine 320 mg. per cent.

Normal saline (100 cc.) injected intravenously.

Infant died 10 hours later.

It seems safe to assume on the strength of the low blood chlorine content that at the commencement of the treatment there was a depletion of tissue chlorine. The first/

TABLE 48.

Showing the Urinary Findings in Case 1. (J.B.)

and the Percentage Retention of NaCl after Intravenous Injection of Sali

Date	Volume cc.	Titr.Acid cc.N/10%	Ammonia cc.N/10	Urea g.%	NaCl g.%	NaCl Total g.	NaCl injected intraven. g.	Percent. Retenti of Chlo
12.11.29	82	13.6	6.8	2.04	0.0	-	-	-
13.11.29	100	6.4	5.2	1.28	.082	.082	0.9	90.9
14.11.29	91	11.2	20.0	1.55	.130	.118	0.9	86.8
15.11.29	105	5.2	15.2	1.12	.374	.393	0.9	56.3
16.11.29	93	Alk.	62.8	0.72	.316	.294	-	-
17.11.29	71	Alk.	96.8	1.75	.246	.175	-	-
18.11.29	60	Alk.	142.8	1.38	.234	.140	-	-
19.11.29	39	Alk.	113.0	1.06	.328	.128	-	-
20.11.29	59	7.6	34.6	0.7	.585	.345	0.9	-

first injection of normal saline led to a retention of 90.9 per cent. of the amount injected and simultaneously to the development of oedema. After twenty four hours there was "puffiness" of the feet and on the day following the second injection sufficient oedema to produce pitting on pressure over the lower limbs. The ensuing injections of saline led to retention of 86.8 per cent. and 56.3 per cent. respectively. In all 2.7 g. of Na Cl were injected and only 0.54 g. excreted. During this period there was an increase in weight of 256 g., which approximates to the weight of saline retained, on the assumption that the Na Cl was held in the body as a 0.9 per cent. solution. Thus, the weight of saline injected was 300 g. and that of the saline excreted was 60 g. leaving 240 g. The urinary output of chloride gradually increased to a maximum on November 15th (Table 48). Thereafter smaller but still appreciable amounts of chloride were found in the urine until death occurred on November 20th. Unfortunately ten hours before death another injection of 100 cc. of normal saline was given. The chlorine contents of the tissues were estimated (Table 47), and it is apparent that with the exception of brain all were above the maximum values/

values for the control series. Since about 38 per cent. of the chlorine of the last injection was excreted in the urine the high values for tissue chlorine can be attributed only in small part to this last administration of saline. The fact that the blood chlorine was as high as 320 mg. per cent. immediately before the last injection is confirmatory evidence that the excess storage of chlorine in the tissues had already taken place prior to this.

This case also raises the problem of low urinary output of chlorine in the presence of oedema. Evidence of impaired renal function could not be obtained: the percentage excretion of urea and ammonia was quite good (Table 48). Indeed, the output of ammonia would appear to be excessive in view of the fact that the urine was alkaline. Fasold has shown that the administration of sodium chloride to healthy infants leads to alkalinisation of the urine with increased output of ammonia and diminution of organic acids. He attributes the increase in ammonia to the production of a mild acidosis. Here, however, one can hardly assume the presence of an acidosis in view of the high carbon dioxide content and slow respiration. With the data at present available it is impossible to offer more than a conjecture as/

as to the significance of this finding. The work of Parnas on the relationship of ammonia to muscle catabolism does, however, suggest that the disorder is one of the tissues generally rather than of the kidney. The picture presented by the clinical and biochemical findings resembles in many respects that seen in nephrosis where the chlorine appears in minimal quantities in the urine although the power of excreting nitrogenous substances seems quite unimpaired. In the present instance the first injection of saline would, owing to the depletion of the tissue chlorine lead to an outpouring of sodium chloride solution from the blood-stream to the tissue. This would, however, tend to go beyond the equilibrium point, a state of affairs that is well-known in in vitro experiments and which would take some time to adjust itself. Next day, in spite of the now partially replete tissue chlorine, the intravenous injection of more saline by suddenly raising the blood chlorine would again lead to the passage of chloride to the tissues beyond the equilibrium point. The third injection would still further increase the amount of chlorine in the tissues. Forty eight hours after the last injection of saline the oedema commenced to disappear but the total amount of sodium chloride excreted

four/

four days after the last administration of saline amounted to 1.33 g., i. e. less than one-half of the quantity injected. Some of the chlorine was lost in the vomitus and some was retained in the tissues as revealed by the post-mortem analysis.

Another feature of interest was the persistence of shallow breathing on November 16th although the chlorine content of the blood was high, indicating that alkalosis can exist without any depletion of blood chlorine. It is possible that a part of the chlorine was not united to base, thereby leaving an excessive amount of the latter to combine with carbon dioxide. Such an assumption that some of the chlorine was not in the form of inorganic chloride, would explain the presence of depressed breathing and high carbon dioxide content as well as the small amount or absence of urinary chloride although the body as judged by both blood and tissue analysis contained its full quota of chlorine.

Case 2. R.M. Male infant: aged six weeks: expulsive vomiting for past four weeks: visible gastric peristalsis: pyloric tumour palpable. Respiration depressed with apnoeic periods.

On admission (28.VIII.29) the blood findings were as follows:-

Carbon/

TABLE 49.

Showing the Urinary Findings in Case 2 (R.M.) and the Percentage Retention of NaCl after Intravenous Injection of Saline.

Date	Volume cc.	Titr.Acid cc.N/10%	Ammonia cc.N/10%	Urea g.%	NaCl g.%	NaCl Total g.	NaCl injected intraven. g.	Percentage Retention of Chlorine
28.8.29	182	4.4	4.0	1.20	.012	.022	-	-
30.8.29	280	3.6	6.4	0.96	.012	.034	0.45	97.3
31.8.29	250	3.6	12.4	-	.012	.030	-	-
6.9.29	170	Alk.	16.4	-	.012	.020	-	-
7.9.29	178	0.8	14.4	-	.018	.031	0.63	98.2
8.9.29	195	4.8	8.8	-	.070	.137	1.08	90.1
9.9.29	96	4.8	16.0	-	.070	.067	0.90	94.8
10.9.29	66	38.4	42.0	-	.093	.062	0.90	95.3
9.10.29	67	44.8	47.2	-	.006	.004	-	-
10.10.29	129	20.8	46.0	-	.059	.076	1.08	92.0
28.10.29	275	8.0	31.6	-	.176	.483	-	-
29.10.29	330	6.0	21.6	-	.211	.696	-	-
2.11.29	106	-	-	-	.094	.100	-	-
3.11.29	75	41.6	83.6	-	.187	.140	-	-
8.11.29	95	64.8	126.4	2.10	.059	.056	-	-
9.11.29	128	42.0	126.4	2.18	.059	.056	-	-
10.11.29	109	30.2	140.8	2.28	.094	.076	-	-
11.11.29	142	21.6	73.2	1.74	.117	.166	-	-
12.11.29	178	20.8	90.4	1.73	.152	.270	-	-
19.11.29	303	26.4	34.4	0.95	.316	.957	-	-
20.11.29	190	42.0	60.8	1.62	.433	.823	-	-

Carbon dioxide	114.8 vol. per cent.
Chlorine	190 mg. per cent.
Non-protein Nitrogen	57.6 mg. per cent.

The injection of 50 cc. normal saline did not cause any change in the chloride concentration of the urine while the total output was only increased by 0.012 g. of Na Cl, i.e. about 2.7 per cent. of the extra intake.

A week later an injection of saline was given every day for four days. Prior to the commencement of this treatment blood analysis gave the following findings:-

Carbon dioxide	121.6 vol. %
Chlorine	230 mg. %
Non-protein Nitrogen	57.6 mg. %

On the day before the last injection the blood-findings were:-

Carbon dioxide	69.6 vol. %
Chlorine	270 mg. %
Non-protein Nitrogen	32.0 mg. %

The body weight had increased by 370 g. from 2.52 kg. to 2.89 kg. and there was considerable oedema (pitting on pressure of skin of feet and legs). The daily output and retention of salt are shown in Table 49. It will be observed that the urinary output of chloride was very low not exceeding 0.137 g.

The/

The total amount of injected chloride which was not excreted by the urine was 3.304 g. which corresponds almost exactly to the amount of extra chloride present in the body when calculated on the assumption that the increase in weight (370 g.) was due to retention of 0.9 per cent. saline. It is obvious therefore, that chlorine is not excreted by the kidney even when the value for blood chlorine is normal (270 mg. per cent.). The other points of interest are the reduction of blood carbon dioxide and non-protein nitrogen following the injections of saline.

One month later the blood analysis gave the following results:-

Carbon dioxide	71.3 vol.%
Chlorine	305 mg. %
Non-protein Nitrogen	50.4 mg. %

Injection of 120 cc. of 0.9 per cent. saline led to an extra excretion of only 0.072 g. Na Cl, i.e., a retention of 92 per cent. This is a further indication of how little chlorine is excreted by the urine even when the blood chlorine is comparatively high.

The infant made a complete recovery without surgical treatment. Table 49 gives the results of urine analysis on various/

various days during the period of convalescence. It shows how much more prolonged the return to normal metabolic conditions takes in medical as opposed to surgical treatment. It further indicates how the chlorine output may rise to a comparatively normal value only to recede within a few days to a very low amount. This latter occurrence is rare after successful operative interference as the increased output of chlorine is noticeable within a week and reaches a normal level within a fortnight.

Case 3. P.T. Male infant: aged thirteen weeks: vomiting for past seven weeks. Visible gastric peristalsis: pyloric tumour palpable.

On admission (23.XII.29) the infant was acutely ill with a broncho-pneumonia involving both lungs. Blood analysis yielded the following figures:-

Carbon dioxide	95.4 vol. per cent.
Chlorine	230 mg. per cent.
Non-protein Nitrogen	68.2 mg. per cent.

Vomiting was marked on the two following days but slight on the third (25.XII.29). Next day (26.XII.29) there was an increase in weight of 115 g. and on 27.XII.29 of 145 g. Simultaneously with the increase in/

in weight the vomiting ceased and did not recur. On 27.XII.29 slight oedema of the feet was noted. The oedema gradually increased and before death which took place on 30.XII.29, was well marked in both feet and the lumbar region. Analysis of the tissues revealed the chlorine content of the tissues to be within normal limits.

This case illustrates the development of oedema without the parenteral introduction of saline, although it is possible that here the pneumonic condition played a part in the production of the oedema. Retention of chlorine and increase in weight is a well recognised phenomenon in lobar pneumonia. Increase in weight has occasionally been noted in cases of broncho-pneumonia in infancy but rarely, if ever, has definite oedema been detected. Nevertheless, in this case the inflammatory process was so widespread in the pulmonary tissue that it is quite conceivable that the excretion of carbon dioxide was affected and that this together with the abnormality of chlorine metabolism associated with pyloric stenosis was sufficient to produce oedema.

CONGENITAL PYLORIC STENOSIS AND TETANY.

In experimental pyloric stenosis the majority of workers/

workers (McCallum et alii, Hastings, Murray and Murray and others) have found tetany a very common occurrence. Dragstedt and Ellis, however, report that when complete loss of gastric juice was induced by a pyloric fistula with anastomosis of oesophagus and duodenum there was no evidence of tetany although the plasma chloride was low and the pH, bicarbonate and non-protein nitrogen of the serum were high. Withdrawal of chlorine from the body by other means has also been found to be a cause of tetany. Thus Gruenwald found that administration of diuretin to rabbits led to a greatly increased output of urinary chlorine together with a diminution of blood chlorine with rise of non-protein nitrogen: associated with this biochemical picture went an increased muscular excitability frequently ending in toxic manifestations and death.

Clinically the association of vomiting with convulsive seizures has been known for a considerable time. Indeed, Fleiner noted an observation of Morgagni in the Epistola anatomico-medica XXX de vomitu of a case of vomiting, (probably gastro-duodenal ulceration with pyloric stenosis), in whom convulsive seizures occurred. Kussmaul in 1869 remarked on the frequent onset of tetany when there was

excessive/

excessive vomiting. In 1891 Korczynski and Jaworski concluded that the chief cause of the nervous symptoms was the improvement of the tissue-chlorine stores. More recently Ross reported four cases of tetany with alkalosis three of which were associated with pyloric obstruction and Gollwitzer-Meier observed the onset of tetany in an adult patient with pyloric obstruction and all the biochemical changes observed in the pyloric stenosis of infancy. Steinitz attributed the occurrence of tetany to parathyroid insufficiency with chlorine loss as the exciting stimulus. Meyer considers that excessive loss of chlorine frequently leads to pseudo-uraemic manifestations and Porges has described such a condition as coma hypochloraemicum.

In view of the clinical findings it is strange that during the past ten years at the Royal Hospital for Sick Children, Glasgow of 384 cases with congenital pyloric stenosis only two had any signs of tetany. Both had convulsive seizures and in one laryngismus was noted. In six cases the electrical excitability of the neuro-muscular system was determined but in none was any increase found. The reason for this absence of signs of tetany despite the existence of a marked degree of alkalosis is possibly the

presence of an increased serum calcium content. In only two instances was this determined but in one it amounted to 14.0 mg. per cent. and in the other to 12.8 mg. per cent. Klingner in four cases of pyloric obstruction found that the serum calcium was normal but that the ultra-filtrable moiety was low during attacks of tetany. In addition, this investigator found that the serum proteins were high. In the two cases of congenital pyloric stenosis in which serum proteins were determined by the refractrometer, the values were slightly lower than the minimum observed for normal infants. It is possible, therefore, that the rise in total serum calcium without increase in the protein-bound fraction produced a moiety of ionised calcium sufficiently large to neutralise the effects of the alkalosis. Peters and Van Slyke also remark that active tetany is a comparatively rare occurrence in clinical pyloric obstruction even in cases with very high carbon dioxide content of the blood. They state, however, that the hypochloraemia renders these patients very susceptible to the effects of overventilation and that slight hyperpnoea is sufficient to precipitate an attack of tetany. In two instances hyperpnoea (40 per minute) has been observed in association with high carbon dioxide values

(81.3 and 95.4 vol. per cent.) without any sign of tetany.

PATHOGENESIS OF THE ALKALOSIS IN CONGENITAL HYPERTROPHIC PYLORIC STENOSIS.

The cause of the alkalosis is undoubtedly the increased amount of available base resulting from a deficiency of chlorine so that an increase in the carbon dioxide content becomes necessary in order to meet the demand for excess acid. Of all the acid radicles carbon dioxide is the most mobile, responding most readily to deficiency or excess of other acid and being very easily retained in greater or less amounts by variations in the activity of the respiratory system.

The most popular view as to the ultimate cause of the chloride deficit is that elaborated by Gamble and his co-workers, namely that the loss of chlorine by the vomitus is the primary factor, leading to a depletion of tissue and blood chlorine. Gatewood, Gaebler, Mantwyler and Myers have maintained that alkalosis is not produced by excessive loss of gastric juice in the vomitus if there is lack of hydrochloric acid in the gastric secretion. This is not in accord with the findings of Ellis who demonstrated alkalaemia in patients who suffered from vomiting associated with achlorhydria/

achlorhydria. Furthermore all the observations made on the vomitus obtained from infants with pyloric stenosis have shown that free hydrochloric acid has not been present and that the pH is usually about 5.7 . The occurrence of alkalosis without loss of free hydrochloric acid can, however, readily be explained by the fact that the store of chlorine in the body is much less than that of fixed base, so that the amount of the former lost is, relative to its reserve in the tissues, much greater than that of the latter and therefore of more importance in determining the direction of the shift in acid-base equilibrium. A more serious objection was the finding of Haden and Orr who produced a condition of alkalosis by experimental pyloric obstruction in rabbits, animals that do not vomit. Gamble and McIver, however, were able to show that in rabbits there followed a very marked dilatation of the stomach into which the chloride was secreted and virtually lost to the body despite the absence of vomiting. In rats, in which the pylorus had been ligated, Drake, McKhann and Gamble found that the total body chlorine was diminished by 28.3 per cent. and that this could be entirely accounted for by loss in the gastric contents.

Still/

Still more support is given to this view by the results obtained when animals are fed for long periods on a chlorine-poor diet. Thus Frouin found that dogs became dull and ultimately suffered from twitchings and convulsions when the intake of chlorine was reduced to a minimum. Gruenwald also observed toxic manifestations with reduction of blood chlorine and increase of non-protein nitrogen when the chlorine stores were depleted. A still more convincing proof of the effect of chlorine loss is the fact noted by Haden and Orr and Gatch, Trusler and Ayers that in experimental pyloric obstruction perenteral administration of saline prevented the onset of symptoms of toxaemia and alkalosis presumably owing to the body store of chlorine being kept normal.

Examination of the evidence satisfies one as to the adequacy of Gamble's view in explaining the causation of the manifestations of experimental pyloric obstruction and probably of pyloric stenosis due to carcinomatous involvement in the adult human subject. In the infant with congenital hypertrophic pyloric stenosis there have been recorded certain facts which cannot be explained in this way. In the present series of cases there have been noted several/

several in whom a typical picture of alkalosis with raised blood carbon dioxide content and depressed breathing was present during a period of several days in which no vomiting occurred. In one instance there had been no vomiting since birth and the suspicion of pyloric stenosis was raised by the clinical observation of slow respiratory rate. In another case there occurred an increase of blood carbon dioxide and decrease of chlorine during a prolonged absence of vomiting. In one patient of the series reported by Maizels, alkalosis increased long after the vomiting had ceased: aspiration of gastric residue and lavage were carried out so that about three ounces of fluid were removed daily, leading to a loss of chlorine which the author thinks might gradually have produced the alkalotic conditions. The few cases of the present series in which alkalosis was present despite the absence of vomiting cannot be explained in this way since lavage was not adopted as a routine treatment. Nor is it at all certain that lavage acts in this way since Salvesen found that in two adult patients with pyloric obstruction, repeated gastric lavage was associated with a fall of the bicarbonate and rise of the chloride of the serum.

If the hypothesis that the cause of the alkalosis is the loss of chlorine by the vomitus one would have expected to find some correlation between the various chemical changes and the duration of the illness. Such was not the case either in this series or that of Maizels. Furthermore, the increase of carbon dioxide content of the blood was not always proportionate to the chlorine deficit. Per contra the raising of the blood chlorine to normal by administration of saline, intravenously or otherwise, was at times not accompanied by a fall in the carbon dioxide content or a restoration of the breathing to normal. Still another point which is difficult to explain on the basis of Gamble's theory is the occasional absence of urinary chlorine despite a value for blood chlorine within normal limits. Maizels also noted the absence of chloride from the urine in two cases with raised plasma chlorine. Apparently dehydration was present so that the total chlorine in circulation was low, and he suggested that this together with a rise in renal threshold was responsible for the absence of urinary chloride. While the increase in renal threshold might conceivably have played a part in the cases reported here, there certainly was no deficiency in body fluid since in several of the/

the cases isotonic saline solution had been given intravenously and in three oedema had been produced.

Another hypothesis which has received considerable support is that the condition is in part due to renal inefficiency. Apart from occasional post-mortem findings of renal damage in adults, the chief evidence in favour of this view is the presence of an increased non-protein nitrogen content of the blood. It has previously been shown, however, that there is no defect in the kidneys' ability to excrete either urea or ammonia. Furthermore, it is well known that in conditions in which there is excess tissue breakdown especially when associated with dehydration, there is frequently an increase of non-protein nitrogen of the blood. Very significant in this connection is the finding of Michelsen that impoverishment of the body chlorine resulting from excessive diuresis induced by repeated doses of diuretin is accompanied by an increase in blood urea. The author attributes this almost entirely to an increased destruction of tissue protein. He also mentions the possibility of a relative incompetence of the kidneys on the strength of a low nitrogen concentration in the urine but this latter finding cannot be interpreted as an indication of renal insufficiency/

insufficiency in view of the unlimited supply of water obtained by the rabbits and the diuresis. It is possible, nevertheless, that there is a partial defect of renal function in so far as base may be excreted with difficulty, although in two cases recorded here the sodium of injected sodium chloride was excreted in greater amount than the chlorine. While therefore the possibility of renal damage acting as a factor in the production of alkalosis cannot be denied, it is necessary to bring forward much stronger evidence than has yet been adduced.

There remains a third hypothesis, that of Haden and Orr who first suggested that the chlorine was fixed in the tissues by some toxin. They supported this view by the fact that beneficial results are obtained from administration of saline in experimental ligation of the pylorus. These can be equally well explained on Gamble's theory which Haden and Orr now accept.

Drake and Tisdall have shown that injection of histamine, a substance which is elaborated in the gut, leads to a reduction of plasma chlorine which cannot be attributed to vomiting. In the human subject it has been noted by Rafflin and Saradjichvili that subcutaneous injections of histamine/

histamine led to a temporary increase of chlorine output in the urine followed quickly by a fall when the gastric secretion increased. Alsina and Pijoan have found that interference with the circulation of ten to twelve centimetres of bowel in dogs produces a fall in plasma chlorine and an increase in alkaline reserve. This suggests the elaboration of some toxic product in the damaged area of bowel wall. McQuarrie and Whipple, indeed, have demonstrated the formation of a toxic proteose in a ligated loop of intestine.

The fact that a high value for blood chlorine has been occasionally found with a high carbon dioxide content and absence of urinary chloride suggests the possibility of chlorine existing in the body in a form which is not united with base. Falta, and Richter-Quittener have reported that ordinarily some of the plasma chlorine is not ionised while Blum, Delaville and Caulaerts found that in cases of acidosis chlorine was not completely ultrafiltrable. Of interest in this connection is the work of Katsu who found that the ionic activity of chlorides in solution was reduced by the addition of glycine. This is in accord with the observations of Morris and Morris that part of the chlorine in the blood and tissues may exist in organic form and/

and that among the factors which determine the amount of this organic chlorine is the concentration of nitrogenous substances such as urea and amino acids. It is feasible, therefore, that the primary cause of congenital hypertrophic pyloric stenosis is a disorder of the metabolic processes taking place in the pyloric musculature and leading to the production of some histamine-like substance, which, on absorption, causes abnormal metabolism of chlorine. This is not to say that the loss of chlorine by the vomitus is not an important, if not the main, factor in diminishing the blood chlorine. Indeed in view of the well-known stimulating action of histamine on the gastric secretion it is possible that a histamine-like substance is responsible for the great loss of chlorine by the loss of gastric juice. Thus it would be responsible for diminishing the ionic chlorine content and therefore for increase of carbon dioxide and alkalosis not only by changing inorganic chlorine into an organic form but also by increasing the loss by the gastric secretion. It may be objected in view of the findings of Michelsen of chlorine deprivation on tissue breakdown that it is unnecessary to postulate anything more than an excessive loss of chlorine which in turn would give rise/

rise to excess protein disintegration and consequent de-ionisation of some of the chlorine. This, however, would not explain the presence of alkalosis in patients who had never lost any chlorine by vomiting.

THERAPEUTIC INDICATIONS.

From the practical point of view it would seem that there is a definite indication for the administration of saline when there has been a considerable amount of vomiting. Care, however, must be taken that oedema should not be caused since, as has already been noted, this can and does occur much more rapidly in congenital pyloric stenosis than in other conditions. The administration of saline is specially necessary when together with the chlorine depletion there is an excess of nitrogenous end-products in the blood since a liberal supply of chlorine is necessary for normal nitrogen metabolism.

Another point in treatment is the avoidance of the use of alkali in gastric lavage or the employment of citrated milk in feeding, since it would appear rational to suppose that the additional alkali would aggravate the condition of alkalosis.

GENERAL CONCLUSIONS.

Congenital/

Congenital hypertrophic pyloric stenosis is associated with an alkalosis characterised by depressed or periodic breathing, increase in the carbon dioxide and non-protein nitrogen contents and decrease in the chlorine content of the blood. The fixed base of the blood is unaltered or at most only slightly diminished.

There is a definite correlation between the rise in blood carbon dioxide and the diminished respiratory volume.

Although generally the severity of the condition is roughly proportional to the variation from normal of the biochemical findings, this is not always the case. In certain instances the loss of chlorine could not have been due to vomiting.

In untreated pyloric stenosis the chlorine content of the tissues is low. This can be corrected by the administration of saline which, if continued, may lead to an excessive retention of chlorine and the production of oedema: in such cases the chlorine content of the tissues is higher than normal.

Restoration of the blood chlorine does not necessarily result in a correction of the alkalosis as is evidenced by the persistence of the high total carbon dioxide content

and/

and the depressed breathing.

A normal blood chlorine may be accompanied by a fractional amount of urinary chloride.

Three theories of the pathogenesis of alkalosis in pyloric stenosis are discussed and it suggested that the primary disturbance may be an excessive production of a histamine-like substance in the pyloric musculature.

---0---

Chapter Five

DISTURBANCE OF ACID-BASE EQUILIBRIUM IN THE PATHOGENESIS OF RICKETS AND INFANTILE TETANY.

Chapter Five.

DISTURBANCE OF ACID-BASE EQUILIBRIUM IN THE PATHOGENESIS OF RICKETS AND INFANTILE TETANY.

For many years opinion has been divided as to the presence of disturbances in acid-base equilibrium in rickets and infantile tetany. The view that rickets is essentially a condition of acid-poisoning was promulgated many years ago and is still held by some workers (Pritchard and Blum, Delaville and Van Caulaert). The latter investigators consider that in rickets there is a state of non-gaseous acidosis. There is, in addition, a large body of authoritative opinion which stresses the association on the one hand of rickets and acidosis, and on the other, of tetany and alkalosis. It is, of course, well-known that conditions of gaseous and non-gaseous alkalosis such as are induced respectively by hyperpnoea and by excess alkali medication of patients with peptic ulcer or pyuria, may precipitate an attack of tetany. In this chapter the investigations deal with/

with the variety of tetany associated with rickets and it is to be understood that unless otherwise indicated the term tetany refers solely to the condition generally known as rachitic tetany.

Liégeois and Lefèvre reported a reduction of total carbon dioxide, relative increase of free carbonic acid to bicarbonate and a reduction of the pH of the blood of dogs with spontaneous rickets. No change in the actual reaction of the blood has, however, been detected in rickets by György, Kappes and Kruse but a low carbon dioxide capacity of the blood has been reported by these same workers, Burgess and Osman, Lenhardt and Chaptal and Blum and his co-workers. Shohl, Brown, Rose, Smith and Cozad, on the other hand, have published results showing that in rats made rachitic on a high calcium low phosphorus diet (Steenbock 2965) serum analyses indicate a state of the acid-base equilibrium bordering on alkalosis. Whether or not a reduction of the alkaline reserve is present in rickets it does not necessarily signify a condition of acidosis, as György himself admits. In tetany the pH of the blood has been reported as high by Turpin and Holló and Weiss but generally, published values have fallen within normal limits (György/

(Gyorgy and co-workers: Drucker and Faber). Decreased values for the alkaline reserve have been reported in infantile tetany by Calvin and Borovsky, Scheer, Rohmer and Woringer, and Drucker and Faber. Anderson and Graham obtained normal values in a large series of cases while Lesné, Turpin and Guillaumin found an increased alkaline reserve. It is clear that attempts to obtain direct proof of change in the acid-base equilibrium of the blood and tissues have met with equivocal results. Certainly one is not justified in drawing any conclusions from the reported changes in the carbon dioxide content of the blood. The fact that reductions have been noted both in rickets and in tetany militates against the view that there are contrary metabolic tendencies in these conditions as far as acid-base equilibrium is concerned.

The evidence obtained indirectly is even more confusing and has given rise to widely varying interpretations. In florid rickets Hodgson, György (1923) and Freudenberg and György have reported a great increase in the urinary acid output. The bulk of this acid is present in combination with ammonia the output of which may be so increased as to render the urine alkaline. This latter finding is certainly not/

not characteristic of acidosis and ought to make one pause before classifying rickets as an acidotic condition, as has been done by Freudenberg and György. In no other acidotic state is the ammonia output so high as to render the urine alkaline. As a matter of fact a very high output of ammonia has been found in cases of congenital pyloric stenosis with all the clinical and biochemical signs of alkalosis (Chapter Four). In tetany, György (1922) and Hottinger have each reported a reduction of titratable acidity and ammonia of the urine: the latter also demonstrated a decrease in the output of organic acids. On the other hand, Zehnter and Foncin found the ammonia excretion to be increased in tetany while Tezner could detect no significant departure from normal either in the reaction or the acid output of the urine. Drucker also was unable to obtain any support for the view that tetany was associated with alkalosis: he states that the diminished acid excretion found by György was due to the relatively low-protein diet which the latter investigator employed.

Recently György (1929) has expressed the opinion that a state of acidosis, while not necessarily the fundamental feature, is certainly associated with rickets and that calcification/

calcification is prevented by the increased acidity of the bone-forming tissues. Freudenberg and György maintain that the condition of acidosis is characterised by an increased acid production due to Stoffwechsel-verlangsamung. As instances of this metabolic retardation they mention the slower rate of glycolysis in the blood and tissues of rachitic rats found by Freudenberg and Welcker and Brock and Welcker, the increased excretion of organic acid demonstrated by Hottinger and the fact that a greater degree of ketonuria appears in rachitic than in non-rachitic children on a fat-rich diet. This evidence is not above criticism since Fasold has shown that the output of organic acid is decreased in acidosis. Furthermore, Bosanyi has found that the pH of rachitic cartilage is more alkaline than the normal, being 7.6 in the former as compared with 7.2 in the latter.

The slowing of the metabolic processes is manifested according to Freudenberg and György by defective oxidation and to this cause is attributed the rachitogenic effect of strontium, thallium and other metals all of which act as cellular poisons. It is further maintained by György (1929) that intracellular oxidation depends in great part on the phosphate ion so that according to this hypothesis "rachitic acidosis"/

acidosis" is due to hypophosphataemia. It is true that acidotic disturbances are accompanied by phosphorus loss and as has been shown in chapter two, the loss may take place either by the urine or the faeces and is often accompanied by a reduction of the serum phosphate. Schloss has objected to the "acidotic" conception of rickets on the grounds that administration of acid leads not to rickets but to osteoporosis and that administration of alkali does not cause healing of rickets. This objection has been countered by György (1929) with the statement that one cannot infer the reaction of the tissues from that of the blood since the two need not run parallel but in fact diverge because of compensatory activity. Brühl indeed states that in rickets the capillary blood is more acid than the venous blood from the longitudinal sinus - a central acidosis.

Alkalosis, on the other hand, is, according to Freudenberg and György, due to a diminished production of acid in the tissues resulting from Stoffwechselbeschleunigung. Hanssen noted the occurrence of spasms in children after the administrations of large amounts of sodium bicarbonate while Howland and Marriott reported the onset of tetany. Anderson and Graham and Frank, Nothmann and Wagner were, however,/

however, unable to increase neuro-muscular excitability in children without tetany by giving large doses of alkali. Johanssen, indeed, produced manifest attacks in children who were the subjects of latent tetany but neither Tezner nor Drucker and Faber could confirm these results. The last-named authors found only a trifling increase in galvanic excitability in one of two patients with tetany who had been given six grams of sodium bicarbonate daily for a week. The weight of the evidence is against any tetanigenic effect of large doses of alkali certainly in normal subjects and most probably in patients with infantile tetany. Nor apart from the urinary changes does alkali produce any obvious alteration in the acid-base equilibrium of these subjects. But in children in whom there is some impairment of renal function especially pyuria or where there has been a recent upset in acid base balance, e.g. acidosis in gastro-enteritis, the administration of sodium bicarbonate may result in the development of a non-gaseous alkalosis, which is occasionally accompanied by manifestations of tetany.

The following case illustrates the development of alkalosis and tetany in an infant with pyuria.

E. McG./

E. McG. female, six months. Admitted with pyuria: was given sodium bicarbonate 1 gram four hourly. After forty eight hours on this treatment an attack of tetany became manifest. Carbon dioxide content was 103.8 vol. %. The sodium bicarbonate was discontinued and calcium chloride commenced. No more convulsions occurred.

The association of tetany with alkali therapy in patients with defective renal function indicates that alkalosis may give rise to tetany but does not justify the conclusion that in infantile tetany there exists a condition of alkalosis.

Most workers believe that hypocalcaemia is an essential concomitant and probably the important factor in the pathogenesis of infantile tetany. Freudenberg and György, however, have pointed out that it is not so much the fall in total serum calcium as the diminution in the amount of ionised calcium that is of importance. They believe that in infantile tetany there is an Entionisierung of the serum calcium resulting from simultaneous alkalosis and phosphate stasis. György (1929) extended the original Rona equation for determining the amount of ionised calcium by introducing the phosphate buffer system.

$$\frac{(Ca^{++})\ (HCO_3^-)\ (HPO_4^{--})}{(H^+)\ (H_2PO_4^-)} = K.$$

On the assumption that the calcium-precipitating power of the phosphorus in the acid phosphate is compensated by the acid nature of the salt György eliminated the H_2PO_4 from the denominator so that the equation becomes

$$\frac{(Ca^{++})\ (HCO_3^-)\ (HPO_4^{--})}{(H^+)} = K.$$

On the basis of this equation the decrease in calcium ionisation in tetany is explained as the result of decrease in the concentration of hydrogen ions and increase in the phosphate concentration of the serum. The validity of this extended Rona equation has, however, been adversely criticised by Holló and Holt, La Mer and Chown, who state that the equilibrium between calcium and phosphate with precipitation of calcium phosphate develops so slowly that this process cannot appreciably influence the amount of ionised calcium in the blood <u>in vivo</u>. Furthermore, high values for serum phosphate are by no means the rule in infantile tetany. A low value is regularly found associated/

TABLE 50.

Showing Low Serum Phosphorus Values

in Children with Tetany.

Name	Age	Diagnosis	Serum Inorg. Phosphorus mg.%	Serum Calcium mg.%
J.C.	1 11/12	Rickets and Tetany	3.4	5.3
R.McA.	2 3/12	Rickets and Tetany	3.0	6.1
J.S.	2 10/12	Coeliac Disease and Tetany	2.5	6.3
E.B.	4 6/12	Coeliac Disease and Tetany	2.3	6.5

associated with tetany in coeliac disease (Table 50) but even in infants with rickets normal values have been found by Iversen and Lenstrup and Hess, Calvin, Wang and Fletcher. György himself found that occasionally the serum phosphate was slightly below normal but attributed this to the fact that in rickets it is very low so that the value obtained in rachitic tetany was really an increase on that present before the onset of tetany. In table 50 are noted low serum inorganic phosphorus values obtained in patients with rickets and tetany.

It has already been shown that the evidence on which the assumption of an alkalotic tendency in tetany is based is equivocal. It is necessary now to examine the data which are brought forward to support the view of phosphate stasis. Since Binger succeeded in producing tetany by the injection of neutral or alkaline phosphates, the retention of phosphorus has been favoured by many as an important etiological factor in the production of tetany. It was natural to attribute its action to the lowering of serum calcium and the fact that this also took place without the symptoms of tetany when acid phosphate was injected, was accounted for by the increased ionisation of the remaining calcium as a result of the/

the rise in hydrogen-ion concentration. Binger thought that the phosphates precipitated the calcium but this is unlikely since one would expect to find some correlation between rise in phosphate and fall in calcium which, as has been shown by Salvesen, Hastings and McIntosh, and af Klercker and Odin, does not exist. Jeppsson and af Klercker produced increased mechanical and galvanic hyperirritability and even manifest tetany in dogs, rabbits and children by the administrati of phosphates. They concluded that the phosphate ion had a specific tetanigenic action, and supported their view by the fact that infants fed on breast-milk which has a much lower phosphorus content than cow's milk, never get tetany and also because they were able to diminish the tetanigenic effect of cow's milk by precipitating the alkaline phosphates. The specific action of phosphate was also favoured by Elias and Kornfeld and Nothmann and Guttmann who stressed the importance of the reaction of the solution used. These results have not, however, been allowed to pass unchallenged. Rohmer and Woringer and af Klercher and Odin state that oral ingestion of phosphate has no specific effect except in subjects with manifest and latent tetany, while Calvin and Borovsky found that administration of alkaline phosphate had no/

TABLE 51.

Showing Partition of Phosphorus in Whole Blood of Children with Infantile Tetany.

(Figures in mg.per 100 cc.)

Name	Age	Inorg.P.	Ester P.	Acid-Soluble P.	Lipoid P.	Total P.
Normal Average		4.2	22.4	26.6	13.7	40.3
J.K.	1 1/12 yr.	5.5	17.4	22.9	8.0	30.9
J.M.	4 2/12 "	3.5	19.9	23.4	10.4	33.8
J.C.	1 6/12 "	4.0	21.2	25.2	11.7	36.9
T.M.	8/12 "	5.7	21.4	27.1	10.4	37.5
G.T.	1 5/12 "	6.1	27.3	33.4	9.3	42.7

no effect in increasing irritability either in rachitic children or even in those with latent tetany.

György (1929) who is one of the chief exponents of the view that phosphate stasis plays an important part in the production of tetany postulates such a stasis on the grounds (1) that urinary phosphate is greatly diminished during the attack of tetany and much increased as a result of acid therapy, and (2) that the acid soluble phosphorus falls during acid treatment synchronously with the rise of serum calcium and disappearance of symptoms. Neither of these reasons is, however, sufficient to justify the conclusion that there is a true increase of phosphate retention in infantile tetany. Sokolovitch, working in my laboratory showed that during active tetany the acid soluble phosphorus is generally not increased and may be lower than normal although the inorganic phosphorus is raised. In a series of five cases the total acid-soluble phosphorus was in only one above the normal range (Table 51). Thus the increase in inorganic phosphorus is probably to be attributed not to increased phosphate retention but to increased break-up of ester phosphorus. It is certainly not permissible to infer an increased phosphorus retention from high values of blood phosphate/

phosphate since Ford has shown that these may be present in cases of chronic interstitial nephritis associated with low phosphorus retention. Although György reported a decreased phosphate output in infantile tetany, Zehnter and Foncin found in twelve children with tetany that the amount of phosphate excreted in the urine was higher during the tetany period than after recovery. The decreased urinary excretion of phosphate, however, when it does occur, probably indicates nothing more than a diversion of the route of elimination from urine to faeces and certainly cannot be taken as an index of phosphate stasis in the body.

Important evidence in support of the view that increased retention of phosphorus plays a part in the pathogenesis of tetany has been brought forward by Shohl, Bennett and Weed, and Hess, Weinstock, Benjamin and Gross. Shohl showed that in rats which had developed rickets on a diet with a calcium-phosphorus ratio of five to one, symptoms of tetany appeared when the phosphorus intake was increased so that the ratio was two to one. Hess obtained similar results with the apparent paradox that rats previously fed on a low phosphorus-high calcium intake developed tetany when the diet was changed to a normal one. Gerstenberger, Hartman, Russell and/

and Wilder noted the occurrence of tetany in infants during the healing of rickets especially when the therapy was interrupted or insufficient so that the healing was incomplete. They attributed this to sudden increase of phosphorus retention. It has long been known that the very early stage of healing in rickets is frequently associated with tetany and it is possible that the prevalence of this latter condition in the spring months of the year is due to the slight healing effect of the comparatively weak sunshine of these months. Rominger, Meyer and Bomskov maintain that this association of rickets and tetany results from the fact that in the very early healing stage of the former disease the retention of phosphorus increases before that of calcium.

A review of the evidence adduced indicates that the role of acid-base disturbance and phosphorus in the pathogenesis of infantile tetany is still in doubt and that while there is no direct proof against the association of rickets and acidosis the trend of the evidence is against such a relationship. The work detailed here was undertaken to throw some light on these problems. It is given under three headings. In the first place are described the changes/

changes in metabolism taking place in rickets with and without tetany both in the active and healing stages. Secondly, the problem of increased retention of phosphorus during the early stage of healing is discussed together with the effect of increased intake and retention as a factor in the causation of tetany. Lastly are detailed the consequences of an artificially induced acidosis on active rickets.

A.

METABOLISM IN RICKETS WITH AND WITHOUT TETANY IN THE ACTIVE STATE AND DURING HEALING.

For this purpose the subjects were two children with the clinical and radiological signs of active rickets. The urine and faeces of each were collected for a period of seven days while aliquot samples were taken of the daily diet on which the child had been fed during the previous weeks. When this seven-day period was completed, medication with vitamins D and A was commenced and the excreta and food collected as before.

The clinical and metabolic findings in each patient are/

are given in detail.

CASE 1. E.B. Female. Aet. 5 years. Healthy at birth, she appeared to thrive well till 4 years old when she had a severe "cold". Since then she has not walked. Three months ago a deformity of the right arm was noticed. She is a very small thin deformed child with fiddle-shaped chest, bowing of both tibial femora and clavicles and a very marked curvature of the right forearm. Height 96 cm. (normal for age - 108 cm.). Weight 8.5 Kg. (normal 18 Kg.). X-ray of both wrists showed active rickets with no indication of healing. Chvostek's sign +. The gastric juice was acid but contained no free hydrochloric acid before or after a test-meal of thin oatflour porridge. After a "metabolism period" of seven days, adexolin (3 minims daily) was given. Within eleven days the X-ray showed evidence of very slight healing although Chvostek's sign was still +. No sign of tetany was present two weeks after the commencement of the vitamin D therapy and healing was marked on the radiograms three days later. Nine weeks after treatment was initiated, a test meal revealed the presence/

TABLE 52.

Showing Composition of Urine for the Various Periods (Average Daily Figures) - Case E.B.

Period	Vol. cc.	Titr.Acid cc.N/10	Ammonia cc.N/10	Urea g.	Total Nitrogen g.	Na Cl g.	Ca O g.	P_2O_5 g.
1	593	40.6	191.0	8.290	4.455	1.258	0.035	0.563
2	686	62.5	167.9	8.736	4.934	1.567	0.290	0.751
3	563	63.9	158.5	7.96	3.996	1.364	0.046	0.753
4	450	89.1	167.1	8.650	4.434	1.275	0.024	0.806
5	445	89.7	148.2	7.962	4.377	0.831	0.030	0.657
6	476	91.3	107.8	7.844	3.946	1.168	0.047	0.751
7	666	118.5	133.6	9.039	4.975	1.366	0.012	0.993
8	647	123.5	89.4	10.999	5.348	1.600	0.028	0.921
9	590	89.3	54.4	7.266	4.090	1.362	0.057	0.785
10	603	104.5	81.2	8.953	4.954	1.469	0.068	0.853
11	678	112.2	69.3	7.771	4.650	1.408	0.131	0.812

Period 1 - No treatment. Signs of Tetany

Periods 2 - 5 Treatment. Signs of Tetany still present.

Periods 6-11 Treatment. No signs of Tetany.

TABLE 53.

Showing Excretion and Retention of Lime and Phosphorus for the Various Periods.

(Average Daily Figures). Case E.B.

Period	CaO Intake	CaO Output Urine	CaO Output Faeces	CaO Retention	P_2O_5 Intake	P_2O_5 Output Urine	P_2O_5 Output Faeces	P_2O_5 Retention
	g.							
1.	1.602	0.035	1.399	+0.168	2.252	0.563	1.406	+0.283
2.	1.755	0.029	2.159	-0.433	2.345	0.751	2.077	-0.483
3.	1.604	0.046	0.903	+0.655	2.024	0.753	0.499	+0.772
4.	1.675	0.024	0.554	+1.097	2.309	0.806	0.450	+1.053
5.	1.582	0.030	0.532	+1.020	2.282	0.657	0.413	+1.212
6.	1.625	0.047	0.534	+1.044	2.206	0.751	0.382	+1.073
7.	1.660	0.012	0.252	+1.396	2.238	0.993	0.205	+1.040
8.	1.302	0.028	0.577	+0.697	2.307	0.921	0.413	+0.973
9.	1.482	0.057	0.279	+1.346	2.306	0.785	0.184	+1.337
10.	1.676	0.068	0.526	+1.082	2.307	0.853	0.364	+1.090
11.	1.679	0.131	0.507	+1.041	2.245	0.812	0.333	+1.100

Period 1. No treatment. Signs of Tetany.

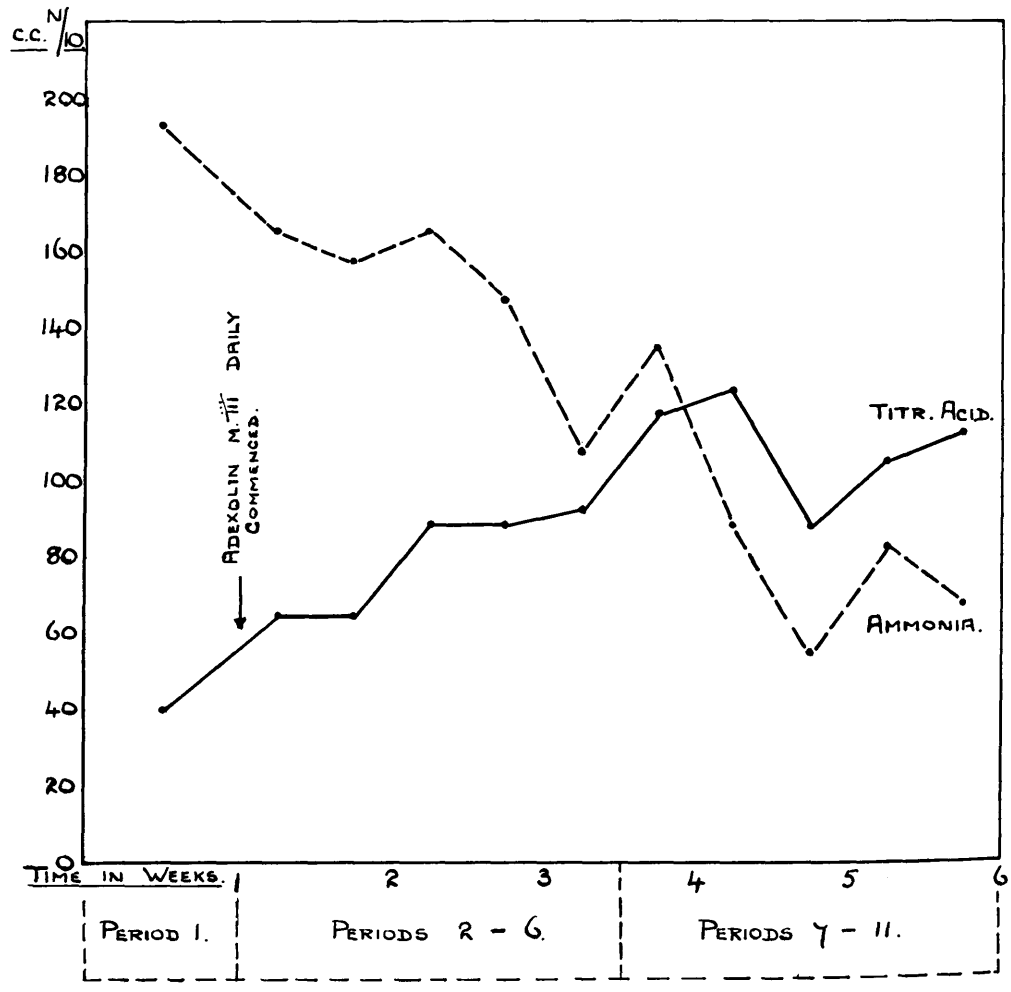
Period 2-5 Treatment. Signs of Tetany still present.

Period 6-11 Treatment. No signs of Tetany.

Fig. XX.

Daily Output of Ammonia and Titratable Acid in c.c. N/10.

E.B.



presence of free hydrochloric acid in the gastric juice although in subnormal amount (10.5 cc. N/10 per 100 cc.). The results of the urinary and faecal analyses are given in Tables 52 and 53.

Immediately after the administration of the adexolin there was a gradual increase in the urinary output of titratable acid and decrease in that of ammonia. (Table 52 and Fig. 20). The urinary output of calcium did not show any significant increase till the end of the fourth week of vitamin D treatment when healing was already marked. The renal excretion of phosphorus, on the other hand, commenced to increase immediately after the initiation of treatment. During the first three days of adexolin administration there was a negative balance of both lime and phosphorus. György (1930) stated that it took ten to fourteen days to produce a demonstrable effect on the mineral metabolism of the healthy organism with irradiated ergosterol but Bauer, Marble and Claflin found that the alteration in mineral metabolism occurred immediately as a rise in faecal, and a fall in urinary, calcium and phosphorus followed by a gradual fall in the faecal output and increase in the urinary excretion. Hottinger had previously described a similar effect and referred/

Fig. XXI.

Retention of CaO and P_2O_5 in mg. per Kilo. per Day.

E.B. (Rickets and Tetany).

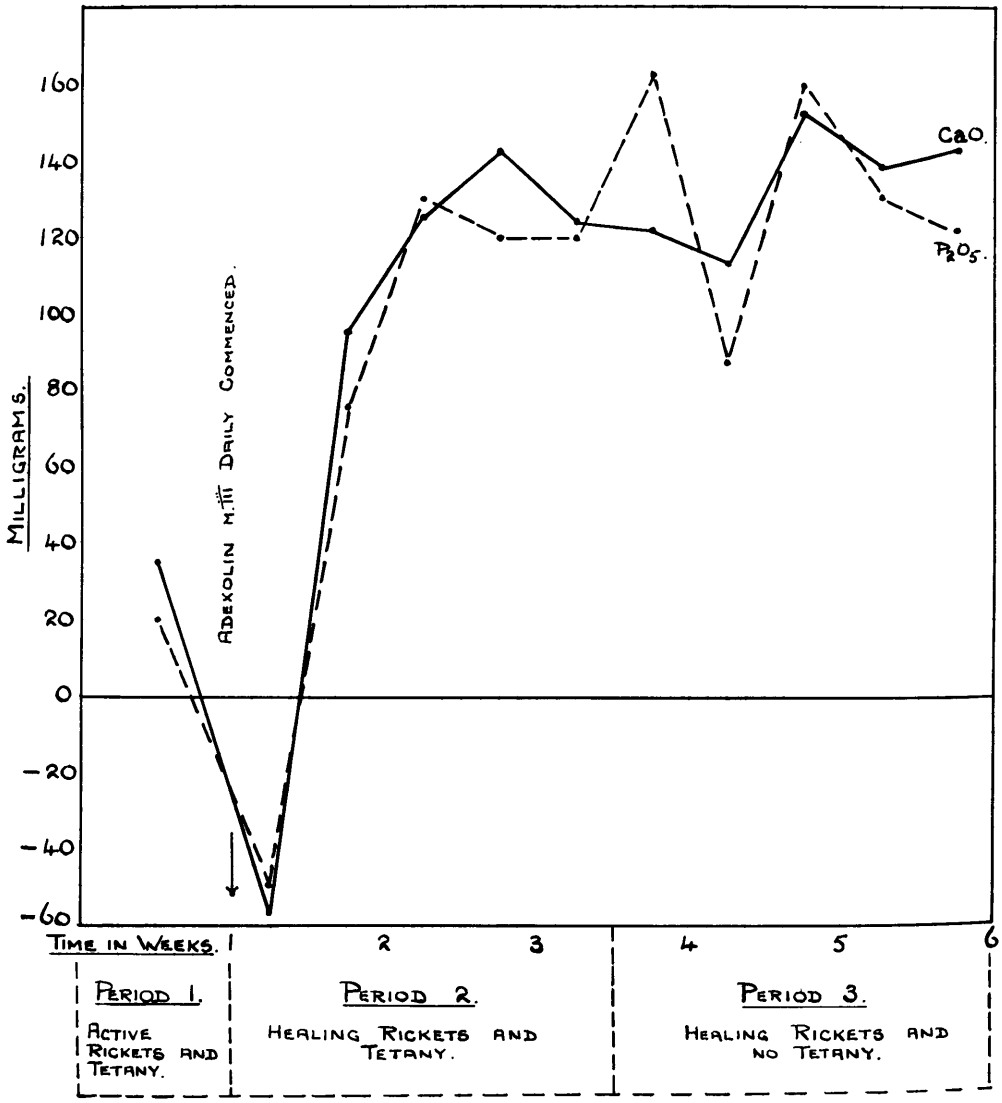
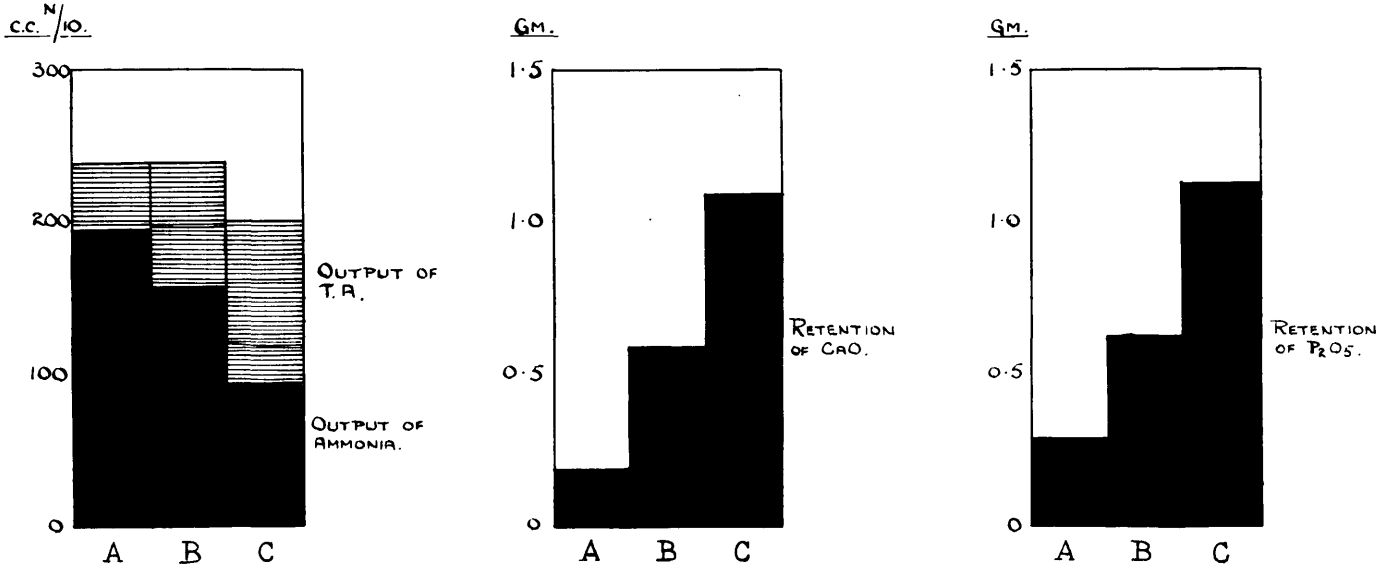


Fig. XXII.

Case E.B.



Period A - Active rickets and tetany.

" B - Healing rickets and tetany.

" C - Healing rickets; no tetany.

referred to it as a biphasic action. It is apparent that this biphasic effect was very well marked in the present case (Table 53 and Fig. 21). After this short period of negative mineral retention the storage of calcium and phosphorus in the body increased. When the metabolic results are computed for three periods (A) before treatment, (B) during treatment and persistence of signs of tetany and (C) during treatment and absence of signs of tetany, it will be observed that there is a fairly close parallelism between the increased excretion of titratable acid, decreased output of ammonia and increased retentions of calcium and phosphorus. (Fig.22).

CASE 2. J.W. Male. Aet. 2½ years. He appeared to thrive normally till 1 year old when he had an attack of broncho-pneumonia. Although he was previously able to walk with support, he has not walked since. He is a small peevish child; Height 66 cm. (normal 86); Weight 6.5 Kg. (normal 13.1), with bossing of the skull, well marked Harrison's sulcus, beading of the ribs and enlargement of the epiphyses. Radiograms of both wrists showed severe rickets with no indication of healing. Chvostek's sign was not present. After a metabolism/

TABLE 54.

Showing Composition of Urine for the Various Periods.

(Average Daily Figures). Case J.W.

Period	Volume cc.	Titr. Acid cc.N/10	Ammonia cc.N/10	Urea g.	Total Nitrogen	NaCl g.	CaO g.	P_2O_5 g.
1.	611	11.5	424.6	8.757	4.969	1.299	0.043	0.617
2.	590	14.2	498.9	8.448	4.459	1.289	0.048	0.608
3.	590	27.5	441.0	8.899	4.716	1.391	0.061	0.811
4.	637	91.5	250.6	8.595	4.469	1.434	0.031	1.107
5.	523	106.6	244.7	8.203	4.429	1.217	0.021	1.100
6.	497	64.3	296.9	8.106	4.458	1.272	0.049	1.092
7.	483	99.6	258.5	8.549	4.531	1.427	0.072	1.120
8.	473	132.3	183.3	8.275	4.634	1.362	0.062	1.013
9.	440	128.3	197.0	8.245	4.598	1.272	0.108	1.190

Fig. XXIII.

Daily Output of Ammonia and Titratable Acid in c.c. N/10.

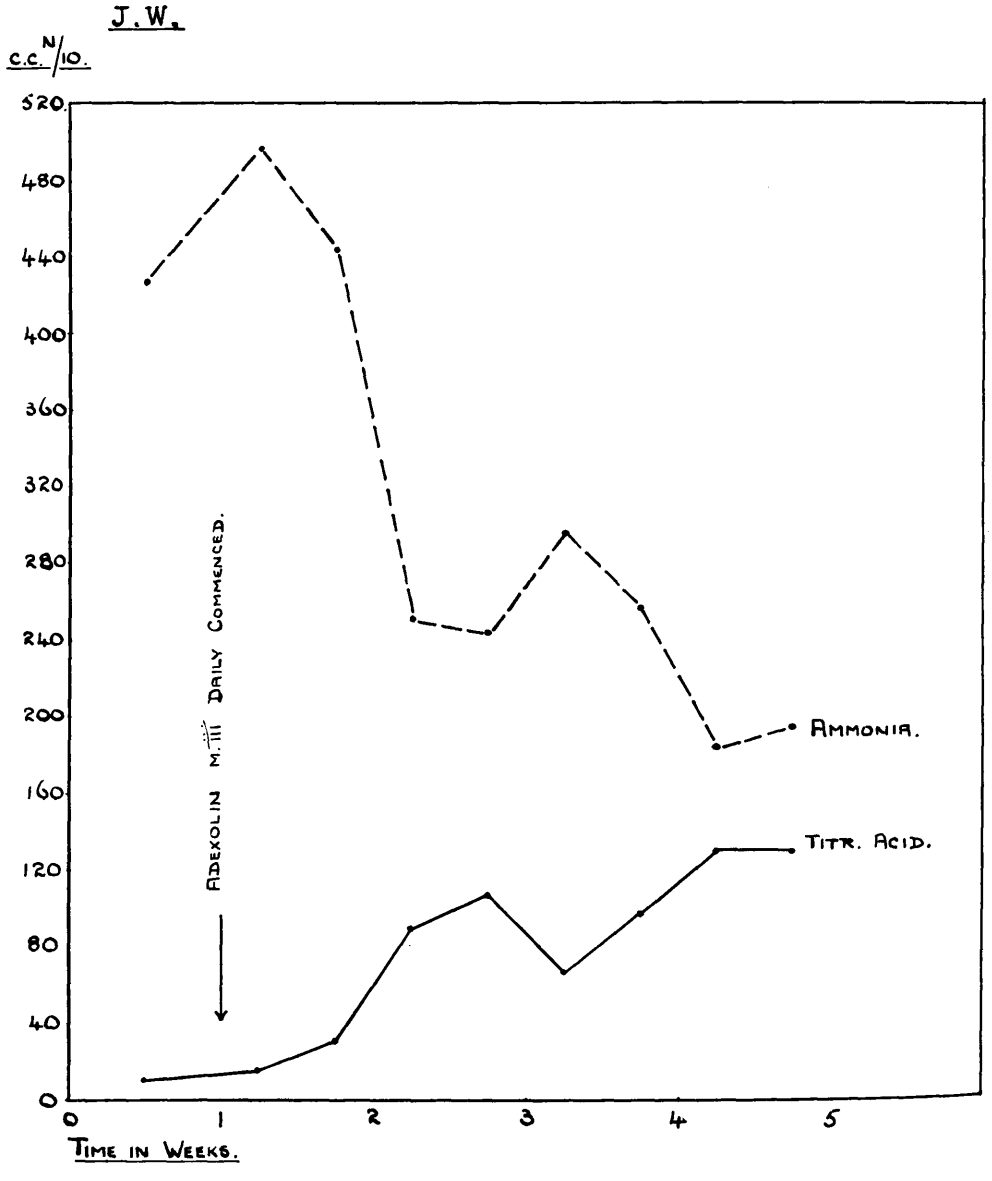


TABLE 55.

Showing Excretion and Retention of Lime

and Phosphorus for the Various Periods. (Average Daily Figures).

Case J.W.

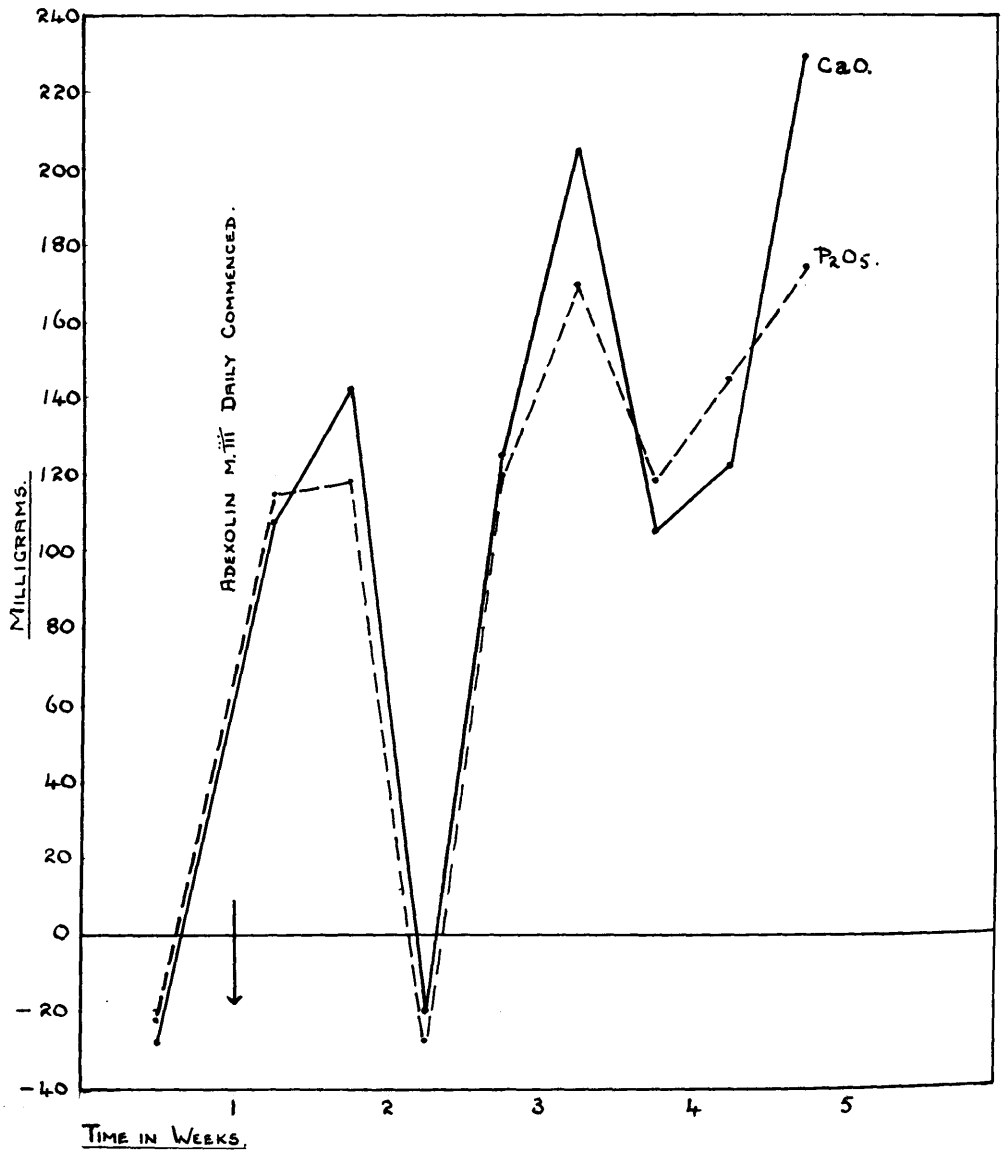
g.

Period	CaO Intake	CaO Output Urine	CaO Output Faeces	CaO Retention	P_2O_5 Intake	P_2O_5 Output Urine	P_2O_5 Output Faeces	P_2O_5 Retention
1.	1.693	0.043	1.817	-0.167	2.343	0.617	1.879	-0.153
2.	1.655	0.048	0.940	+0.667	2.307	0.608	0.992	+0.707
3.	1.665	0.061	0.735	+0.870	2.287	0.811	0.733	+0.743
4.	1.702	0.031	1.800	-0.129	2.353	1.107	1.419	-0.173
5.	1.679	0.021	0.877	+0.781	2.340	1.100	0.480	+0.760
6.	1.680	0.049	0.374	+1.257	2.291	1.092	0.149	+1.050
7.	1.695	0.072	0.973	+0.650	2.268	1.120	0.408	+0.740
8.	1.680	0.062	0.863	+0.755	2.397	1.013	0.481	+0.903
9.	1.682.	0.108	0.141	+1.433	2.324	1.190	0.053	+1.081

Fig. XXIV.

Retention of CaO and P_2O_5 in mg. per Kilo. per Day.

J.W. (Rickets: No tetany).

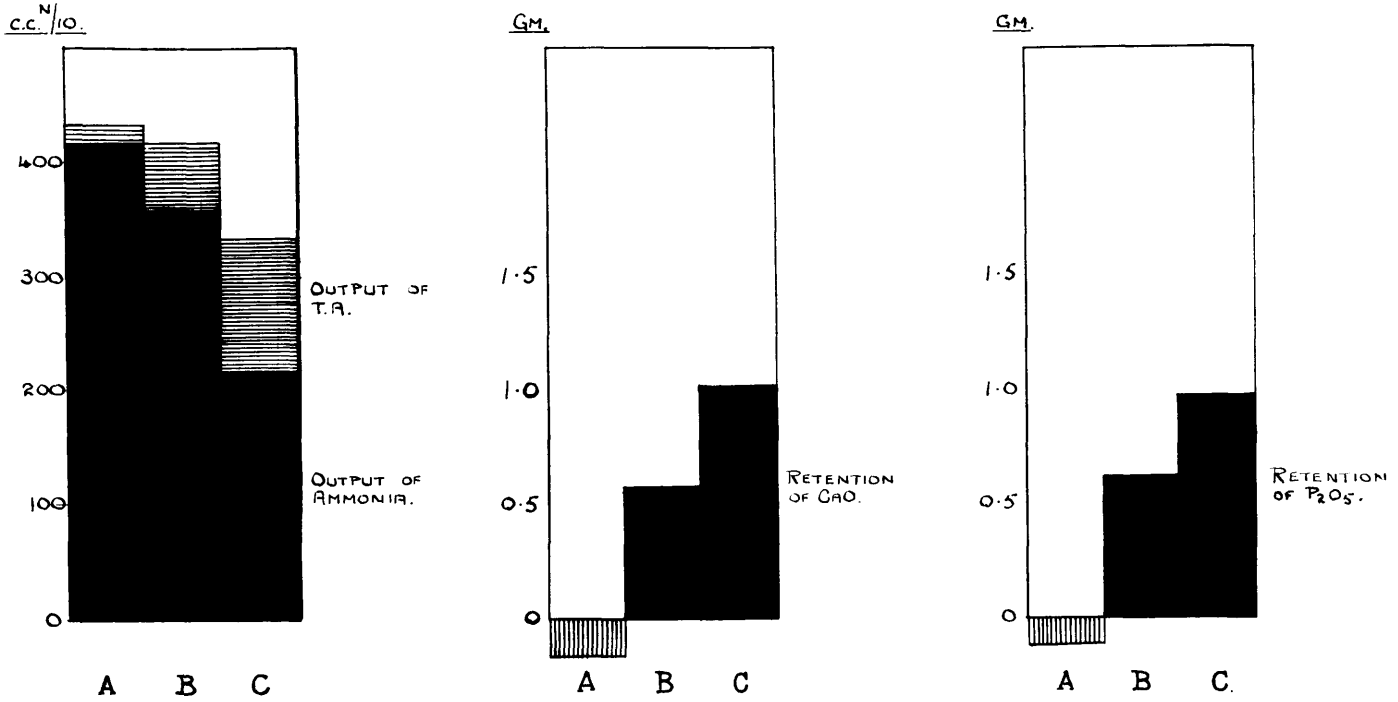


"metabolism period" of seven days, adexolin (3 minims daily) was given. Within two weeks evidences of healing were present in the radiogram. During the period of investigation which lasted for five weeks no signs of tetany were present.

The results of the analyses of urine and faeces are given in Tables 54 and 55. Following the commencement of vitamin D therapy the urinary output of titratable acid was increased while that of ammonia was diminished (Table 54 and Figure 23). The renal excretion of phosphorus was also immediately increased whereas that of calcium was not significantly altered throughout the course of the investigation (Table 55). The biphasic effect of Hottinger was also noticed in this case (Figure 24), but the negative balance of minerals did not occur till the second week of vitamin D administration, coinciding with a very marked increase in titratable acidity of the urine and diminution in ammonia output. Here also when the metabolic results are computed for (A) the pre-treatment, (B) early healing (2 - 5) and (C) later healing (6 - 9) periods the parallelism between increase in titratable acidity diminution in urinary ammonia and increase in mineral retention is apparent/

Fig. XXV.

Case J.W.



Period A - Active rickets: no tetany.

" B - Healing rickets (early): no tetany.

" C - Healing rickets (later): no tetany.

TABLE 56.

Showing Output of Urinary Ammonia and Titratable Acid and Retention of Minerals in Case E.A.(Average Daily Figures).

Period	Urine			Faeces		Retention		
	Titr.Acid cc.N/10	Ammonia cc.N/10	CaO g.	P_2O_5 g.	CaO g.	P_2O_5 g.	CaO g.	P_2O_5	Fat %
1. No Rickets	105.8	165.8	.026	1.042	1.690	1.430	0.059	-0.185	68.7
2.Active Rickets	31.9	253.3	0.052	0.884	1.843	1.670	-0.110	-0.100	77.3
3.Healing Rickets	196.2	148.0	0.043	1.286	0.130	0.040	+1.910	+1.530	97.7

apparent (Fig. 25).

In view of the correlation between the urinary output of titratable acid and the retention of minerals during the healing of rickets the following data are recorded from a case of coeliac disease before the onset of coeliac rickets, during the active stage of the rachitic process and during the healing period.

CASE 3. E.A. Female. She was first admitted to hospital when 4 years old. She was healthy at birth but at 3½ months weighed only 6¼ lbs.: motions at this time were bulky and pale. On admission she was small, and spare. Height 78 cm. (normal 96.7), weight 8.48 Kg. (normal 15.41). The abdomen was prominent and the gluteal regions wasted. She was kept in hospital for 3½ months: stools continued pale and offensive with short periods of intermission. At 5¼ years during a second readmission when there was no radiographic evidence of rickets but manifest symptoms of coeliac disease, the mineral retention was determined (Period 1). Nine months later at 6 years the signs of rickets were present. The mineral retention was again determined before (Period 2) and one month after (Period 3) the commencement/

commencement of adexolin treatment. The results are given in Table 56.

As far as utilisation of fat and minerals is concerned there is a close resemblance between the results obtained in periods 1 and 2, both being characterised by poor retentions. But the output of ammonia and titratable acid in period 1 resembles that of period 3 where the retentions of fat and minerals were good. This would indicate that the high ammonia and low titratable acid output found in active rickets is not due directly to the low mineral retention but rather that both are associated with some metabolic disturbance in the tissues. This view is supported by the finding that while plasma phosphatase is increased in rickets it is not outwith normal limits in coeliac disease unless rickets is also present.

Discussion - The results of the investigations on these three patients may now be briefly summarised.

The administration of a preparation containing vitamins D and A to children suffering with active rickets was followed by parallel increases in the retentions of calcium and phosphorus. These parallel increases occurred whether or not the signs of tetany were present. During the active stage of rickets the urinary titratable acid was greatly reduced/

reduced and the renal excretion of ammonia increased whether or not tetany was present. During the process of healing the changes in titratable acidity (increase) and ammonia (decrease) were roughly parallel with the increased mineral retentions.

The greatly increased output of ammonia is a common occurrence in acidotic conditions where there is not impairment of renal function. Satke and Bartolomey, however, after determining the ammonia coefficient and cH of urines of patients with varied types of disease concluded that an increased urinary excretion of ammonia was not necessarily evidence of an acidotic state. Hasselbalch further showed that in acidosis the rise of ammonia coefficient was accompanied by an increase in acidity of the urine. Accordingly in view of the frequent occurrence of an alkaline urine during active rickets it is hardly probable that the stimulus leading to increased ammonia formation is the necessity for neutralising and excreting excessive amounts of acid. Kroetz and Hottinger go so far as to suggest because of the increased output of acid that a state of acidosis is produced in the early stages of irradiated ergosterol treatment. This, however, is unlikely/

unlikely since synchronous with the increase in titratable acid goes a decrease in the ammonia-combined acid. Much more feasible is the possibility that ammonia has to be formed in order to transport the normal amounts of acid because the fixed base is retained in excess in the tissues. While, therefore, the increased output of ammonia in a urine the alkalinity of which frequently exceeds a pH of 8, cannot be taken as evidence of acidosis it is conceivable that there may be some condition which immobilises the supply of fixed base for renal excretion. It is possible, moreover, that this state of affairs is due to an excessive formation of acid substances in the tissues or tissue fluids with consequent retention of fixed base for purposes of neutralisation. The possibility of tissue acidosis, which has been suggested by György as a factor of some importance in rickets will be further discussed in the third section of this chapter.

It may be argued that the poor absorption of calcium by reducing the urinary output of fixed base may be a cause of the high ammonia excretion. The low urinary calcium cannot, however, be held responsible since in all three cases there was a considerable fall in urinary ammonia before any significant/

Fig. XXVI.

Urinary Excretion of P_2O_5 and Titratable Acid (Average Daily Figures).

E.B.

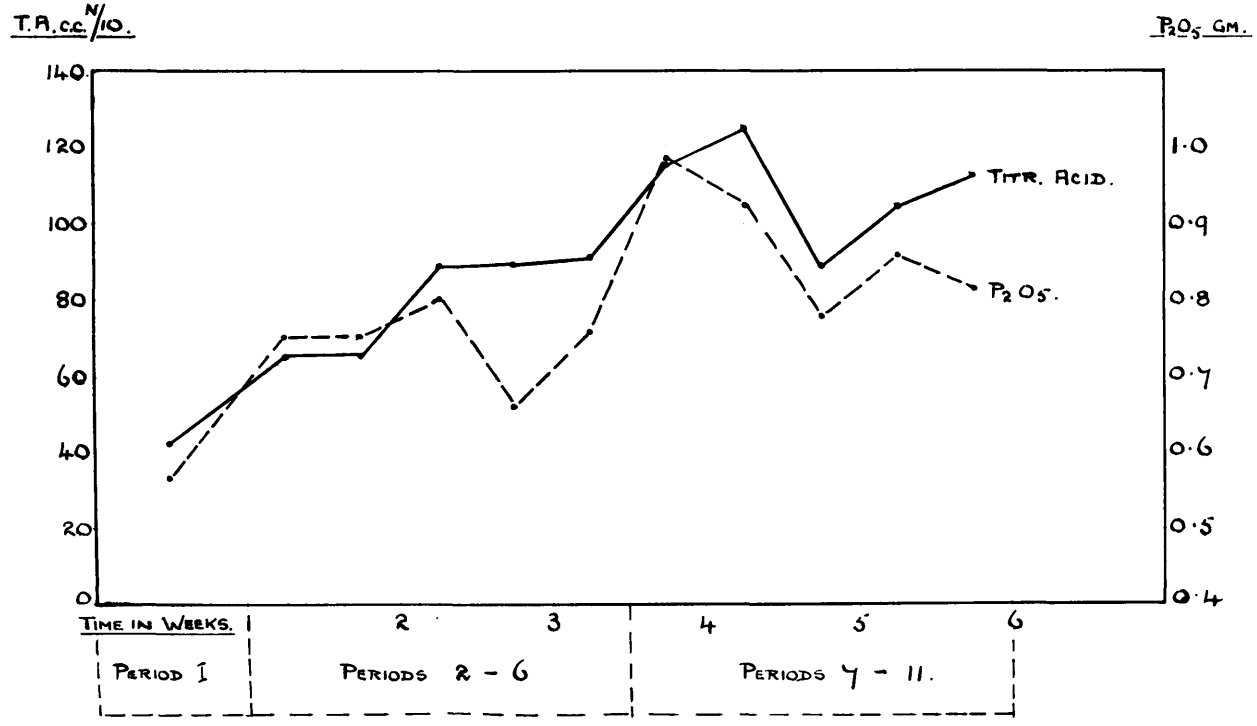
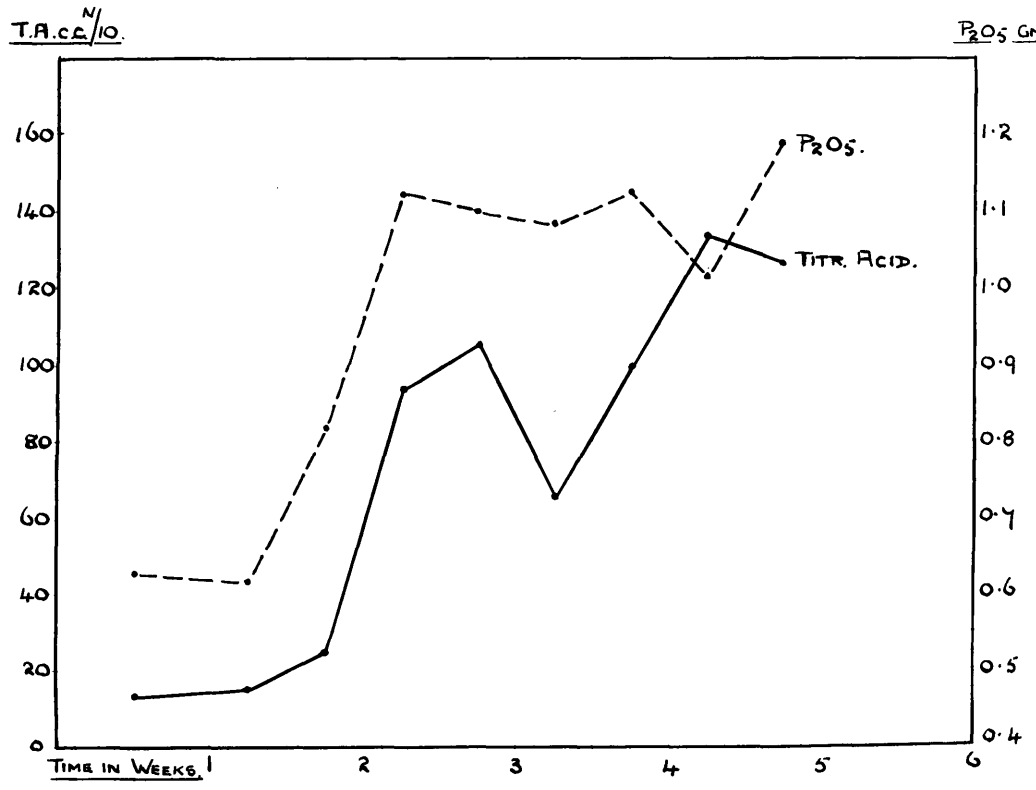


Fig. XXVII.

Urinary Excretion of P_2O_5 and Titratable Acid (Average Daily Figures).

J.W.



significant change was noted in the renal excretion of calcium. Nor can the high urinary output of ammonia be attributed to defective absorption of fixed base other than calcium since apart from the calcium and phosphorus there was but little variation in the ash content of the faeces and certainly not sufficient to account for the great excess in ammonia.

Underhill, Tileston and Bogert report a case of tetany in whom there was a steady loss of weight with poor utilisation of fat to which they attribute the high urinary output of ammonia. There was, however, no evidence of ketonuria or other indication of defective oxidation of fats in the three subjects of the present investigation.

As regards the low titratable acidity of the urine an explanation that merits consideration is the greatly reduced renal excretion of phosphate. Figures 26 and 27 show the relationship of the output of urinary phosphorus to the titratable acidity. The trend of both curves is the same so that it is possible that the reduction in titratable acidity is in part due to the reduced excretion of phosphorus. The latter factor cannot, however, explain the accompanying increase in ammonia since with the reduction in phosphate there must be an extra supply of base available/

TABLE 57.

Showing Analyses of Urine and Faeces and Retentions of Lime and Phosphorus in Patients with Manifest Tetany and Healing Rickets.(Average Daily Figures).

Case	Urine				Faeces		Retention per Kilo	
	Vol. cc.	Titr.Acid cc.N/10	Ammonia cc.N/10	CaO g.	P_2O_5 g.	CaO g.	P_2O_5 g.	CaO g.	P_2O_5 g.
J.F.	508	72.3	145.0	0.009	0.680	1.272	1.184	0.050	0.074
J.C.	372	81.2	430.7	0.031	1.392	0.657	0.258	0.124	0.098.

available.

The similarity of the metabolic results in active and healing rickets whether or not associated with tetany shows that György's hypothesis of opposite metabolic tendencies is not justified. Similar conclusions must be drawn from the results of Nitschke and Schneider who found that the basal metabolic rate was reduced in active rickets whether or not tetany was present. Certainly there is no evidence that alkalosis is a necessary concomitant of infantile tetany. It is true that the urinary acidity was greatly diminished but this finding is characteristic of active rickets and not of tetany as is apparent from the results (Table 57) obtained from two patients with symptoms of tetany, and definite radiographic evidence of healing rickets. In both of these subjects the urinary acidity was not greatly reduced. Zehnter and Foncin, indeed, found that in eight out of twelve children with tetany the urinary ammonia was increased although the true reaction of the urine was unchanged. This, however, was probably the result of associated rickets. Tezner could detect no significant difference in the pH or titratable acidity of the urines of healthy children and those with tetany. Admittedly the production of an acidosis/

acidosis by ammonium or calcium chloride or the occurrence of acidosis in the course of gastro-enteritis rapidly banishes the symptoms and signs of tetany but this is due not to any previous alkalosis but rather to the fact that acidosis renders the body store of available calcium more readily available and increases the ionised moiety of serum calcium.

B.

PHOSPHATE RETENTION IN THE PATHOGENESIS OF INFANTILE TETANY.

Hess, Weinstock, Benjamin and Gross have shown that in animals in which rickets has been induced by a high calcium, low phosphorus diet, tetany can be produced by a sudden increase of the phosphorus intake. Karelitz and Shohl and Shohl, Bennett and Weed found that in animals with rickets due to a diet poor in phosphorus the administration of alkaline or neutral phosphates led to attacks of tetany. These results cannot, however, be applied to the pathogenesis of infantile tetany since rickets in the infant is not the result of an insufficient intake of phosphorus. Rominger, Meyer and Bomskov consider that the rachitic process whether occurring in/

in the infant or produced experimentally may be divided into four stages. (1) Poor phosphorus, and fairly good calcium, retention with moderate clinical and X-ray signs. (2) Poor phosphorus and calcium retention with marked clinical and X-ray signs. (3) High phosphorus but low calcium retention - the stage of commencing healing where more phosphorus is retained than can be deposited in the bones. (4) High phosphorus and calcium retention - healing more advanced. They have given figures to show that in infancy the first phase of healing rickets is associated with a great increase in phosphorus retention without a simultaneous rise of calcium storage and state that the frequent occurrence of tetany in this phase is the result of the unbalanced increase of phosphorus retention which leads to a fall in serum calcium owing to deposition of calcium phosphate in the bones. Hottinger reported cases of rickets in whom the calcium retention increased more rapidly than that of phosphorus but Rominger explained this away by stating that these patients had already reached stage three before the investigations were commenced.

In experimental work also, Huldschinsky and Ullrich were able to demonstrate ossification radiographically in a stage/

TABLE 58.

Showing Retentions of Lime and Phosphorus during Early Stages of Healing of Rickets.

Name	Age yr.	Weight Kg.	Retentions g.per day CaO	Retentions g.per day P_2O_5	Signs of Healing (Radiographic)	Signs of Tetany
J.P.	1 5/12	8.50	0.549	0.637	+	0
D.B.	3 9/12	10.58	1.438	1.363	0	0 Serum Ca7.3mg.%
H.W.	1 1/12	8.53	0.459	0.693	0	0
J.P.	1 1/12	6.84	1.058	1.062	0	0 Serum Ca 9.1mg.%
J.McN.	2 2/12	11.80	0.673	0.558	+	0
R.F.	3 2/12	14.08	0.829	0.719	0	0
M.R.	2 1/12	9.84	0.786	0.513	+	0 Serum Ca 8.5mg%
J.F.	2 2/12	8.90	0.461	0.656	0	0 Serum Ca 8.7 mg.% " P 4.8mg.%
A.G.	2 6/12	9.10	0.595	0.693	+	+
J.M.	10/12	7.40	0.361	0.578	0	+ Serum Ca 7.9mg.% " P 4.0 mg%
J.C.	1 11/12	8.92	1.111	0.870	+	+ *Serum Ca 5.3mg.% and 4.0mg.% *Serum P 3.4mg.% and 4.1mg.%

*First figure is value obtained two days prior to commencement of metabolic period: second figure obtained on last day of period.

In all cases period of investigation lasted seven days.

stage of rickets associated with tetany. In rachitic animals the high phosphorus metabolism of fasting was shown by Cavins, Wilder and Shohl, Brown, Chapman, Rose and Saurwein, to be frequently associated with tetany.

The view that rachitic tetany is the result of phosphate stasis is thus very attractive since it fits in with a large number of well-authenticated findings such as the frequent occurrence of tetany immediately after the commencement of vitamin D therapy and would explain the reduction of serum calcium and increase of serum phosphorus. Nevertheless the conclusion that excessive phosphorus retention is the cause of tetany is not, however, justified unless it can be shown first, that tetany in association with rickets only occurs when there is excessive retantion of phosphorus and second, that tetany occurs when excessive retention of phosphorus without simultaneous increase of calcium storage is induced in a child with depleted mineral stores.

In a series of eleven rachitic patients showing indications of very early healing six had a larger retention of P_2O_5 and five of CaO (Table 58). Three had signs of manifest tetany during the period of investigation: of these, two had a greater retention of P_2O_5 and the third of CaO.

The/

The latter (J.C.) is worthy of special mention since during the period of investigation the CaO retention was as high as 124 mg. per kilo body weight per day, whereas that of P_2O_5 was 98 mg. Despite this excess retention of CaO the serum calcium fell from 5.3 mg. % to 4.0 mg. % and the serum phosphorus rose from 3.4 mg. % to 4.0 mg. %. Furthermore, the retentions of lime and phosphorus ran parallel (Figs. 21 and 24) in the two subjects whose investigation was reported in the previous section of this chapter, although only one had any signs of tetany. This is in accord with the observations of Schabad and Berend that in tetany as in rickets the increased excretion of phosphorus ran parallel with that of ~~phosphorus~~ calcium. An even more striking criticism of the role of phosphate stasis in the pathogenesis of infantile tetany is afforded by the undernoted analytical data taken from the series of Rominger, Meyer and Bomskov. The following is a summary of the essential details.

H.G. aet. 1 year. Over a period of 12 days the daily retentions were Ca 390 mg.: P_2O_5 150 mg., and the serum Ca and P 7.6 mg. % and 7.6 mg. %. The signs of tetany were present throughout. Thus tetany can exist although the retention of phosphorus is much lower than that of calcium. Furthermore/

Furthermore, the serum phosphorus was high although the retention was poor, indicating that a high serum value is no indication of "stasis".

Data are also available to show that during the healing of rickets a great excess retention of phosphorus over calcium may occur without the appearance of tetany.

The following are the details of a case which illustrates this point.

W.R. Male. Aet. 4⅛ years. He throve well till 2 years old when he had a febrile illness. Since then he has been unable to walk. On admission he was a small underweight child, height 76 cm. (normal 101 cm.), weight 9.3 kg. (normal 17 kg.) with enlargement of the epiphyses, beading of ribs, and bowing of femora and tibiae. Radiograms showed severe rickets with multiple fractures. A test meal revealed free hydrochloric acid in the gastric juice but in subnormal amount (15.6 cc. N/10 per 100 cc.). Prior to the first metabolsim period the X-ray showed callus formation at the site of fracture, but at the end of the week the X-ray showed signs of early healing. Vitamin D (Ostelin 9 minims daily) was then given for three weeks at the end of which/

TABLE 59.

Showing Excretion and Retention of Lime and Phosphorus for the Various Periods.(Average Daily Figures). Case T.R.

g.

Period	Intake	Urine	Faeces	Retention	Intake	Urine	Faeces	Retenti
1.	1.946	0.044	1.126	+0.776	2.851	0.918	0.863	+1.070
2.	1.937	0.030	1.925	-0.018	2.899	0.850	1.479	+0.570
3.	1.960	0.036	0.726	+1.198	2.931	0.835	0.546	+1.550
4.	1.947	0.031	1.112	+0.804	2.901	1.107	0.877	+0.917
5.	1.951	0.020	0.818	+1.113	2.865	0.542	0.620	+1.703
6.	1.951	0.038	1.041	+0.872	2.885	0.702	0.826	+1.357
7.	1.945	0.038	1.030	+0.877	2.828	0.789	0.856	+1.183
8.	1.964	0.043	0.873	+1.048	2.865	0.965	0.607	+1.293
9.	1.948	0.043	0.770	+1.135	2.898	0.917	0.548	+1.433
10.	1.954	0.034	0.773	+1.143	2.811	0.891	0.493	+1.427
11.	1.950	0.038	0.261	+1.651	2.776	0.878	0.111	+1.787
12.	1.950	0.032	0.232	+1.686	2.831	1.041	0.173	+1.617

Period 1 - No treatment.

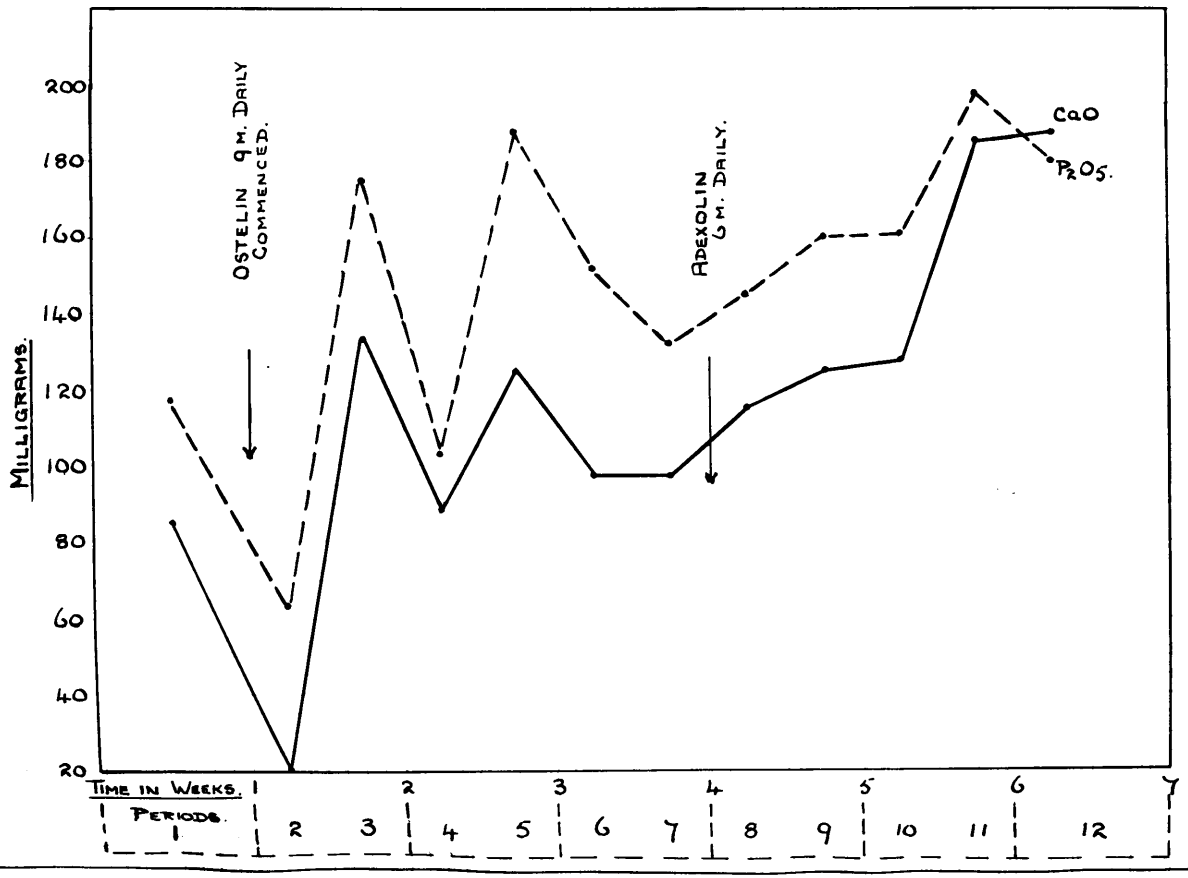
Period 2-7 Ostelin 9 min. daily

Periods Adexolin 3 min. daily.

Fig. XXVIII.

Retention of CaO and P_2O_5 in mg. per Kilo. per Day After Administration of Vitamins A and D.

T.R. (Healing Rickets).



which time healing was slightly more marked. Thereafter, a mixture of vitamin A and D (6 minims daily) was given and within eighteen days healing was much more marked.

The results of the urinary and faecal analyses are given in Table 59 and the retentions of lime and phosphorus are graphically represented in Figure 28. Throughout the whole investigation until the last three days the retention of P_2O_5 was higher, frequently in a very marked degree, than that of lime but at no time was there any suspicion of tetany latent or manifest. One other point is worth mentioning, namely, the sudden negative balance of calcium (biphasic effect) immediately after the commencement of vitamin D therapy.

But the data of Rominger, Meyer and Bomskov also provide good examples of high phosphorus low calcium retention without tetany. The following two instances, taken from their work, are worth quoting.

(1) L.S. Aet. 5/12. Daily retention 6 days before commencement of vigantol treatment CaO 85 mg.: P_2O_5 420 mg. Daily retention for 9 days after commencement of vigantol treatment CaO 250 mg. : P_2O_5 510 mg. This prolonged excess retention/

retention of phosphorus was not associated with any of the manifestations of tetany.

(2) W.A. aet. 8/12.

Date	Retentions (mg.) CaO	P_2O_5	Signs of Tetany.
9-12.12.30	50	100	++
12-15.12.30	0	-390	++
15-18.12.30	-280	430	++
18-21.12.30	-280	530	∓
21-24.12.30	-140	380	∓
24-27.12.30	-70	270	∓
27-30.12.30	-210	670	0

Two points are worthy of note. In the first place during the period 9-15.12.30 signs of tetany were present despite the loss of phosphorus. Secondly in the period 27-30.12.30 the signs of tetany had gone although for the previous fifteen days there had been quite a marked retention of phosphorus associated with continued loss of calcium.

Opportunity was taken in two cases to determine the effect/

TABLE 60.

Showing Effect of Administration of Phosphate on Excretion and Retention of Lime and Phosphorus. (Average Daily Figures).

g.

Name	Phosphate given	CaO				P_2O_5			
		Intake	Output Urine	Output Faeces	Retention	Intake	Output Urine	Output Faeces	Retention
W.B.	-	.973	0.065	1.577	-0.669	1.495	0.562	0.998	-0.064
	NaH_2PO_4	.976	0.126	0.823	+0.026	3.007	0.967	0.756	+1.284
J.W.	-	1.678	0.044	0.871	+0.763	2.299	0.355	0.911	+1.033
	Na_2HPO_4	1.721	0.038	1.783	-0.100	4.024	0.541	1.993	+1.490

effect of increasing the intake of phosphorus when the retention of calcium was poor. The first patient W.B. was the subject of coeliac disease with very marked osteoporosis while the second J.W. showed indications of healing rickets but in the very early stage. Phosphorus was administered as the dihydrogen salt in the first case and as the disodium salt in the second. The mineral retention was estimated for one week before administration of phosphate and from the fourth to tenth days (inclusive) of phosphate ingestion. The results are given in Table 60.

In neither case was there any manifestation of tetany although the administration of phosphate was followed by a marked increase in phosphorus retention. In case J.W. the phosphate was given regularly for five weeks: despite this extra intake no symptoms or signs of tetany made their appearance. Salter, Farquharson and Tibbets found that administration of either the acid or alkaline phosphate led to retention of about twenty five per cent of the extra phosphorus and the appearance in the faeces of another twenty five per cent. In the patient with coeliac disease who was given acid phosphate 84.9 per cent. was retained, the remainder being excreted in the urine. Administration

TABLE 61.

Case J.W. Showing Effect of Administration of Na_2HPO4 (3g.daily) on the Composition of the Urine. (Daily Figures).

Period	Vol.	Titr.Acid cc.N/10	Ammonia ccN/10	Urea g.	NaCl g.	Ca O g.	P_2O_5 g.	Total N. g.
1.	483	29.0	249.4	6.016	.945	. 044	.355	3.519
2.	653	20.2	299.6	6.762	-	-	-	3.719
3.	707	0.0	462.7	8.215	1.299	.038	.541	4.634

of alkaline phosphate to the patient with healing rickets led to retention of 26.5 per cent. and the appearance of 62.7 of the extra phosphorus in the faeces. 15.1 and 10.8 per cent. respectively was found in the urine. Farquharson, Salter, Tibbets and Aub found that neither the acid nor the alkaline phosphate produced any change in the urinary or faecal excretion of lime but Orr, Holt, Wilkins and Boone stated that in children ingestion of excess phosphorus caused an increased loss of calcium in the faeces. In the present two cases the acid salt prevented a fairly large negative balance while the alkaline phosphate converted a large positive, to a slight negative, retention of lime.

The urinary output of ammonia and titratable acid was estimated in the second case (Table 61). Although the salt given was the disodium phosphate, with its excess of base the ammonia excretion was increased indicating that the fixed base was retained. It might be suggested that during the phosphate period there was defective absorption of fixed base other than calcium. This, however, could not have been significant since the total ash content of the faeces after deduction of calcium and phosphorus was almost exactly the/

TABLE 62.

Showing Retention of "Excess Phosphorus"

in Rachitic Patients with and without Tetany.

Name	Weight Kg.	Retention g. per day Ca O	P_2O_5 Total	P_2O_5 Amount required for deposition of Ca in bone	Excess	Signs of Tetany
W.B.	12.3	0.026	1.284	0.020	1.264	0
J.W.	6.5	0.763	0.911	0.572	0.339	0
6 "	"	-0.100	1.490	0.000	1.490	0
E.B.	8.5	0.168	0.283	0.126	0.157	+
"	"	0.593	0.639	0.445	0.194	+
"	"	1.101	1.103	0.826	0.277	0
T.R.	9.3	1.187	1.576	0.890	0.686	0
"	"	0.611	0.972	0.458	0.514	0
"	"	0.776	1.069	0.582	0.487	0
"	"	0.808	1.213	0.606	0.607	0
"	"	1.332	1.512	0.999	0.513	0
A.G.	9.10	0.595	0.693	0.446	0.247	+
J.M.	7.40	0.361	0.578	0.271	0.307	+
J.C.	8.92	1.111	0.870	0.833	0.037	+

the same (0.42 g. per day) in both periods. The increase in urinary ammonia is in contradistinction to what happens in healthy subjects in whom Farquharson, Salter and Aub found a reduction of urinary ammonia after the ingestion of disodium phosphate. Even with the acid phosphate Farquharson, Salter, Tibbets and Aub could detect no appreciable effect on the ammonia output.

It is apparent from the results of the present work as well as the data obtained from the work of Rominger, Meyer and Bomsker that tetany complicating rickets may be present without excessive retention of phosphorus over calcium and that excessive retention of phosphorus may occur without any manifestation of tetany. This conclusion is strengthened by a consideration of the distribution of the retained phosphorus.

It is generally held that calcium is deposited in bone as a mixture of carbonate and phosphate and that the ratio of calcium to phosphorus is approximately 2.20 to 1. Thus every gram of CaO retained requires about 0.75 gram of P_2O_5. In table 62 are shown the amounts of P_2O_5 retained in excess of that necessary for deposition in the bone as calcium phosphate. It is evident that this "excess phosphorus/

phosphorus" may be considerably increased without any appearance of tetany, latent or manifest. Indeed in case E.B. in period three the retention of "excess phosphorus" was about forty per cent. greater than in period two although the signs of tetany had completely disappeared. Of even greater interest is case J.C. where the excess phosphorus was only 37 mg. per day, the smallest amount recorded in the series, although the signs of tetany were manifest,

It may therefore be stated that there is a large body of evidence which is against the view that increased retention of phosphorus plays an important immediate role in the pathogenesis of tetany. Cavins and Wilder observed in animals rendered rachitic by a phosphorus-poor diet, that complete inanition led to an increase of blood phosphate and reduction of serum calcium with the appearance of tetany: each concluded that it is not so much the increase in phosphorus retention as the rise in the inorganic moiety of phosphorus of the plasma which produced tetany, presumably by inactivating some of the calcium. This view is hardly tenable in view of the fact that tetany frequently occurs when the serum phosphorus is much below normal (Table 50). Thus it appears justifiable to conclude that while there is a disturbance/

disturbance of phosphorus metabolism in infantile tetany the relationship is not one of cause and effect.

C.

THE EFFECT OF AMMONIUM CHLORIDE ADMINISTRATION ON THE METABOLISM IN RICKETS ASSOCIATED WITH TETANY.

It has been shown in the first section of this chapter that active rickets is characterised by the urinary excretion of large amounts of ammonia with a diminished output of titratable acid. Although this is an anomalous finding in acidosis it is possible that in rickets there exists a tissue acidosis which is responsible for the retention of fixed base and for the formation of ammonia to transport such acid that is excreted. If this hypothesis is correct one would naturally infer, as indeed György does, that anything which increases the acid content of the body will aggravate the rachitic condition.

The effect of the acid-base content of the diet on the development of experimental rickets has been studied by several/

several workers. Jones found that in experimental rickets the administration of hydrochloric acid had a favourable therapeutic effect. Hess and Matzner observed no such action when citric acid was given as lemon juice which, however, acts as an alkali after absorption. McClendon observed that the addition of alkali to a diet increased its power to produce rickets. Zucker, Johnston and Barnett also found that in rats a rachitic condition supervened much more quickly and to a more marked degree when the diet was less acid-forming in nature. Thus the substitution of calcium chloride for calcium lactate in a typical rachitogenic dietary led to the development of a milder type of rickets. Similarly the addition of two per cent. ammonium chloride to a rachitogenic diet prevented rickets while the administration of two per cent sodium bicarbonate even when the régime was less injurious, led to marked rickets. Hess, Weinstock, Rivkin and Gross found that when rickets was induced with a diet containing 11.5 times as much calcium as phosphorus, the administration of cod liver oil or irradiated ergosterol or the exposure to ultra-violet light produced healing of the rachitic state only when there was an excess of acid over basic elements in the/

the diet such as occurred when calcium chloride was substituted for calcium carbonate in equi-molar amounts. Flood found that the addition of hydrochloric acid to milk caused a reduced excretion of lime by rachitic infants. On the other hand, Samuel and Kugelmass reported experiments on rats in which rickets developed much more markedly when the vitamin D-free diets contained an excess of acid elements. Shelling, also, found that ceteris paribus acid diets were more rachitogenic than alkaline. Similarly Shohl, Brown, Chapman, Rose and Saurwein found that a diet which produced mild rickets when acid, caused no rickets when neutral. Shohl in a bried review, however, concludes that "obviously, the acid-base nature of the diet is secondary to other factors". Mellanby after a review of the evidence came to the conclusion that the acid-base factor was unimportant. Hess argues that if rickets is due to acidosis administration of alkali should result in cure but that such is not the case. Wilder and Shohl and Brown, indeed, have shown that inanition which tends to produce an acidotic condition produces signs of healing in rachitic animals. Shohl, Brown, Rose, Smith and Cozad found that rickets in rats is not necessarily associated with acidosis nor tetany with alkalosis/

alkalosis although an alkaline or neutral diet seemed to favour the production of tetany.

Most workers are now agreed that the excessive loss of minerals in the faeces in rickets is the result not of increased excretion through the gut-wall but of defective absorption in which the reaction of the bowel contents plays an important part. McClendon, Bessell, Lowe and Meyer in 1919 showed by direct electrometric investigation that normally the reaction of the small intestine in dogs and cats is on the acid side of neutrality. They also reported results indicating that in man the contents of the duodenum and jejunum have normally a pH below 7.0. In 1927 Lloyd Arnold confirmed these findings in dogs. As far as rickets is concerned Schloss in 1917 showed that in the active stage the reaction of the faeces is alkaline while it tended to become acid in the healing phase. Redman confirmed this by noting a certain correlation between the mineral content and pH of the faeces of the rachitic children. Experimentally it has been shown by Zucker and Matzner and Jephcott and Bacharach that in rats the active stage of rickets is associated with a definite alkaline reaction of the faeces, while during the process of healing induced by cod/

cod liver oil or ultra-violet light the reaction moves to a pH well below 7.0. In dogs Grayzell and Miller were able to show by direct determination of the pH of the contents of the small intestine that the rickets producing diet of Mellanby caused a change in reaction from acid to alkaline while the addition of cod liver oil to the food or exposure of the animal to ultra-violet light produced a return to an acid reaction of the contents. Similar findings were reported by Tisdall and Price in rats in which rickets had been produced by rachitogenic dietaries. On the other hand Oser and Shohl and Bing have reported that experimental rickets has been cured without any change in intestinal pH.

A change in the pH of the intestinal contents to the alkaline side would certainly account for defective absorption of calcium since, as has been shown by Telfer, the solubility of calcium salts is dependent on the degree of acidity in the alimentary tract. Flood found that rachitic infants healed much faster on hydrochloric acid milk than on any other food and attributed this to the fact that the acid overcame the high buffer content of milk which is of special importance in rickets where there is usually hypochlorhydria. Flood pointed out that elimination of the buffer effect rendered/

rendered the calcium of the milk more accessible for absorption and cited the results of Zottermann who was able to increase the diffusible calcium of cow's milk by the addition of hydrochloric acid. It is necessary, however, to stress the fact that the rachitic process depends upon more than mere inability to retain lime since in coeliac disease which is characterised by a poor mineral retention there is only osteoporosis unless growth is taking place when rickets supervenes.

The following investigation was undertaken to determine the effect of the administration of an acid-producing substance on the mineral metabolism in rickets and tetany. In order to minimise changes due to alterations in intestinal reaction ammonium chloride was chosen because of its neutral character in the gut. In this way it was hoped that the influence of acidosis on the tissues could be studied without any direct action on intestinal absorption. It has already been shown in chapter one that calcium and phosphorus play an important part in dealing with excess of acid in the body, at any rate until ammonia formation has increased to such a pitch as to neutralise the extra acid. If the rachitic condition is associated/

associated with a state of tissue acidosis it should be less capable than the non-rachitic of dealing with the extra acid especially as the bones are deficient in calcium and phosphorus.

The effect of ammonium chloride administration was studied in four children who showed evidence of rickets. In three there was a marked degree of active rickets indicated by radiograms taken at weekly intervals for three weeks prior to the commencement of the investigation; in addition, signs of tetany were present, in three a positive Chvostek's sign and in one laryngismus as well. In the fourth child the radiograms showed that early spontaneous healing was taking place before the investigation was commenced. In each case two metabolic studies of seven days' duration each were made. The diets were kept constant for three days prior to the commencement of the first seven day period and remained unchanged until the completion of the investigation. In the second period ammonium chloride was given in one gram doses five times daily.

CLINICAL FEATURES.

As in non-rachitic children, no clinical signs of acidosis/

acidosis were manifest during the period of ammonium chloride administration. To Case 1 (R. McA.) the administration of five grams of ammonium chloride daily was continued for thirty eight days without any obvious clinical indication of acidosis. It is clear, therefore, that in active rickets the power of the individual to deal with acid is at least as good as normal so far as the prevention of clinical manifestations of acidosis is concerned. During the administration of the ammonium chloride Chvostek's sign disappeared in all the cases. In case 3 (J.M.) the laryngeal spasm also disappeared during ammonium chloride administration but both it and Chvostek's sign reappeared on the day after the ammonium chloride ingestion ceased: in the other two patients there was no return of any sign of tetany.

Perhaps the most remarkable feature noted was the appearance of healing in the radiogram of case 1 (R. McA.) within three weeks of the commencement of the ammonium chloride. A series of weekly radiograms indicated that in case 1 (R. McA.) healing became apparent two weeks after the initiation of ammonium chloride ingestion, and gradually increased until at the end of thirty eight days it was quite definite/

TABLE 63.

Showing Effects of Ammonium Chloride Composition on Blood-Chemistry in Active Rickets.

Case	Stage	Serum Cal. mg.%	Serum Inorganic Phosphorus mg.%	Blood-Carbon Dioxide Vol.%	Blood Non protein Nitrogen mg.%
1.R.McA.	Before NH_4Cl	-	3.0	50.7	-
	3/52 on NH_4Cl	6.1	3.4	40.3	39.8
	4/52 on NH_4Cl	6.5	4.8	-	-
	5/52 on NH_4Cl	7.4	4.2	37.8	40.9
	1/52 after NH_4Cl	6.9	2.8	59.6	31.6
2.R.P.	Before NH_4Cl	7.6	5.6	55.6	34.8
	1/52 on NH_4Cl	8.0	3.6	44.7	44.5
3.J.M.	Before NH_4Cl	7.6	6.2	52.5	36.3
	1/52 on NH_4Cl	8.9	4.6	41.4	43.1

definite although not nearly so marked as that which occurs with vitamin D preparations or ultra-violet light. The fact that healing took place during the administration of an acid-producing substance is strong evidence against the view that there is in rickets a shift of acid-base equilibrium to the acid side. It is true that the patient had signs of tetany but the fact that healing continued after these had disappeared indicates that the rachitic process can be healed even during the ingestion of a great excess of acid-producing substance.

BLOOD CHEMISTRY.

The findings are given in Table 63. The serum calcium which was abnormally low in all three patients (cases 1, 2 and 3) with rickets and tetany increased during ammonium chloride administration although the normal level was not quite reached while the patients were under observation. The inorganic phosphorus content of the serum which was high in two patients (cases 2 and 3) before the commencement of ammonium chloride fell during that period, while in the third (case 1) where it was initially low, an increase was noted followed by a marked fall when the ammonium chloride administration ceased. The carbon dioxide content of the blood was lowered and the non-protein/

TABLE 64.

Showing Effect on Ammonium Chloride Administration on Excretion and Retention of Lime and Phosphorus.

(Average Daily Figures in g.)

Case	Period	Ca O				P_2O_5			
		Intake	Output		Retention	Intake	Output		Reten
			Urine	Faeces			Urine	Faeces	
1.R.McA	A.	1.173	0.010	0.988	0.175	1.543	0.528	0.757	0.258
	B.	1.173	0.012	0.899	0.262	1.543	0.663	0.534	0.346
2.R.P.	A.	2.16	0.017	1.977	0.166	3.024	1.110	1.630	0.284
	B.	2.16	0.029	1.392	0.739	3.024	1.205	1.203	0.616
3.J.M.	A.	1.80	0.013	1.426	0.361	2.52	0.331	1.765	0.424
	B.	1.80	0.021	1.307	0.472	2.52	0.393	1.659	0.468
4.M.R.	A.	1.80	0.025	1.011	0.764	2.34	0.930	0.707	0.703
	B.	1.86	0.102	1.326	0.432	2.40	1.097	1.049	0.254

Period A - Without Ammonium Chloride

Period B - With Ammonium Chloride

Cases 1, 2. 3- Active Rickets.

Case 4 - Healing Rickets.

protein nitrogen showed a slight rise. Generally it may be said that the changes in blood chemistry following the administration of ammonium chloride were the same in rachitic as in normal children, indicating what Van Slyke has termed a compensated acidosis.

METABOLIC CHANGES.

Calcium and Phosphorus - In all four cases the urinary excretion of lime and phosphorus was increased during the ammonium chloride period (Table 64). The increase was very marked in case 4 (healing) particularly as regards calcium, the urinary output of which was four times greater: in the others the increase was but slight. The faecal output of both calcium and phosphorus was increased in case 4 as a result of the rise in faecal weight and the percentage of minerals in the faeces. In the patients with active rickets the output of lime and phosphorus in the faeces was greatly reduced. In case 1 the reduction was due both to a fall in faecal weight and to a reduction in its ash content while in cases 2 and 3 the main factor was the smaller percentage content of ash in the faeces. The most striking difference, however, between the patient with active rickets and that of the normal child or the patient with/

TABLE 65.

Showing Effect of Ammonium Chloride Administration on Excretion and Retention of Chlorine (as Sodium Chloride) in Rickets.

(Average Daily Figures).

Case	Period	Intake g.	Output g.	Retention Total g.	% of Intake	% of Excess Chlorine Excreted
1.R.McA	A.	1.519	0.963	0.556	36.6	
	B.	6.987	2.005	4.982	71.3	--19.0
2. R.P.	A.	2.390	1.791	0.599	25.1	
	B.	7.858	5.572	2.286	40.9	-30.8
3. J.M.	A.	2.023	1.519	0.504	24.9	
	B.	7.491	2.692	4.799	65.9	-21.4
4. M.R.	A.	2.004	1.652	0.352	17.5	
	B.	7.472	5.966	1.506	20.1	-78.9

Period A. Without Ammonium Chloride

Period B. With Ammonium Chloride.

Cases 1,2,3. Active Rickets

Case 4 Healing Rickets.

with healing rickets was the increased retention of calcium and phosphorus in the former during ingestion of ammonium chloride and the decrease in the latter. This is in accord with the radiographic evidence of definite healing in case 1 which has already been recorded.

Chlorine - The retention of chlorine in the subjects with active rickets and tetany showed a distinct variation from normal both in the control and ammonium chloride periods (Table 65). In non-rachitic children on an adequate milk diet the retention of chlorine varies from zero to about 12 per cent of the intake, whereas in all four of the present subjects the chlorine retained exceeded this figure. In case 1 as much as 36.6 per cent of the chlorine ingested over a six day period was not excreted in the urine. Since the faecal output of chlorine is negligible and as there was no sensible perspiration it is unlikely that the diminished urinary excretion was due to the chlorine being diverted to other routes. During the ammonium chloride period the tendency to retain chlorine became even more apparent. In normal children over 80 per cent of the chlorine contained in the ingested ammonium chloride was found in the urine whereas in the present series the percentage excreted fell

TABLE 66.

Showing Effect of Ammonium Chloride Administration on the Composition of Urine in Rickets.

(Average Daily Figures).

Case	Period	Volume cc.	Titr. Acidity cc.N/10	Ammonia cc.N/10	NaCl g.	Ca O g.	P_2O_5 g.
1.R.McA.	A.	300	35.3	204.1	0.963	0.010	0.528
	B.	310	75.0	287.2	2.005	0.012	0.663
2. R.P.	A.	784	101.7	337.4	1.791	0.017	1.110
	B.	787	203.4	785.2	5.572	0.029	1.205
3. J.M.	A.	613	57.8	149.0	1.519	0.013	0.331
	B.	337	23.1	513.0	2.691	0.021	0.393
4. M.R.	A.	576	112.5	226.4	1.652	0.025	0.930
	B.	691	216.5	788.0	5.966	0.102	1.897

Period A - Without Ammonium Chloride.

Period B. - With Ammonium Chloride.

Cases 1,2,3.- Active Rickets.

Case 4 - Healing Rickets.

TABLE 67.

Showing Effect of Ammonium Chloride Administration on Fat Content of Faeces in Rickets (Average Daily and Percentage Figures).

Case	Period	Faecal Wt. g.	Ash	Fat		Soaps		Free Fatty Acid		Neutral Fat	
				Total g.	%	Total g.	%	Total g.	%	Total g.	%
1.R.McA.	A.	7.7	31.4	3.322	43.2	2.552	33.2	0.550	7.2	0.219	2.
	B.	7.2	29.7	3.143	43.4	2.092	28.9	0.977	13.5	0.074	1.
2. R.P.	A.	10.5	40.0	2.991	28.5	1.803	17.2	0.851	8.1	0.336	3.
	B.	7.6	40.0	2.506	33.2	1.503	19.9	0.715	9.5	0.288	3.
3. J.M.	A.	8.9	45.0	2.502	28.1	0.232	2.6	1.757	19.7	0.513	5.
	B.	10.4	45.0	2.630	25.4	0.195	1.9	1.540	14.9	0.895	8.
4. M.R.	A.	5.2	38.2	0.963	18.7	0.643	12.5	0.140	2.7	0.181	3.
	B.	6.4	42.1	1.044	16.3	0.634	9.9	0.252	3.9	0.158	2.

Period A - Without Ammonium Chloride.

Period B. - With Ammonium Chloride.

Cases 1,2,3. - Active Rickets.

Case 4 - Healing Rickets.

far below that figure except in case 4 (healing) in whom percentage amounted to 78.9 per cent of the intake, which is practically within normal limits.

Urinary Acidity and Ammonia (Table 66). - The increase of titratable acidity and ammonia output in the urine during the ammonium chloride period was not as great in the active cases as in the patient with healing rickets or as has been observed in non-rachitic children. It is of interest to note here the finding of Farquharson, Salter, Tibbets and Aub in a patient with scoliosis where the administration of ammonium chloride produced an increase of ammonia output although the urine remained alkaline.

Faecal Fat (Table 67). - The only constant change observed in the excretion of faecal fat was the diminution in the amount of calcium soaps during the ammonium chloride period. This decrease was quite insignificant in the patient with healing rickets but was quite definite in the others, amounting to between 15 and 18 per cent.

The chief points of difference between the metabolic reactions of the rachitic patient to ammonium chloride and those of the normal child are noted in the following schema:-

		Normal	Healing Rickets	Active Rickets & Tetany.
Urine	Increase in titratable acidity	++	++	+
	Increase in ammonia	++	+++	+
	Percentage of excess chlorine excreted.	+++	++	+
	Excretion of CaO	+	++	+
	Excretion of P_2O_5	+	+	+
Faeces	Excretion of CaO	+	+	−
	Excretion of P_2O_5	+	+	−
Retention	CaO	−	−	+
	P_2O_5	−	−	+

The use of acid producing salts as a decalcifying agent is quite well known as a therapeutic measure for the correction of deformities. In active rickets, however, the increased retention of lime and phosphorus following ammonium chloride administration is in marked contrast to what occurs in health and healing rickets. Although ammonium chloride was chosen as the acid-producing substance because of its neutrality in the gut, the possibility must be conceded of a decrease in the pH of the intestinal/

intestinal contents due to alteration of the composition of the body fluids. It may further be urged that if the absorption of minerals is thus facilitated, the increased retention in active rickets is merely due to the fact that the lime-starved bones utilise so much of the extra calcium and phosphorus available that the normal withdrawal of these substances is completely overshadowed. This contention cannot, however, be upheld in view of the results obtained in the case of healing rickets. The mineral retention was in this child reduced during the ammonium chloride period just as in the normal subject in spite of the fact that, healing having just commenced, there was still a marked degree of osteoporosis shown in the radiogram. To explain the effect of ammonium chloride in increasing the retention of lime and phosphorus in active rickets it therefore seems necessary to assume that some change has been effected enabling the tissues of the patient with active rickets to fix calcium and phosphorus. If this assumption be granted, the corollary must be drawn that in addition to a defect in the absorption of minerals from the intestine there also exists in or around the tissues a condition which prevents the fixation of lime and phosphorus.

As/

As regards the nature of the abnormal state of affairs in the tissues there is little that can be said. Nevertheless the fact that an acid-producing salt with ammonium chloride can during the active stage of rickets, lead to an increased fixation of lime and phosphorus makes it highly improbable that in active rickets acidosis is either a causal or an associated factor. In this connection two other findings, the subnormal increase in urinary acidity and the high retention of chlorine, are of some interest.

The subnormal increase in urinary acidity might at first glance be attributed to the diminished output of urinary phosphorus providing a smaller amount of acid phosphate which is responsible for a large amount of titratable acidity. This explanation is not, however, very satisfactory since the renal excretion of phosphorus during ammonium chloride ingestion is high relative to the increase in titratable acid. It would appear, therefore, that in these cases there is a tendency for acids to be retained.

The high retention of chlorine has probably little to do with the rachitic process <u>per se</u> but is almost certainly associated with tetany. In any case the healing of rickets during/

during retention of large amounts of an acid radicle is important evidence against the view that acidosis is associated with rickets.

A disturbance in chlorine metabolism has long been associated as an etiological factor in the production of certain forms of tetany. MacCallum and his co-workers attributed the onset of tetany during experimental pyloric obstruction to the loss of vomiting of the hydrochloric acid secreted in the gastric juice with the consequent production of an alkalosis. Grant found in one of six cases of adult tetany a marked reduction in the blood chlorine. Iversen and Hansborg showed that during tetany following complete thyro-parathyroidectomy more sodium chloride than usual was retained in the tissues although the amount of chlorine in the blood was reduced. Parhon, Ballif and Derevici also reported a fall in the serum chlorine. Dragstedt was able to prevent or control symptoms of tetany in parathyroidectomised animals by the intravenous injection of Ringer's solution in which strontium chloride replaced the calcium salt. In a series of investigations on the metabolic changes produced by parathyroidectomy and injections of guanidine Morris, Watson and/

and Morris found that during the tetany period there was a great increase in the amount of chlorine retained in the tissues even although in most instances the intake was well below that of the control period. They further were able to abolish all signs of tetany by intraperitoneal injections of isotonic sodium chloride solution. The suggestion was put forward that the increased retention of chlorine was associated with an increased tissue catabolism leading to the appearance in the urine of greater amounts of amino-acid and purine bodies. While the rise in retention of chlorine has also been found in infantile tetany no evidence of an increased protein breakdown has been obtained.

It is unjustifiable to draw too close a comparison between parathyroidectomy or guanidine poisoning and infantile tetany, but it would appear that closely associated with tetany is a disturbance of chlorine metabolism. Nor is this entirely due to an alkalosis resulting from loss of chlorine since Michelsen has recently shown that chlorine depletion produced by low chlorine intake and diuretin is in rabbits accompanied by an acidosis and symptoms not unlike tetany.

GENERAL CONCLUSIONS.

The work presented in this chapter shows that tetany may/

may be present in association with rickets although the increases in the retentions of calcium and phosphorus run parallel. Furthermore, the phosphorus retention may be proportionately greater than that of calcium either during spontaneous healing or following vitamin D therapy without any manifestations of tetany. Even the administration of large doses of phosphate leading to a great increase in the retention of phosphorus with a diminution in that of lime does not lead to the appearance of tetany although the calcium stores of the body are previously greatly depleted. These results indicate that tetany complicating rickets may occur without an excessive retention of phosphorus over calcium and that excessive retention of phosphorus may take place without any manifestation of tetany. It therefore seems justifiable to conclude that phosphorus retention is not the dominating factor in the pathogenesis of infantile tetany.

The increased urinary output of ammonia observed during the stage of rickets occurs whether or not signs of tetany are present. The fact that it is associated with a diminution in the titratable acidity of the urine renders it unlikely that there is a condition of acidosis present.

This/

This conclusion is further strengthened by the fact that healing of rickets as evidenced by increased retention of minerals and X-ray signs, may take place during prolonged administration of an acid-producing substance.

Infantile tetany is characterised by an increased retention of chlorine but there is no evidence of any disturbance of acid-base equilibrium as a causal or associated factor.

---0---

BIBLIOGRAPHY

Chapter One

Haldane, J.B.S., 1921. J. Physiol. 55. 265.

Haldane, J.S., 1922. Respiration. Yale Univ. Press, New Haven.

Henderson, L.J., 1928. Blood: A Study in General Physiology, Yale Univ. Press, New Haven.

Van Slyke, 1921. Physiol. Reviews. 1. 141

Chapter Two.

Albright, Bauer and Ropes, 1929. J. Clin. Inv. 7. 139.

Albright and Ellsworth, 1929, J. Clin. Inv. 7. 183

Aub, Fairhall, Minot and Reznikoff, 1925. Medicine. 4. 1.

Baird, Douglas, Haldane and Priestley, 1923, J. Physiol. 57. Proc. Physiol. Soc. 41.

Bauer, Albright and Aub. 1929. J. Clin. Inv. 7. 75.

Benedict and Nash, 1926. J. Biol. Chem. 69, 381.

Berg, 1911. Bioch. Ztschr. 30. 107.

Bernhardt/

Bernhardt, 1924. Ztschr. Klin. Med. 100. 735.

Bogert and Kirkpatrick, 1922. J. Biol. Chem. 54. 375.

Dagois and Stolte, 1913. Jahrb. f. Kinderheilk. 77. 21

Farquharson, Salter, Tibbets and Aub, 1931. J. Clin. Inv. 10. 221.

Ferrier , 1907. Compt. rend. 145. 95.

Fiske, Goodall, Hathaway and West, 1926. J. Biol.Chem. 67. 385.

Fitz, Alsberg and Henderson, 1907. Amer.J. Physiol. 18. 113.

Flood, 1926. Amer. J. Dis. Child. 32. 550.

Fölling, 1929. Acta med. Scand. 71. 221.

Gamble, Blackfan and Hamilton, 1925. J. Clin. Inv. 1. 359.

Gamble and Ross. 1923. Amer. J. Dis. Child. 25, 470.

Gamble, Ross and Tisdall, 1923. Amer. J. Dis. Child. 25, 455.

Givens, 1918. J. Biol. Chem. 35. 241.

Givens and Mendel, 1917. J. Biol. Chem. 31. 421.

Gollwitzer-Meier, 1925. Bioch. Ztschr. 160. 433.

Ztschr. f. d. ges. exp. Med. 46. 15.

Goto. 1918. J. Biol. Chem. 36. 355.

Grosser, 1920. Ztschr. f. Kinderheilk. 1920. 25. 141.

Haldane, 1921. J. Physiol. 55. 265.

1925. Biochem. J. 19. 249.

Haldane, Hill and Luck. 1923. J. Physiol. 57. 301.

Haldane, Wigglesworth and Woodrow, 1924. Proc.Roy.Soc.B. 96. 15.

Hamilton/

Hamilton and Moriarty, 1928. Amer.J.Dis.Child. 36. 450.

Hjort, 1926. J. Biol. Chem. 65. 783,

Irving, 1926. J. Biol. Chem. 68, 513.

Katzenstein, 1928. Ztschr. f. d. ges. exp. Med. 60. 532

Keith and Whelan, 1926. J. Clin. Inv. 3. 149.

Koehler, 1927. J. Biol. Chem. 72. 99.

Mainzer, 1927. Klin. Wchnschr. 6. 1689.

Mainzer and Joffe, 1928. Ztschr. f. d. ges. exp. Med. 59. 492.

Nelson, 1928. Amer. J. Dis. Child. 36. 716.

Percival and Stewart, 1927. Bioch. J. 21. 301.

Ross and Scriver, 1926. Amer.J.Dis.Child. 32. Trans. Amer. Pediatr. Soc. 637.

Salvesen, 1923, J. Biol. Chem. 56. 443.

Sato. 1918. Amer. J. Dis. Child. 16. 293.

Sawyer, Baumann and Stevens, 1918. J. Biol. Chem. 33. 103.

Scheer. 1928. Klin. Wchschr. 7. 835.

Schwenckenbecker and Spitta. 1907. Arch.f.exp.Path.u.Pharm.56.284.

Shohl and Sato, 1923. J. Biol. Chem. 58. 235: 257.

Steenbock, Nelson and Hart. 1914. J. Biol. Chem. 19. 399.

Stehle, 1917. J. Biol. Chem. 31. 461.

Stehle and McCarty, 1921. J. Biol. Chem. 47. 315.

Stewart and Haldane. 1924. Bioch. J. 18. 855.

Telfer/

Telfer. 1922-23. Quart. J. Med. 16. 45.

1923-24. ibid. 17. 245.

White. 1927. Amer. J. Physiol. 80. 82.

Zucker. 1921. Proc. Roy.Soc.Exp.Biol.and Med. 18. 272.

Chapter Three

Barcroft, Dryerre, Meakins, Parsons, T.R., and Parsons,W.

1925-26. 19. 257

Bernard and Merklen, 1931. Compt.rend.Soc.de Biol. 107. 973.

Denis, 1915. J. Pharm. Exp. Therap. 7. 256.

Denis and Means, 1916. J. Pharm. Exp. Therap. 8. 273.

Ehrmann. 1907. Münch.med. Wchnschr. 54. 2595.

Fleischer, 1875. Berlin Klin. Wchnschr. 12. 529: 547.

Grabfield and Knapp. 1928. J. Pharm. Exp. Therap. 32. 341.

Hanzlik, 1926. Medicine. 5. 197.

Hanzlik and Karsner. 1917. Arch. Int. Med. 19. 1016.

Hartley and Trevan. 1915-16. J. Physiol. 50. Proc. Physiol.Soc.49.

Johnston, 1930. J. Amer. Med. Ass. 94. 784.

Lees. 1908-9. Proc.Roy.Soc.Med. 2. Pharmac.Sect. 34.

1912. Brit. Med. J. 2. 930.

Langmead. 1906. Lancet. 1. 1822.

Maclagan/

Maclagan. 1876. Lancet. 1.349.

Meara. 1910. Amer. J. Med. Sci. 139. 328.

Meyer. 1883. Arch. f. exp. Path. u. Pharm. 17. 304.

Myers and Ferguson, 1929. J. Pharm. Exp. Therap. 35. 313.

Scott, Thorburn and Hanzlik, 1917. J. Pharm. Exp. Therap.9. 247.

Walter, 1883. Arch. f. exp. Path. u. Pharm. 17. 304.

Chapter Four.

Alsina and Pijoan, 1928. Compt. rend. Soc. de Biol. 99. 1278.

Atchley and Benedict. 1927. J. Biol. Chem. 75. 697.

Atchley, Palmer, Loeb and Benedict. 1923. Arch.Int.Med. 31. 606.

Barcroft, Dryerre, Meakins, Parsons, T.R., and Parsons, W.
Quart. J. Med. 1925-26. 19. 257.

Blum, Delaville and Van Caulaert. 1925. Compt.rend.Soc.de Biol.
93. 295.

Boldt, Brahm and Andresen, 1929. Arch. Kinderheilk. 87. 277.

Brown,Eusterman,Hartmann and Rowntree 1923. Arch.Int.Med.32. 425.

Christiansen, Douglas and Haldane. 1914. J. Physiol. 48. 244.

Cooke, Rodenbaugh and Whipple 1916. J. Exp. Med. 23. 717.

Dragstedt. 1927-28. Proc.Soc.Exp.Biol. and Med. 25. 239.

Dragstedt/

Dragstedt and Ellis 1928. Proc.Soc.Exp. Biol.and Med. 26. 305.

Drake, McKhann and Gamble, 1930. J.Exp.Med. 51. 867.

Drake and Tisdall, 1926. J. Biol. Chem. 67. 91.

Ellis, 1923-24. Quart. J. Med. 17. 405.

Essen,Kauders and Porges, 1923. Wien.Arch.inn.Med. 5. 499.quoted by Peters and Van Slyke.

Falta and Richter-Quittener, 1918. Bioch. Ztschr. 91. 381.

Fasold 1931. Ztschr. Kinderheilk. 51. 541.

Felty and Murray, 1923. J. Biol. Chem. 57. 573.

Fleiner, 1903. Münch. med. Wchnschr. 50. 409.

Foster and Hausler, 1925. Arch. Int. Med. 36. 31.

Frouin, 1922. Presse méd. 30. 1096.

Gamble and McIver, 1924-25. J. Clin. Inv. 1. 403.

Gatch, Trusler and Ayers, 1927. Amer.J. Med.Sci. 173. 649.

Gatewood, Gaebler, Muntwyler and Myers, 1928. Arch.Int.Med.42. 79.

Gollwitzer-Meier, 1924. Ztschr. f. d. ges. exp. Med. 40. 83.

Gram. 1924. J. Biol. Chem. 61. 337.

Gruenwald. 1909. Arch.f.exp.Path.u.Pharm. 60. 360.

Haden and Orr, 1923. J. Exp. Med. 37. 377.

1926. ibid. 44. 435.

1927. ibid. 45. 433.

1928. ibid. 48. 591.

1928. J. Amer. Med. Ass. 91. 1529

Hardt and Rivers. 1923. Arch. Int. Med. 31. 171

Hartmann and Smyth. 1926. Amer. J. Dis. Child. 32. 1.

Hastings, Murray and Murray, 1921. J. Biol.Chem. 46. 223.

Hutchison, 1898. J. Path. and Bacter. 5. 406.

Ingvaldsen, Whipple, Baumann and Smith. 1924. J.Exp.Med.39. 117.

Katsu, 1927. Jap.J. Biophysics. 2. 151. quoted by Klinke (1931).

Katz. 1896. Arch. f. d. ges. Physiol. 63. 1.

Klingner, 1933. Ztschr. f. d. ges. exp. Med. 92. 129.

Klinke, 1931. Der Mineralstoffwechsel. Leipzig.

Korczynski and Jaworski, Deutsch.Arch.f.Klin.Med. 47. 578.
quoted by Klingner (1933).

Kussmaul, 1869. Deutsch. Arch.f. Klin.Med. 6. 455 quoted by
Klingner (1933).

McCallum,Lintz,Vermilye,Leggett and Boas 1920. Bull.Johns
Hopk. Hosp. 31. 1.

McCann. 1918. J. Biol.Chem. 35. 553.

McIver and Gamble 1928. J. Amer.Med.Assoc. 91. 1589.

McQuarrie and Whipple, 1919, J. Exp. Med. 29. 421.

Maizels, 1931. Arch.Dis.in Child. 6, 293 .
1929-30. Quart. J. Med. 23. 171.

Maizels and McArthur, 1930. Amer.J. Dis. Child. 41. 35.

Maizels, McArthur and Payne, 1930. Lancet 1. 286.

Meyer/

Meyer. 1931. Klin. Wchnschr. 10. 155.

1932. ibid. 11. 1383.

Michelsen, 1933. Arch. f. exp. Path. u. Pharm. 173. 737:746:750.

Moraczewski. 1897. Ztschr. f. physiol. Chem. 23. 483.

Morris and Graham. 1929. Arch. Dis. in Child. 4. 299.

1930. Brit. J. Exp. Path. 11. 322.

Morris,N, and Morris, S. 1930. Biochem. J. 24. 1716.

1932. ibid. 26. 2015.

Myers and Booher, 1925. Proc.Soc.Exp.Biol.and Med. 22. 512.

Parnas, 1929. Bioch. Ztschr. 206. 16.

Peters, Barr and Rule. 1921. J. Biol. Chem. 45. 489.

Peters and Van Slyke, 1931. Quantitative Clinical Chemistry.

Volume One. Interpretations.

Phélizot. 1931. Rev. franç. Pédiatr. 7. 560.

Porges. 1932. Klin. Wchnschr. 11. 186.

Rafflin and Saradjichvili, Compt. rend. Soc.de Biol. 102. 559.

Rominger and Meyer, 1927. Arch. Kinderheilk. 80. 195.

1929. Ztschr. Kinderheilk. 47. 721. 48. 43.

Ross 1923. Canad. Med. Ass. J. 13. 97.

Salvesen, 1927. Acta med. Scand. 66. 518.

Shohl, 1922. Proc. Soc. Exp. Biol. and Med. 20. 139.

Shohl, Wakeman and Shorr, 1928. Amer.J. Dis. Child. 35. 576.

Steinitz/

Steinitz, 1928. Ztschr. Klin. Med. 107. 560.

Straub. 1923. Deutsch. Arch. f. Klin. Med. 142. 145.

Tileston and Comfort, 1914. Arch. Int. Med. 14. 626.

Van Slyke. 1923. J. Biol. Chem. 58. 523.

Venables, 1925. Guy's Hosp. Med. Rep. 75. 152.

Von Noorden, 1907. Metabolism and Practical Medicine, London. 1. 416.

Wendt. 1925. Handb. Biochem. 8. 183. quoted by Klinke (1931).

White and Bridge. 1927. Boston Med. and Surg. J. 196. 893.

Wildman, 1929. Arch. Int. Med. 43. 615.

Chapter Five.

Anderson and Graham. 1924. Quart. J. Med. 18. 62.

Bauer, Marble and Claflin. 1932. J. Clin. Inv. 11. 391.

Berend.1913. Monatsschr. Kinderheilk. quoted by Aschenheim 1919.
Erbebn. inn. Med. u. Kinderheilk. 17. 153.

Binger, 1917. J. Pharm. Exp. Therap. 10. 105.

Blum, Delaville and Van Caulaert 1925. Presse méd. 33. 801.

Bosanyi, 1927. Jahrb. f. Kinderheilk. 117. 240.

Brock and Welcker, 1927. Ztschr. Kinderheilk. 43. 193.

Brühl. 1932. Monatsschr. Kinderheilk. 53. 1. quoted by Shohl.(1933).

Burgess and Osman. 1924. Lancet. 1. 281.

Calvin/

Calvin and Borovsky. 1922. Amer. J. Dis. Child. 23. 238.

Cavins 1924. J. Biol. Chem. 59. 237.

Dragstedt, 1925. Amer. J. Physiol. 72. 205.

Drucker. 1927. Acta Paediatr. 6. Supplem.

Drucker and Faber, 1926. J. Biol. Chem. 68. 57.

Elias and Kornfeld. 1923. Klin. Wschr. 2. 1206.

Farquharson, Salter and Aub. 1931. J. Clin. Inv. 10. 251.

Farquharson, Salter, Tibbets and Aub. 1931. J. Clin.Inv. 10. 221.

Fasold. 1931. Ztschr. Kinderheilk. 50. 651.

Flood. 1926. Amer. J. Dis. Child. 32. 550.

Ford. 1931. Arch. Dis. in Child. 6. 209.

Frank, Nothmann and Wagner, 1923. Klin. Wschr. 2. 405.

Freudenberg and György. 1922. Münch.med. Wschr. 69. 422.

Freudenberg and Welcker, 1926. Ztschr. Kinderheilk. 41. 466.

Gerstenberger, Hartman, Russell and Wilder, 1930. J. Amer. Med. Ass. 94. 523.

Grant, 1922. Arch. Int. Med. 30. 355.

Grayzell and Miller, 1928. J. Biol. Chem. 76. 423.

György, 1922. Jahrb. Kinderheilk. 99. 104.

1923. Ztschr. ges. exp. Med. 38. 9.

1929. Ergebn. inn. Med. u. Kinderheilk. 36. 752.

1930. Klin. Wschr. 9. 102.

György/

György, Kappes and Kruse. 1926. Ztschr. Kinderheilk. 41. 700

Hanssen. ----------quoted by Drucker (1927),

Hasselbalch, 1916. Bioch. Ztschr. 74. 18: 78.112.

Hess. 1929. Rickets, Osteomalacia and Tetany. Phila. 133: 168.

Hess, Calvin, Wang and Fletcher. 1923. Amer.J. Dis. Child. 26. 271.

Hess and Matzner, 1924. J. Amer. Med. Ass. 82. 1604.

Hess, Weinstock, Benjamin and Gross, 1931. J. Biol. Chem. 90. 737.

Hess, Weinstock, Rivkin and Gross. 1930. J. Biol. Chem. 87. 37.

Hodgson, 1921. Lancet. 2. 945.

Hollό, 1924. Bioch. Ztschr. 150. 496.

Hollό and Weiss, 1926. Wien. Klin. Wschr. 2. 1422 quoted by
György (1929).

Holt, La Mer and Chown, 1925. J. Biol. Chem. 64. 509: 567.

Hottinger . 1925. Monatsschr. f. Kinderheilk. 30. 497. quoted
by György (1929).

1929. Ztschr. Kinderheilk. 47. 341.

Howland and Marriott, 1918. Bull. J. Hopk. Hosp. 29. 235.

Huldschinsky, 1920. Deutsch. med. Wschr. 45. 712.

Iversen and Hansborg. 1922. Act. med. Scand. 57. 95.

Iversen and Lenstrup. quoted by Drucker (1927).

Jephcott and Bacharach, 1926. Bioch. J. 20. 1351.

Jeppsson and af Klercker, 1921, Ztschr. Kinderheilk. 28. 71.

Johannsen/

Johannsen, 1924. Act. Paediatr. 3. 168.

Jones, 1924. J. Amer. Med. Ass. 82. 439.

Karelitz and Shohl, 1927. J. Biol. Chem. 73. 655.

af Klercker and Odin, 1925. Act. Paed. 5. 79.

Kroetz, 1927. Klin. Wschr. 6. 1171.

Lenhardt and Chaptal, quoted by György (1929).

Lesné, Turpin and Guillaumin, 1925. Rev.franç. de Pédiatr.1.40.

Liégeois and Lefèvre. 1928. Compt. rend. Soc.de Biol. 98. 1445.

1931. ibid. 102. 957.

Arnold. 1927. Klin. Wschr. 6. 607.

McCallum, Lintz, Vermilye, Leggett and Boas 1920, Bull.J.

Hopk. Hosp. 31. 1.

McClendon, 1922. Amer. J. Physiol. 61. 373.

McClendon, Bessell, Lowe and Meyer. 1920. J. Amer. Med. Ass.75.1638

Mellanby, 1925. Med.Res. Council Spec. Report. 93.

Michelsen, 1933. Arch.f. exp. Path.u. Pharm. 173. 737: 746: 750.

Morris, Watson and Morris 1931. Bioch. J. 25. 786.

Nitschke and Schneider, 1932-33. Ztschr.f.Kinderheilk. 54. 1.

Nothmann and Guttmann, 1924. Arch. f. exp. Path.u.Pharm. 101. 28.

Orr, Holt, Wilkins and Boone, 1924.Amer.J. Dis. Child.28. 574.

Oser, 1928. J. Biol. Chem.80. 487.

Parhon, Ballif and Dérévici, 1926. Compt.rend.Soc.de Biol.95.786:787.

Pritchard/

Pritchard, 1923. Brit. Med. J. 1. 887.

Redman, 1929. Bioch. J. 23. 256.

Rohmer and Woringer, 1921. Rev.franç.de Pédiatr. 1. 290.

1923. Compt.rend.Soc.de Biol. 89. 575.

Rominger, Meyer and Bomskov. 1930. Ztschr.f.d.ges.exp.Med.73. 343.

1931. Klin. Wschr. 10. 1342.

1931. Ztschr.f.d.ges.exp.Med.78. 259.

Salter, Farquharson and Tibbets. 1932. J. Clin. Inv. 11. 391.

Salvesen, Hastings and McIntosh, 1924. J. Biol. Chem. 60. 311.

Samuel and Kugelmass. 1930, Amer.J. Dis. Child. 39. 687.

Satke and Bartolomey, 1930, Wien.Arch.f.inn.Med. 20. 121:

abstract in Amer. J. Dis. Child. 1930. 40. 863.

Schabad, 1909. Ztschr. Kinderheilk. 67. 454.

Scheer, 1922, Jahrb. Kinderheilk. 97. 130.

Schloss, 1917.Ergebn. inn. Med. u. Kinderheilk. 15. 55.

Shelling, quoted by Shohl et alü J. Biol. Chem.1932. 98. 215.

Shohl, 1933. Annual Review of Biochemistry, 2. 207.

Shohl, Bennett and Weed, 1928. J. Biol. Chem.78, 181.

Proc. Soc. Exp. Biol. and Med. 25. 669.

Shohl and Bing, 1928. Amer. J. Physiol. 86. 633.

Shohl and Brown, 1929. J. Biol. Chem. 84. 501.

Shohl,Brown,Chapman,Rose and Saurwein, 1932.J.Biol.Chem.98.215.

Shohl/

Shohl,Brown,Rose,Smith and Cozad, 1931,J.Biol.Chem.92. 711.

Sokolovitch, 1931, Arch. Dis. in Child. 6. 183.

Telfer, 1922-23. Quart. J. Med. 16. 45: 63.

1923-24. ibid. 17. 245,

Tezner, 1925. Monatsschr.Kinderheilk. 29. 207 quoted by Drucker (1927)

Tisdall and Price, 1927. Bull.Johns Hopk. Hosp. 41. 432.

Turpin, La Tétanie infantile. quoted by Drucker (1927).

Ullrich, 1929. Ztschr. Kinderheilk. 47. 105: 581.

Underhill, Tileston and Bogert, 1922, J. Metab. Res. 1. 724.

Wilder, 1929. J. Biol. Chem. 81. 65.

Zehnter and Foncin, 1924. Arch.de Méd.des Enfants, 27. 11.

Zucker, Johnson and Barnett, 1922, Proc.Soc.Exp.Biol.and Med.20. 20.

Zucker and Matzner, 1923. Proc.Soc.Exp.Biol.and Med. 21. 186.

--0--

METHODS.

Blood.

Carbon Dioxide.	Haldane 1920.J.Path.and Bact.23.443.
Chlorine.	Whitehorn, 1921.J.Biol.Chem.45.449.
	Van Slyke 1923. ibid. 58. 523.
Fixed Base	Stadie and Ross 1925.J.Biol.Chem.65.735.
Calcium	Kramer and Tisdall 1921,J.Biol.Chem.47.475.
Phosphorus	Tisdall 1922.J.Biol.Chem. 50. 329.
Non-protein Nitrogen.	Folin and Wu ,1919.J.Biol.Chem.38.81.
Salicylate.	Thorburn and Hanzlik,1915.J.Biol.Chem.23.163.
Sugar.	Maclean,1919.Biochem.J. 13. 135.
pH	Dale and Evans 1920.J.Physiol.54.167.

Urine and Faeces.

Ammonia	Folin Method.Cathcart,Paton and Pembrey. Practical Physiology, 1929. 330.
Titratable Acid.	Ibid. 306.
Urea	Urease method.
Creatinine	Folin Method. Cathcart,Paton and Pembrey. Practical Phsyiology. 1929. 328.
Total Nitrogen	Kjeldahl Method.

Chlorine/

Chlorine .	Volhard's Method.
Fixed Base.	Stadie and Ross, 1925. J. Biol. Chem. 65, 735.
Calcium.	Gravimetric Method.
Phosphorus.	Gravimetric Method.
Salicylate.	Thorburn and Hanzlik, 1915. J. Biol. Chem. 23. 163.

---O---

From the Transactions of the Third International Pediatric Congress.
ACTA PÆDIATRICA. VOL. XVI, 1933.

H: 3.

Phosphorus Retention and Alkalosis in the Pathogenesis of Infantile Tetany.

By **NOAH MORRIS**, M.D.

From the Dept. of Paediatrics, University of Glasgow, and the Biochemical Laboratory, Royal Hospital for Sick Children, Glasgow.

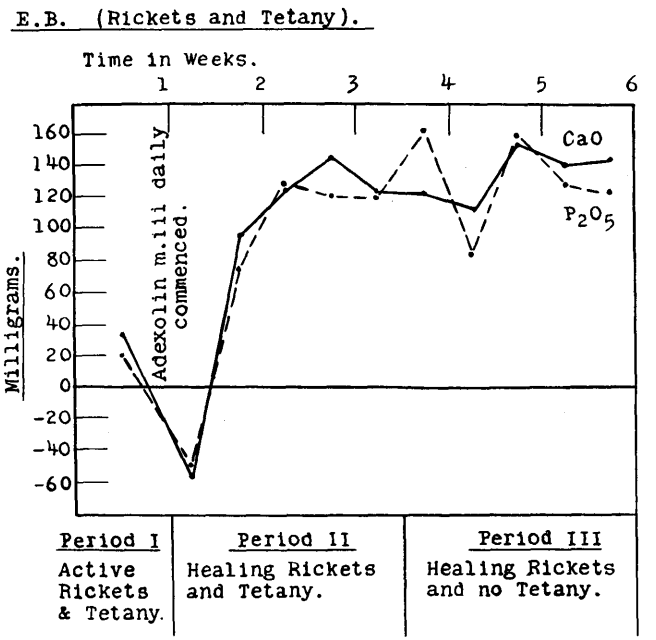
Of late years it has been alleged that the onset of tetany in the rachitic state has been associated with a supernormal retention of phosphorus and a subnormal retention of calcium. This state of affairs is said to occur as the initial phase in the passing of active into healing rickets. It is well known that tetany frequently occurs in the early stages of healing rickets and that the blood phosphorus suddenly changes from a subnormal to a high value at or before the onset of tetany. This latter observation, however, cannot be taken as an indication of increased phosphorus retention since in cases such as chronic nephritis the serum phosphorus is high although the retention of phosphorus may be very low. HESS and others have shown that in animals in which rickets has been induced by a high calcium-low phosphorus diet tetany can be produced by a sudden increase of the phosphorus-moiety of the diet. There is an objection in applying this evidence to the pathogenesis of infantile tetany since rickets in the infant is not the result of an insufficient intake of phosphorus.

ROMINGER and his co-workers, however, have given figures to show that in infancy the first phase of healing rickes is associated with a great increase in phosphorus retention but a poor retention of calcium simultaneously with the appearance of the phenomena of tetany. From the results of these metabolic investigations supported by experimental work on animals, they drew the conclusion that excessive phosphorus retention unaccompanied by corresponding increase in calcium is the important factor in the production of tetany. This conclusion is not, however, justified unless it can be shown 1) that tetany in association with rickets only occurs when there is excessive retention

of phosphorus and 2) that when excessive retention of phosphorus without simultaneous increase in calcium retention is induced in a child suffering from active rickets, tetany occurs.

The following results show 1) that the administration of a vitamin D preparation to a child suffering with active rickets is followed by parallel increases in the retentions of calcium

Fig. I a. *Daily Retention of CaO and P_2O_5 in mg. per kilo per day.*

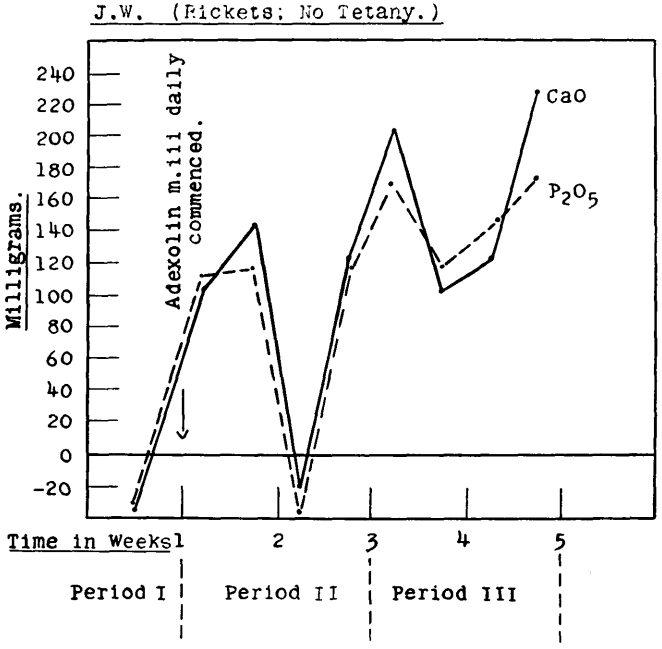


and phosphorus (Fig. 1), 2) that these parallel increases occur whether or not the signs of tetany are present (Fig. 1), 3) that administration of phosphate to children with active rickets can lead to a very marked increase in the retention of phosphorus without any increase in calcium retention and in such circumstances that the phenomena of tetany even when the administration of the phosphate is prolonged over a considerable period, do not appear (Table 1).

It would, therefore, seem justifiable to conclude that tetany complicating rickets may occur without an excessive retention of phosphorus over calcium and that excessive retention of phosphorus may occur without any indication of tetany.

It has long been known that conditions of alkalosis may be accompanied by signs of tetany. GYÖRGY among others has

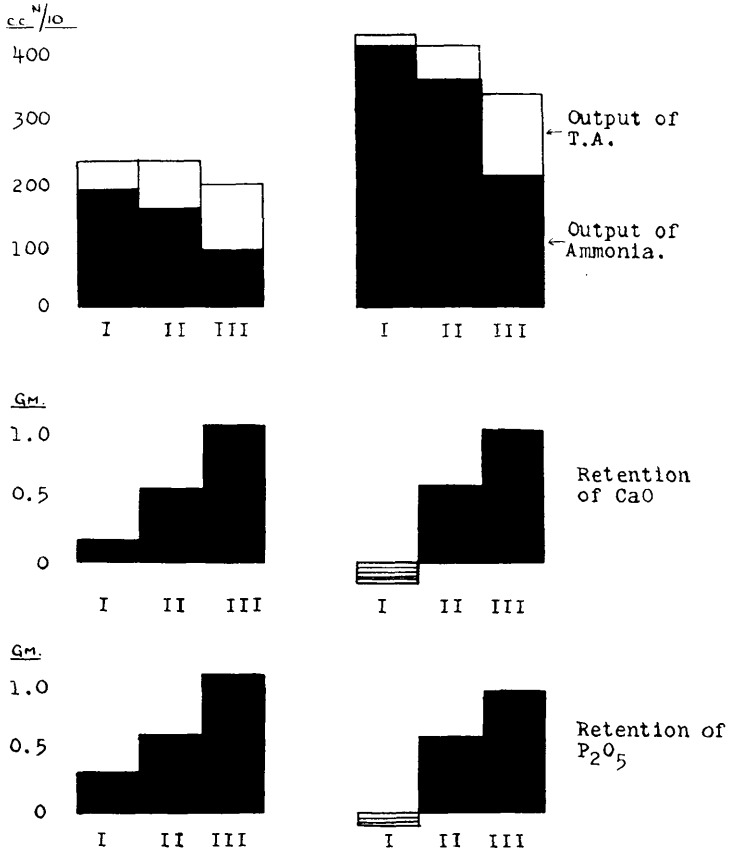
Fig. I b. *Retention of CaO and P_2O_5 in mg. per kilo per day.*



suggested that in rickets there is a state of acidosis and that in tetany there is a swing in the direction of alkalosis. This view is based in part on evidence derived from a biochemical study of the urine: in rickets uncomplicated by tetany the urinary output of ammonia is high while in the presence of tetany the output is said to be low.

The following results (Fig. 2) show that the changes in output of titratable acid and ammonia by the urine follow the

Fig. II.



E.B.

Period I - Active rickets and tetany.

" II - Healing rickets and tetany.

" III - Healing rickets. No tetany.

J.W.

Period I - Active rickets. No tetany.

" II - Healing rickets (early). No tetany.

" III - Healing rickets (later). No tetany.

Table I. *Effect of Phosphate Administration on CaO and P_2O_5 Retention in Rickets.*

	Retention in mg. per kg. per day	
	CaO	P_2O_5
Case I. (W.B.)		
Before giving phosphate	— 93	— 9
During administration of NaH_2PO_4 gm. 2.5 daily	+ 4	+178
Case II. (J.W.)		
Before giving phosphate	+123	+166
During administration of Na_2HPO_4 gm. 3.0 daily	— 16	+207

same course whether or not there are signs of tetany. They further indicate that there is a direct relationship between the urinary output of titratable acid and the retentions of Ca and P and an inverse relationship between the urinary output of ammonia and the retentions of calcium and phosphorus. Since the urea output was practically at the same level in the active as in the healing state, it would appear that the excess urinary ammonia in the active state cannot be due to defective liver function. These facts would suggest that fixed base is retained in the tissues and tissue fluids thus necessitating the formation of ammonia by the kidneys to transport acid.

The only difference observed between the metabolic results obtained in rickets with tetany and in rickets without tetany was the diminished output of chlorine when signs of tetany were present. There is no evidence from these results apart from the great reduction in urinary titratable acid that alkalosis is a necessary concomitant of infantile tetany. Admittedly the production of an acidosis by ammonium chloride cures tetany but that is probably because it renders the body store of calcium more readily available.

The following results (Table 2) show that administration of an acid producing salt to children showing signs of tetany and the radiological and metabolic characteristics of active rickets

Table II. *Effect of Administration of NH_4Cl (5 gm. daily) on CaO and P_2O_5 in Active Rickets.*

	Retention in mg. per Kg. per day			
	Before NH_4Cl		During NH_4Cl	
	CaO	P_2O_5	CaO	P_2O_5
R.M.	30	44	45	49
J.M.	12	21	57	47
C.P.	37	44	50	57

led to an increased retention of calcium and phosphorus which in one instance became manifest on X-ray examination. It would appear, therefore, that the increased output of ammonia observed in untreated active rickets cannot be a manifestation of acidosis since the production of acidosis may lead to an increased mineral retention.

The author desires to thank the Medical Research Council for a personal grant.

Reprinted from the "Glasgow Medical Journal," March, 1932, vol. cxvii, p. 126.

CONVULSIONS IN INFANCY.*

PART II.

BY NOAH MORRIS, M.D.

DR. GRAHAM has dealt with the clinical aspect of convulsions, and I should like to discuss some recent biochemical findings which seem to throw light on the pathogenesis and may be of some service as indicating suitable lines of treatment.

For many years attention has been directed to a deficiency in calcium as the primary factor in the causation of convulsive disorders. Crude attempts at the precipitation of calcium in the blood and in the nervous system by means of such salts as oxalates led to the production of convulsions. Within the past fifteen years it has been definitely shown that frank infantile tetany is associated with marked lowering of the serum calcium. In addition, however, we have at the Royal Hospital for Sick Children obtained results showing that in a series of 37 infants suffering with convulsions, but without clinical signs of tetany or rickets, the serum calcium was diminished in 21. *E.g.*:—

P. McL. 9/52. Normal labour, healthy infant till a week ago, when he commenced to have very frequent convulsions affecting especially the left side (nystagmus, eyelids, face, arm, leg). No cyanosis or crowing. Serum Ca 5·2. W.R. negative.

Treatment.—$CaCl_2$ gr. 20 6x. Within three days of commencing treatment no convulsions. Dismissed well.

This case shows the association of a low serum calcium with convulsions, although there was no evidence of any clinical manifestation of tetany. It is tempting to assume that the low serum calcium is in this group of cases the cause of the convulsions. Dr. Graham has pointed out that there is an increase in the frequency of convulsive disorders during the winter six months, the period during which there is a minimum of sunlight and a consequent decrease in calcium retention and serum Ca.

* A communication made to the Royal Medico-Chirurgical Society of Glasgow at a meeting held on 20th November, 1931.

In several instances, however, the onset of convulsions seems to have been precipitated by a febrile illness or some metabolic disorder. *E.g.*:—

R. E. 2/12. Normal labour, healthy infant till six days ago, when he became highly fevered with onset of convulsions. Two days later another convulsion. Temperature, 105·4°. On admission temperature normal, physical examination negative. On seventh day in hospital convulsions again commenced; next day fever. Serum Ca 6·7 mgm. per cent.

Treatment.—At first chloral for five days—no effect; then $CaCl_2$ gr. 30 6x —immediate cessation of convulsions. Dismissed well.

There also occurred in our series several cases of inflammatory mischief of the central nervous system characterized by convulsions and associated with a low serum calcium. *E.g.*:—

J. F. 8/52. Normal labour; apart from vomiting seemed quite healthy till a week ago, when he commenced to have fits, which have continued since. On admission slight fever, otherwise physical examination negative.

Next day (27th December, 1930).—Ca 3·7.

28th December, 1930.—Four fits—twitching of face, fingers and toes. Lumbar puncture—excess of cells, especially lymphocytes (blood in cerebro-spinal fluid).

29th December, 1930.—Ca 6·4.

17th January, 1931.—Ca 7·3.

7th March, 1931.—Head noticeably bigger. Injection of phenol sulphone phthalein into the ventricle—in 5 mins. at lumbar region—only 2 per cent in urine after two hours.

18th March, 1931.—Lumbar puncture—Cerebro-spinal fluid contained 22 cells; phenol sulphone phthalein injected—only 6 per cent in urine in six hours. Serum Ca 10·6.

15th May, 1931.—Definite hydrocephalus—circumference of head 43·5 cms.

A similar case could be quoted in which the lesion was a pneumococcal meningitis. The question arises as to whether the low serum Ca was produced by the metabolic disturbances associated with meningitis or whether there was pre-existent instability of the calcium metabolism. Certainly the serum Ca is not always low in meningitis even when it is accompanied by convulsions. *E.g.*:—

G. D. 9/52. Meningoc. meningitis, convulsions, serum Ca 9·7.

Similarly there are cases of convulsions occurring at the onset of a febrile attack where the serum Ca is within normal limits,

A normal serum Ca is regularly found in certain types of tetany, such as bicarbonate and gastric. The onset of convulsions in these conditions of tetany without diminution of serum Ca has been explained by relative excess of some of the other basic ions or by an alteration in the physico-chemical state of calcium.

It is well known that in many ways Ca and Na are antagonistic ions. In the perfusion of hearts and other physiological experiments it is of the greatest importance to use a solution that is properly balanced as regards its inorganic constituents. Apart from experiments on isolated tissues and organs there has been no direct proof of the correctness of this view. Some recent dietetic work in Germany is based on this hypothesis. The Na salts are in great part replaced by a salt mixture of K, Ca and Mg salts, and it is claimed that this mixture exerts a parasympathicotonic action instead of the sympathicotonic effect of Na. Irritative disorders such as neuro-muscular hyperexcitability and even epilepsy are said to undergo definite improvement under this modified salt régime.

The serum Ca is said to exist in three moieties—(*a*) indiffusible, not passing through a collodion membrane; (*b*) diffusible and not ionized; (*c*) diffusible and ionized. It is clear that a reduction may occur in any of these without any necessary change in the total amount of Ca in the serum. The amount of Ca which is ionized is the important fraction in the part this element plays in the regulation of neuro-muscular excitability; it is dependent on the serum contents of phosphate and carbonic acid which in turn are dependent in great part on the reaction of the serum. The greater the tendency to acidosis the greater the amount of Ca in the ionized form, while in alkalosis the ionized Ca will become less.

It will be convenient now to consider some of the conditions that have been put forward as the cause of convulsions met with clinically, viz., alkalosis, hyperphosphatæmia and hypoglycæmia.

It has been shown that intravenous injection of alkali leads to increase of neuro-muscular excitability. Administration of alkali orally, however, to the normal individual, even in very large doses, does not lead to convulsions. If, however, renal efficiency is impaired as in some cases of pyuria, excessive

alkaline therapy may lead to convulsions and even to frank tetany. *E.g.*:—

E. McG. 27/52. Because of pyuria given sod. bic. gr. 15 6x; two days later convulsions—Ca 5·1, CO_2 103·8, N.P.N. 50. After the sod. bic. was stopped and $CaCl_2$ commenced, there were no more convulsions.

It may be urged that the alkaline treatment of hydræmic nephritis where the serum Ca is low does not induce convulsions. In this connexion it must be remembered that in such a condition the renal function, as judged by the ammonia production, is not impaired. In uncomplicated experimental alkalotic tetany, although the serum Ca is not reduced, the ionized moiety is much below normal. If the alkalosis is produced by, or in association with, such a condition as fever or pyelonephritis there may be a definite reduction of the serum Ca.

Excess of serum inorganic phosphate has also been credited with the causation of convulsions. The injection of alkaline phosphate leads to tetany, whereas the use of the acid salt unless in very large doses does not produce tetany although the serum Ca falls. Phosphoric acid and ammonium phosphate not only do not cause tetany but have been used successfully for its treatment. Both lead to an increase in acid radicles: the ammonium moiety is changed to the neutral urea, leaving phosphoric acid as an addition to the amount of acid in the body. It would appear, therefore, that administration of phosphorus will cause a state of spasmophilia only when the reaction of the blood is moved toward the alkaline side. In gastric tetany and tetany associated with rickets the serum phosphate is increased, whereas in the form due to overbreathing and that accompanying cœliac disease it is diminished. The conclusion of Peters and Van Slyke in this connexion seems to give a summary of the state of affairs. They state that "the available data taken together indicate that there is probably no relationship between phosphates and tetany except such as may be exerted indirectly through the influence of phosphates on the reaction and the calcium content of the blood serum."

It would thus appear that a large number of cases of convulsions is associated with a low value for ionic serum

calcium and frequently a shift of the reaction to the alkaline side. By making the serum less alkaline and thus increasing the amount of ionic calcium convulsions can be prevented. These facts strongly suggest that one or both of these conditions (low ionic Ca and alkalæmia) is responsible for the state of increased neuro-muscular excitability leading to convulsions.

Another type of convulsion is the hypoglycæmic variety. The hypoglycæmic syndrome following overdosage of insulin is well known, and there are being described in the literature cases of idiopathic hyperinsulinism characterized by definite increase in neuro-muscular excitability. Several authors attribute many of the convulsions of infancy to idiopathic attacks of hypoglycæmia. There are several findings, however, which militate against the acceptance of such a view. In the first place, we have found quite low values for blood-sugar in children who had not convulsions or any state of increased neuro-muscular excitability. Secondly, the treatment of epilepsy with high-fat diet has shown that the low level of blood-sugar produced and maintained for weeks certainly does not increase neuro-muscular excitability. Indeed, great benefit is said to be obtained from the institution of this treatment. It is to be noted, however, that some convulsion-producing drugs, such as guanidine, cause lowering of the blood-sugar, and it is possible that this reduction is the result of some interaction between the sugar and the guanidine or the products of its action on the body. Furthermore, there is a relationship between phosphorus and carbohydrate metabolism, and it is feasible that the association of hypoglycæmia with convulsions may be due to this relationship having an effect on the physical state of calcium.

It is evident from the data presented that calcium metabolism plays an important part in the regulation of neuro-muscular excitability. Even in those cases where the serum Ca was within normal limits it is difficult to determine whether the normality obtained at the onset of the fit. Serum Ca has been shown to vary very rapidly, and it is often impossible to obtain serum for analysis until several hours after the convulsion. It is probable that the moiety of calcium specially responsible is the ionized fraction, and it is possible that where the total serum Ca was normal there may have been a diminution in the ionized fraction. It must be admitted, however, that there

is one series of findings which prevents us from fixing the sole responsibility for convulsions on a low value for ionized calcium. The normal level for the ionized calcium of serum is about one-third of the total, *i.e.*, about 3 to 3·5 mgm. per 100 c.c. Occasionally we have come across values for total calcium approximating this without any evidence of increased neuro-muscular excitability. Indeed, Howland and Marriott quote a figure of 1·5 mgm. total calcium in a child without history or presence of any " convulsive " manifestation. *E.g.*:—

1. G. L. 6/52. Congenital atresia of bile-ducts. No convulsions. Serum Ca 7·3.

2. T. N. 9/12. ? Mental defective. No convulsions in hospital. Serum Ca 4·2.

3. J. W. 12 years. Acute exacerbation of chronic nephritis. No convulsions although F.P.++. Serum Ca 3·4.

The conclusion seems forced on us that although ionized calcium plays an important part in the prevention of hyper-excitability there must be some other factor or factors which influence the onset of convulsions.

That calcium is one of the important, if not the all-important, substance in the prevention of convulsions is made evident by the success of calcium therapy. By this is not necessarily meant the administration of calcium but rather its mobilization and thus the raising of the serum content of calcium, more particularly the ionized fraction. Any treatment that will increase the content of ionized calcium will achieve the desired result. Calcium has been given intravenously in combination with an organic acid which is quickly oxidized, so leaving the calcium to exert its effect. Perhaps the simplest method is the oral administration of calcium chloride. There is still much confusion about its mode of action, and the lactate is frequently recommended as if the action depended on absorption of calcium. Paradoxically the superiority of calcium chloride is due to the fact that the bulk of the calcium is left unabsorbed in the gut, allowing the chlorine to enter the blood-stream, produce a tendency to acidosis and thus increase the serum content of total calcium and more especially the ionized fraction. Other acid-producing substances, such as NH_4Cl, HCl, ammonium phosphate, which do not contain calcium are

equally efficacious in raising the serum calcium and preventing convulsions. Probably the chlorine-containing substances are most effective, and it is possible that the chlorine ion exerts apart from its acid-producing effect a special action on waste products of metabolism. The dangers of acidosis resulting from the use of acid-producing salts are generally much overrated. With the dosage advised it is not often encountered, and when it does occur it is easily remedied by substituting an alkali (sod. bic.) for the calcium or ammonium chloride.

Finally, I would like to point out that this acid treatment is curative in many cases of convulsions not associated with other manifestation of tetany, and frequently when chloral or other sedative has been of no avail.

I might recall the case of R. E. already mentioned, where five days of chloral treatment were of no avail while administration of $CaCl_2$ led to an immediate cessation of convulsions.

It is clear, therefore, from a consideration of the biochemical evidence that a considerable number of the convulsions in infancy, although not revealing other signs of frank tetany, are spasmophilic in nature, or at anyrate associated with hypocalcæmia. There must exist a state of metabolism where there is liable to occur a diminution in active calcium and where consequently any treatment capable of counteracting this diminution is likely to prove successful.

The author wishes to acknowledge the assistance of the Medical Research Council from whom a grant was received.

GLASGOW: PRINTED BY ALEX. MACDOUGALL

[*From the Proceedings of the Physiological Society*, June 11, 1932.]
Journal Physiology, Vol. LXXV.

Difference between the retentions of calcium and phosphorus as a factor in the production of infantile tetany. By F. J. Ford, S. G. Graham and Noah Morris. (*Biochemical Laboratory, Department of Medical Pædiatrics, University of Glasgow.*) (*Preliminary communication.*)

Recent experimental work by Hess and others [1931] seems to indicate that an important factor in the onset of tetany in rachitic animals is an increase in the retention of phosphorus without corresponding increase in that of calcium. Rominger and his co-workers [1931] assert that infantile tetany, so commonly associated with rickets in the young child, is also due to relative increase of phosphorus over calcium retention. In two children, E. B. and J. W., both the subjects of

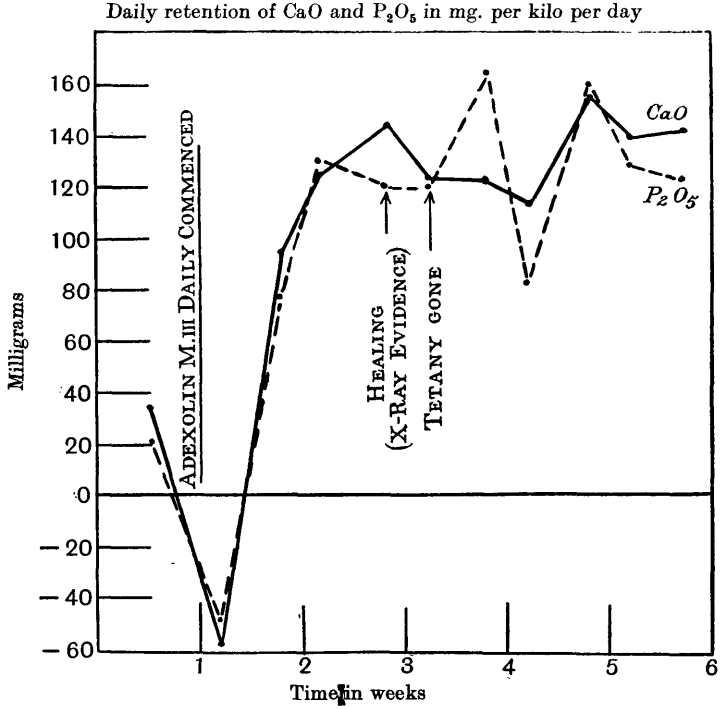


Fig. 1.

active rickets, and E. B. with latent tetany, calcium and phosphorus retentions were determined before and after the administration of vitamin D (adexolin). The retentions of calcium and phosphorus, which in both instances were increased, ran parallel to one another, although

in E. B. signs of latent tetany did not disappear till 16 days after vitamin D was commenced. Fig. 1 shows the result in E. B. Phosphate was administered to two children: W. B. who had marked osteoporosis, was given NaH_2PO_4 and J. W. with healing rickets received Na_2HPO_4 over a period of 3 weeks. In both cases the retention of phosphorus was markedly increased, while the retention of calcium remained subnormal, but there was no evidence of tetany latent or active.

TABLE I. Effect of phosphate ingestion on calcium and phosphorus retention.

	Retention in mg. per kg. per day	
	CaO	P_2O_5
Case I (W. B.)		
Before giving phosphate	− 93	− 9
NaH_2PO_4 g. 2·5 daily	+ 4	+178
Case II (J. W.)		
Before giving phosphate	+123	+166
Na_2HPO_4 g. 3·0 daily	− 16	+207

From these results it would seem that in the pathogenesis of infantile tetany associated with rickets, excess retention of phosphorus is not a prime factor.

Two of us (N. M. and S. G.) are in receipt of personal grants from the Medical Research Council to whom we express our thanks for defraying the expenses of this research.

REFERENCES.

Hess, A. F., Weinstock, M., Benjamin, H. P. and Gross, J. (1931). *J. Biol. Chem.* **90**, 737.

Rominger E., Meyer, H. and Bomskov, C. (1931). *Klin. Wochenschr.* **10**, 1342.

Reprinted from the British Journal of Experimental Pathology, 1932, Vol. XIII, p. 132.

ON THE EFFECT OF INTRAVENOUS INJECTION OF GLYCINE ON THE SERUM CALCIUM.

NOAH MORRIS, J. BASIL RENNIE* AND SAMUEL MORRIS.

From the Biochemical Laboratory, University Department of Pædiatrics, Royal Hospital for Sick Children, Glasgow; and the Hannah Dairy Research Institute, Ayr.

Received for publication February 15th, 1932.

IT has long been known that both in tetania parathyreopriva and that due to guanidine poisoning there occurs an increased catabolism of nitrogen (Berkely and Beebe, 1909, Burns, 1917). After parathyroidectomy Justschenko (1913) found an increase in the output of amino-acids, and Burns (1917) reported the same finding after the injection of guanidine. Morris, Watson and Morris (1931) confirmed these findings both for parathyroidectomy

* Carnegie Research Scholar.

and guanidine poisoning. Their results seemed to indicate that there was some relationship between the excess amino-acid nitrogen of the urine and the decrease in the amount of calcium retained. The following series of experiments were undertaken to determine whether amino-acid injected directly into the blood-stream had any effect on the serum calcium.

Glycine was the amino-acid used : 2·5 c.c. of a 20 per cent. solution per kilo body-weight were injected intravenously. In two instances the glycine solution was administered intraperitoneally. Blood was withdrawn for analysis before the injection and once or oftener thereafter. Rabbits and goats were the animals used, the latter so that changes in blood volume, produced by withdrawal of blood, should be kept at a minimum.

TABLE I.—*Effect of Glycine Injection on Serum Calcium and Non-Protein Nitrogen of Whole Blood in Rabbits.*

Rabbit No.	Serum calcium (mgm. per 100 c.c.)	Non-protein nitrogen of blood (mgm. per 100 c.c.)	
1	7·9	42·7	
	6·7	100·0	10 mins. after intravenous injection.
2	12·2	40·3	
	9·0	59·9	1 hour after 1st ,, ,,
	7·3	56·2	1 ,, 2nd ,, ,, (2 hours after 1st injection.)
3	11·2	..	
	8·1	..	½ hour after intravenous injection.
4	10·5	29·4	
	9·5	49·1	½ ,, ,, ,,
5	9·7	20·0	
	10·1	50·0	1 ,, 1st intraperitoneal injection.
	10·3	66·3	1 ,, 2nd ,, ,, (3 hours after 1st injection.)
6	9·3	34·2	
	9·8	46·9	1 hour after intraperitoneal injection.
	8·7	48·7	2 hours ,, intravenous ,, (4 hours after intraperitoneal injection.)
A	11·3	40·1	
	12·2	33·0	½ hour after intravenous injection of saline.

In no instance was there noted any manifestation of increased neuromuscular excitability or any other abnormality. The intravenous administration of glycine led to a fall in serum-calcium of 1·0 to 3·1 mgm. per 100 c.c. in the rabbit (Table I), and of 2·4 to 2·9 mgm. per 100 c.c. in the goat (Table II). Two hours after the injection the serum calcium had not yet reached its normal value. The non-protein nitrogen was increased more markedly in the rabbits than in the goats, but in only one rabbit (No. 1) when the blood was withdrawn within 10 minutes of the glycine injection did the increase account for even 50 per cent. of the amount of glycine nitrogen injected. In the goats both the serum protein and the chlorine content of the whole blood were slightly diminished, while the inorganic phosphorus of the serum was very slightly increased. When the glycine solution was injected intra-peritoneally in rabbits the calcium content of the serum was practically unchanged.

TABLE II.—*Effect of Glycine Injection on Composition of Blood in Goats.*

Goat No.		Before.	½ hour after injection.	1 hour after injection.	2 hours after injection.
1	Serum calcium	11·3	8·6	8·8	10·4
	,, inorg. P.	3·5	3·8	3·8	3·6
	,, protein	8·68	8·49	8·58	8·56
	Blood N.P.N.	28·5	35·2	32·9	29·9
	,, Cl	315	308	309	313
2	Serum calcium	10·7	8·3	9·0	10·0
	,, inorg. P.	3·5	3·8	3·7	3·6
	,, protein	8·52	8·40	8·45	8·51
	Blood N.P.N.	31·0	36·1	36·0	33·5
	,, Cl	324	310	312	320
3	Serum calcium	10·9	8·0	8·7	9·3
	,, inorg. P.	3·6	3·9	3·7	3·7
	,, protein	9·05	8·56	8·82	9·0
	Blood N.P.N.	31·1	36·1	32·0	32·0
	,, Cl	313	303	304	312

Results in mgm. per 100 c.c. except protein, where figures represent gm. per 100 c.c.

From these results it is clear that the injection of glycine directly into the blood-stream leads to a fall in serum-calcium. From the non-protein-nitrogen values it is evident that comparatively little of the amino-acid nitrogen remains in the blood. It seems feasible, therefore, that the glycine carried calcium with it into the tissue-cells. The tendency of the serum calcium to return to its normal value is probably due to the deaminization of the glycine with consequent release of the calcium. When the glycine is slowly absorbed, as happens when the injection is into the peritoneal cavity, it will be deaminized as quickly as it is absorbed, with the result that serum calcium is not affected. Greenwald (1925) has suggested that in tetania parathyreopriva calcium is precipitated in the tissues as calcium phosphate. In view of our results it seems that excess production of amino-acids also leads to a withdrawal of calcium from the serum into the tissues.

SUMMARY.

Intravenous injection of glycine solution leads in rabbits and goats to a diminution in serum calcium. It is suggested that excess amino-acid production after parathyroidectomy plays a part in lowering the serum calcium.

We are indebted to Drs. A. McLeod Watson and Jordan for performing the injections on rabbits and goats respectively. It is a pleasure to acknowledge the advice and helpful criticism of Prof. G. B. Fleming and Dr. Norman Wright. Thanks are due to the Medical Research Council for defraying a portion of the expenses of the investigation and for a personal grant to one of us (N. M.).

REFERENCES.

BERKELY, W. N., AND BEEBE, S. P.—(1909) *J. Med. Res.*, **20**, 149.
BURNS, D.—(1917) *Quart. J. Exp. Physiol.*, **10**, 361.
GREENWALD, I., AND GROSS, J.—(1925) *J. Biol. Chem.*, **66**, 217.
JUSTSCHENKO, A. S.—(1913) *Biochem. Z.*, **48**, 61.
MORRIS, S., WATSON, A., AND MORRIS, N.—(1931) *Biochem. J.*, 25, 786.

Made and printed in Great Britain for
H. K. Lewis & Co. Ltd., by
Adlard & Son, Ltd., 21, *Hart Street, London, W.C.* 1.

EFFECT OF AMMONIUM CHLORIDE ADMINISTRATION ON METABOLISM IN INFANTILE TETANY AND RICKETS

BY

NOAH MORRIS, M.D.,
and
OLIVE MACRAE, M.D.

(From the Department of Pædiatrics, Glasgow University, and the Biochemical Laboratory, Royal Hospital for Sick Children, Glasgow.)

For many years various opinions have been expressed as to disturbances in acid-base equilibrium in rickets and infantile tetany. The view that rickets is essentially a condition of acid poisoning was promulgated many years ago and some workers[1] still hold this view. Recently György[2] has expressed the opinion that a state of acidosis, while not necessarily the fundamental feature, is certainly associated with rickets, and that calcification is prevented by the increased acidity of the bone-forming tissues. With reference to this latter statement, however, Bosanyi[3] found that the pH of rachitic cartilage was more alkaline than the normal, being 7·6 in the former as compared with 7·2 in the latter. Most efforts to obtain direct proof of change in the acid-base equilibrium of the blood or tissues have met with equivocal results, and from the evidence indirectly obtained widely varying conclusions have been drawn. Schabad[4] maintained that the very low urinary excretion of lime and phosphorus in the early stage of rickets was against the theory of increased acid production. Recent investigations in general support this conclusion. Zucker, Johnston and Barnett[5] found that in rats a rachitic condition supervened much more quickly, and to a more marked degree, when the diet was less acid-forming in nature. Thus the substitution of calcium chloride for calcium lactate in a typical rachitic dietary led to the development of a milder type of rickets. Similarly, the addition of 2 per cent. ammonium chloride to the diet prevented rickets in rats, whereas the administration of 2 per cent. sodium bicarbonate even to a well-balanced diet led to marked rickets. Hess[6] found that when rickets was induced with a diet containing 11·5 times as much calcium as phosphorus, the administration of cod-liver oil or irradiated ergosterol, or exposure to ultra-violet light, only produced healing of the rachitic state when there was an excess of acid over basic elements in the diet, as occurred when calcium chloride was substituted for calcium carbonate in equi-molar amounts. On the other hand, Samuel and Kugelmass[7] report experiments on rats in which rickets developed much more markedly on diets free of vitamin D when there was an excess of acid elements,

The urinary excretion of acid and ammonia in rickets has been determined by several workers. Hodgson[8] in 13 patients found an increase in the renal output of both acid and ammonia: Burgess and Osman[9] also reported an increased excretion of ammonia. Freudenberg and György[10], while confirming the increased output of ammonia, found that in some cases the urine was neutral or even alkaline.

Most workers are now agreed that the excessive loss of calcium and phosphorus in the fæces in rachitic conditions is the result, not of increased excretion through the gut-wall, but of defective absorption. The reaction of the alimentary tract has frequently been investigated during the past decade. McClendon[11] and his co-workers in 1919 by direct electrometric investigation showed that normally the reaction of the whole of the small intestine in dogs and cats is on the acid side of neutrality. A year later they reported results indicating that in man the contents of the duodenum and jejunum have normally a pH below 7·0. In 1927 Lloyd Arnold[12] confirmed these findings in dogs. As far back as 1917 Schloss[13] pointed out that the reaction of the fæces was definitely alkaline in the active stage of rickets, and tended to become acid in the healing phase. Redman[14] noted a certain correlation between the mineral content and pH of the fæces of rachitic children thus confirming the observations of Schloss. Experimentally it has been shown in rats that the active stage of rickets is accompanied by a definite alkaline reaction of the fæces, while during the process of healing induced by cod-liver oil or ultra-violet light there is a definite increase in acidity (Zucker and Matzer[15], Jephcott and Bacharach[16], etc.). Direct determinations of the pH of the contents of the small intestine in dogs show that on a rickets-producing dietary (Mellanby's) the reaction changed from acid to alkaline, while addition of cod-liver oil to the food or exposure to ultra-violet light produced a return to an acid reaction (Grayzell and Miller[17]). Similar findings are reported by Tisdall and Price[18] in rats in which rickets had been produced. The consistency of these results provides almost conclusive proof that there is an actual change in the pH of the intestinal contents to the alkaline side in the active phase of rickets. While such a change in reaction would account for the defective absorption of calcium and phosphorus it is not sufficient to explain all the manifestations of the rachitic condition. Thus it has been shown experimentally that simple deprivation of lime, such as would occur if the sole abnormal factor were defective absorption, leads not to rickets but to osteo-porosis.

Present investigation.—The following investigation was undertaken to determine the effect of the administration of an acid-producing salt on the mineral metabolism in rickets and tetany. Ammonium chloride was the substance chosen to produce the acidosis, chiefly because in the gut it is neutral and therefore it was hoped that the influence of acidosis on the tissues could be studied without any direct action on the intestinal contents. It has already been shown by Gamble[19] and his co-workers and ourselves[20] that

calcium and phosphorus play an important part in dealing with a sudden excess of acid radicle in the tissues, at any rate until the ammonia formation has increased to such a pitch as to neutralize the extra acid. If the rachitic condition is accompanied by a state tending to acidosis it seems natural to expect that the organism will be less capable of dealing with the extra acid, especially when it is remembered that the bones are deficient in calcium and phosphorus.

The effect of ammonium-chloride administration on the calcium, phosphorus and chlorine metabolism was studied in four children who showed evidence of rickets. In three, in addition to a marked degree of active rickets shown by radiogram, there were present signs of tetany manifested by Chvostek's sign and in one laryngismus was also noted. In the fourth child the radiogram indicated that spontaneous healing was taking place during the period of the investigation. Two metabolic studies of seven days' duration were made in each patient. The diets were kept constant for three days prior to the commencement of the investigation and remained unchanged until the completion of the studies. In the second of the two ' metabolic ' periods ammonium chloride was given in one gramme doses five times daily.

Clinical features.—No clinical signs of acidosis were manifest during the period of ammonium-chloride administration. In Case 1, 5 grm. of ammonium chloride was given daily for 38 days without any obvious clinical indication of acidosis. In a previous investigation[20] we noted that in the normal child a similar dosage of ammonium chloride was also without apparent clinical effect, and we attributed this result to the fact that the acid-producing salt when given in small amounts frequently, and not in one massive dose daily, allowed the metabolic processes to deal with the influx efficiently and thus prevent manifest disturbance. It is clear that in active rickets the power of the individual to deal with acid is at least as good as normal so far as the prevention of clinical manifestations of acidosis is concerned. During the administration of the ammonium chloride Chvostek's sign disappeared in all the cases. In Case 3, the laryngeal spasm also disappeared during the administration of ammonium chloride, but both it and Chvostek's sign returned on the day after the ammonium-chloride ingestion ceased, although in the other two patients there was no return of any sign of tetany.

Perhaps the most remarkable clinical feature noted was the appearance of healing in the radiogram of Case 1 after 38 days' administration of ammonium chloride although the environmental and dietetic conditions remained constant throughout this period. A series of weekly radiograms (Table 6, p. 57) indicate that the healing became apparent two weeks after the initiation of ammonium-chloride ingestion, and gradually increased until at the end of 38 days it was definite, although not nearly so marked as that which occurs with vitamin-D preparations or ultra-violet light. The fact

that healing can take place during the administration of an acid-producing salt is in itself strong evidence against the view that there is in rickets a shift of acid-base equilibrium to the acid side. It may be urged that this patient was suffering from tetany. Nevertheless the occurrence of healing after the disappearance of Chvostek's sign indicates that in rickets the deposition of calcium can take place during the ingestion of a great excess of acid-producing substance.

Blood chemistry (Table 1).—The serum calcium which was abnormally low in all three patients (Cases 1, 2 and 3) with rickets and tetany showed a marked increase during ammonium chloride administration although the

TABLE 1.

CHANGES IN CHEMICAL COMPOSITION OF BLOOD.

Case No	Stage.	Calcium mgrm. %	Phosphorus mgrm. %	CO_2 Vol. %	N.P.N. mrgm. %
1	Before NH_4Cl administration	—	3·0	50·7	—
	3 weeks on NH_4Cl	6·1	3·4	40·3	39·8
	4 ,, ,, ...	6·5	4·8	—	—
	5 ,, ,, ...	7·4	4·2	37·8	40·9
	1 week after NH_4Cl stopped	6·9	2·8	59·6	31·6
2	Before NH_4Cl administration	7·6	5·6	55·6	34·8
	1 week on NH_4Cl	8·0	3·6	44·7	44·5
3	Before NH_4Cl administration	7·6	6·2	52·5	36·3
	1 week on NH_4Cl	8·9	4·6	41·4	43·1

normal level was not quite reached while the patients were under observation. The inorganic phosphorus content of the serum which was high in two patients (Cases 2 and 3) before the commencement of the ammonium chloride fell during that period, while in the third (Case 1), where it was initially low, an increase was noted for three weeks followed by a slow decline in its value. The total CO_2 of the blood was lowered during the ammonium chloride administration while the chlorine tended to rise in value and the non-protein nitrogen showed no change. Generally it may be said that the changes in blood chemistry following the administration of ammonium chloride are the same in rachitic patients as they are in normal children. Further, it may be said that the results of blood analysis indicate the presence of what van Slyke has termed a compensated acidosis, just as occurs in healthy children under similar conditions.

Metabolism.

It will be convenient first to summarize the metabolic results for each substance individually and thereafter to correlate the various findings.

Volume, titratable acidity and ammonia of urine (Table 2).—There was a slight increase in urinary volume in two of the cases during the ammonium-chloride period. In Case 3 the amount of urine was only half of that of the control period and in Case 4 (early healing) it was reduced by about one-tenth. The titratable acidity of the urine during the ammonium-chloride period was in none of the cases as greatly increased as it is in the

TABLE 2.

SHOWING URINARY OUTPUT OF WATER, TITRATABLE ACID, AMMONIA AND CHLORIDE.

Case.	Period.	Volume c.cm.	T.A. c.cm. N/10.	Ammonia c.cm. N/10.	NaCl. grm.
1	1st. Normal. 6 days	1800	211·6	1224·4	5·780
	2nd. NH_4Cl. 6 days	1860	450·0	1723·2	12·028
2	1st. Normal. 7 days	5485	711·6	2362·0	12·5341
	2nd. NH_4Cl. 7 days	5510	1424·0	5496·2	39·0078
3	1st. Normal. 7 days	4290	404·8	1042·8	10·6307
	2nd. NH_4Cl. 7 days	2355	162·0	3591·0	18·8400
4 Healing rickets	1st. Normal. 7 days	4030	787·6	1584·8	11·5673
	2nd. NH_4Cl. 7 days	4849	1516·0	5515·0	41·7650

normal child: in Case 3 there was an actual decrease. There was a normal rise in the ammonia output in all except Case 1, where the increase was much less than normal.

Chlorine (Table 2).—The retention of chlorine in these four subjects shows a distinct variation from normal both in the control and ammonium-chloride periods. In normal children on an adequate milk diet the retention of chlorine varies from zero to about 12 per cent. of the intake. In all the four patients studied the chlorine retained amounted to over 12 per cent. of the intake. In Case 1 36·6 per cent. of the chlorine ingested over a six-day period was not excreted in the urine. Practically no chlorine was found in the fæces and as there was no noticeable perspiration, it is unlikely that the diminished urinary excretion was due to excess being passed out in the sweat. When the results of the ammonum-chloride periods are examined this tendency to retain chlorine becomes even more apparent. In normal

children over 80 per cent. of the extra chlorine ingested is found in the urine. In the present series the percentage excreted fell far below that figure, except in Case 4 in which the rickets was healing and in whom the percentage excreted amounted to 78·9 per cent. of the extra intake.

Calcium and phosphorus (Tables 3 and 4).—In all instances administration of ammonium chloride led to an increased urinary output of lime and phosphorus. The increase was very marked in the case of healing rickets particularly in the output of calcium which was increased fourfold: in the others the increase was slight. The fæcal output of both lime and

TABLE 3.

SHOWING INTAKE, FÆCAL AND URINARY OUTPUTS AND RETENTION OF CALCIUM AND PHOSPHORUS (IN GRAMMES). (7-DAY PERIODS).

Case.	Period.	Intake.		Output.				Retention.			
				Fæces.		Urine.		Total.		Daily per kgrm.	
		CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO mgrm.	P_2O_5 mgrm.
1	1. Normal	8·208	10·80	6·915	5·301	·067	3·696	1·226	1·803	30	44
	2. NH_4Cl	8·208	10·80	6·293	4·644	·084	3·740	1·831	2·416	45	59
2	1	15·12	21·168	13·837	11·408	·116	7·772	1·167	2·996	12	21
	2	15·12	21·168	9·743	8·419	·205	8·436	5·172	5·321	57	47
3	1	12·60	17·64	9·984	12·355	·092	2·320	2·524	3·805	37	44
	2	12·60	17·64	9·148	11·616	·146	2·754	3·306	4·110	50	57
4	1	12·60	16·38	7·076	4·946	·176	6·512	5·348	3·922	75	55
Healing	2	13·02	16·80	9·284	7·346	·713	7·676	3·023	1·778	41	17

phosphorus was increased in Case 4 (healing), as a result of rise in fæcal weight and percentage of minerals in the fæces. In the patients with active rickets the output of lime and phosphorus in the fæces was greatly reduced; in Case 1 the reduction was due both to a fall in fæcal weight and in percentage content, while in Cases 2 and 3 the main factor was the reduction in the percentage content of lime and phosphorus of the fæces. It is of interest to note that in the patient (Case 3) who had the signs of tetany most marked, the phosphorus content of the fæces was very high and the combined fatty acids very low in both periods. The most striking difference between the behaviour of the patient with active rickets and that of the normal child or one with healing rickets is the increased retention of calcium and phosphorus in the former during ingestion of ammonium chloride and the decrease in the later. This is in accord with the radiographic evidence of definite healing in Case 1 already mentioned.

TABLE 4.

SHOWING ASH, CALCIUM, PHOSPHORUS AND FAT CONTENT OF FÆCES (7-DAY PERIODS).

Case	Condition.		Period 1 (Normal). Total quantity in fæces grm.	Period 1 (Normal). % in fæces	Period 2 (NH_4Cl). Total quantity in fæces grm.	Period 2 (NH_4Cl). % in fæces.
1	Active rickets and tetany	Fæcal weight ...	46·1	—	43·40	—
		Ash	—	31·4	—	29·7
		CaO	6·915	15	6·293	14·5
		P_2O_5	5·301	11·5	4·644	10·7
		Total fat	19·929	43·23	18·857	43·45
		Combined fatty acids	15·314	33·22	12·551	28·92
		Free fatty acids ...	3·298	7·155	5·859	13·50
		Neutral fat	1·316	2·855	0·447	1·03
2	Active rickets and tetany	Fæcal weight ...	73·6	—	52·95	—
		Ash	—	40	--	40
		CaO	13·837	18·8	9·743	18·4
		P_2O_5	11·408	15·5	8·419	15·9
		Total fat	20·939	28·45	17·542	33·13
		Combined fatty acids	12·622	17·15	10·521	19·87
		Free fatty acids ...	5·961	8·1	5·004	9·45
		Neutral fat	2·355	3·2	2·017	3·81
3	Active rickets and tetany	Fæcal weight ...	62·4	—	72·6	—
		Ash	—	45	—	45
		CaO	9·984	16·0	9·148	12·6
		P_2O_5	12·355	19·8	11·616	16·0
		Total fat	17·515	28·07	18·411	25·36
		Combined fatty acids	1·622	2·60	1·365	1·88
		Free fatty acids ...	12·299	19·71	10·781	14·85
		Neutral fat	3·594	5·76	6·265	8·63
4	Healing rickets	Fæcal weight ...	36·1	—	45·07	—
		Ash	—	38·2	—	42·1
		CaO	7·076	19·6	9·284	20·6
		P_2O_5	4·946	13·7	7·346	16·3
		Total fat	6·743	18·68	7·310	16·22
		Combined fatty acids	4·498	12·46	4·439	9·85
		Free fatty acids ...	0·975	2·7	1·767	3·92
		Neutral fat	1·270	3·52	1·104	2·45

Discussion.

The following differences are to be noted in the reaction of the rachitic patient to ammonium chloride from that of the normal child (Table 5).

1. There is an increase in the retention of lime and phosphorus in active rickets. When the rickets is healing ammonium chloride leads to a decrease in the retention of minerals as in health.

2. A much smaller proportion of the extra chlorine ingested is excreted by the rachitic than by the normal individual.

3. The increase in the urinary excretion of titratable acid and ammonia is not so marked in active rickets as in health or healing rickets.

TABLE 5.

COMPARISON OF THE METABOLIC REACTIONS OF NORMAL AND TETANY-RICKETS GROUPS TO ACIDOSIS PRODUCED BY AMMONIUM CHLORIDE.

		Normal.	Healing rickets.	Tetany-rickets.
Urine	Increase in titratable acidity	+ +	+ +	+
	Increase in ammonia	+ +	+ + +	+
	% of excess chlorine excreted	+ + +	+ +	+
	Excretion of CaO	+	+ +	+
	Excretion of P_2O_5	+	+	+
Fæces	Excretion of CaO	+	+	—
	Excretion of P_2O_5	+	+	—
Retention ...	CaO	—	—	+
	P_2O_5	—	—	+

The increased retention of lime and phosphorus following ammonum-chloride administration in active rickets is in marked contrast to what occurs in health and healing rickets. The use of acid-producing salts therapeutically as a softener of bones is well-known. Ammonium chloride was chosen in this study as an acid-producing substance because of its neutrality in the gut. Nevertheless the possibility must be conceded of its being able to decrease the pH in the intestine secondarily by its effect on the composition of the body fluids. In this way absorption of the mineral elements would be facilitated. It may further be urged that the increased retention resulting from ammonium-chloride administration is due to the fact that the mineral-starved bones utilize so much of the extra lime and phosphorus absorbed that the slight increase in the amount excreted is completely overshadowed. This contention cannot be upheld in view of the results recorded in the case of healing rickets. The mineral retention was here reduced during the

ammonium-chloride period just as in the normal child in spite of the fact that, healing having just commenced, there was still osteo-porosis shown in the radiogram at the time of the study. To explain the effect of ammonium chloride in increasing the retention of lime and phosphorus in active rickets, it therefore seems necessary to assume that some change has been effected enabling the tissues of the patient with active rickets to fix calcium and phosphorus. If this assumption be granted the corollary must be drawn that in addition to a probable defect in the absorption of minerals from the intestine there also exists in or around the tissues a condition which prevents the fixation of lime and phosphorus. As soon as healing commences, although the bones are still very defective in mineral content, the state of the tissues is such that administration of ammonium chloride leads to increased mineral excretion just as occurs in the healthy child. The fact that an acid-producing salt like ammonium chloride can during the active stage of rickets lead to an increased fixation of lime and phosphorus makes it highly improbable that in active rickets acidosis is either a causal or an associated factor. In this connection two other findings in the present investigation are of importance.

1. Subnormal increase in urinary acidity. This might at first glance be attributed to the diminished output of urinary phosphorus providing a smaller amount of phosphate for conversion from the mono- to the di-hydrogen salt. This explanation, however, is not satisfactory since the increase in the renal excretion of phosphorus during ammonium chloride ingestion is high relative to the increase in titratable acid. It would appear, therefore, that in these cases the tendency is for acids to be retained.

2. Subnormal increase in the excretion of chlorine. Defective renal function might be suggested as the explanation of this finding. There was, however, in none of the cases any impairment of kidney function. It is true that the ammonium output did not rise to the extent found in healthy children after ammonium-chloride administration; but this is more readily explained on the assumption that acid being retained, there was obviously no need for as much extra alkali to be formed. Further, if in active rickets there is relative excess of fixed base that excess will be used in preference to ammonia for the transport of acid.

The fact that an acid radicle such as chlorine is retained to an extent far exceeding the normal is strong supporting evidence of the view that if there is any change in acid-base equilibrium in active rickets that change is towards the alkaline and not the acid side.

In connection with the retention of chlorine it is of interest to mention the findings of Morris, Watson and Morris[21] in experimental tetany induced by thyro-parathyroidectomy or injection of guanidine. During both the latent and active periods of these types of tetany there occurred a very marked retention of chlorine. Analysis of the tissues showed that the tissue content of chlorine was markedly increased. Further, there was noted a

relationship between the amount of chlorine retained, the quality and amount of protein breakdown, and the development of convulsions. When the protein katabolism was in great excess relative to the amount of available chlorine, active tetany ensued. This suggests an explanation of the effect of high-protein intake in the form of meat in the conversion of a latent to an active tetany. It will be seen, therefore, that our results in the three children with tetany and rickets corroborate these experimental findings.

The clinical findings show that ammonium chloride did not in any way aggravate the rachitic condition. As in normal individuals receiving similar dosage, there was no apparent symptom of acidosis. It is admitted that in three patients there was tetany, in which condition the beneficial action of acid-producing substances has long been known. Nevertheless, radiographic evidence of healing was marked in the one patient who was given ammonium chloride for a prolonged period. And in the other two subjects of tetany, although all signs disappeared within two days, continued administration of ammonium chloride did not lead to any untoward manifestation. If rickets were accompanied by an increased acid formation the accumulation of acid resulting from ammonium chloride should lead to an aggravation of the condition, or at least prevent healing. The clinical, radiographic and metabolic results all show exactly the opposite. It therefore seems justifiable to conclude that there is no tendency to acidosis in rickets.

Shohl[22] and his colleagues have recently published results showing that in rats made rachitic on a high-calcium low-phosphorus diet (Steenbock 2965) serum analyses indicate a state of the acid-base equilibrium bordering on alkalosis. Thus, although there is no conclusive evidence in the work detailed in this paper that a condition of alkalosis exists, it is tempting to draw this conclusion.

Summary.

The administration of ammonium chloride to three patients with active rickets and latent tetany, and to one patient with early healing rickets produced no apparent signs of acidosis.

In the tetany-rickets group there was a definite increase in the retention of calcium and phosphorus, a subnormal increase in the output of titratable acid and a marked retention of the excess chlorine. In healing rickets there was a definite decrease in the retention of calcium and phosphorus, but a retention of the excess chlorine slightly above normal.

One patient who was given ammonium chloride over a period of five weeks showed radiographic and metabolic evidence of healing, although the dietetic and environmental conditions were kept unchanged.

The findings are discussed and it is concluded that there is no evidence of any tendency to acidosis in rickets.

We desire to thank Dr. Leonard Findlay, at whose suggestion this research was undertaken, for his very helpful criticism during the course of the work. Thanks are also due to the Medical Research Council for defraying the expenses of the investigation and for a personal grant to one of us (N.M.).

TABLE 6.

PROGRESS OF HEALING IN CASE I AS INDICATED BY RADIOGRAMS.

(R.M., aet. 2 years, 1 month).

Date.	
8.5.29	Admitted to hospital.
9.5.29	X-ray of wrist: active rickets.
16.5.29	,, ,, I.S.Q.
23.5.29	,, ,, I.S.Q.
1.6.29	,, ,, I.S.Q.
6.6.29	,, ,, I.S.Q.
18.6.29	,, ,, I.S.Q.
19.6.29	Commencement of administration of NH_4Cl 1 grm. 5 times daily.
29.6.29	X-ray of wrist: I.S.Q.
30.6.29	Administration of NH_4Cl ceased.
10.7.29	X-ray of wrist: definite signs of early healing.
24.7.29	,, ,, ? further advance in healing process.
31.7.29	Administration of NH_4Cl 1 grm. five times daily again started.
12.8.29	X-ray of wrist: definite advance in healing process.
22.8.29	,, ,, further healing.
29.8.29	,, ,, very slow healing progressing.
8.9.29	Administration of NH_4Cl ceased.
9.9.29	X-ray of wrist: further healing.

REFERENCES.

1. Pritchard, E., *Brit. Med. J.*, London, 1923, i, 887.
2. György, P., *Ergebn. d. inn. Mediz. u. Kinderheilk.*, Berlin, 1929, XXXVI, 752.
3. Bosanyi, A., *Jahrb. f. Kinderheilk.*, Berlin, 1927, CXVII, 240.
4. Schabad, *Arch. f. Kinderheilk.*, Stuttgart, 1910, LIII, 380; 1910, LIV, 83.
5. Zucker, T. F., Johnson, W. C., & Barnet, M., *Proc. Soc. Exp. Biol. & Med.*, N.Y., 1922, XX, 20.
6. Hess, A. F., Weinstock, M., Rivkin, H., & Gross, J., *Ibid.*, 1929, XXVII, 140.
7. Samuel, E. L., & Kugelmass, I. N., *Amer. J. Dis. Child.*, Chicago, 1930, XXXIX, 687.
8. Hodgson, A., *Lancet*, London, 1921, ii, 945.
9. Burgess, N., & Osman, A. A., *Ibid.*, 1924, i, 281.
10. Freudenberg, E., & György, P., *Münch. med. Wochen.*, München, 1922, XII, 422.
11. McClendon, J. F., Bessell, F. S., Lowe, E. R., & Meyer, P. F., *J. Amer. Med. Assoc.*, Chicago, 1920, LXXV, 1638.
12. Lloyd, A., *Klin. Wochenschr.*, Berlin, 1927, i, 607.
13. Schloss, E., *Ergeb. d. inn. Mediz. u. Kinderheilk.*, Berlin, 1917, XV, 55.
14. Redman, T., *Biochem. J.*, Cambridge, 1929, XXIII, 256.

15. Zucker, T. F., & Matzner, M. J., *Proc. Soc. Exp. Biol. & Med.*, N.Y., 1923, XXI, 186.
16. Jephcott, H., & Bacharach, A., *Biochem. J.*, Cambridge, 1926, XX, 1350.
17. Grayzell, D. M., & Miller, E. G., *J. Biol. Chem.*, Baltimore, 1928, LXXVI, 423.
18. Tisdall, F., & Price, H. W., *Bull. Johns. Hopk. Hosp.*, Baltimore, 1927, XLI, 432.
19. Gamble, J. L., Ross, S. G., & Tisdall, F., *Amer. J. Dis. Child.*, Chicago, 1923, XXV, 455.
20. Morris, N., & MacRae, O., *Arch. Dis. Childh.*, London, 1930, V, 207.
21. Morris, S., Watson, A. M., & Morris, N., *Biochem. J.*, Cambridge, 1931, XXV, 786.
22. Shohl, A. T., Brown, H. B., Rose, C. S., Smith, D. N., & Cozad, F., *J. Biol. Chem.* Baltimore, 1931, XCII, 711.

METABOLIC REACTIONS TO ACIDOSIS PRODUCED BY AMMONIUM CHLORIDE

BY

NOAH MORRIS, M.D., B.Sc.,

and

OLIVE MACRAE, M.B., Ch.B. (Faulds Fellow in Medicine, University of Glasgow.)

(From the Department of Pædiatrics, Glasgow University, and the Biochemical Department, Royal Hospital for Sick Children, Glasgow.)

Acidosis is so frequently put forward as the underlying pathological condition in such a variety of disorders that it is important to appreciate what actually are the metabolic manifestations of the acidotic state. Clinically one seldom if ever has an opportunity of studying acidosis uncomplicated by some such other factor as inanition or toxæmia. It is therefore advisable in an investigation of the metabolic reactions to a disturbance in acid-base equilibrium to have these secondary factors as far as possible excluded. This can only be done when the acidosis is induced in a healthy individual with the minimal amount of upset especially as regards food-intake, and such a condition is most nearly attained in the acidosis produced by the ingestion of ammonium chloride. Haldane[1] was the first to show that ammonium chloride taken in large amounts led to a marked acidosis owing to its ammonium moiety being converted to urea. Since then many papers have been published dealing with changes following the administration of this substance.

The present research was undertaken primarily with the object of studying the changes in mineral metabolism during acidosis and, if possible, of correlating these changes with other metabolic phenomena. Most workers are agreed that there is an increased output of lime in the urine during acidosis. The effect on the fæcal excretion of lime and the influence of the acidotic state on phosphorus metabolism have not, however, been clearly determined. Steenbock, Nelson and Hart[2] have pointed out the detrimental effect of acid-forming diets on calcium retention and calcification in animals. Sawyer, Bauman and Stevens[3] found an increased urinary output of calcium and phosphorus in two children during a period of high fat intake : in only one, however, was there an increase in the fæcal amounts of these substances, while in the other there was a decrease. In a study of acid and base-forming diets in adult women Bogert and Kirkpatrick[4] did not obtain a constant change in the amount of fæcal calcium during the period of acid-forming diet although the urinary lime was always increased.

In infants Flood[5] found that administration of N/10 HCl led to no alteration in the retention of calcium, although this substance always appeared in slightly increased amount in the urine. An accurate knowledge of the changes occurring in mineral metabolism during acidosis would, for instance, be invaluable in throwing further light on the pathogenesis and chemical pathology of such a condition as rickets. Freudenberg and Gyorgy[6] claim, indeed, that the fundamental factor in the hindrance of calcification in this disease is an increased acidity of the tissue fluids which prevents the precipitation of calcium salts.

It has been shown by Haldane[1], and Gamble, Ross and Tisdall[7] that salts such as calcium and ammonium chloride produce their diuretic effect in virtue of their acid-producing powers. This relationship between water-loss and acidosis has frequently been commented upon in states of dehydration accompanying gastro-enteritis. The opportunity was therefore taken in this study to attempt a correlation between the various metabolic changes following on the production of an acidosis by ammonium chloride administration. Gamble, Blackfan and Hamilton[8], and Følling[9] have shown the close relationship between the extra loss of water by the kidney and the excess excretion of fixed base. In this research it is hoped to bring forward evidence as to the part played by calcium and phosphorus.

Present investigations.

The subjects of the study were four apparently normal children—N.G., female aged 11 years ; W.C., male, aged 9 years ; N.M., female aged 10 years ; and J.F., male, aged 9¼ years. Each had recovered from a mild attack of rheumatism. The diet throughout the period of the investigation was constant, consisting of cow's milk with sugar sufficient to satisfy the caloric requirements of the child. After at least three days on the arranged diet the urine and fæces were collected with the usual precautions for periods of seven or six days as stated. Thereafter 1 grm. of ammonium chloride was administered 5 times daily in capsule form. The excreta were again collected for a period of 7 days. In the case of N.M. and J.F. the ammonium chloride was continued so as to include a third period of 5 and 6 days respectively. In the case of N.M. 1 drm. of cod-liver oil was given thrice daily during this last period.

The individual metabolic changes will first of all be discussed separately. Thereafter an attempt will be made to correlate the various findings with special reference to the defence of the organism against acidosis.

Clinical features.—No apparent change was produced in the appearance of any of the children during or following the ingestion of the ammonium chloride. In two cases the administration continued for a period of eighteen days without any sign of circulatory disturbance. The daily intake varied from 0·166 to 0·247 grm. per kgrm. of body weight. Haldane produced in himself marked respiratory distress by taking one dose of 25 grm. of ammonium chloride, equivalent to 0·25 grm. per kgrm. of body weight. Koehler[10] found that administration of 10–15 grm. of ammonium chloride daily to well-developed adults produced definite symptoms of listlessness, thirst, diuresis and muscular

aches: these subjects, however, were all patients recovering or recovered from lead-poisoning. Three explanations may be offered for the difference between our results and those recorded elsewhere. First, children may not be as susceptible as adults to the action of ammonium chloride. It is well known that children tolerate a much larger dose per kilogramme of body weight than adults of such a drug as salvarsan. It seems strange, however, that this should be the case with an acid-producing substance when the peculiar susceptibility of the young to disturbances of acid-base equilibrium is remembered. Secondly, the difference in the diets of our subjects and those of the adults may be of importance since milk contains an excess of fixed base over mineral acid. It is possible that this excess base enabled our subjects to withstand the acidosis more effectively than would otherwise have been the case. Thirdly, the division of the daily dose into five portions may have allowed the compensating reactions of the body to come into play before there was any necessity for visible extra effort on the part of the respiratory or other system. It seems to us that the last is the most likely explanation; but whatever the cause may have been, the absence of clinical manifestations of acidosis in no way invalidates this study for, as we shall show later, the blood analyses were indicative of a disturbance of the acid-base equilibrium towards the acid side. Our principal object was to study the changes over a period of at least several days, and it would have been manifestly impossible to have accomplished this in the presence of respiratory distress or other evidence of acute acidosis.

Changes in chemical composition of the blood.—The changes in the chemical composition of the blood found during the ammonium chloride period are in close agreement with those reported by Haldane[1], and Gamble, Blackfan and Hamilton[8]. The changes in the individual constituents will be discussed in turn.

A. CARBON DIOXIDE. (See Table 1, column 1.) The total CO_2 of the blood was reduced in every case. Keith and Whelan[11] found that the plasma CO_2 dropped about the 4th or 5th day of the administration of ammonium chloride. In the last three subjects the CO_2 content was estimated two or more times during the ammonium chloride period. From these results it is evident that the reduction in the CO_2 content reached what was practically its maximum, comparatively early in the reaction to ammonium chloride. Continued administration of the acid-producing substance had but little further effect on the CO_2 content of the blood. This is probably due to the fact that the other regulating mechanisms came into play, and thus protected the CO_2 contents, and almost certainly the pH, from further reduction. Accordingly, if in any case the CO_2 content of the blood persistently falls from day to day, it would indicate that the other regulating reactions are unable to cope with the amount of acid produced.

B. CHLORINE. (See Table 1, column 2.) The chlorine was moderately increased. The increase in chlorine when calculated in milli-equivalents of bicarbonate (Table 2) did not compensate for the decrease in milli-equivalents of bicarbonate. Baird, Douglas, Haldane and Priestley[12] found that the carbonate ion of the plasma and tissues is partly replaced by the chlorine ion in

TABLE 1.

CHANGES IN CHEMICAL COMPOSITION OF BLOOD.

Name	Stage	(1) CO_2 Vol. %	(2) Cl′ mgrm. %	(3) Fixed base c.cm. N/10%	(4) N.P.N. mgrm. %	(5) Calcium mgrm. %	(6) Phosphorus mgrm. %
N.G.	Normal ...	55·1	300	158	36·5	—	—
	7 days NH_4Cl	38·7	350	154	46·1	—	—
W.C.	Normal ...	68·2	340	148	37	10·6	5·2
	4 days NH_4Cl	41·4	360	157	—	—	—
	8 „ „	40·3	340	157	44	10·1	6·5
J.F.	Normal ...	66·7	240	154	35·5	9·1	—
	9 „ „	49·1	360	159	48·5	9·1	—
	19 „ „	45·8	320	154	40·1	8·80	—
N.M.	Normal ...	60·6	285	147	35	9·25	4·2
	3 days NH_4Cl	45·1	290	—	—	—	—
	6 „ .	41·8	—	—	—	—	—
	9 „ „	43·4	280	132	46	9·8	4·1
	13 „ „	41·5	320	137	32	—	—

TABLE 2.

SHOWING COMPENSATORY DECREASE AND INCREASE OF CHLORINE AND BICARBONATE IONS RESPECTIVELY.

Name	Stage	HCO'_3 c.cm. N/10%	Cl′ c.cm. N/10%	HCO'_3+Cl' c.cm. N/10%	Change of HCO'_3 & Cl′ from normal	Change in fixed base
N.G.	Normal ...	24·6	84·5	109·1	—	—
	7 days NH_4Cl	17·3	98·6	115·9	+6·8	—4
W.C.	Normal ...	30·4	95·7	126·1	—	—
	4 days NH_4Cl	18·5	101·4	119·9	— 6·2	+9
	8 „ „	18·0	95·7	113·7	—12·4	+9
J.F.	Normal ...	29·8	70.0	99·8	—	—
	9 days NH_4Cl	21·9	101·4	123·3	+23·5	+5
	19 „ „	20·4	90·1	110·5	+10·7	0
N.M.	Normal ...	27·1	80·3	107·4	—	—
	3 days NH_4Cl	20·1	81·7	101·8	—5·6	—
	9 „ „	19·4	80·3	99·7	—7·7	—15
	13 „ „	18·5	90·1	108·6	+1·2	—10

conditions of acidosis. Gamble, Blackfan and Hamilton[8] also reported similar results with both NH_4Cl and $CaCl_2$. In our cases, however, this replacement was by no means exact even when allowance was made for the change in fixed base. In W.C. there was an actual increase in fixed base accompanied by a deficit in the sum of bicarbonate and chloride. In J.F., on the other hand, the increase in chloride over-compensated the loss in bicarbonate: in this case the control period was characterized by a very low chlorine content. An objection may be raised that these analyses were performed on whole blood and not on plasma, but it should be remembered that the cell walls are equally permeable to chlorine and carbonic acid. It would seem, therefore, that all the acid ions take part in mutual compensation. As will be indicated later, there is but little change in the inorganic phosphorus of the blood. Indeed, the possible limits of the amounts of this substance and sulphate render a change in either almost negligible as a compensatory factor. It is probable that the organic acids of the blood play an important part in balancing excess or deficit of the other acids, as has been suggested by Gamble and his co-workers in alkalosis resulting from experimental obstruction of the pylorus. We have no data on the change in base-combining power of the plasma proteins, but the findings of Keith and Whelan[11], and Følling[9] suggest that such change is trifling.

C. FIXED BASE. (See Table 1, column 3.) Gamble, Blackfan and Hamilton[8] state that there is a very slight reduction in the fixed base of the serum, and Følling reports a fall of 15·4 milli-equivalents per litre. In two of our cases there was a decrease in the fixed base content of the serum: in one of these (N.M.) the reduction amounted to 15 milli-equivalents per litre, resulting in a value outside the normal limits. In the other two cases there was an increase in fixed base but the raised values fell within normal limits.

D. CALCIUM AND PHOSPHORUS. (See Table 1, columns 5 and 6.) In 1924 Stewart and Haldane[13] noted a 10 per cent. rise in the serum calcium of a healthy adult, following the administration of 25 grm. NH_4Cl. Haldane, Wigglesworth and Woodrow[14] found no significant change in the inorganic phosphorus of the serum during acidosis but observed a slight fall as the acidosis was passing off. In the results recorded in Table 1, where the estimations were made after a variable period from the commencement of the acidosis, no constant change in either calcium or phosphorus was observed. In the other two subjects (Fig. 1 and 2) more frequent analyses were made. In both, the serum calcium showed an initial rise which persisted till the fifth day, thereafter falling below the normal level: on the cessation of ammonium chloride administration the calcium immediately rose somewhat above the 'control' level and then returned to normal. The serum phosphorus moved in the inverse direction to calcium.

E. NON-PROTEIN NITROGEN. (See Table 1, column 4.) In every case this was found to be increased. In the two cases in which it was determined twice during the administration of ammonium chloride, the value had fallen at the second estimation.

FIG. 1. CASE E. C. CHANGES IN CALCIUM AND PHOSPHORUS CONTENT OF SERUM.

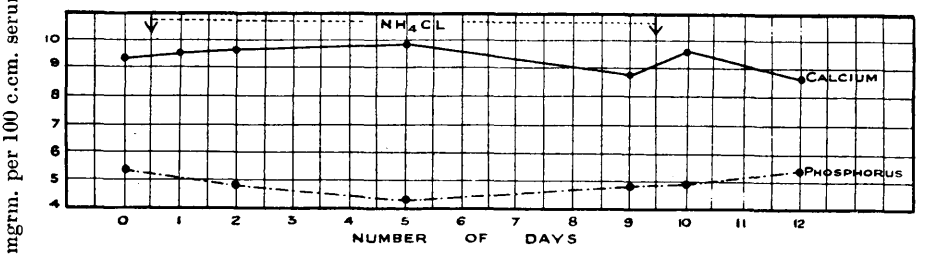
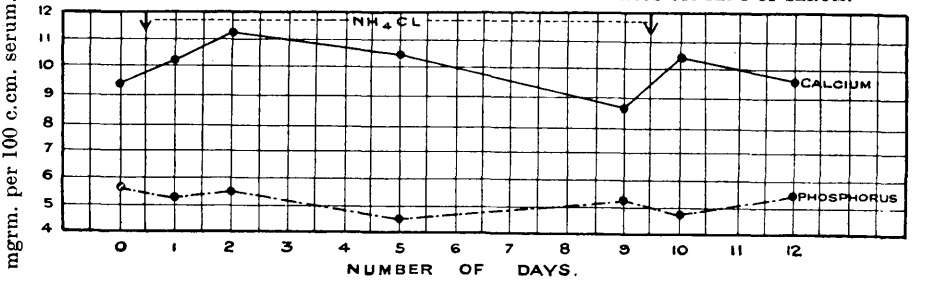


FIG. 2. CASE A. M. CHANGES IN CALCIUM AND PHOSPHORUS CONTENT OF SERUM.



Keith and Whelan[11] have shown that there is no increase in the ammonia content of the blood after ingestion of ammonium chloride. In our cases if the increase in non-protein nitrogen had been due to ammonia there would have been a rise in the ammonia value of from 8·5 to 15·8 mgrm. per cent.

TABLE 3.

BLOOD ANALYSES IN CASES OF CLINICAL ACIDOSIS.

Name	Condition	CO_2 Vol. %	N.P.N. Mgrm. %
J.H.	Acidosis; cyclical vomiting	36·5	60·0
	Recovered 2 days	48·8	40·0
H.P.	Gastro-enteritis	37·6	76·4
	Recovered	57·0	38·0
H.F.	Acidosis; ? gastro-enteritis	28·7	78·0
	Recovered	89·0	47·3
McP.	Acidosis; gastro-enteritis	26·8	52·1
E	Acidosis	36·1	109·1
E.F.	Gastro-enteritis	28·7	78·1
C.	Ileo-colitis	32·7	53·1
McG.	Gastro-enteritis	27·5	86·1

Such an amount of circulating ammonia would almost certainly have produced marked symptoms of poisoning, as ammonia is a very toxic substance. It is therefore highly improbable that the increase in non-protein nitrogen is due to ammonia nitrogen. A more reasonable explanation is that the rise is the result of increased breakdown of tissue proteins consequent on the disturbance of acid-base equilibrium. This view is supported by blood analyses in some cases of frank clinical acidosis (Table 3). It will be seen that in three cases during the stage of acidosis the non-protein nitrogen was increased, to fall to normal in the post-acidotic period. In cases of alkalosis where it also rises, the suggestion has been put forward that the cause is renal inefficiency. This can hardly have been the case in our subjects since, as will be seen later, there was a definite increase in the urinary output of ammonia, a good indication of unimpaired renal function.

Metabolism of calcium and phosphorus.—The intake and output of calcium and phosphorus in each case is detailed in Table 4 which also shows the retention and the partition of these substances between urine and fæces. In Table 5 are given the results of the fæcal analyses for calcium, phosphorus and fat.

It will be convenient to give a brief account of the results in each case, and thereafter to summarize and discuss the bearing of these findings on the general problem of mineral metabolism.

TABLE 4.

SHOWING INTAKE, FÆCAL AND URINARY OUTPUTS, AND RETENTION OF CALCIUM AND PHOSPHORUS (GRM.).

Name	Period		Total intake		Fæcal output		Urinary output		% of total output in urine		Total Retention		Retention per kgrm. per day	
	Intake	Days	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5
N.G.	Normal	7	20·16	27·72	9·508	6·818	1·406	11·55	12·8	62·8	9·246	9·352	·05	·05
	NH_4Cl	7	20·16	27·72	12·445	8·984	2·949	12·222	19·0	57·6	4·766	6·514	·025	·034
W.C.	Normal	7	20·16	27·72	14·983	14·390	1·521	9·464	9·2	39·7	3·66	3·87	·018	·019
	NH_4Cl	7	20·16	27·72	15·938	14·564	2·60	12·600	14·0	46·4	1·62	0·56	·008	·002
J.F.	Normal	6	13·44	18·48	8·621	7·494	0·683	7·686	7·3	50·6	4·136	3·30	·034	·027
	NH_4Cl	6	13·44	18·48	11·158	9·564	2·133	7·812	16·0	44·9	0·149	1·104	·001	·009
	NH_4Cl	6	13·44	18·48	8·823	7·728	2·099	7·392	19·0	48·9	2·518	3·36	·021	·028
N.M.	Normal	7	19·60	26·95	10·959	9·627	1·784	11·102	14·0	53·5	6·857	6·121	·033	·029
	NH_4Cl	7	19·60	26·95	19·649	17·445	3·192	11·928	13·7	40·7	—3·241	—2·759	—·015	—·013
	NH_4Cl & cod-liver oil	5	14·00	19·25	17·799	16·425	2·829	10·212	13·7	38·3	—6·608	—7·387	—·044	—·050

TABLE 5.

SHOWING ASH, CALCIUM, PHOSPHORUS AND FAT CONTENT OF FÆCES.

Name		Period 1 (normal)		Period 2 (NH_4Cl)	
		Total quantity in fæces grm.	% in fæces	Total quantity in fæces grm.	% in fæces
N.G.	Fæcal weight	62·55		66·55	
	Ash		31·8		36·2
	CaO	9·508	15·0	12·445	18·0
Weekly	P_2O_5	6·818	10·9	8·984	13·0
figures	Total fat	21·736	34·75	19·486	29·28
	Combined fatty acids	17·795	28·45	15·659	23·53
	Free fatty acids	1·189	1·9	1·637	2·46
	Neutral fat	2·752	4·4	2·189	3·29
W.C.	Fæcal weight	84·65		88·40	
	Ash		40·8		41·0
	CaO	14·983	15·0	15·938	18·0
Weekly	P_2O_5	14·390	14·0	14·564	14·0
figures	Total fat	29·527	33·7	24·699	27·94
	Combined fatty acids	17·437	20·6	14·003	15·84
	Free fatty acids	6·833	8·19	7·284	8·24
	Neutral fat	4·156	4·91	3·412	3·86

Name		Period 1 (normal)		2 (NH_4Cl)		3 (NH_4Cl)	
		Total quantity in fæces grms.	% in fæces	Total quantity in fæces grms.	% in fæces	Total quantity in fæces grms.	% in fæces
J.F.	Fæcal weight	56·35		79·70		60·85	
	Ash		33·2		31·5		32·8
	CaO	8·621	15·3	11·158	14·0	8·823	14·5
6-day	P_2O_5	7·494	13·3	9·564	12·0	7·728	12·7
figures	Total fat	20·70	36·79	33·386	41·89	20·00	32·92
	Combined fatty acids ...	14·01	24·95	18·18	22·81	12·64	20·81
	Free fatty acids	4·89	8·652	13·047	16·37	5·57	9·18
	Neutral fat	1·80	3·188	2·16	2·71	1·78	2·93
N.M.	Fæcal weight	8·65		13·69		18·05	
	Ash		40·8		44·7		43·4
	CaO	1·565	18·0	2·807	20·0	3·556	19·0
Daily	P_2O_5	1·375	16·0	2·492	18·0	3·285	18·0
figures	Total fat	2·85	33·03	3·936	28·75	6·58	36·4 5
	Combined fatty acids ...	2·204	25·48	2·909	21·25	5·008	27·75
	Free fatty acids	0·236	3·43	0·602	4·40	0·826	4·58
	Neutral fat	0·376	4·12	0·424	3·10	0·743	4·12

N.G. The retentions of CaO and P_2O_5 were reduced. The output of lime in the fæces was increased as the result of an increased percentage of CaO in the fæces and also a rise in the weight of the dried fæces. The urinary excretion of lime was also increased. The output of P_2O_5 by the urine was likewise raised, but the decreased retention of P_2O_5 was mainly the result of an increased fæcal content of this substance. As with lime the rise in fæcal phosphorus was consequent on an increased percentage output together with a rise in the fæcal weight. The percentage of ash was also increased, while the percentage and absolute amounts of total fat and combined fatty acids were decreased.

W.C. The results here were practically identical with those recorded above. One point of difference may be noticed, namely, the fact that there was but little increase in the fæcal output of P_2O_5, the lowering of the retention value being due to a fairly marked rise in the urinary content.

J.F. In this case there were two successive periods on NH_4Cl. The retentions of CaO and P_2O_5 were reduced during the first of the two periods but returned practically to normal in the second. There was a rise in the urinary CaO in both periods, and the urinary P_2O_5 in each was practically unchanged. The reduced retentions of lime and phosphorus were due entirely to the increased fæcal output resulting from a rise in the fæcal weight; the percentages of ash, calcium and phosphorus were all reduced. The percentage of total fat in the fæces was increased during the first period because of the marked increase in free fatty acids, while in the second period the value for total fat was slightly below that of the normal. The percentage of combined fatty acid was reduced during both periods.

N.M. With this subject there were two periods on NH_4Cl, the first for 7 days and the second for 5 days during which time one dram of cod-liver oil was given three times daily in addition. A negative balance of both lime and phosphorus was found during each NH_4Cl period, being much more marked on the second. The urinary output of lime and phosphorus was increased on both occasions, the increase in urinary phosphorus being much more marked in the second of the two periods. The percentages of lime phosphorus and ash in the fæces were increased, but the marked rise in the total fæcal output of CaO and P_2O_5 was chiefly the result of the striking increase in the weight of dried fæces. In the first period the percentages of total fat and combined fatty acids were reduced but in the second, during which cod-liver oil was also administered, these percentages were increased slightly above those outlined in the normal period.

SUMMARY.—There was in all cases a reduced retention of lime and phosphorus resulting from an increased output of these substances in both urine and fæces.

(a). Calcium.—Goto[15] found in acidosis an increased excretion of calcium by the urine but Keith and Whelan [11] observed but little change in the urinary excretion of calcium during administration of ammonium chloride. In our cases, however, the urinary calcium was at least doubled and in one instance trebled. The increased fæcal excretion of calcium was due to an increase in the total fæcal weight and, with the exception of J.F., an increase in the percentage of lime in the fæces. Generally the increase in fæcal calcium was much greater than that obtained in the urine. In W.C., however, the urinary and fæcal increases were approximately equal, and in J.F. (2nd period) the urinary increase exceeded that in the fæces.

(b). Phosphorus.—The extra output of phosphorus was even less consistently distributed between urine and fæces than was the extra lime. In only one case (W.C.) was the increase in urinary phosphorus marked. Apart from this case, in which the rise in fæcal phosphorus was insignificant, the main increase in excretion was by the fæces.

(c). Weight of dried fæces.—In all cases there occurred an increase.

(d). Ash.—With the exception of J.F. (1st period) there was always a rise in the percentage of ash in the dried fæces.

(e). Fæcal fat.—The percentage of total fat in the fæces was reduced in all cases except J.F. (1st period) and N.M. (2nd period). The percentage of combined fatty acids was reduced in all cases except N.M. (2nd period).

Discussion.—In spite of the large amount of work done in connection with calcium metabolism there is as yet no definite information as to the extent of absorption of this element, and it is still a matter for conjecture how much of the fæcal calcium has been absorbed and excreted through the bowel wall, and how much has passed through the gut unabsorbed. Grosser[16] found that subcutaneous injection of calcium salts led to an increased excretion by the bowel, and Salvesen[17] showed that in parathyroidectomized dogs, of calcium chloride injected intravenously, nine-tenths was excreted in the fæces and one-tenth in the urine. Percival and Stewart[18] isolated the large intestine in cats and found that the intravenous administration of calcium chloride was followed by a marked increase in the excretion of calcium by the large intestine, but no change in the urinary output. Recently Bauer, Allbright and Aub[19] have published the results of an investigation of the calcium metabolism on a very low calcium intake in 13 normal adults. On these 13 subjects there were 46 three-day periods of investigation. With the exception of a single period in one case, they found in all a negative balance of calcium, and with the exception of three periods there occurred in the fæces a greater amount of lime than had been ingested. In one case (N.M.) of our series during each of two periods on ammonium chloride there was a greater amount of calcium in the fæces than had been ingested. From these results it is justifiable to conclude that in ammonium-chloride acidosis excretion of calcium through the bowel wall can occur. Our results also contradict the statement of Givens and Mendel[20] that the increase in urinary calcium in acidosis is the result of diversion of lime from stools to urine. In our cases both the urinary and fæcal lime was increased in amount.

In one period at least (N.M., 2nd period) there was unequivocal evidence of calcium excretion by the bowel wall. Since, however, there was no alteration in the lumen of the gut other than the temporary presence of ammonium chloride which we shall show was practically completely absorbed, it seems reasonable to assume that absorption of lime was unaltered during the ammonium chloride period. The excess of fæcal calcium must therefore have been the result of excretion by the bowel wall. On similar grounds it would appear that the excess of fæcal phosphorus in the ammonium chloride period was the result, not of decreased absorption, but of increased excretion through the wall of the intestine.

The investigations of Nelson[21] on the mineral metabolism of patients suffering from diabetes and of epileptic subjects fed on ketogenic diets have shown that the kidneys are capable of excreting large amounts of lime. Indeed, more than half the total excretion of calcium may take place through the urinary system. In these cases there is also a slight increase in the fæcal output of lime. The presence of an acidosis, therefore, leads to an increased

excretion of lime both by urine and fæces. In our series, with the exception of N.M., the percentage of lime excreted by the urine is always increased during the ammonium chloride period. This would suggest that the amount of calcium excreted by the kidneys is to some extent dependent on the degree of acidosis. Additional support would at first glance seem to be lent to this view by the results of Shohl and Sato[22], and Bogert and Kirkpatrick[4]. The former found that the addition of sodium bicarbonate to the diet decreased the urinary output of CaO and P_2O_5, but increased their content in the fæces to such an extent that the retention of each was reduced. Bogert and Kirkpatrick record similar results with a base-forming diet. The addition of sodium bicarbonate to the diet brings in another factor, namely, a change in the reaction of the lumen of the gut. The decreased urinary output of calcium during administration of bicarbonate may be entirely due to the local effect of this salt in interfering with the absorption of lime by increasing the pH of the intestinal contents.

Freudenberg and György[6] have advanced the theory that the decreased retention of calcium in rickets is due to an acidosis of the tissues which interferes with the precipitation of calcium salts. If this view were correct one would expect the mode of excretion of lime and phosphorus to be similar to that found during ammonium chloride acidosis. Quite the reverse obtains, since the output of lime in the urine is markedly diminished, as is also the urinary phosphorus; and the very low retention of these minerals is caused entirely by the large fæcal content of CaO and P_2O_5. In other words, the partition of calcium and phosphorus in the excreta in rickets closely resembles that found in conditions tending to alkalosis rather than acidosis.

TABLE 6.

SHOWING INTAKE, OUTPUT AND RETENTION OF CHLORINE (C.CM. N/10 Cl).

Name	Period	Intake	Output		Retention	
			Urine	Fæces	Total	Per kgrm. per day
N.G.	Normal	4133	3585	36	+512	+2·8
	NH_4Cl	10675	9518	40	+1117	+6·1
W.C.	Normal	4133	3815	15	+303	+1·6
	NH_4Cl	10675	9853	17	+805	+4·3
J.F.	Normal	2755	2829	—	—74	—0·6
	NH_4Cl	8365	7299	—	+1066	+8·8
	NH_4Cl	8365	7656	—	+709	+5·8
N.M.	Normal	4018	3706	—	+312	+1·5
	NH_4Cl	10560	10040	—	+635	+3·0
	NH_4Cl ... ... (5 days)	7545	7678	—	—133	—0·9

Chlorine.—The excretion of chloride normally takes place through the urine and the sweat. The fæcal output is practically negligible. It was estimated in two of our cases, and as will be seen from the results in Table 6 the fæcal excretion of chloride was relatively minute both during the control and ammonium chloride periods.

During the control period there was a small retention of chlorine except in the case of J.F. where there was a very slight negative balance. While ammonium chloride was being administered the retention was increased in every case except during the second period of N.M., which was characterized by a slight negative balance.

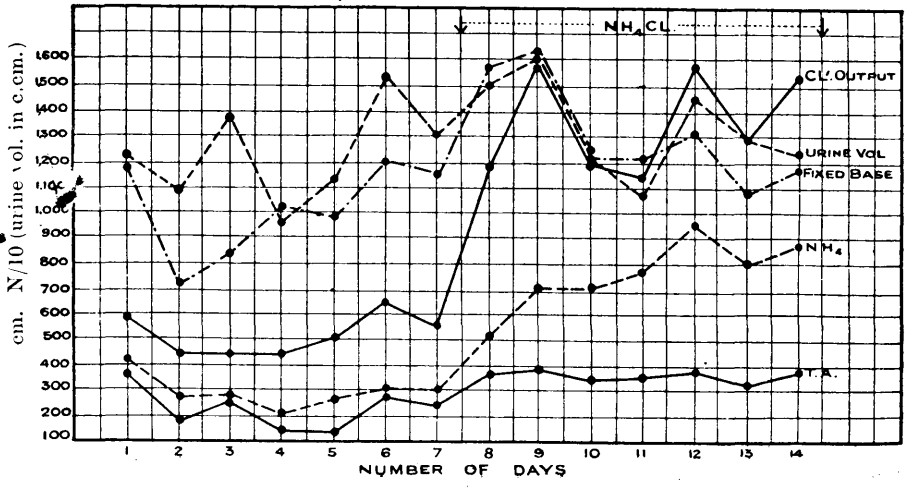
TABLE 7.

PERCENTAGE EXCRETION OF CHLORINE IN FIRST 24 HOURS FOLLOWING INGESTION OF SODIUM CHLORIDE.

Amt. of NaCl given	Form in which NaCl given	Diet	% Excretion of extra salt during 1st 24 hr.
4·5	Saline	Salt poor	53
4·5	Saline	Salt rich	62
10·0	Solid in capsule	Salt poor	50
10·0	Solid in capsule	Salt rich	67

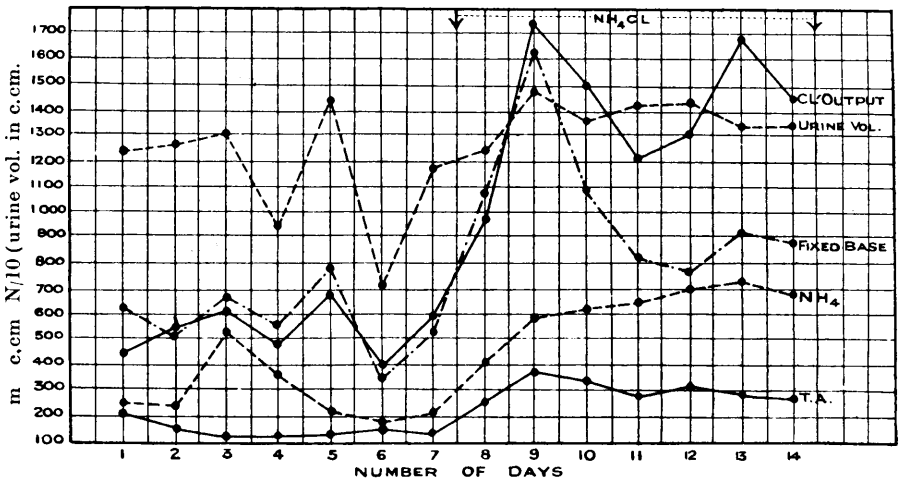
When one comes to examine the daily figures it is plain that all the subjects reacted immediately to the extra chlorine by the excretion of a greatly increased amount of this substance in the urine. In N.G. the output was doubled on the first day, so that only about 30 per cent. of the extra chlorine had been retained. This corresponds to what happens when sodium chloride is given. Table 7 indicates the percentage excretion of chlorine in the first 24 hours following ingestion of sodium chloride: the subject was a healthy boy aged 11 years.

FIG. 3. CASE N. G. GRAPH SHOWING DAILY URINARY OUTPUT OF WATER, CHLORINE, FIXED BASE, AMMONIA AND TITRATABLE ACID



With the exception of N.M. the daily curve of NaCl output (Fig. 3—6) during administration of ammonium chloride shows that the peak of chloride excretion occurred on the second or third day. This was followed by a drop lasting over two or three days, which was succeeded on the fifth or sixth day by a peak reaching almost to the level of the first. In the case of N.M. there was a complete absence of the first peak but the second was quite marked. It will be noted in every case that this second peak corresponded with the maximum rise in the output of ammonia. It is therefore fair to conclude that this secondary rise in the excretion of chlorine was due to the increased ability of the kidney to supply ammonia. Indeed, a glance at the chlorine and ammonia curves following this second peak shows in every case a fairly marked parallelism indicating a correlation between these two substances. This parallelism is not noticeable during the first five days of ammonium chloride administration. There is also a fairly marked correlation between the amount of urinary chloride and fixed base both during the control and the ammonium chloride periods, with the exception of the control period of N.G. The urinary volume and chlorides also show some parallelism especially during the ammonium chloride periods.

FIG. 4. CASE W. C. GRAPH SHOWING DAILY URINARY OUTPUT OF WATER, CHLORINE, FIXED BASE, AMMONIA AND TITRATABLE ACID.



Urinary volume.—Gamble, Ross and Tisdall[7] reported an increase of urinary volume in children during the administration of either calcium or ammonium chloride. Keith and Whelan[11], however, found no change in the volume of urine excreted by a normal individual during ingestion of ammonium chloride. During such administration there was in all our cases except the first period of N.M. an increase in urinary volume, not, however, as marked as might have been expected. The daily output of urine varied greatly, frequently falling much below the maximum observed in the control period.

Ammonia and titratable acidity of the urine.—The output of ammonia and titratable acid was increased in every case during the administration of ammonium chloride. The maximum output of titratable acid was reached

by the second day, following which there was usually a very gradual decline in the output. The ammonia content of the urine did not attain its greatest value till the 5th or 6th day, and in the case of J.F. the 9th day. Thereafter the output of ammonia remained at a constant level, except in the case of N.M. where considerable variations were observed from day to day. The ammonia output was not estimated in the days following the ammonium chloride period, but Gamble and others have shown that the output remains definitely above normal for some days following the administration of an acid salt.

TABLE 8.

SHOWING INTAKE, URINARY AND FÆCAL OUTPUTS AND RETENTION OF FIXED BASE (C.CM. N/10 MONOVALENT BASE).

Name	Period	Intake	Output			Retention	
			Urine	Fæces	Total	Total	Per kgrm. per day
N.G.	Normal ...	16254	7172	4561	11733	4521	24·5
	NH_4Cl ...	16254	9105	5397	14502	1752	9·4
W.C.	Normal ...	16254	5442	6579	12021	4233	22·4
	NH_4Cl ...	16254	8434	6695	15129	1125	6·0
J.F.	Normal ...	10836	4494	4135	8629	2207	18·1
	NH_4Cl ...	10836	5465	5036	9501	1335	11·0
	NH_4Cl ...	10836	5841	4069	9910	926	7·5
N.M.	Normal ...	15803	5726	5563	11289	4514	21·5
	NH_4Cl ...	15803	8229	8039	16268	—465	— 2·2
	NH_4Cl ...	11290	6616	7617	14233	—2943	—19·6

Fixed base.—The output of fixed base (Table 8) was with the exception of the control period of W.C., and the second ammonium chloride period of N.M., somewhat greater by the urine than by the fæces. The fæcal output of base was chiefly composed of calcium which constituted from 70 to 90 per cent. in the control periods and 77 to 99 per cent. in the ammonium chloride periods (Table 9). Thus, not only did ammonium chloride increase the output of fixed base by the fæces, but it also raised the proportion of calcium to other base. In the urine the calcium formed 5·4 to 11·1 per cent. of the fixed base in the control periods and 11 to 15 per cent. in the test periods. The urine, therefore, showed during administration of ammonium chloride an increase in total fixed base, and a slight rise in the relative proportion of calcium to other base. The urinary fixed base reached its maximum within three days of the commencement of administration, thereafter falling to slightly above the average level of the control period. This is in agreement with the findings of Gamble, Blackfan and Hamilton[8] with several acid-producing salts. The retention of fixed base varied from 18·1 to 24·5 c.cm. N/10 per kgrm. of body

TABLE 9.

SHOWING RELATIONSHIP OF OUTPUTS OF CALCIUM AND TOTAL FIXED BASE.

Name	Period	% of calcium to output of total fixed base	
		Urine	Fæces
N.G.	Normal	7·0	74·3
	NH_4Cl	11·5	82·4
W.C.	Normal	10·0	89·8
	NH_4Cl	11·0	99·0
J.F.	Normal	5·4	74·1
	NH_4Cl	14·0	79·0
	NH_4Cl	12·8	77·5
N.M.	Normal	11·1	70·1
	NH_4Cl	13·8	87·6
	NH_4Cl	15·0	83·3

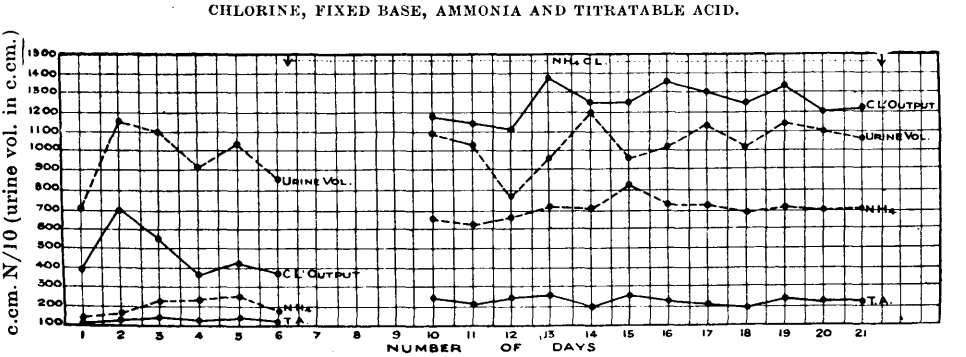
weight per day during the control periods. It was always diminished during ammonium chloride administration, and was negative during both the periods of N.M., the loss being entirely accounted for by calcium.

Metabolic reactions to acidosis.

Against the production of a non-gaseous acidosis such as is produced by ammonium chloride the organism has the following general defences :—(1) an increase in the available base of the blood ; (2) an increased excretion of volatile acid by the lungs ; and (3) an increased supply of base for neutralizing acids that are to be excreted.

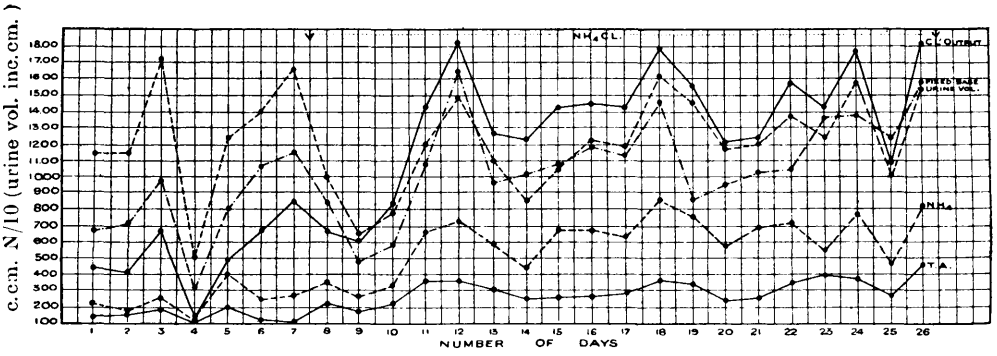
(1) **Increase in available base of the blood.**—The fixed base is maintained at a fairly constant level. By a reduction in the CO_2 content of the

FIG. 5. CASE J. F. GRAPH SHOWING DAILY URINARY OUTPUT OF WATER CHLORINE, FIXED BASE, AMMONIA AND TITRATABLE ACID.



blood a certain amount of base is freed and rendered available for the neutralization of other acid radicles. It has been shown that the base-combining powers of protein and inorganic phosphorus are reduced with a fall in the pH of the blood, but the amount of base released by these changes in such a condition as prevails in the experiments detai ed here is practically negligible. The fall in blood-CO_2, therefore, undoubtedly constitutes the chief immediate response to acidosis of the non-gaseous variety. In the subjects of this study the increase in available base produced by the reduction in CO_2 could not have amounted to more than 230 c.cm. N/10 (on the assumption that the blood-volume was one-thirteenth of the body weight). One gramme of ammonium chloride (i.e., one-fifth of the daily intake) contains 187 c.cm. N/10 acid. Two such doses are therefore much more than sufficient to use up all the base made available by the reduction in CO_2. The relief afforded by this means is only temporary, since prolonged administration of ammonium chloride does not to any extent further reduce the CO_2 content of the blood. The state of affairs produced in our subjects corresponds according to the nomenclature of Van Slyke to the condition of compensated acidosis (i.e., a fall in bicarbonate without symptoms of acidosis or change in the pH of the blood.) Although the pH was not actually estimated it is safe to assume that the absence of

FIG. 6. CASE N.M. GRAPH SHOWING DAILY OUTPUT OF WATER CHLORINE, FIXED BASE, AMMONIA, AND TITRATABLE ACID.



noticeable respiratory changes indicated an absence of any change in the pH of the blood that would be detected by physico-chemical means.

In clinical acidosis figures for total CO_2 have been noted much lower than the lowest in the present series. Such low figures supply definite evidence of an inability of the other compensatory mechanisms to deal with the situation either because of the suddenness of the demands (as in Haldane's case with an avalanche of 25 grm. of ammonium chloride), or because of the functional inefficiency, relative or absolute, of the other defensive reactions, as in diabetic or uræmic coma.

(2) **Increased excretion of volatile acids by the lungs.**—Respiratory changes leading to an increased output of CO_2 must naturally follow the displacement of CO_2 from its union with base. Otherwise the tension of CO_2 in the blood would increase and lead to the production of a CO_2 acidosis

(gaseous). In our cases, as would be expected from the results of the CO_2 analyses of the blood, there were no marked respiratory alterations. The excretion of the extra CO_2 must have been of such relatively small amount that no apparent strain was put on the respiratory system.

(3) **Supply of base for the excretion of acid.**—The kidney is undoubtedly the principal organ for the excretion of the non-volatile acid radicles. The sweat glands may play an important part in the metabolism of chlorine when large amounts of sweat are produced, although Schwenkenbecker and Spitta[24] conclude that not more than one gramme of sodium chloride is excreted daily even during profuse sweating. In the absence of hyperidrosis, at any rate, it is justifiable to assume that the amount of electrolyte lost in this way is practically negligible. The bowel certainly plays a part in mineral metabolism, but as far as the actual excretion of chlorine is concerned the intestinal output is negligible. Accordingly we may conclude that the extra acid supplied in these experiments must have been excreted by the kidneys.

At the lowest possible value of the urinary pH chlorine cannot be excreted as a free acid, requiring therefore a full equivalence of base. This base can be obtained in three ways. (a) Base may be released from weak acids which can be excreted either free or with only a partial complement of base. (b) Extra ammonia may be formed. (c) Fixed base may be supplied from the tissues and tissue-fluids.

(A) RELEASE OF BASE FROM WEAK ACIDS.—This is, of course, an accompaniment, if not the result, of increased acidity of the urine, which decreases the base-combining powers of the weaker acids. Change of phosphate from the mono-hydrogen to the di-hydrogen variety forms the best example of the saving of base effected in this way.

If we assume that during the control period the pH of the urine was 6·81, and during the NH_4Cl period 5·91, the amount of base saved by change of phosphate from Na_2HPO_4 to NaH_2PO_4 may be calculated as follows :—

at pH 6·81—50 per cent. of phosphorus is in the form of NaH_2PO_4

„ „ 5·91 90 „ „ „ „ „

Of 1000 mgrm. phosphorus.

at pH 6·81 500 mgrm. are present as NaH_2PO_4

„ „ 5·91 900 „ „ „ „

In changing, therefore, from pH 6·81 to pH 5·91, 400 mgrm. are converted from the mono-to the di-hydrogen variety. Since one H-ion is involved in the change of each phosphate molecule it would require 1 litre of normal acid to change 1 litre of normal phosphorus (i.e., 31 grm. phosphorus) from the mono-to the di-hydrogen phosphate.

To change 400 mgrm. P. would require $\frac{0{\cdot}400 \times 1000}{31}$ c.cm. N/1 acid, (i.e., 13 c.cm. N/10 acid).

By change of urinary pH from 6·81 to 5·91 there will be a saving of 130 c.cm. N/10 base per every gramme of phosphorus excreted.

This saving is indicated by the increase in titratable acidity. The response of the urinary system in this direction reaches its maximum within a very short time of the commencement of ammonium chloride administration. The base so released amounts, however, to only a small part of that likely to be required in any but the very mildest forms of increased acid excretion, and it certainly would be hopelessly inadequate to meet the requirements of even the smallest degree of acidosis that could be recognised clinically.

(B) INCREASE IN AMMONIA FORMATION.—The work of Benedict and Nash[25] has shown that ammonia is formed in the kidney. In cases of marked renal inefficiency the ammonia output is low: this must play an important part in the production of renal acidosis. In the subject with normal renal function the supply of ammonia forms a most important bulwark against acidosis. The increase in ammonia formation takes some time to reach its maximum. Some mechanism is therefore required to tide over the needs of the excretory system for more base until the supply of ammonia is sufficient to meet the demands. This mechanism will be discussed in the next section, but before leaving the question of increased ammonia production it is well to remember that the increase in ammonia output is continued after the need for increased acid excretion has ceased. As Gamble and his co-workers have pointed out this continued formation of ammonia is of vital importance in restoring the depleted stores of body base to normal.

(C) SUPPLY OF FIXED BASE FROM TISSUES AND TISSUE-FLUIDS.—This method provides the chief immediate means whereby excess anions are excreted. The base may be derived from the bones or from the other tissues in which latter case it must be accompanied by fluid in order to prevent disturbances of osmotic equilibrium.

In the bones calcium is found as phosphate with a small amount of carbonate. Accordingly the release of calcium entails the freeing of phosphorus which must also be excreted. In this transaction, however, there is a distinct saving of base. In bone two equivalents of phosphorus neutralize three equivalents of calcium (i.e., six equivalents of monovalent base). As excreted, however, the phosphorus in the urine is monovalent while in the fæces it has probably about the same valency as in the plasma, namely, 1·8. Accordingly for every equivalent of bone phosphorus excreted in the urine we have the saving of two equivalents of base, while for every equivalent in the fæces the saving effected is 1·2 (=3—1·8).

If it is assumed that the excess of calcium excreted is derived in proportionately equal amounts from the carbonate and phosphate of the bone, then one-fifth of this excess comes from the carbonate. Therefore the amount of base rendered available by the release of calcium carbonate may be calculated as the equivalents of monovalent base contained in one-fifth of the total excess calcium found in the excreta. The amount of base obtained from the phosphate may be calculated from the excess phosphorus as follows:—

(Excess urinary P. in c.cm. N/10 × 2) + (Excess fæcal P. in c.cm. N/10 × 1·2).

It may be objected that not all of the extra calcium and phosphorus come from bone. As the calcium content of the non-osseous tissues is relatively minute, bone must form the chief source of calcium. Phosphorus, however, plays an important part quantitatively in practically all metabolic processes. In three of our cases there was more extra phosphorus than extra calcium found in the excreta, thus showing that bone is not the source of all the extra phosphorus. The amount of extra phosphorus excreted from the extra-osseous source must form, however, only a small fraction of the total, and can only modify slightly the saving of base as calculated from the above formula.

The fixed base from the non-osseous tissues consists almost entirely of sodium and potassium, the former derived chiefly from extra-cellular, the latter being mainly an intra-cellular constituent. In either case, however, the base is associated with an equivalent amount of acid. If this base is excreted, some means must be found for dealing with its anions. Of these CO_2 is excreted by the lungs, while protein which holds about ten equivalents of base is probably katabolized. With the exception possibly of some of the organic acid, the remaining anions demand their full quota of base for neutralizing purposes. The water carrying the base must also be got rid of in order to prevent an upset of osmotic equilibrium. Accordingly for every 150 c.cm. N/10 base there will be rendered available only about 40 c.cm. N/10 for neutralizing extra acid. This 40 c.cm. is made up of 24 c.cm. from $B.HCO_3$ and the remainder from B. protein and organic salts. The efficiency in supplying base is thus only 26 per cent., even when blood plasma is the fluid called upon, and must be less in the case of the tissue-juices where the protein content is lower. Accompanying this 150 c.cm. N/10 base will be 100 c.cm. water which will contain its normal quota of fixed acid (Cl',SO''_4). The amount of tissue fluid excreted during the ammonium chloride period is indicated by the increase in urinary volume. The chlorine content of this excess in urinary volume should, if the hypothesis put forward be correct, approximate to that of the plasma.

TABLE 10.

SHOWING THE MEANS EMPLOYED IN NEUTRALISING THE EXCESS ACID.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Name	Incr. in Cl'	Incr. in T.A.	Incr. in NH_4	Incr. base derived from			Tissue fluid base	Incr. in urinary volume	Calculated c.cm. N/10 B.Cl per 100 c.cm. excess H_2O
				$CaCO_3$	Fæcal P'	Urinary P'			
N.G.	5933	917	3298	320	370	192	837	850	98·4
W.C.	6038	1172	2452	145	30	896	1343	1475	91·1
J.F.	4470	527	3051	285	355	36	216	231	93·5
	4827	503	3670	116	40	nil	498	526	94·7
N.M.	6334	1150	2768	721	1340	236	121*	nil	—

*This amount of fixed base was probably supplied without the accompaniment of fluid by the reduction of the fixed base content of the blood by 15 c.cm. N/10 per 100 c.cm.

In Table 10 are given the figures indicating the methods whereby the excess of excreted acid (chlorine) has been neutralized. Column 1 gives the values for the excess excretion of chlorine. Columns 2 to 7 indicate the amount of base derived from the sources indicated for the neutralization of this excess chlorine. The figures in columns 2 to 6 are calculated from the

results of the analyses, but those in column 7 have been obtained as follows :— Cl—(T.A. $+NH_4$ +Base derived from bone). Our results do not contain values for sulphates and organic acids which, as has been shown by Gamble, are also excreted in excess during acidosis. These acid radicles probably come from tissue juices and carry down with them their quota of base from the tissue-juices. Thus by calculating the figures in column 7 in the manner stated, we obviate any error due to the presence of excess sulphates. The base figures given in column 7 indicate the amount of tissue-fluid base combined with chlorine, in other words the amounts of B.Cl. Accordingly if these figures are divided by the corresponding increase in urinary volume (column 8) the results obtained should give the value for the percentage of B.Cl. in the tissue fluid (column 9). It is evident that these values lie between 90 and 100, therefore within the normal limits of chlorine in plasma and presumably in tissue juice. Despite the fact that the method of calculation is and must be one of comparatively rough averages, we feel that the consistency of the values so obtained is sufficiently striking to afford strong support to the thesis which has been advanced.

Several further points may be noted. If the view proposed is correct, one would have expected that the bulk of the excess phosphorus would have been excreted in the urine, since output by the kidneys effects a saving of two equivalents of base per equivalent of phosphorus, whereas for fæcal excretion the economy achieved is only 1·2. The fact that the bulk of the excess phosphorus is not excreted in the urine is probably related to the fact that there is a close association between the calcium and phosphorus in the fæces.

Administration of hydrochloric acid has been shown to increase the urinary output of phosphorus. This has been attributed to a change of reaction in the intestinal lumen producing a better absorption of phosphorus. In the light of our results, which also show an increased fæcal excretion, it would seem reasonable to refer both the increased urinary and fæcal excretion to the necessity for providing more base in the manner indicated.

Another point of interest is the different extent to which the various mechanisms are brought into play in reacting to excess acid. For the immediate supply of base both bone and tissue-juices are called upon. The one exception to this is the ammonium chloride period of N.M., where practically all the necessary fixed base was derived from bone ; less urine was passed than in the control period indicating a lack of response on the part of the tissue fluids. As to the rapidity with which the osseous tissue responds to the stimulus of an acidosis we have no data. H. L. White[26] obtained an increased urinary output of phosphorus within four hours of the administration of acid, but considered this merely a temporary phenomenon. Haldane, Hill and Luck[27], however, found a definite increase in the urinary output of phosphorus for 24 hours following the ingestion of large amounts of $CaCl_2$. On the other hand Gamble, Blackfan and Hamilton[8] observed no significant change in phosphorus excretion after the intake of moderately large amounts of acid.

Summary.

1. The effect of prolonged administration of ammonium chloride on the metabolism was studied in four apparently normal children, in none of whom was produced any clinical manifestation of acidosis.

2. Chemical changes in blood. (a). The CO_2 was reduced ; the reduction almost always reached its maximum early in the administration of ammonium chloride.

(b). The chlorine was moderately increased. The increase in chlorine did not exactly balance the deficiency in CO_2.

(c). The fixed base remained within normal limits except in one case where it was reduced by 15 milli-equivalents.

(d). The calcium was slightly increased and the phosphorus slightly diminished.

(e). The non-protein nitrogen was slightly increased but within normal limits.

3. Metabolism. (a). There was an increased output of calcium both by urine and fæces and consequently a decreased retention. Evidence is adduced in favour of the excretion of calcium through the bowel wall.

(b). There was an increased excretion of phosphorus by urine and fæces and a diminished retention.

(c). There was a slightly increased retention of chlorine. There were usually two peaks in the excretion of chloride : they occurred about the second and sixth days. The second peak usually coincided with the maximum output of ammonia.

(d). There was an increased excretion of fixed base both by urine and fæces. The extra fæcal base consisted chiefly of calcium.

4. The metabolic reactions to acidosis are discussed, and it is suggested that the increase in the output of calcium and phosphorus is the result of the response of the osseous tissues and forms a reaction of prime importance in the defence of the organism to acidosis. Evidence is brought forward from the result of the study in support of this thesis.

We desire to express our thanks to the Medical Research Council for assistance in this work, and for a personal grant to one of us (N.M.).

Analytical methods used :—Total CO_2—Haldane : Chlorine—Whitehorn : Non-protein nitrogen—Folin and Wu : Fixed base—Stadie and Ross : Calcium—Kramer and Tisdall : Phosphorus—Tisdall : Titratable acidity of urine—using phenolphthalein as indicator.

REFERENCES.

1. Haldane, J. B. S., *J. Physiol.*, Lond., 1921, LV, 265.
2. Steenbock, H., Nelson, M. V., & Hart, E. B., *J. Biol. Chem.*, Baltimore, 1914, XIX, 399.
3. Sawyer, M., Baumann, L., & Stevens, F., *Ibid.*, 1918, XXXIII, 103.
4. Bogert, L. J., & Kirkpatrick, E. E., *Ibid.*, 1922, LIV, 375.
5. Flood, R. S., *Amer. J. Dis. Child.*, Chicago, 1926, XXXII, 550.
6. Freudenberg, E., & György, P., *Münch. med. Woch.*, München, 1922, XII, 422.
7. Gamble, J. L., Ross, S. G., & Tisdall, F., *Am. J. Dis Child.*, Chicago, 1923, XXV, 455.
8. Gamble, J. L., Blackfan, K. D., & Hamilton, B., *J. Clin. Investig.*, Baltimore, 1925, I, 329.

9. Følling, A., *Acta. Med. Scand.*, Stockholm, 1929, LXXI, 221.
10. Koehler, A. E., *J. Biol. Chem.*, Baltimore, 1927, LXXII, 99.
11. Keith, N. M., & Whelan, M., *J. Clin. Investig.*, Baltimore, 1926-7, III, 149.
12. Baird, M. M., Douglas, C. G., Haldane, J. B. S., & Priestley, J. G., *Proc. Physiol. Soc.* p. xli, *J. Physiol.*, Lond., 1923, LVII.
13. Stewart, C. P., & Haldane, J. B. S., *Biochem. J.*, Lond., 1924, XVIII, 856.
14. Haldane, J. B. S., Wigglesworth, V. B., & Woodrow, C. E., *Proc. Roy. Soc. B.*, Lond., 1924, XCVI, 1.
15. Goto, K., *J. Biol. Chem.*, Baltimore, 1918, XXXVI, 355.
16. Grosser, P., *Ztschr. f. Kinderh.*, Berlin, 1920, XXV, 141.
17. Salvesen, H. A., *J. Biol. Chem.*, Baltimore, 1923, LVI, 443.
18. Percival, G. H., & Stewart, C. P., *Biochem. J.*, Lond., 1927, XXI, 301.
19. Bauer, W., Albright, F., & Aub, J. C., *J. Clin. Investig.*, Baltimore, 1929, VII, 75.
20. Givens, M. H., & Mendel, L. B., *J. Biol. Chem.*, Baltimore, 1917, XXXI, 421.
21. Nelson, M. V., *Am. J. Dis. Child.*, Chicago, 1928, XXXVI, 716.
22. Shohl, A. T., & Sato, A., *J. Biol. Chem.*, Baltimore, 1923, LVIII, 235.
23. Telfer, S. V., *Quart. J. Med.*, Oxford, 1922, XVI, 63.
24. Schwenckenbecker & Spitta., *Arch. f. Exp. Path. u. Pharm.*, Leipzig, 1907, LVI, 284.
25. Benedict, S. R., & Nash, T. P., *J. Biol. Chem.*, Baltimore, 1926, LXIX, 381.
26. White, H. L., *Amer. J. Physiol.*, Baltimore, 1927, LXXX, 82.
27. Haldane, J. B. S., Hill, R., & Luck, J. M., *J. Physiol.*, Lond., 1923, LVII, 301.

[From THE BIOCHEMICAL JOURNAL, Vol. XXV, No. 3, pp. 786–798, 1931]

LXXXIX. METABOLISM STUDIES IN TETANY.

By SAMUEL MORRIS, ALEXANDER McLEOD WATSON and NOAH MORRIS.

From the Institute of Physiology, University of Glasgow, The Biochemical Laboratory of the University Department of Paediatrics, Royal Hospital for Sick Children, Glasgow, and The Hannah Dairy Research Institute, Ayr.

(*Received April 17th, 1931.*)

A DISTURBANCE in chlorine metabolism has long been associated as an etiological factor in the production of certain forms of tetany. MacCallum and his co-workers [1920] attributed the onset of tetany in cases of pyloric obstruction to the loss of the gastric hydrochloric acid by the vomitus with the consequent production of an alkalosis. Grant [1922] found in one of six cases of adult tetany a marked reduction in the blood-chlorine. Iversen and Hansborg [1922] showed that after thyroidectomy sodium chloride was retained in the tissues to an abnormal extent although the amount of chlorine in the blood was reduced. Parhon, Ballif and Derevici [1926] also reported a fall in the serum-chlorine. Rockemann [1923] found that tetany following injection of sodium dihydrogen phosphate was accompanied by decreased excretion of urinary chloride.

The present investigation was undertaken primarily to study the changes in chlorine metabolism during conditions of tetany not associated with vomiting. Much work, however, has already been done indicating that in many forms of tetany there is a profound disturbance in calcium metabolism. Numerous experimental studies by Berkeley and Beebe [1909], Cooke [1911] and others showed that after parathyroidectomy there occurs a definite increase in the output of nitrogen, while Burns [1917] recorded a similar finding following guanidine injection. The first section of this paper accordingly deals with the effect of tetany on chlorine metabolism, while succeeding parts describe the alterations in nitrogen and mineral metabolism.

Methods.

Tetany was induced in cats by removal of the parathyroids and in rabbits by injection of guanidine. The parathyroids were first removed under chloroform anaesthesia; this usually produced a condition of latent tetany indicated by sluggishness and the other characteristic manifestations. A second operation of complete thyroidectomy led to the development of active tetany. The effect of guanidine was investigated by the subcutaneous injection of a 6 % solution of guanidine carbonate. 5 cc. were used in each case except rabbit 2

where the dose was 7 cc. An interval of 6 days elapsed between succeeding injections. The food was weighed and aliquot portions of each article of diet were analysed. The unused residues of the diet were kept, thoroughly mixed and analysed. Accordingly the intake during each period of the experiment was accurately known. The rabbits were fed on a mixture of equal parts of oats, bran and linseed, while the cats received oatmeal porridge and milk. Urine and faeces were collected separately. The urine was analysed daily while the faeces for each period were collected, dried on a steam-bath and analysed. The methods of analysis employed were: chloride [Van Slyke, 1923]; calcium [McCrudden, 1911]; phosphorus, Neumann, total nitrogen, Kjeldahl; purines, modified Camerer method [Cathcart *et al.*, 1925], creatine and creatinine, Folin; ammonia, Folin aeration method; amino-acids, Sørensen.

Chlorine.

Results.

A. *Urine* (Table I). It is clear that the urinary output of chlorine was diminished after removal of parathyroids or injection of guanidine much more than can be accounted for by the decreased intake. Accordingly there

Table I. *Showing intake, output and retention of chlorine in mg. daily.*

Cat	Period	Intake	Output	Re-tention	% re-tention
1	1. Normal	790·6	672·5	118·1	14·9
	2. Latent tetany	718·7	496·0	222·7	30·9
	3. Recovery	830·4	529·4	301·0	36·3
	4. Active tetany	194·2	Nil	194·2	100·0
	5. Recovery	706·0	1267·0	Negative	Negative
2	1. Normal	919·0	906·7	12·3	1·3
	2. Latent tetany	1000·0	642·0	358·0	35·8
3	1. Normal	707·4	583·6	123·8	17·5
	2. Latent tetany	528·0	157·4	370·6	70·1
	3. Active tetany	676·3	148·4	527·9	78·0
	4. Recovery	707·4	564·0	143·4	20·2
4	1. Normal	400·7	379·6	21·1	5·2
	2. Latent tetany	400·4	260·4	140·0	34·9
5	1. Normal	431·4	372·7	58·7	13·6
	2. Latent tetany	430·0	287·0	143·0	33·2
Rabbit					
1	1. Normal—7 days	30·6	30·1	0·5	1·63
	2. Guanidine—5 days following 1st injection	18·2	8·6	9·6	52·7
	3. Guanidine—5 days following 2nd injection	8·55	3·0	5·55	64·9
	4. Guanidine—10 days following 3rd injection	7·1	1·4	5·7	80·2
2	1. Normal—7 days	28·4	31·3	12·9	—
	2. Guanidine—5 days after 1st injection ...	7·1	4·04	3·06	43·1
3	1. Normal—7 days	42·6	41·2	1·4	3·3
	2. Guanidine—5 days following 1st injection	16·05	13·33	2·72	16·9
	3. Guanidine—5 days following 2nd injection	10·2	4·76	5·44	53·3
	4. Guanidine—10 days following 3rd injection	14·2	5·1	9·1	64·1
4	1. Normal—7 days	44·4	42·9	1·5	3·4
	2. Guanidine—5 days following 1st injection	8·66	4·54	4·12	47·5
	3. Guanidine—5 days following 2nd injection	7·1	2·44	4·66	65·6
	4. Guanidine—10 days following 3rd injection	8·6	2·40	6·20	72·1

was actually an increased retention of chlorine during both latent and active tetany periods although the intake was in most cases much less than that of the normal. If sufficient chlorine had been ingested the retention was more marked in the active stage.

B. *Blood* (Table II). The immediate effect of an injection of guanidine on the total chlorine content of the blood was with one exception (rabbit 11) a reduction of 8 to 18 milli-equiv. per litre. This reduction was followed within

Table II. *Showing total and volatile chlorine of blood in normal condition and active and latent tetany (m.-eq. per litre).*

	Cat 1		Cat 3		Cat 5		Cat 6		Cat 7		Cat 8	
Period	Total Cl	Vol. Cl	Total Cl	Vol. Cl	Total Cl	Vol. Cl	Total Cl	Vol. Cl	Total Cl	Vol. Cl	Total Cl	Vol. Cl
Normal	102·6	14·6	101·8	11·4	96·2	10·4	98·6	11·8	104·7	12·2	98·0	10·0
	—	—	100·4	10·4	97·1	11·2	100·1	11·8	106·0	12·0	100·2	10·4
	—	—	—	—	—	—	—	—	106·2	11·7	100·3	10·5
Latent tetany	—	—	—	—	—	—	91·2	12·9	93·1	16·3	92·5	12·1
	122·1	19·8	—	—	109·5	20·4	110·5	18·1	113·2	17·4	112·6	14·6
	—	—	—	—	108·7	18·3	112·8	20·6	115·9	17·3	115·9	19·8
	—	—	—	—	110·5	12·3	112·8	20·3	113·8	20·0	115·4	19·2
Active tetany	—	—	—	—	110·0	0·2	—	—	102·8	0·0	107·5	1·0
Recovery	105·8	12·6	103·0	11·6	—	—	—	—	—	—	—	—
	Rabbit 1		Rabbit 2		Rabbit 3		Rabbit 4		Rabbit 5		Rabbit 6	
Normal	80·2	8·6	74·6	12·2	85·0	8·6	77·6	11·2	82·5	10·0	67·3	11·2
	80·0	10·2	75·1	12·6	84·8	10·4	78·9	10·8	80·6	10·0	67·4	11·5
1st day after 1st injection	70·3	0·0	75·5	0·7	76·8	0·8	62·5	3·6	70·3	2·1	50·8	3·5
4th day after 1st injection	81·8	17·4	80·4	15·3	88·2	19·3	80·9	20·0	80·6	17·8	75·5	16·3
1st day after 2nd injection	72·9	−0·1	—	—	75·8	2·3	70·7	2·2	68·9	4·5	58·8	5·1
4th day after 2nd injection	86·6	17·7	—	—	80·4	19·6	86·5	18·6	78·8	18·4	75·9	16·1
1st day after 3rd injection	78·8	−0·2	—	—	75·9	1·1	80·4	4·9	75·5	0·3	70·2	0·3
8th day after 3rd injection	73·0	12·6	—	—	73·0	9·6	78·1	13·3	67·7	7·2	75·5	10·7

3 to 4 days by a return to a normal or slightly super-normal figure except in the period following the third injection. In two cases after the third injection a return to normal was recorded while in the other three the total chlorine was still further reduced. The volatile chlorine of the blood almost vanished immediately after administration of guanidine, only to return 4 days after the injection to a value exceeding the normal figure.

Similar results were noted following the removal of parathyroids. The onset of latent tetany was accompanied by a decrease of the total chlorine of the blood with a rise in the volatile portion. As the state of latent tetany was prolonged the total chlorine rose to a value 10–15 % in excess of the normal, while the volatile chlorine became almost twice as great as in health. When active tetany was induced by a complete thyreo-parathyroidectomy the total chlorine still remained above normal in two of the three cases, while

the volatile form almost completely disappeared. In two cats in which as the result of treatment with saline there were no evident symptoms the blood-chlorine values were normal.

C. *Tissues* (Table III). Analysis of the tissues showed that in both the latent and active stages of tetany due either to parathyroidectomy or guanidine poisoning there occurred a marked increase of the total Cl of muscle, heart

Table III. *Showing total and volatile chlorine of tissues in normal condition and active and latent tetany (m.-eq. per litre).*

		Muscle		Heart		Liver		Kidney		Lung	
Cat	Condition	Total Cl	Vol. Cl	Total Cl	Vol. Cl	Total Cl	Vol. Cl	Total Cl	Vol. Cl	Total Cl	Vol. Cl
A	Normal	15·0	1·8	32·1	6·1	27·2	3·4	41·0	7·0	61·1	11·1
B	,,	13·6	4·1	—	—	26·3	7·8	40·0	4·4	—	—
C	,,	14·2	2·2	26·6	6·2	31·5	2·3	35·0	5·0	62·5	11·7
	Average	14·3	2·7	29·4	6·2	28·3	4·5	38·7	5·5	61·8	11·4
6	Latent tetany	51·0	28·2	67·0	36·4	34·5	19·7	51·2	9·2	57·7	22·0
2	Active tetany	63·6	2·1	77·8	0·0	62·2	0·1	56·3	0·0	42·0	0·2
5	,,	63·8	3·1	86·0	0·7	66·0	0·0	63·3	0·0	51·2	0·0
7	,,	54·0	0·2	85·0	0·1	40·0	1·7	64·0	0·0	57·2	1·1
8	,,	76·5	0·7	48·4	0·4	60·4	0·2	40·8	0·8	55·9	2·9
	Average	65·0	1·5	74·3	0·3	57·2	0·5	56·1	0·2	51·6	1·1
1	Recovered	46·0	5·6	50·5	20·4	28·2	5·8	50·0	5·0	54·0	4·9
3	,,	23·0	3·0	40·0	16·0	30·0	5·2	53·0	4·0	51·0	21·4
	Average	34·5	4·3	45·3	18·2	29·1	5·5	51·5	4·5	52·5	13·2
Rabbit											
7	Normal	7·3	3·7	34·2	9·9	27·6	3·5	—	—	48·8	16·2
8	,,	11·0	3·9	36·0	9·6	22·0	2·0	48·6	14·1	50·0	15·9
9	,,	15·6	4·7	34·8	8·5	23·0	5·1	53·8	19·6	49·2	13·0
	Average	11·3	4·1	35·0	9·3	24·2	3·5	50·7	16·9	49·3	15·0
3	Latent tetany	35·0	25·2	55·0	12·5	39·5	24·3	37·8	10·4	43·5	14·7
4	,,	31·0	19·9	52·0	7·0	41·4	14·9	44·0	9·9	41·4	11·6
5	,,	41·0	32·8	55·4	12·2	42·4	24·1	43·2	25·6	47·7	17·7
	Average	35·7	26·0	54·1	10·6	41·1	21·1	41·7	15·3	44·2	14·7
1	Active tetany	46·0	0·2	54·6	0·6	40·1	1·5	23·7	0·8	46·3	0·3
2	,,	42·4	0·5	56·4	0·4	38·9	0·1	29·8	1·0	47·2	0·4
6	,,	40·0	2·8	55·7	1·9	37·8	1·8	28·5	1·4	41·2	1·2
	Average	42·8	1·2	55·6	1·0	38·9	1·1	27·3	1·1	44·9	0·6

and liver. Following injection of guanidine the total Cl of kidney and lung was reduced, while after removal of the parathyroids the Cl was increased in the kidney but reduced in the lung. During the latent tetany stage the volatile Cl was increased markedly in muscle and liver and slightly in kidney in both series of experiments. In heart and lung the volatile portion was unchanged in the guanidine-treated group but definitely increased in the parathyroidectomy series. During active tetany in both groups there was almost complete disappearance of volatile Cl. Two cats, which were killed during a period of freedom from all manifest signs of tetany, showed a moderate increase in the total Cl content of heart and skeletal muscle but otherwise practically normal values.

Discussion.

Guanidine. From these results it is permissible to conclude that guanidine poisoning creates a demand for chlorine in the tissues. The early fall in the total chlorine content of the blood coupled with the diminution in the urinary excretion is clearly a consequence of the withdrawal of chlorine to the tissues. The subsequent rise of the blood-chlorine is in all probability the result of gradual repletion of the blood from the chlorine ingested.

It has already been shown that chlorine exists in part as a volatile organic compound which has the power of forming a complex with substances containing the amino-group [Morris and Morris, 1930]. When so combined the power of volatilisation at 100° is lost. The amount of non-volatile amino-chlorine complex depends on the proportion of volatile organic chlorine to amino-groups, closely following the adsorption formula. The changes in the content of volatile chlorine of blood and tissues are strongly suggestive of the formation of amino-compounds in the tissues. Within 24 hours of the injection of guanidine the volatile chlorine of the blood has completely disappeared while the non-volatile fraction remains practically unaltered in amount. As there is no excessive excretion of chlorine it must be concluded that the volatile chlorine has gone to the tissues. Four days after the injection the volatile chlorine in the blood has increased above its normal value. Meanwhile in order to satisfy the extra demands there is an excessive retention of the chlorine ingested. The results of tissue analysis during the latent tetany stage conform with this view. It is seen that during that period there is a great increase in the total chlorine, affecting in muscle and liver only the volatile form. Renal tissue, however, contains rather less chlorine in this period, the result probably of less chlorine having been carried to the kidney for excretion. It is possible that the increase of volatile chlorine is due to the presence of guanidine itself. If that were so it would be reasonable to expect either that it should be more or less uniformly distributed over the tissues or that excess should be found only in the liver, the chief seat of deamination. It is significant, however, that skeletal muscle, one of the chief centres of metabolic activity, shows equally with liver a marked increase in the amount of volatile chlorine.

In the stage of active tetany the striking feature of the results of tissue analysis is the great diminution in volatile chlorine, which must be due to the presence of excessive amount of amino-compound. The total chlorine of all the tissues except kidney remains high as in the latent tetany stage.

Parathyroidectomy. The results show that chlorine metabolism was affected in almost the same manner as in guanidine poisoning In several instances intraperitoneal injection of sodium chloride led to a rapid disappearance of the symptoms. This was shown by Paton and Findlay [1917] who attributed the beneficial action of the saline to a dilution of the poison. It seems from the present study more correct to refer the effect of administered

chloride to a neutralisation of the protein breakdown product by the formation of an amino-chlorine complex.

It appears justifiable, therefore, to conclude that the production of tetany by guanidine poisoning or by parathyroidectomy is the result of excessive proteolysis leading to the liberation of a large amount of amino-nitrogen which unites with the volatile chlorine. As long as the ratio of volatile chlorine to amino-nitrogen is maintained above a certain value manifest symptoms of tetany do not appear. When the liberation of amino-nitrogen proceeds apace and reduces this ratio the volatile chlorine disappears owing to the formation of a non-volatile complex, and active tetany ensues. Should the ratio again be raised by the parenteral administration of chlorine, owing to the rapid formation of volatile chlorine the symptoms again become latent. It is well known that the feeding of meat hastens the onset of tetany, a result to be expected in view of the increased amount of circulating amino-nitrogen. This view would explain the discordant results recorded of guanidine poisoning. Obviously the production of manifest tetany would depend on two factors, (*a*) the extent of protein breakdown and (*b*) the available supply of chlorine. The greater the supply of chlorine the larger would be the dose of guanidine necessary to produce symptoms. Further, the guanidine having itself an amino-group might conceivably be detoxicated before it could act on the tissues, provided there was available an abundance of chlorine.

Still further support is lent to our hypothesis by a consideration of gastric tetany. In this form there is a great loss of chlorine by the vomitus leading to a great deficiency in the tissues, which coincides with normal or slightly excessive liberation of amino-nitrogen. Further, it is of interest to state that in the idiopathic tetany of children there also occurs a marked retention of chlorine [Morris and MacRae, 1931].

Nitrogen.

The results noted in Table IV indicate that, following parathyroidectomy, in spite of decreased intake the urinary output of nitrogen was markedly increased so that the retention was greatly diminished, there being in four instances a net loss. The faecal nitrogen content varied but little. This is in accord with the numerous experimental studies previously recorded by Berkeley and Beebe [1909] and others for tetania parathyreopriva and by Burns [1917] for guanidine tetany. The partition of nitrogen in the urine is of importance in indicating the source of the excess nitrogen. The results heretofore recorded in the literature are conflicting. Berkeley and Beebe [1909] found an increase in the ammonia of the blood and urine and because of this and of the effectiveness of calcium therapy in parathyroid tetany and ammonia intoxication concluded that the excess of ammonia was the causal agent in the production of tetany. Falta and Kahn [1912] and Underhill *et al.* [1922] also found an increase in ammonia whereas Carlson and Jacobson [1911], Greenwald [1913], Albertoni [1914] could only show a slight rise in the ammonia

Table IV. *Showing intake, output and retention of nitrogen in g. daily.*

Cat	Period	Intake	Output			Retention	NH_3	Total* creatinine	Amino-* acid-N	Purine*-N
			Urine	Faeces	Total					
1	Normal	3·5200	1·2400	1·6209	2·8600	0·6592	—	167·40	101·52	3·478
	Latent	3·2700	1·9725	1·7100	3·6825	0·4125	—	50·20	359·56	31·500
	Active	0·7205	1·5170	0·0000	1·5170	0·7965	—	70·70	203·60	30·080
	Recovery	2·9100	0·8824	0·6911	1·5174	1·3926	—	124·40	172·02	3·500
2	Normal	5·7370	1·7130	3·5400	5·2530	0·4840	—	234·36	113·40	6·456
	Latent	5·7000	2·8180	3·7610	6·5790	0·8790		66·75	344·25	20·410
	Normal	3·9000	0·6886	0·2781	0·9667	2·9333	0·1294	137·06	63·95	2·514
	Latent	1·3730	0·8770	0·0370	0·9140	0·4590	0·0346	39·36	301·25	37·829
	Active	0·7080	2·1443	0·0000	2·1443	1·4363	0·0239	53·96	302·00	105·148
	Recovery	3·9000	0·6540	0·2581	0·9121	2·9879	0·0681	110·92	62·83	9·201
	Normal	3·5300	0·5960	0·2820	0·8780	2·6520	0·1074	220·32	43·38	1·538
	Latent	2·2800	0·8430	0·1280	0·9710	1·3090	0·0507	135·66	47·54	41·025
5	Normal	2·6140	0·7306	0·0900	0·8206	1·7934	0·1279	243·00	70·65	1·032
	Latent	2·0500	0·9512	0·0821	1·0333	1·0167	0·0515	157·00	60·13	44·600
Rabbit										
1	Normal	1·4301	0·7767	0·3240	1·1007	0·3294	0·0296	24·50	61·30	9·87
	1st injection	0·7322	0·3986	0·3329	0·7315	0·0007	0·0165	25·10	38·60	11·80
	2nd ,,	0·3000	0·2310	0·2225	0·4535	−0·1535	0·0014	4·90	82·10	31·72
	3rd ,,	0·2860	0·3137	0·2005	0·5142	−0·2282	0·0004	—	131·40	33·87
2	Normal	1·1072	0·5997	0·2490	0·8487	0·2585	0·0370	24·50	22·00	12·04
	1st injection	0·2860	0·2460	0·1380	0·3840	−0·0980	0·0086	25·00	53·40	12·01
3	Normal	1·7875	0·8857	0·6000	1·4857	0·3018	0·0096	17·46	27·75	12·90
	1st injection	0·6464	0·3852	0·2082	0·5934	0·0530	0·0076	18·05	17·58	10·20
	2nd ,,	0·4118	0·3148	0·1071	0·4219	−0·0101	0·0018	5·18	41·84	30·70
	3rd ,,	0·5720	0·5839	0·1109	0·6948	−0·1228	0·0008	—	174·40	25·40
4	Normal	1·8000	0·8420	0·5850	1·4270	0·3730	0·0113	13·53	20·00	9·87
	1st injection	0·3489	0·1870	0·1800	0·3670	−0·0181	0·0123	13·00	16·08	10·00
	2nd ,,	0·3000	0·3201	0·1075	0·4276	−0·1276	0·0068	5·80	81·00	24·27
	3rd ,,	0·3000	0·4333	0·1069	0·5402	−0·2402	0·0016	—	152·80	22·89

* The figures for creatinine, amino-acid-N and purine-N are in mg. daily.

excreted in the urine and no excess in the blood. Carlson and Jacobson [1911] also showed that there is a difference in the therapeutic actions of calcium salts in ammonia poisoning and tetany. Wilson, Stearns and Thurlow [1915] found a lowering of the ammonia coefficient during latent, but a rise during active tetany. MacCallum and Voegtlin [1911] on the other hand obtained a definite increase immediately after parathyroidectomy. Cooke [1910] and Palladin and Grilliches [1924] reported in tetania parathyreopriva an increase in the total creatinine output whereas Burns [1917] obtained the opposite result after parathyroidectomy but a slight increase in guanidine tetany. Esau and Stoland [1930] concluded that the initial effect of parathyroidectomy is on tissue metabolism, the substance chiefly affected being phosphagen. Justschenko [1913] found an increase in the output of amino-acids, while Falta and Kahn's [1912] results indicated no change in the excretion of amino-acids but a rise in the peptide-nitrogen of the urine. Burns [1917] reported a slight rise in the output of amino-acids after one injection of guanidine.

Our results demonstrate the following points.

Total creatinine. After removal of the parathyroids there was a definite decrease in the amount of total creatinine during latent and active tetany with a tendency to return to normal in the recovery stage. After the first

injection of guanidine, however, the output was unchanged or slightly increased but following the later injections it was markedly diminished. After the third injection creatinine could not be detected in the urine.

Ammonia. The amount excreted was diminished, the decrease becoming more marked as the tetany became more manifest.

Amino-acids. After parathyroidectomy the amino-acid content of the urine was markedly increased in all the tetany periods except two (in one it was practically unchanged and in the other somewhat reduced). Following the first injection of guanidine there occurred except in rabbit 2 a decrease in the amount of amino-acid excreted. The later injections of guanidine led to a marked increase in the output.

Purine-nitrogen. In all cases the output of purine-nitrogen was definitely increased.

From these results it seems fair to conclude that an immediate and important effect of parathyroidectomy and guanidine poisoning is an excessive tissue catabolism. It also seems that the effect on nitrogen metabolism is roughly parallel with the degree of tetany produced. This is more apparent in the guanidine series where it is evident that the effect of guanidine is dependent on the dosage. The second and third injections produced more marked effects.

Minerals.

It is clear that comparison between the retentions of calcium and phosphorus in the various periods is difficult owing to the difference in intakes. Accordingly we have estimated the retention as a percentage of the intake. In calculating the difference in the amount of calcium or phosphorus excreted we have calculated the expected excretion of the normal period on the same calcium or phosphorus intake as that of the tetany period. This admittedly is not satisfactory, but the fact that we obtain such close correlation between the excess calcium calculated on this basis and the excess amino-acids seems to afford some justification for this method of computation. The only other course is to withhold food entirely during all the periods. Such a procedure is open to the very serious objection that ketosis with all its concomitant metabolic effects complicates the picture.

Calcium.

Our results (Table V) may be summarised as follows.

After removal of the parathyroids the urinary output of calcium was increased in the stage of latent tetany and still more in the active period, the faecal output was decreased but not markedly so and the retention of calcium calculated as a percentage of the intake was decreased.

After the first injection of guanidine there occurred a decrease of urinary calcium. The second and still more the third administration of guanidine led to a rise in the amount of calcium in the urine. The percentage retention was

Table V. *Showing intake, output and retention of calcium and phosphorus in g. daily.*

		Calcium						Phosphorus					
			Output			Retention			Output			Retention	
Cat	Period	Intake	Urine	Faeces	Total	Total	%	Intake	Urine	Faeces	Total	Total	%
1	Normal	0·3309	0·0311	0·1034	0·1345	0·1964	59·3	0·4107	0·1545	0·1325	0·2870	0·1237	30·1
	Latent	0·2512	0·0388	0·0878	0·1266	0·1246	49·6	0·3678	0·1667	0·0876	0·2543	0·1135	30·8
	Active	0·0765	0·0410	0·0000	0·0410	0·0355	46·4	0·1824	0·1210	0·0000	0·1210	0·0614	33·9
	Recovery	0·1960	0·0354	0·0512	0·0866	0·1094	55·9	0·1900	0·0800	0·0438	0·1238	0·0662	82·7
2	Normal	0·3417	0·0620	0·1450	0·2070	0·1347	39·4	0·4715	0·2017	0·2200	0·4217	0·0498	24·7
	Latent	0·3635	0·1480	0·1076	0·2556	0·1079	29·7	0·4300	0·2250	0·1302	0·3552	0·0748	33·2
3	Normal	0·2030	0·0140	0·1322	0·1462	0·0568	28·0	0·2933	0·1090	0·1972	−0·3063	−0·0130	—
	Latent	0·1270	0·0113	0·1021	0·1134	0·0136	10·9	0·1530	0·0792	0·0831	0·1623	−0·0093	—
	Active	0·0570	0·0640	0·0000	0·0640	−0·0070	—	0·1100	0·1628	0·0000	0·1628	−0·0528	—
	Recovery	0·2030	0·0100	0·1411	0·1511	0·0519	25·0	0·2933	0·0705	0·2324	0·3029	−0·0096	—
4	Normal	0·2010	0·0068	0·1021	0·1089	0·0921	45·8	0·2830	0·0925	0·1001	0·1926	0·0904	31·9
	Latent	0·1650	0·0113	0·0786	0·0899	0·0751	45·5	0·2500	0·1074	0·0625	0·1699	0·0801	32·0
5	Normal	0·1690	0·0089	0·0885	0·0974	0·0716	42·4	0·2280	0·0935	0·0870	0·1805	0·0475	20·8
	Latent	0·1350	0·0085	0·0614	0·0699	0·0651	48·2	0·1650	0·0910	0·0390	0·1300	0·0350	21·2
Rabbit													
1	Normal	0·0904	0·0094	0·0304	0·0398	0·0506	56·0	0·9010	0·5737	0·1555	0·7292	0·1718	19·0
	1st injection	0·0474	0·0046	0·0151	0·0197	0·0277	58·4	0·4500	0·2870	0·0780	0·3650	0·0850	18·9
	2nd ,,	0·0242	0·0041	0·0085	0·0126	0·0116	47·9	0·2002	0·1403	0·0295	0·1698	0·0304	15·2
	3rd ,,	0·0185	0·0092	0·0061	0·0153	0·0032	17·3	0·1802	0·1413	0·0188	0·1601	0·0201	11·1
2	Normal	0·0700	0·0073	0·0236	0·0309	0·0391	55·8	0·6660	0·3000	0·1270	0·4270	0·2390	35·9
	1st injection	0·0201	0·0022	0·0070	0·0092	0·0109	54·2	0·1800	0·1168	0·0464	0·1182	0·0618	34·3
3	Normal	0·1140	0·0120	0·0401	0·0521	0·0619	45·7	1·1340	0·7632	0·2195	0·9827	0·1513	13·3
	1st injection	0·0380	0·0039	0·0128	0·0167	0·0213	56·0	0·3868	0·2608	0·0740	0·3348	0·0480	12·4
	2nd ,,	0·0282	0·0047	0·0096	0·0143	0·0139	49·3	0·2600	0·2015	0·0325	0·2340	0·0260	10·0
	3rd ,,	0·0370	0·0201	0·0122	0·0323	0·0047	12·6	0·3600	0·2888	0·0408	0·3296	0·0304	8·4
4	Normal	0·1135	0·0289	0·0305	0·0594	0·0541	47·6	1·1409	0·7856	0·1740	0·9596	0·1813	15·9
	1st injection	0·0224	0·0060	0·0054	0·0014	0·0110	49·0	0·2196	0·1380	0·0444	0·1824	0·0462	21·0
	2nd ,,	0·0242	0·0118	0·0070	0·0188	0·0054	22·3	0·2002	0·1478	0·0286	0·1764	0·0238	11·9
	3rd ,,	0·0242	0·0181	0·0066	0·0247	−0·0005	—	0·2002	0·1563	0·0205	0·1768	0·0234	11·7

unchanged or slightly increased after the first injection of guanidine but diminished after the second and third doses.

Cybulski [1906], Haskins and Gerstenberger [1909] and Salvesen [1923] found a decreased retention of calcium in tetany, the increased output being both by urine and faeces. Cooke [1910] failed to obtain an increase of calcium either in the urine or faeces and maintained that tetany was produced by an altered salt equilibrium brought about by accumulation of acid. Greenwald [1929] although he upholds the low calcium theory of the causation of tetany, found with Hastings and Murray [1921] a retention of calcium. Several investigators have mentioned the possibility of acid substances being involved in the increased loss of lime. Thus Salvesen attributed the loss of lime to the high phosphate content of the blood. Table VI shows the close relationship between the excess excretion of total calcium and that of amino-acids. The excess amino-acid/excess calcium ratio in the parathyroidectomy series varied from 10·3 to 10·8 in all but one instance and in all but two of the guanidine series from 9·5 to 11·0. In the exceptions it will be noted that there was an actual increase in the retention of calcium and a reduction in the output of amino-acids. The other results which show such a close correlation, seem to

Table VI. *Calcium and amino-acid excretion.*

Cat	Period	Excess Ca over normal excretion in g.	Excess amino-acid over normal excretion in g.	Amino-acid Calcium
1	Latent	0·0245	0·25804	10·53
	Active	0·0099	0·10210	10·31
2	Latent	0·0220	0·23805	10·82
3	Latent	0·0220	0·23730	10·78
	Active	0·0230	0·23805	10·35
4	Latent	0·0004	0·00416	10·4
5	Latent	−0·0080 (retention)	−0·01052 (retention)	1·32
Rabbit				
1	1st injection	−0·0058	−0·0227	3·91
	2nd ,,	0·0020	0·0208	10·4
	3rd ,,	0·0071	0·0701	9·9
2	1st ,,	0·0031	0·0314	10·1
3	1st ,,	−0·0007	−0·01017	14·5
	2nd ,,	0·0014	0·01409	10·6
	3rd ,,	0·0154	0·14665	9·5
4	1st ,,	−0·0004	−0·00392	9·8
	2nd ,,	0·0061	0·06100	10·0
	3rd ,,	0·0120	0·13280	11·0

indicate that there is some etiological relationship between the excess production of amino-acids and the decreased retention of calcium. These findings lead one tentatively to suggest that the decalcification of the bones which has been shown radiologically to occur after parathyroidectomy [Erdheim, 1911] may be due to the formation of amino-acids in excessive amounts.

Phosphorus.

Greenwald [1922] found a decreased retention of phosphorus during the latent stage following parathyroidectomy and a marked increase during the active period. Recently Greenwald [1929] concluded that the only two constant changes following parathyroidectomy were a fall in serum-calcium and a retention of phosphorus and maintained that the phosphorus is precipitated in the tissues as calcium phosphate. The increase in blood-phosphorus he attributed to a breakdown of lipoids and proteins containing phosphorus. Esau and Stoland [1930] have elaborated this theory on the basis of the recent work carried out on the relationship of phosphocreatine to muscle metabolism.

The results in connection with phosphorus metabolism are given in Table V. They indicate that after parathyroidectomy there is but slight change in the retention of phosphorus. After injection of guanidine the percentage retention of phosphorus is diminished, the diminution becoming more marked after succeeding injections. Owing to the marked reduction in the intake it may be urged that the decrease in the percentage is a result not of abnormal metabolic processes but simply of the smaller amounts ingested. In rabbit No. 4, however, the intake of phosphorus was increased in the periods following the second and third injections and despite this the absolute amount of phosphorus retained was appreciably less. This lends support to the

view that there is an increased catabolism of phosphorus-containing substances as a result of guanidine poisoning.

In view of the diminished retention of calcium and the fact that four-fifths of the calcium of the body is united with phosphorus it seems at first glance strange that the phosphorus retention should not run parallel with that of calcium. It has to be remembered, however, that whereas practically the sole depôt of calcium is bony tissue, considerable amounts of phosphorus are also found in combination with lipoids and nitrogenous substances.

On the basis of the results recorded here it seems possible to formulate a hypothesis as to the chemical pathogenesis of tetany produced either by parathyroidectomy or by injection of guanidine. The primary effect in both cases is to produce an abnormal protein catabolism resulting in the formation of some toxic amino-compound which immediately combines with an organic chlorine compound. Simultaneously there occurs an excessive catabolism of phosphorus-containing substances and formation of large amounts of amino-acids. The effect on calcium and phosphorus suggests that the excess amino-acid carries away calcium from the bone leaving phosphorus either to be excreted or to form a phosphocreatine compound. Whether the extra phosphocreatine formed in the body is the result of excessive guanidine formation following parathyroidectomy we have no means of deciding from the data presented here. It is certainly suggestive that injection of guanidine produces metabolic results bearing a striking resemblance to those following removal of the parathyroids.

There is not the same gradation in the results observed following guanidine injection as there is after parathyroidectomy. It must be remembered, however, that in the latter case the abnormality produced leads either to continuous production of some abnormal substance or to an equally continuous process of excessive protein breakdown. In guanidine poisoning under the conditions of the experiment there is but one massive injection of poison. If this dose is too great death is likely to ensue, whereas if the body is able to deal with it a return to normal is likely to follow provided a second injection is not given too quickly. The fact that chlorine is retained in such large amount after even a single injection of guanidine, and this despite the fact that there is comparatively little upset of nitrogen metabolism, suggests that guanidine is detoxicated by a chlorine compound. If the available chlorine is sufficient to detoxicate the guanidine there will be but little disturbance. This would explain the variability in the results recorded of guanidine injection. The immediate effect at any rate seems to depend not simply on the amount of guanidine injected but on the amount of available chlorine. The fact that succeeding doses of guanidine of the same amount as the first produce effects quite out of proportion to the first indicates that the amount of available chlorine progressively decreases.

The actual onset of active tetany, if this hypothesis be correct, does not occur until the amount of toxic substance exceeds the amount of available

chlorine. As already mentioned this would explain the conditions of so-called tetania chloropriva. Furthermore, the carrying away of the calcium by the excess amino-acids would account for the low serum-calcium. The rise in inorganic phosphorus which tends to occur could be explained by the extra production of acid-soluble phosphorus resulting from abnormal nucleoprotein catabolism.

SUMMARY.

Following parathyroidectomy or injection of guanidine there occur the following metabolic disturbances.

A. *Chlorine.* There is a marked retention of chlorine in the tissues. In the latent tetany stage there is an increase of volatile chlorine both in the blood and tissues while in the active period there is almost complete absence of volatile chlorine.

B. *Nitrogen.* There is an increased catabolism of tissue protein associated with a rise in the urinary output of total nitrogen, amino-acids and purine-nitrogen and a fall in creatinine and ammonia.

C. *Calcium.* The percentage retention is decreased as a result of relative excess in the urinary excretion. There is a correlation between the excess of calcium and amino-acids excreted.

D. *Phosphorus.* The retention is either slightly diminished or unchanged.

It is suggested (1) that the primary effect in both parathyroidectomy and guanidine poisoning is an abnormal breakdown of tissue proteins and that the changes in calcium, phosphorus and chlorine are secondary to the disturbance in nitrogen metabolism, and (2) that the appearance of active tetany is in part at any rate due to a relative deficiency of chlorine compared with some toxic amino-compound occurring in the course of protein breakdown.

It is a pleasure to acknowledge the advice and helpful criticism given by Prof. E. P. Cathcart, Prof. G. B. Fleming, Dr Stanley Graham, and Dr Norman C. Wright. Thanks are also due to Mr John Bell, head attendant at the Institute of Physiology, for his helpful assistance. We are indebted to the Medical Research Council for defraying the expenses of the investigation and for a personal grant to one of us (N.M.).

REFERENCES.

Albertoni (1914). *Centr. exp. Med.* **5**, 163.
Berkeley and Beebe (1909). *J. Med. Res.* **20**, 149.
Burns (1917). *Quart. J. Exp. Physiol.* **10**, 361.
Carlson and Jacobson (1911). *Amer. J. Physiol.* **28**, 633.
Cathcart, Paton and Pembrey (1925). Practical physiology, p. 332. (E. Arnold and Co.)
Cooke (1910). *J. Exp. Med.* **12**, 45.
—— (1911). *J. Exp. Med.* **13**, 439.
Cybulski (1906). *Monatsh. Kinderheilk.* **5**, 1906.
Erdheim (1911). *Z. Path.* **7**, 175.
Esau and Stoland (1930). *Amer. J. Physiol.* **92**, 1.
Falta and Kahn (1912). *Z. klin. Med.* **74**, 105.
Grant (1922). *Arch. Int. Med.* **80**, 355.
Greenwald (1913). *J. Biol. Chem.* **14**, 363.
—— (1922). *J. Biol. Chem.* **54**, 285.
—— (1929). *J. Biol. Chem.* **82**, 531.
Haskins and Gerstenberger (1909). *Cleveland Med. J.* **8**, 671.
Hastings and Murray (1921). *J. Biol. Chem.* **46**, 233.
Iversen and Hansborg (1922). *Acta Med. Scand.* **57**, 95.
Justschenko (1913). *Biochem. Z.* **48**, 61.
MacCallum, Lintz, Vermilye, Leggett and Boas (1920). *Bull. Johns Hopkins Hosp.* **31**, 1.
—— and Voegtlin (1911). *J. Pharm. Exp. Ther.* **2**, 447.
McCrudden (1911). *J. Biol. Chem.* **10**, 187.
Morris and MacRae (1931). Unpublished observations.
—— and Morris (1930). *Biochem. J.* **24**, 1710.
Palladin and Grilliches (1924). *Biochem. Z.* **146**, 258.
Parhon, Ballif and Derevici (1926). *Compt. Rend. Soc. Biol.* **95**, 786.
Paton and Findlay (1917). *Quart. J. Exp. Physiol.* **10**, 315.
Rockemann (1923). *Arch. Kinderheilk.* **72**, 161.
Salvesen (1923). *Acta Med. Scand.* **60**, Suppl. 6.
Underhill, Tileston and Bogert (1922). *J. Med. Res.* **1**, 724.
Van Slyke (1923). *J. Biol. Chem.* **58**, 523.
Wilson, Stearns and Thurlow (1915). *J. Biol. Chem.* **23**, 89.

THE CARBON DIOXIDE DISSOCIATION CURVE OF THE BLOOD IN INFANCY AND CHILDHOOD.

BY

NOAH MORRIS, M.D., and STANLEY GRAHAM, M.D.

(From the Dept. of Pædiatrics, Glasgow University, and Biochemical Dept Royal Hospital for Sick Children, Glasgow.)

Since 1914 much work has been done on the CO_2 dissociation curve in health and disease. So far, with the exception of a few results published in 1923 by Conway-Verney[1] all the observations reported have been made on the blood of adults. The work described in this paper deals with the CO_2 dissociation curves of the blood of a group of normal children as well as a few with disturbances of acid-base equilibrium.

Methods of Investigation.

The method employed was essentially the same as that originally described by Christiansen, Douglas and Haldane[2]. For the analysis of the gas-mixtures, Haldane's air-gas analysis apparatus was used, while the Haldane blood-gas apparatus was employed for the blood. The water-bath accessories were slightly modified. Instead of an electric thermostat, a mercury one was used which controlled the temperature to within 0·5°C. quite satisfactorily. The apparatus was designed to hold two saturating flasks at once, so arranged as to revolve about a central axis, in this way obviating the necessity for a fan to keep the temperature constant throughout the bath. An approximately known amount of CO_2 was introduced into the saturating flask by means of a graduated burette to the lower end of which was attached a mercury reservoir. The upper end of the burette was fitted with a two-way stop-cock and two arms, one connecting with the reservoir of CO_2 and the other for attaching by means of rubber tubing to the saturating flask. After the gases in the flask had become mixed, the pressure in the flask was equalized with atmospheric pressure by a momentary release of the clip on the attached rubber tube.

In all our experiments fully oxygenated venous blood was used. Potassium oxalate and sodium fluoride in minute amounts were used in the syringe, the former to prevent clotting and the latter to prevent possible conversion of glucose into lactic acid, which by combining with base lowers the carbon-dioxide combining capacity of the blood (Lovatt-Evans[5]). It has been shown by several investigators that neither of these substances affects the CO_2 dissociation curve. The determinations were carried out in all cases within two hours of the withdrawal of the blood.

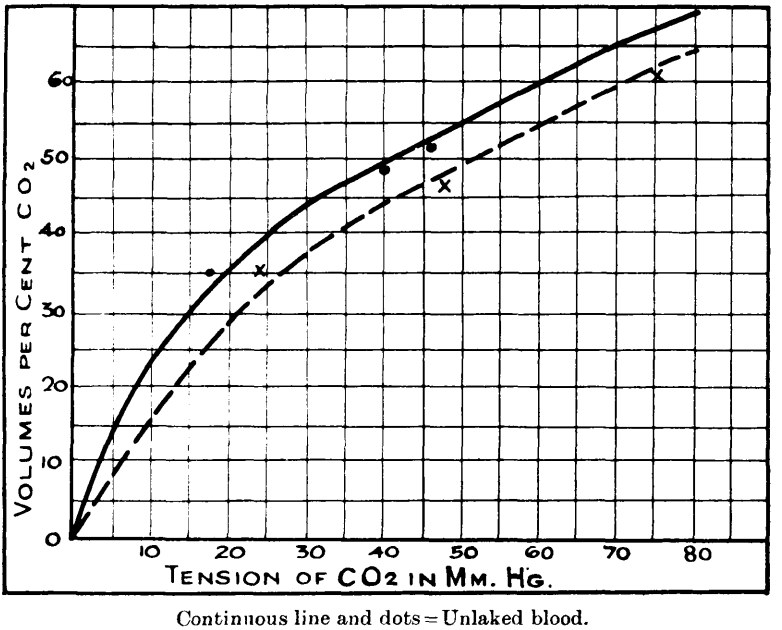
At least three determinations were made on each sample of blood. In every case the first tension of CO_2 to which the blood was exposed was between 35 and 40 mm. Hg. The gas mixture in the flask was then enriched with CO_2 so as to produce a tension of over 60 mm. Hg. Lastly, the blood was exposed to a low tension at least below 25 mm. of CO_2. Haggard and Henderson[4] found that exposure of blood to low pressures of CO_2 led to an irreversible alteration of the H_2CO_3 : $NaHCO_3$ equilibrium. Lovatt-Evans[3] showed that this change was not necessarily due to low tensions of CO_2 but merely that it took place more rapidly under these conditions. It has been shown that blood exposed to a low partial pressure of CO_2 undergoes partial hæmolysis. The hæmoglobin thus liberated into the plasma probably acts as a weak acid and hinders the fixation of CO_2 by the base, with the result that the amount of CO_2 taken up at the various tensions of CO_2 is distinctly less in a hæmolysed than in a non-hæmolysed sample. Figure 1 illustrates an experiment which demonstrates the effect of hæmolysis. It will be seen that at a tension of 40 mm. Hg. laked blood takes up about 10 per cent. less CO_2 than does unlaked blood.

As laking is likely to occur at any tension less than 25 mm. Hg., and since the complete sample of blood is put into the saturating flask at the beginning of the experiment, it is desirable that the exposure to low tensions of CO_2 should be performed last. Otherwise hæmolysis may occur and vitiate the results.

The Normal Carbon Dioxide Dissociation Curve.

In Fig. 2 are plotted the points obtained at various tensions of CO_2 from the bloods of ten normal children whose ages varied from three months to ten years. The abscissa represents the CO_2 tension in mm. Hg. at 37·5° C. to which the blood was exposed. The ordinate represents the total amount of CO_2 (free and combined) taken up at any particular tension of CO_2. Twenty-three points

Fig. 1



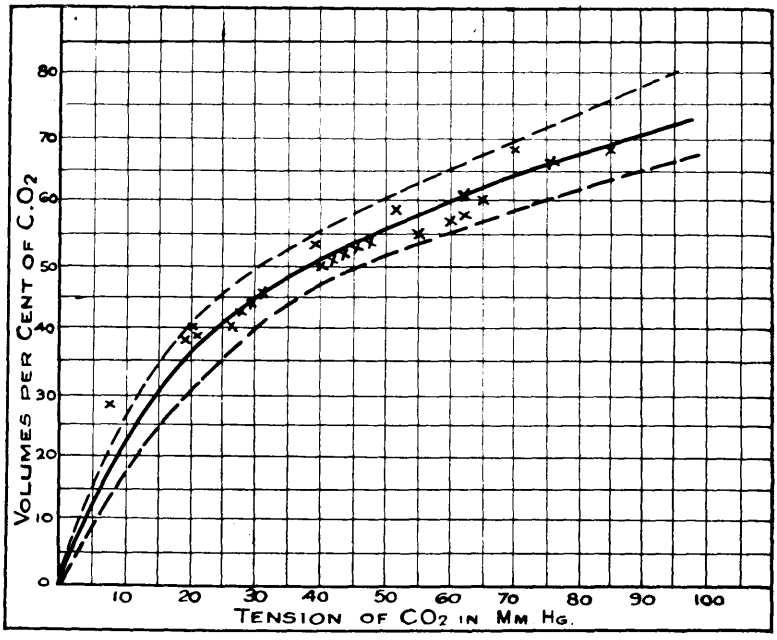
Continuous line and dots = Unlaked blood.
Broken line and crosses = Laked blood.

are marked and with the exception of one point at the low tension of 8 mm. (probably an error) all are within the limits indicated by the dotted lines. At a CO_2 tension of 40 mm. Hg., the total CO_2 content varied from 47 to 56 volumes per cent. with an average of 51·5 volumes per cent. which falls almost exactly on the curve for normal blood as determined by Christiansen, Douglas and Haldane[2]. It is convenient in describing CO_2 dissociation curves to indicate the position of each with reference to that of a standard at a tension of CO_2 equivalent to 40 mm. Hg. The standard generally used is the curve given by Haldane's blood which contains 51 volumes per cent. at 40 mm. Hg. tension of CO_2. If a curve shows 56 volumes per cent at this tension, this will be indicated by saying that it is 9·8 per cent. $\frac{56-51}{51} \times 100$ above the standard. The limits for our series of normals would thus be −8·9 to +9·8. In other words,

if the blood absorbs more CO_2 than normal, the curve is shifted to the left and may be spoken of as hypercapnic. If the blood absorbs less CO_2 than normal, it is shifted to the right and is therefore hypocapnic.

Peters, Barr and Rule[5] analysed the CO_2 dissociation curves in a series of normal adults, in all 18, 3 of their own and 15 collected from the literature. At a tension of 40 mm. Hg., they define the limits of normal as being from 43 to 56 vol. per cent. with an average of 49 vol. per cent., or in terms of Haldane's curve, −15·6 per cent to +9·8 per cent. In children in whom there is generally found evidence of a more unstable acid-base equilibrium, one might have expected that the limits of normal would at least have been wider than in

FIG. II



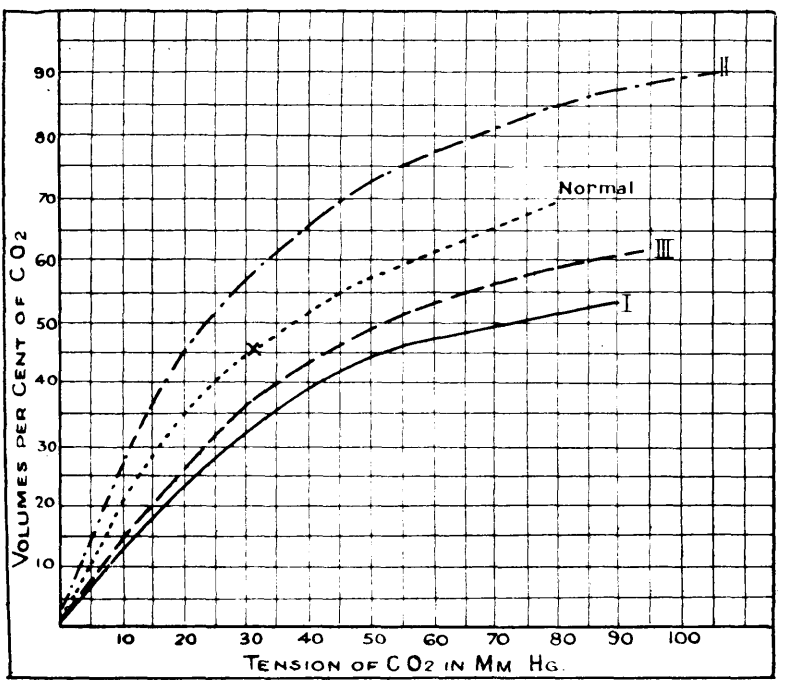
adults. Such, however, was not our experience in this series. Straub, Meier and Schagintweit[6] found that there was a greater variation in the CO_2 dissociation curves in Germany during the hunger years 1917 to 1919 than in 1921, and they attributed this directly to the poor nutritional conditions leading to an unstable acid-base equilibrium.

SIGNIFICANCE AND DISCUSSION OF ABNORMAL CURVES.

It must be emphasized at this point that the direction of the deviation of the curve from the normal does not determine whether there is a state of acidosis or alkalosis. An increase in the CO_2 capacity may be due to a primary decrease in the non-gaseous acid radicles such as Cl, PO_4, etc. (non-gaseous alkalosis), or to an increase in the CO_2 which has led to a secondary migration of these non-gaseous acid radicles (gaseous acidosis). Similarly a decrease in the capacity

of the blood to hold CO_2 may be due either to a primary increase in the non-gaseous acid radicles (non-gaseous acidosis), or to a primary decrease in the CO_2 (gaseous alkalosis). *Thus, by itself, a CO_2 dissociation curve does not give any more information about the pH of the blood than can be determined by a single determination of its total CO_2 content.* If, however, in addition to the dissociation curve, the total CO_2 content of the arterial blood be known, a figure representing the pH of that sample of blood can readily be ascertained. For example, the CO_2 dissociation curve of a sample of blood is as shown in Fig 3 (normal curve). The total CO_2 content of the arterial blood in this case was 45 vol. per cent.

FIG. III.



I Salicylate poisoning.
II Pyloric stenosis.
III Lobar pneumonia.

Therefore the tension of CO_2 necessary to enable the blood to take up 45 vol. of CO_2 is 31 mm. Hg. This point marked x on the graph (Fig. 3) is known as the arterial point. By means of Bohr's coefficient of solubility for CO_2 in whole blood (0·51), the tension is equivalent to 2·08 vol. per cent., which represents the free CO_2 content of the blood. The combined CO_2 is then 42·92 vol. per cent. (45·0—2·08). Hasselbalch's formula $pH = pk_1 + \log \frac{[BHCO_3]}{[HCO_3]}$, pk_1 being a constant equal to 6·1— can now be used to determine the pH, which is thus equal to $6{\cdot}1 + \log \frac{42{\cdot}92}{2{\cdot}08}$, i.e., 7·415.

It is still under discussion whether the dissociation curve determined from fully oxygenated venous blood gives a correct picture of the conditions in arterial blood. Acid substances are produced by the tissues especially during work, and lower the CO_2 combining capacity of the blood. These acid substances, however, are not given off in the lungs but are carried over into the arterial system. Eppinger and Schiller[7] have shown that the CO_2 curves of arterial and venous blood differ but little, such differences as there are being well within the limits of experimental error, but Fraser, Graham and Hilton[8] maintain that such is not the case in the majority of cases. It must be admitted that in disease, where there may be pathological conditions such as a marked relative increase in the corpuscular volume of venous blood, the CO_2 curves of arterial and venous bloods may show marked differences.

If the total CO_2 content of the blood has been determined from a venous sample there will be introduced an error in the determination of the pH owing to the fact that venous blood contains 2 to 4 volumes of CO_2 more than the arterial, the exact amount depending roughly on the degree of venosity. The figure derived from the venous CO_2 and the dissociation curve indicates the pH neither of the arterial nor of the venous blood. To obtain the venous pH it would be necessary to construct a CO_2 dissociation curve at the tension of oxygen which is present in the venous blood. Despite this error the figure obtained by using the CO_2 content of the venous blood and the CO_2 dissociation curve of fully oxygenated blood does yield a figure which is of some value in assessing the state of the acid-base balance.

The curves obtained from the bloods of three children suffering from conditions in which it appeared probable that there was some disturbance in acid-base balance are given in Fig. 3.

Curve I was obtained from the blood of a child suffering from salicylate poisoning. The curve is shifted to the right of normal and is thus hypocapnic. This might be due either to the over-production of acid substances such as occurs in diabetic coma with the consequent diminution in the available alkali (non-gaseous acidosis), or to the over-stimulation of the respiratory centre with washing-out of the CO_2 by over-breathing (gaseous alkalosis). The total CO_2 content of the venous blood was 32·4 vol. per cent.; by using this figure in conjunction with the dissociation curve (admittedly an inaccurate method for reasons already given) we find that the pH is 7·28 which is to the acid side of normality.

Curve II was obtained from the blood of an infant with congenital pyloric stenosis. It is to the left of normal (hypercapnic). This might be due either to a diminution of the acid radicles (non-gaseous alkalosis), or to depression of the respiratory centre with decreased output of CO_2 (gaseous acidosis). The volume of breathing would be diminished in both conditions. In the first instance (non-gaseous alkalosis), the combined CO_2 is primarily increased and the breathing would be diminished in an effort to keep the free CO_2 as high as possible and so cause a minimal disturbance to the normal $HCO_3 : BHCO_3$ ratio of 1 : 20. In the case of the gaseous acidosis, the primary change is in the free CO_2 which is conserved because of the diminished sensitivity of the

respiratory centre by some poison or toxin (morphine). The combined CO_2 is secondarily raised in order to keep the ratio as constant as possible. The work of other investigators as well as some unpublished work of our own shows that in pyloric stenosis, the pH of the blood is frequently on the alkaline side of normality. This indicates that the hypercapnic nature of the CO_2 dissociation curve in this condition is due to a diminution of the acid radicles, that is, a non-gaseous alkalosis. It must be emphasized that such a conclusion is not justified by a consideration of the dissociation curve alone.

Curve III is the curve obtained from the venous blood of a boy on the fourth day of a lobar pneumonia. The curve is hypocapnic and the total CO_2 content of the venous blood was slightly diminished, namely 43·3 vol. per cent. On the curve the CO_2 tension at this volume of CO_2 is 40 mm. Hg. which is the equivalent of a free CO_2 content of 2·69 vol. per cent. The combined CO_2 would then be 40·61 vol. per cent., and by applying Hasselbalch's formula, the venous pH works out at 7·27. As this patient was seven years old, it was possible to obtain the figure for the free CO_2 in another way, namely by the analysis of air equilibrated with the arterial blood in the rectum. By this method the tension of CO_2 was found to be 37·7 mm. Hg. the equivalent of 2·53 vol. per cent. Employing this figure, the combined CO_2 would be 40·77 vol. per cent., and the pH 7·29 which is more accurate than the venous pH of 7·27. It is of interest to note that on the curve at a tension of 37·7 mm. Hg., the total CO_2 is 42 vol. per cent., whereas that determined in the venous blood was 43·3 vol. per cent.

These figures suggest a mild degree of non-gaseous acidosis. One must, however, be cautious about drawing conclusions. Oxygen desaturation of varying degree is always present in lobar pneumonia and must be taken into account in a consideration of the state of the acid-base balance. Meakins and Davies[9] believe that the condition usually present in lobar pneumonia is a partially compensated gaseous alkalosis. Such a condition would of course, also shift the CO_2 dissociation curve to the right, *i.e.*, render it hypocapnic and cause a diminution in the total CO_2 content as occurred in this case. At the same time lobar pneumonia is usually associated with a markedly acid urine and an increase in the excretion of ammonia. There is a retention of chlorine, which is not, however, evidenced by a high blood chloride content. In addition an increase in some unknown organic acid radicle has been reported in the blood. All these facts tend to support the view that the condition is an acidosis. On the other hand Binger, Hastings and Neill[10] have reported a case in which the administration of sodium bicarbonate had a distinctly harmful effect by producing a marked alkali excess in the blood, although the urine remained acid throughout. Another factor of importance is the anoxæmia which *per se* induces an alkalosis. Many years ago, Bohr, Hasselbalch and Krogh[11] demonstrated that lowered tensions of CO_2 caused the oxyhæmoglobin dissociation curve to shift to the left, that is to hold on more firmly to its oxygen. It is difficult, therefore, to reconcile the association of the diminished oxygen saturation with a condition of alkalosis. It must be admitted that the question is one of extreme complexity, and it cannot be finally decided from the findings so far obtained.

Conclusions.

1. The limits of the CO_2 dissociation curves obtained from the bloods of ten normal children at 40 mm. Hg. are from 47 to 56 vol. per cent. (−8·0 to +9·8 per cent).

2. Haggard and Henderson's observations on the occurrence of hæmolysis at low tension with consequent reduction in the CO_2 combining capacity are confirmed.

3. The CO_2 dissociation curve in one case of pneumonia and one case of salicylate poisoning is shown to be hypocapnic and in one example of pyloric stenosis to be hypercapnic. The significance of these findings with reference to acid-base equilibrium is briefly discussed.

We desire to express our thanks to the Medical Research Council by whom the expenses of this work were defrayed.

REFERENCES.

1. Conway-Verney, R., *Brit. Med. Jour.*, London, 1923, ii, 866.
2. Christiansen, J., Douglas, C. G., & Haldane, J. S., *J. of Phys.*, Lond., 1914, XLVIII, 244.
3. Lovatt-Evans, C., *J. of Phys.*, Lond., 1922, LVI, 146.
4. Haggard, H. W., & Henderson, Y., *J. Biol. Chem.*, N.Y., 1922, LIV, 149.
5. Peters, J. P., Barr, D. P., & Rule, F. D., *J. Biol. Chem.*, N.Y., 1921, XLV, 489.
6. Straub, H., Meier, Kl., & Schagintweit, E., *Zeit. f. d. ges. exp. Med.*, Berlin, 1923, XXXII, 229.
7. Eppinger, H., & Schiller, W., *Wien Arch. f. kl. Med.*, Vienna, 1921, II, 3.
8. Fraser, F. R., Graham, G., & Hilton, R., *J. of Phys.*, Lond, 1924, LIX, 221.
9. Meakins, J. C., & Davies, H. W., *Respiratory Function in Disease*, Edinburgh, 1925, 238.
10. Binger, C. A. L., Hastings, A. B., & Neill, J. M., *Arch. Int. Med.*, Chic., 1923, XXXI, 145.
11. Bohr, C., Hasselbalch, K. A., & Krogh, A., *Skandinav. Arch. f. Physiol.*, Berlin & Leipzig, 1907, XVI. 412.

THE THEORETICAL CARBON DIOXIDE DISSOCIATION CURVE IN ACID-BASE DISTURBANCE OF CHILDHOOD.

NOAH MORRIS, M.D., AND STANLEY GRAHAM, M.D.

From the Department of Pædiatrics, Glasgow University, and the Biochemical Department, Royal Hospital for Sick Children, Glasgow.

Received for publication May 10th, 1930.

THE capacity of the blood for holding carbon dioxide depends upon two factors, which may be briefly described as the available alkali and the degree of buffering. The relative importance of these two factors in maintaining acid-base equilibrium is revealed by the theoretical dissociation curve described by Barcroft, Dryerre, Meakins, Parsons and Parsons. Such a curve may be constructed if the carbon dioxide content of a sample of blood be known for each of two tensions of carbon dioxide together with the cH of the blood at one of these tensions. The theoretical curve differs from that obtained in the usual way only at tensions of carbon dioxide below 10 mm. Hg., in that it cuts the y-axis some distance above the origin, which distance represents the volumes per cent. of combined carbon dioxide that would be present were it not for the action of the released hæmoglobin. The theoretical amount of combined carbon dioxide at zero tension is thus really an expression of the amount of base left over after the affinities of all the other (non-gaseous) acid radicles are satisfied, *i. e.*, the minimal amount of base available for combination with carbon dioxide. The theoretical curve also affords information as to the extra amount of carbon dioxide which can be taken up by the blood as a result of unit increase of cH ($cH \times 10^8$).

The results here recorded were obtained during the course of an investigation into disturbance of acid-base equilibrium in childhood. Several

curves were constructed as described by Barcroft. It is not necessary here to refer to the details of the method of construction of such curves, as these are fully given in the paper already quoted. Three graphs are discussed, one from the blood of a "normal child," one from the blood of an infant suffering from congenital pyloric stenosis, and the third from the blood of a child showing symptoms of salicylate poisoning.

NORMAL.

The subject was an apparently normal boy, B. H—, 7 years of age. The following information was obtained in the usual way:

CO_2 tension.	CO_2 in vol. %.	cH × 10^8.
41·3	52·3	3·16
27·0	43·3	...

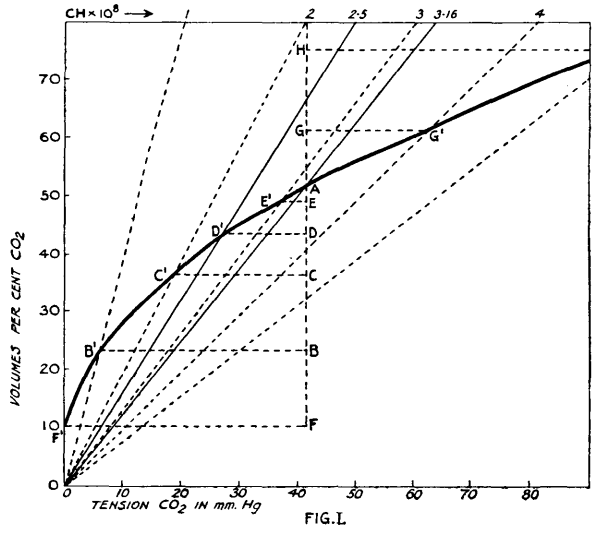


FIG. 1.

Fig. 1 shows the theoretical curve as constructed, and that this curve agrees with that actually determined is well shown in Table I:

TABLE I.

CO_2 tension.	CO_2 vol. % (determined).	CO_2 vol. % (calculated).
27·0	43·3	43·3
30·6	44·3	45·4
41·3	52·3	52·3
51·3	57·7	57·0
62·7	62·0	62·4
76·2	66·6	67·4

In addition to the relationship between tension and volume of carbon dioxide, three other relationships can be determined from this curve:

(1) The relationship between the volume of carbon dioxide and cH, expressed by the equation $vCO_2 = c + b(cH \times 10^8)$, can easily be deduced, where c is the distance between the origin and the point where the curve meets the y-axis (*i. e.*, the amount of available alkali at zero tension of carbon dioxide), and b the degree of buffering representing the increase in the volume per cent. per unit increase of cH. The equation for this blood is graphed in Fig. 3, line 1; it falls almost but not quite within the normal limits given by previous workers. They give the variations for b from 6·5 to 10·1 and for c from 27 to 9. It has been pointed out that b and c generally vary inversely, that is, the greater the amount of available alkali, the less the degree of buffering.

In the case of B. H—, b was 13·3 and c was 10.

(2) The relationship between pressure of carbon dioxide and cH is indicated in Fig. 4. The gradient of the graph indicates the resistance of the blood reaction to increased pressure of carbon dioxide. Thus, in the case of B. H— (line 1), an increase in the tension of carbon dioxide from 37 to 64 mm. Hg. causes the cH to rise from 3×10^8 to 4×10^8.

(3) A modification of Hasselbalch's equation correlates both volume and pressure of carbon dioxide with the cH in the one equation, $cH \times 10^8 = X \times \frac{pCO_2}{vCO_2}$. For normal blood X is recorded by Barcroft as 4·7. In the case of B. H— its value was 4·0.

PYLORIC STENOSIS.

In congenital pyloric stenosis the CO_2 dissociation curve is definitely hypercapnic. Table II and Fig. 2A show the data obtained from the bloods of three infants suffering from pyloric stenosis:

TABLE II.

Name.	CO_2 tension in mm. Hg.	CO_2 vol. %.	Percentage difference from standard curve at a tension of 40 mm. Hg.
H. L—	44·8	67·8	...
	105·5	89·0	...
	17·8	46·1	...
	...	...	+ 25·5
W. B—	42·7	67·6	...
	79·8	80·9	...
	23·5	53·2	...
	...	...	+ 30·0
J. L—	42·1	64·2	...
	90·8	81·1	...
	15·3	46·8	...
	...	...	+ 23·5

In all it is apparent that the volume of CO_2 at any tension of the gas is much higher than normal. This can be more readily seen from the figures in the third column of the table, which indicate the percentage difference between the CO_2 curves and the standard (Haldane's) at a tension of 40 mm. Hg. According to Peters, Barr and Rule, the normal limits are + 9·8% to — 15·6%, while in a series of ten normal children we found that the limits were + 9·8% to — 8·9%.

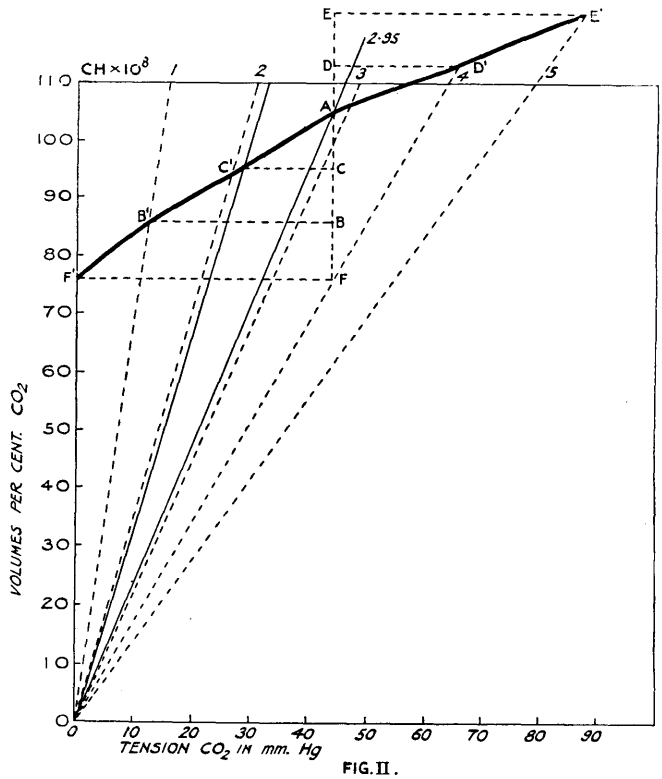


FIG. II.

In order to throw more light on the factors involved in the production of this abnormality a theoretical dissociation curve was constructed from the blood of a patient showing very marked symptoms. The infant, W. R—, aged 7 weeks, had the usual manifestations of congenital pyloric stenosis to a very marked degree—vomiting, visible gastric peristalsis, palpable pyloric tumour, very slow, shallow breathing and a CO_2 content (venous blood) of 110 volumes per cent. The points used in the construction of the curve are as follows:

CO_2 tension.	CO_2 in vol. %.	cH × 10^8.
28·5	96·0	...
43·4	103·8	2·95

It will be seen from Fig. 2 that the curve is much higher and flatter than normal, indicating that the blood contains much more than the usual amount of carbon dioxide at any given tension. The slight flattening is due to the low degree of buffering, while the height of the curve is the result of the large amount of available alkali. In this case b is 9·1 and c 76·8. These results are apparently due to the impoverishment of the blood of its chlorine content. The chlorine ordinarily holds more than half of the total base of the blood, and the chlorine shift between plasma and corpuscles contributes significantly to the degree of buffering. The relationship between tension of carbon dioxide

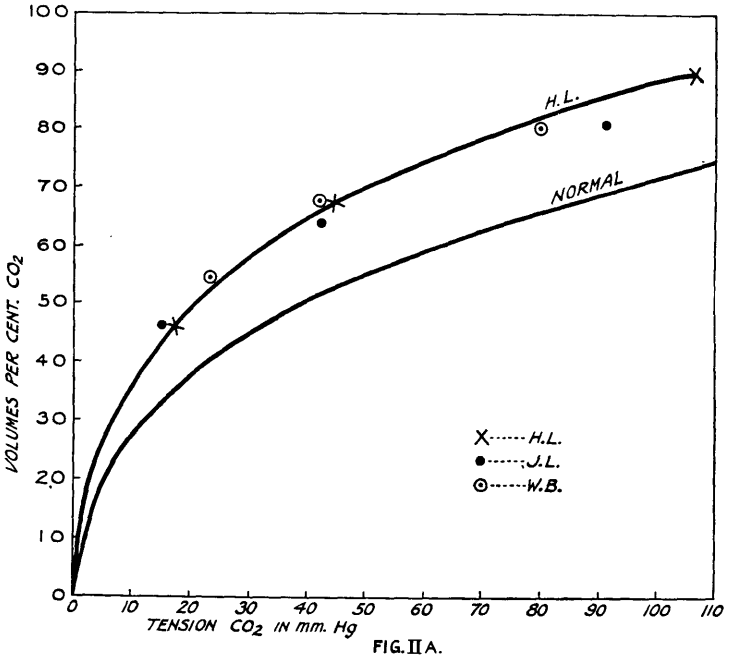


FIG. II A.

and cH (Fig. 4, line 2), roughly parallel to but at a lower level, indicates that for the maintenance of any given cH the blood in this case must have a higher tension of carbon dioxide than normal. This is obviously associated with the slow, shallow breathing, although whether in the relationship of cause and effect it is impossible to infer from the data given. The total carbon dioxide content of the venous blood was 110 volumes per cent. From line 2, Fig. 3, one sees that the cH must be about $3·6 \times 10^8$, and from Fig. 4 that the tension of carbon dioxide must be about 56 mm. Hg. These last two findings are of course only approximate, since the curve has been constructed from fully oxygenated blood. The increased tension of carbon dioxide probably facilitates the dissociation of oxyhæmoglobin and possibly minimizes the symptoms of anoxæmia, since the tissues would thus receive a fair if not

entirely adequate supply of oxygen despite the depressed ventilation of the lungs.

The value of X relating pressure and volume of CO_2 to the cH is in this case 7·05. This high value tends to increase the cH for any given tension of

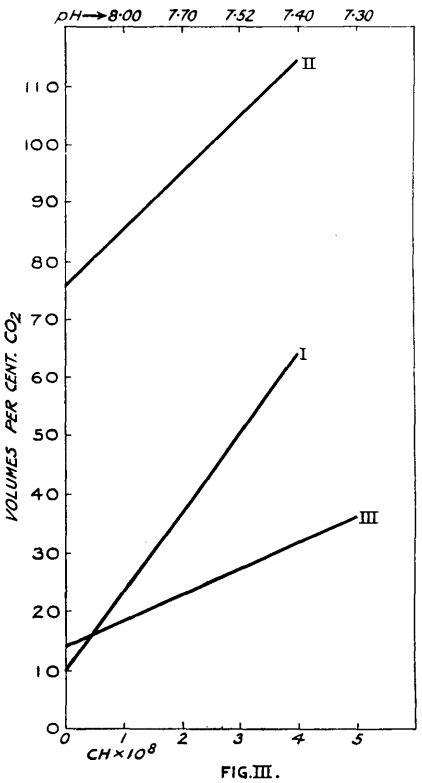


FIG. III.

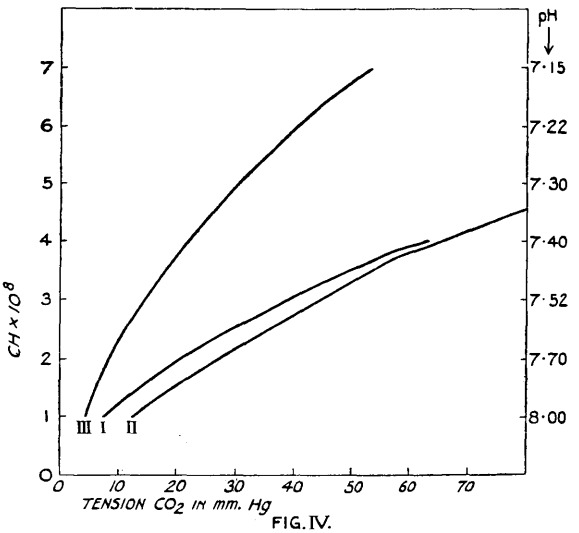


FIG. IV.

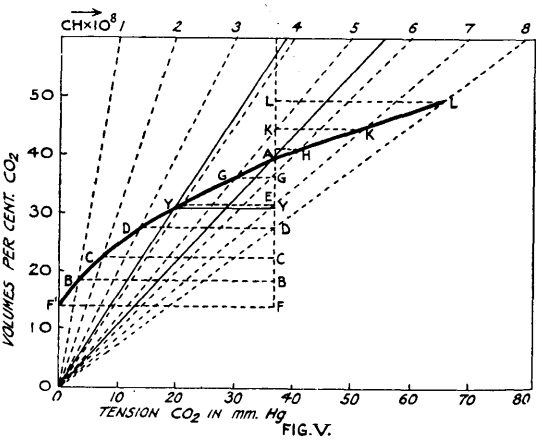


FIG. V.

carbon dioxide. Thus, if X had had the normal value of 4·7, the cH at a tension of 43 mm. Hg. would have been 1·96 (pH 7·70) instead of 2·95 (pH 7·53). This high value of X, together with the low degree of buffering, both tend to bring the cH more rapidly to normal limits when the tension of

carbon dioxide is increased. In other words, it helps to neutralize the effect of the excessive amount of alkali.

SALICYLATE POISONING.

The child, R. H—, aged 5 years, convalescing from a mild attack of rheumatism, had been receiving sod. salicylate gr. x with sod. bicarbonate gr. xx six times daily for four days when he suddenly became dyspnœic. The CO_2 content of the venous blood was found to be 32·4 volumes per cent. A theoretical curve (Fig. 5) was constructed from the following data:

CO_2 tension.	CO_2 in vol. %.	cH × 10^8.
36·3	38·4	5·6
20·0	31·0	...

This curve is definitely lower than normal. At a tension of 40 mm. Hg. the blood contains only 39·7 volumes per cent. of CO_2, *i.e.* 24% below the standard. It is evident from the curve and from lines 3 in Figs. 3 and 4 that the lowness is due solely to the diminution in the degree of buffering ($b = 4·4$). The amount of available alkali ($c = 14·0$) is well within the limits of normality already mentioned. The value of X relating pCO_2 and vCO_2 to the cH is 6·0, which is a further indication of the low degree of accommodation to increase in tension of CO_2. Were it not for the normal level of available alkali the curve would be very low and flat. The fault therefore lies in the buffering, and is possibly due to the toxic action of the salicylic acid on the biological processes constituting the so-called buffering mechanism.

The authors desire to express their gratitude to the Medical Research Council, by whom the expenses of this work were defrayed.

REFERENCES.

Barcroft, J., Dryerre, H., Meakins, J. C., Parsons, T. R., and Parsons, W.—(1925-26) *Quart. J. Med.*, **73**, 257.
Morris, N., and Graham, S.—(1929) *Arch. Dis. Childhood*, **4**, 299.
Peters, J. P., Barr, D. P., and Rule, F. D.—(1921) *J. Biol. Chem.*, **45**, 489.

Made and printed in Great Britain for
H. K. Lewis & Co. Ltd., by
Adlard & Son, Ltd., 21, *Hart Street, London, W.C.* 1.

CHANGES IN THE BLOOD CHEMISTRY IN CONGENITAL HYPERTROPHIC PYLORIC STENOSIS AND THEIR CLINICAL SIGNIFICANCE.

BY

STANLEY GRAHAM, M.D., and NOAH MORRIS, M.D.
(From the Dept. of Pædiatrics, Glasgow University, and the Biochemical Dept., Royal Hospital for Sick Children, Glasgow.)

A fairly extensive literature is accumulating regarding the clinical symptoms and chemical findings in cases of high intestinal obstruction, and particularly when the obstruction is situated at the pylorus. Hartmann and Smyth[1] have recently published an exhaustive report of a series of investigations in infants suffering from vomiting due to various causes. More observations, however, have been made in the adult, but the bulk of the published work is a record of the study of the condition as experimentally produced in animals.

In 1918, McCann[2] showed that the plasma CO_2 combining power of the blood was greatly increased after experimental obstruction of the pylorus. Two years later, McCallum[3] and his co-workers demonstrated the same phenomenon and indicated its association with a fall in the chloride content of the blood. This they explained as being due to loss of chloride by the vomiting of the gastric juice which in turn led to an increase of the plasma CO_2 to make good the deficiency of acid radicles. These same investigators found that the onset of tetany, which invariably occurred, could be prevented by intravenous administration of sodium chloride. Hastings, Murray and Murray[4], Haden and Orr[5] and Gamble[6] have also published papers throwing further light on the condition. Brown, Eusterman, Hartmann and Rowntree[7] described similar chemical findings in duodenal obstruction in the adult and Ellis[8] demonstrated the presence of alkalæmia in two cases of intestinal obstruction. An identical picture was shown to be present in cases of duodenal ulcer after intensive alkali treatment especially in the presence of impaired renal function (Hardt and Rivers[9]).

In the present communication we wish to record the observations both clinical and chemical made during a study of congenital hypertrophic pyloric stenosis extending over the past four years. It is obvious that because of the age of the patients and the severity of the condition, the investigations had of necessity to be less complete than desired. In all, over fifty cases were studied but we propose to give only such details as are relevant for correlating the various findings.

Before we proceed to analyse our results, it will be of advantage to give a brief resumé of these findings which, on the whole, are in accord with those of other workers. A most striking and probably the only important clinical manifestation of the presence of an alkalosis in these cases is the depressed breathing. This may be evidenced in one or more of the following three ways. First, there may be a shallow type of respiration, so shallow in fact that even with the bell of the stethoscope placed in front of the nose and mouth, it is

often very difficult to hear the respirations. Secondly, the rate is frequently diminished, often to six or eight times per minute, and thirdly, there are well-marked and often alarming periods of apnœa followed by three or four shallow respirations producing a typical Biot type of respiration. Most often, all three manifestations are present at the same time. At this point we would mention that we believe this condition to be present at some time in practically every case of pyloric stenosis in infancy. Coupled with this respiratory depression there exists a general lethargy, giving one the impression that the infant is under the influence of a hypnotic drug. In one instance, a baby was brought to hospital because the mother had noticed undue quietness. Recognition of the depressed breathing raised the suspicion of pyloric stenosis although the infant had not vomited on any single occasion. On examination typical gastric peristalsis was evident together with a palpable pyloric tumor. The diagnosis of pyloric stenosis was ultimately confirmed post mortem by the presence of a hypertrophied pylorus.

The biochemical examination of the blood shows usually a decrease in chlorides and an increase in the total CO_2 content and, less frequently, in the non-protein nitrogen. As a rule, these three findings are present together but the variations from the normal in each are not necessarily of the same degree. Table I gives some results which will serve to indicate this variation. In several cases, the fixed base of the serum was also determined (Table 2), and it will be seen that it is usually within normal limits (149—162 mM per litre) although occasionally it is slightly reduced.

TABLE 1

GENERAL BIOCHEMICAL FINDINGS IN THE BLOOD IN PYLORIC STENOSIS.

Name	Age in weeks	Total CO_2		Chlorine (Cl)		Non-protein nitrogen	Excess non-prot. nitrogen	CO_2+Cl (mM. per litre)	CO_2+Cl + excess N.P.N. (mM. per litre)	Respir. rate (per min.)
		Vol. per cent.	mM. per litre	mgm. per cent.	mM. per litre	mgm. per cent.	(mM. per litre)			
R. C. ...	8	140·5	63	140	40	46	2	103	105	18
N. R. ...	4	140·0	63	180	51	80	8	114	122	16
A. M. ...	8	83·1	37	210	60	32	—	97	97	40
W. G. ...	6	85·0	39	190	54	35	—	93	93	24
M. M. ...	12	62·5	28	330	94	60	5	122	127	40
M. A. ...	8	125·6	56	110	31	145	20	87	107	—
A. W. ...	7	110·2	50	170	49	63	5	99	104	26
E. M. ...	8	100·5	45	160	46	86	9	91	100	12
J. K. ...	6	91·8	41	160	46	50	3	87	90	24
J. McK. ...	8	112·0	50	220	63	32	—	113	113	—
A. C. ...	3	113·0	50	240	69	34	—	119	119	10
G. H. ...	7	95·8	43	190	54	75	7	97	104	14
W. A. ...	3	103·7	46	300	86	38	—	132	132	—
W. A. ...	4	148·0	66	130	37	183	27	103	130	—

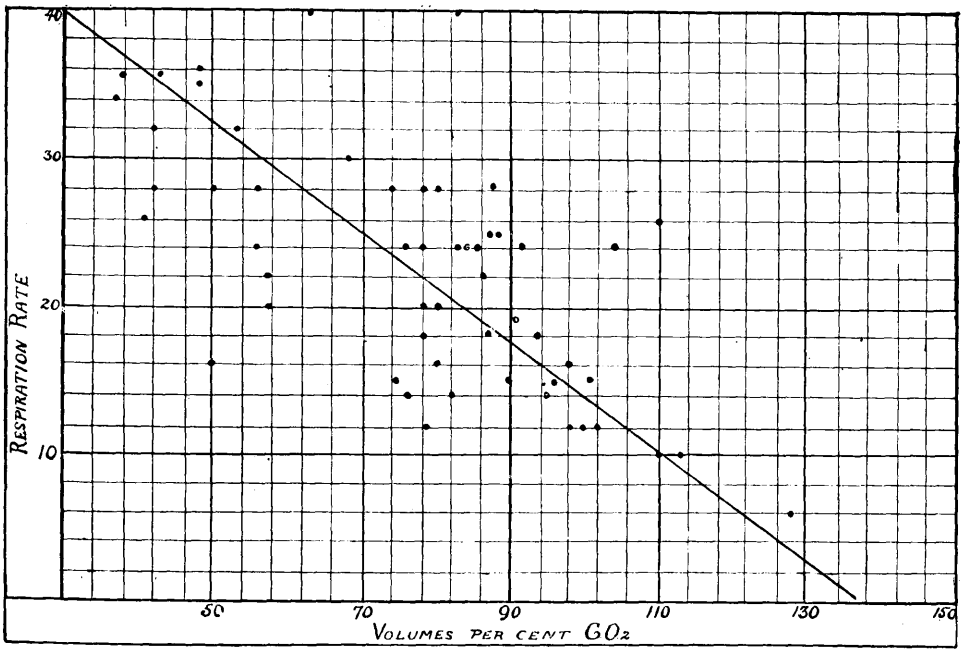
TABLE 2.

SHOWING RELATIONSHIP BETWEEN CO_2, CHLORIDE AND FIXED BASE.

Name	J.McK.	J.P.	T.S.	G.C.	W.B.	A.B.	M.McP.	D.F.	R.S.	E.F.
CO_2 (mM. per litre)	51	53	56	21	50	54	28	49	45	46
Cl (mM. per litre)	63	54	—	70	—	34	83	—	—	60
Fixed base (mM. per l.) ...	144	151	150	160	159	152	154	153	143	161

CHART I.

Showing Relationship between Respiratory Rate and Total CO_2 Content of Blood.



Relationship of the total CO_2 Content and Respiration. One of the most striking correlations among our findings is that between the total CO_2 content of the blood and the respiratory rate. This is all the more striking when one remembers that it is the total respiratory exchange and not merely the rate of breathing which is of significance. Further, it is not the total CO_2 but rather the ratio of the free and combined CO_2, which is the regulating factor in respiration. In Chart I are plotted individual observations of the total CO_2 content of the blood against the respiratory rate at the time of withdrawal of the blood. The slower the respiratory rate, the greater is the increase in CO_2. There are certain anomalous findings on the chart but when one remembers the possibility

of tachypnœa resulting from oxygen deficiency, it can be readily understood that such results are only to be expected. In Table 3, these results are grouped into six averages which indicate clearly the strong correlation.

TABLE 3.

SHOWING CORRELATION BETWEEN CO_2 CONTENT OF BLOOD AND RESPIRATORY RATE IN CASES OF PYLORIC STENOSIS.

Group	1	2	3	4	5	6
Average respiration rate (per min.) ...	8	13·8	18·1	23·4	27·8	38·9
Average CO_2 content (vol. per cent.)	138·8	108·6	99	99	87·7	65·2

This relationship between the CO_2 capacity and slow breathing apparently only holds good in patients suffering from pyloric stenosis. During the past three years, it has been our custom to pay particular attention to respiratory rates in other conditions than pyloric stenosis, and not infrequently we have observed great reductions in rate, but in no instance was the CO_2 content of the blood raised appreciably above the normal. These findings are seen in Table 4. In the case of J.L. suffering from encephalitis with a respiratory rate of 8 per minute, the chlorides (C^1) were 240 mgm. per cent., the non-protein nitrogen was 35 mgm. per cent. and the pH was 7·25 (i.e. an acidosis). One important point in the clinical differentiation of these two types of slow breathing is the degree of cyanosis present. The cases in Table 4 invariably showed a fair degree of cyanosis while the cases of pyloric stenosis as a rule none at all. The explanation of this is not clear. Theoretically, the high CO_2 content should predispose to anoxæmia, since CO_2 shifts the oxyhæmoglobin dissociation curve to the right, that is, the more CO_2 present the less the degree of oxygen saturated, and hence the greater the amount of reduced hæmoglobin.

TABLE 4.

TOTAL CO_2-CONTENT OF BLOOD IN NON-OBSTRUCTIVE CASES WITH REDUCED RESPIRATORY RATE.

Case	Diagnosis	Respiratory rate (per min.)	Total CO_2 (vol. per cent.)
J.L.	Encephalitis Lethargica	14	56·5
,,	,, ,, ...	8	62·7
,,	,, ,, ...	8	67·0
M.M.	Cerebral hæmorrhage	10	57·5
R.M.	Prematurity	10	55·0
W.D.	Congenital heart disease	4	52·7
J.R.	Prematurity	12	60·6
J.G.	Meningitis	12	57·1
M.C.	Pyuria	18	55·4

Relationship of the CO_2 Content and Vomiting. The relationship between the total CO_2 content of the blood and the severity of the vomiting is not close. It is, however, admittedly difficult to gauge the amount of vomitus and, what is even more important, the character of the vomitus, for example, the amount of chlorine present. An attempt has been made in Table 5 to correlate these two factors but we feel that too much stress should not be laid on it. When the records of individual patients are examined, it is found that, occasionally, the total CO_2 content of the blood may remain high despite the fact that there had been no vomiting for several days prior to its estimation. This might be explained by the length of time which it takes for the blood to return to its normal composition. But in one patient, vomiting can have played absolutely no part in producing the increase in the CO_2. This was the case already referred to, who, although suffering from pyloric stenosis, did not vomit either previous to admission or during a four weeks' stay in hospital. The total CO_2 content of the blood reached the value of 102 vol. per cent., and the CO_2 dissociation curve showed clearly its increased capacity of holding CO_2.

TABLE 5.

SHOWING CORRELATION BETWEEN CO_2 CONTENT, CHLORIDE CONTENT, AND VOMITING IN CASES OF PYLORIC STENOSIS

Severity of vomiting	Nil	Slight	Moderate	Severe
Total CO_2 vol. % (average)	102·9*	77·2	97·6	103·2
Cl mgrm. % (average)	——	260	250	180

* Only two cases.

In patients suffering from vomiting, and even from severe vomiting, due to causes other than pyloric stenosis, one rarely if ever finds the CO_2 content raised. It must be remembered, however, that it is not the mere presence of vomiting but rather the nature and amount of the material vomited which is of significance. One nevertheless feels justified in concluding from a study of this series of pyloric stenosis that the severity of the vomiting is no measure of the metabolic disturbance as evidenced by the increase in the total CO_2 content of the blood.

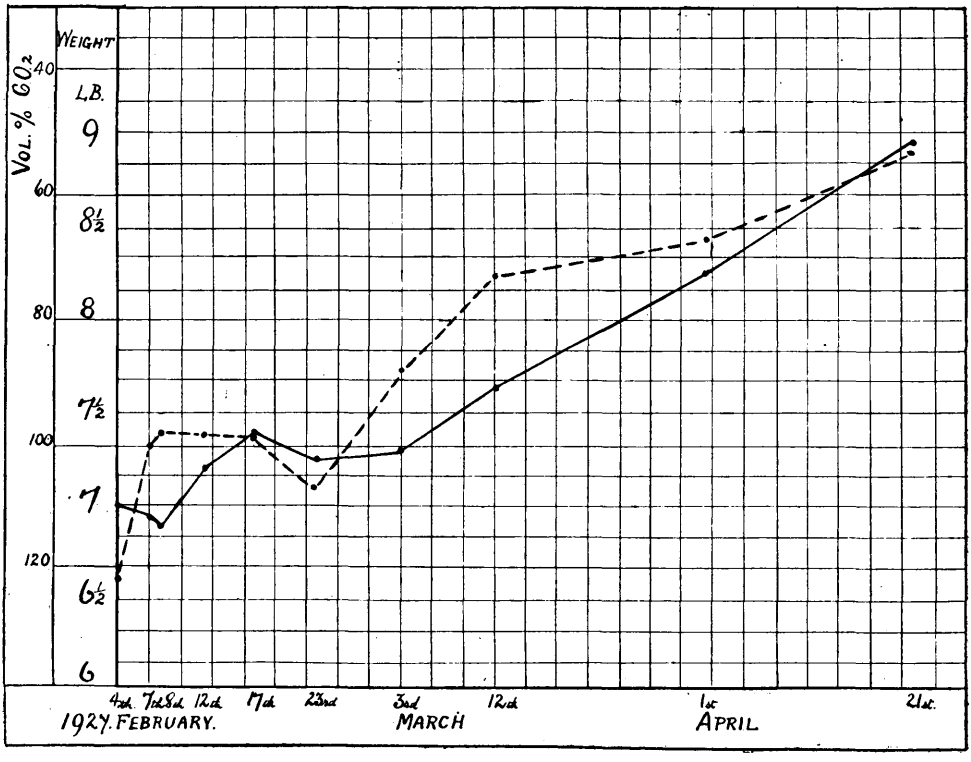
The total CO_2 content of the blood and the general nutrition. An association between the fall of the CO_2 to normal and the increase in the weight of the infant is illustrated in Chart II. This correlation holds good only for the same individual and therefore an isolated observation on the CO_2 content cannot be taken as a prognostic sign. A great increase in the CO_2 content does not necessarily mean a worse prognosis than a moderate rise. Furthermore, there does not seem to be any relationship between the level of the CO_2 and the length of time the vomiting has existed.

The association between the fall in CO_2 and the improvement in general nutrition was recognized to be simply the result of concomitant events and not dependent on cause and effect. Nevertheless, an attempt was made in a few

instances to influence the CO_2 content of the blood and determine whether such change resulted in clinical improvement. In one case, by means of transfusion, the CO_2 value was reduced from 98 vol. per cent. before transfusion to 70·2 vol. per cent. five minutes after transfusion. The donor's blood, of which 110 c.cm. were given along with 40 c.cm. normal saline, contained 62·1 vol. per cent. CO_2. Assuming the volume of the infant's blood to be 200 c.cm. (weight of infant 3·1 kilos) then 75·5 vol. per cent. represents the value one would calculate from a mixture of the two bloods and the saline. There was no apparent change in

CHART II.

To show increase in Weight and Fall in total CO_2 content of Blood in case of infant J.B.



the clinical condition of the infant and this lowering of the CO_2 content was not permanent, as within four days it had risen again to 97·8 vol. per cent. In another case, the infant's blood was withdrawn twenty hours after transfusion but the CO_2 content was practically unaltered from the pre-transfusion value. In two cases calcium chloride was given. In one the CO_2 content was reduced from 110 vol. per cent. to 62·3 vol. per cent. although the clinical condition was unchanged, while in the other, the CO_2 content was unaffected.

The CO_2 content of the blood and its reaction. The question arises whether the increase in the CO_2 content is indicative of a non-gaseous alkalosis or of a gaseous acidosis. The CO_2 dissociation curve of the blood shows a shift

to the left, *i.e.*, the capacity to hold CO_2 is increased, a finding equally characteristic of either of the above two conditions. It is obvious that if the depressed breathing is primary, the condition is one of gaseous acidosis such as occurs in subnormal activity of respiratory function. If, however, the increase in CO_2 content is the cause of the diminished respiratory exchange, the condition must be one of non-gaseous alkalosis. Ellis[8] has given details of the findings in two cases of high intestinal obstruction in both of whom there was an alkalæmia, as indicated by the pH of the blood. In a few instances in our series, the pH of the blood has been determined. The figures obtained are found in Table 6. It will be seen that if any change from the normal does occur, it is toward the alkaline side. In contrast with this finding, it is interesting to note the pH figure in the case of J.L. suffering from encephalitis. Here, although the respiratory rate was only eight per minute, the total CO_2 content was 67 vol. per cent. and the pH 7·25. This slightly increased CO_2 value and the pH on the acid side of normality indicate a gaseous acidosis. One can, therefore, conclude that in pyloric stenosis, the disturbance of the acid base balance is toward the alkaline side and that the change in the respiratory volume is secondary.

TABLE 6.

pH FINDINGS AND THE RELATIONSHIP TO CO_2 CONTENT OF BLOOD.

Name	Diagnosis	Respiratory rate (per min.)	Total CO_2 (vol. %)	pH
J.C.	Pyloric stenosis	?	140·0	7·76
R.R.	,, ,, ...	15	110·0	7·33
W.B.	,, ,, ...	?	73·2	7·35
McL.	,, ,, ...	?	53·4	7·39
M.R.	,, ,, ...	24	110·0	7·52
J.L.	Encephalitis lethargica...	8	67·0	7·25

The presence of such a degree of alkalosis as suggested by the high CO_2 values would lead one to expect the frequent occurrence of tetany. Signs of increased muscular irritability, as a matter of fact, have been reported frequently in high intestinal obstruction in the adult, as well as that produced experimentally in dogs. Convulsions, although said to be common by some observers, are in our experience rare in pyloric stenosis of infancy. In only two cases was there any such history obtained. In one of these laryngismus was an associated symptom. In no case tested was the neuro-muscular excitability increased as one might have expected. The reason for this absence of the signs of tetany may in part be due to the increased blood calcium content which we have found as high as 14 mgrm. per cent.

The cause of the alkalosis presumably is the increased amount of available base resulting from a deficiency of chlorine. It becomes necessary for the CO_2 content to rise in order to combine with this base.

Fig. I (a) adapted from Gamble's paper shows that CO_2 is one of the acid radicles combined with fixed base, the others being Cl, HPO_4, SO_4, organic acid and protein. Of all these acid radicles, CO_2 is the most mobile and elastic, being very easily retained or dispensed with by the activity of the respiratory centre. In Fig. I (b) is represented the state of affairs found in pyloric stenosis. It will be seen that the excess of CO_2 makes up for the deficit of Cl. The results recorded in Table I indicate that this balancing of diminished Cl by increase of CO_2 although generally complete is not necessarily the rule. Unfortunately, we have not obtained complete data in any one case. The figures for fixed base indicate that it is practically unchanged. Our data for acid radicles other than

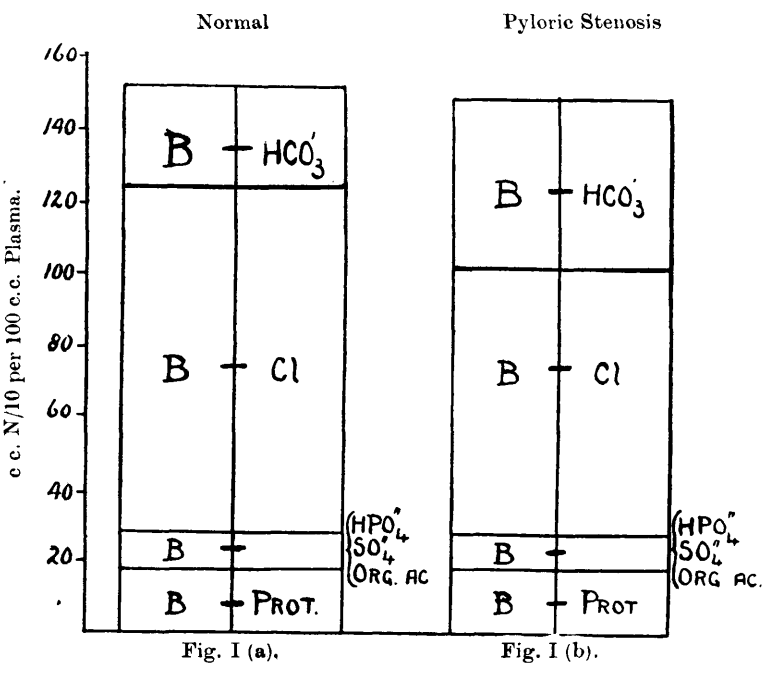


Fig. I (a). Fig. I (b).

Cl and CO_2 are scanty. In two cases we found values for inorganic phosphorus of 10·5 and 11·6 mgm. per cent. respectively. Atchley and Benedict[10] maintain that they can demonstrate an increase in the phosphate, sulphate and protein of the blood sufficient to account for the diminution in the ($Cl+CO_2$) value. Gamble[6] postulates an increase in organic acid. The ketone content of the blood has not been estimated in this series but frequently we have noted a fair degree of ketonuria. This may be consequent on the inanition (starvation), or due to alkalæmia or to both factors, and it is quite probable that these ketone acids take part to a certain extent in the compensation for the diminished $Cl+CO_2$ content.

The behaviour of the chlorides:—The fall in the blood chloride is one of the most constant changes in the biochemical findings. The reduction seems to

bear some relationship to the severity of the vomiting (Table 5), even although this relationship is masked by the inability to determine the actual amount of vomitus and its chloride content. The urine contains but a trace of chlorine, which is to be expected if chlorine is a threshold substance. Following the intravenous injection of a solution of normal saline practically all the water is excreted whereas approximately 80 per cent. of the chlorine is retained. In infants suffering from vomiting from other causes, only about 10 per cent. is retained. These results are shown in Table 7. In the two infants who were vomiting from causes other than pyloric stenosis, the amount of Cl retained was 11·8 and 7·0 per cent. respectively. In the cases of pyloric stenosis, 80·7 and 81·8 per cent. were retained. After operation in one case only 2 per cent. was retained which indicates that the condition was relieved. This retention of Cl in the examples of pyloric stenosis indicates definitely a chlorine hunger of the tissues. Hartmann and Smyth[1] maintain that severe vomiting may also lead to a depletion of the blood chloride. While it is true that the chloride may be somewhat reduced, it appears evident that the metabolism of chlorine in non-obstructive cases presents a different picture from that in pyloric stenosis.

TABLE 7.

CHLORIDE CONTENT OF URINE AND PERCENTAGE ABSORPTION OF CHLORIDE IN CASES OF PYLORIC STENOSIS AND NON-OBSTRUCTIVE VOMITING.

Name	Volume of urine	Amount of saline injected (cc.)	Chloride in urine		Percentage of chloride retained	Remarks
			Per cent.	Total		
W.B.	91	—	0·585	0·532	—	Feeding case.
	205	100	0·647	1·326	11·8	Vomiting +
R.W.	370	—	0·303	1·120	—	Meningitis.
	450	80	0·420	1·890	7·0	Vomiting +
J.R.	265	—	0·048	0·128	—	Pyloric stenosis.
	330	60	0·07	0·232	80·7	
C.C.	232	—	nil	nil	—	Pyloric stenosis
	350	100	0·047	0·164	81·8	(before operation)
	784	—	0·738	5·786	—	(After operation).
	920	100	0·725	6·670	2·0	

DISCUSSION.

Several views have been put forward as to the cause of the biochemical picture in high intestinal obstruction. That most commonly held is the one suggested by MacCallum[3] and elaborated by Gamble[6] and his colleagues. They believe the primary factor to be the loss of chlorine, base, and water by vomiting. This impoverishment of body chlorine is compensated for by the retention of

CO_2 with consequent production of an alkalosis. Haden and Orr[5], however, previously pointed out that a similar condition could be produced by experimental pyloric obstruction in rabbits, animals which do not vomit. But Gamble and McIver[11] were able to show that in rabbits there followed a very marked dilatation of the stomach into which the chloride was secreted and virtually lost to the body despite the absence of vomiting. This view certainly seems to explain all the experimental findings in animals. Ellis records the case of a patient suffering from carcinomatous obstruction of the pylorus with symptoms of tetany and biochemical evidence of a severe alkalæmia. There was no free hydrochloric acid in the gastric juice. The vomiting was severe, entailing in all probability a great loss of neutral chlorides. An explanation to fit this case is found in the fact that the reserve of fixed base is greater than that of chlorine so that the former is maintained at a more or less normal level in the blood while the chlorine content falls despite the fact that equivalent amounts of base and chlorine are lost in the vomitus.

In the analysis of the results in this series there is undoubtedly some relationship between the depletion of the blood chloride and the severity of the vomiting, and this in spite of the difficulty in estimating the latter factor and the great variation which is bound to exist in the chloride content of the tissues of different individuals. There is, however, one case in our series which does not permit of explanation on the basis of this hypothesis. This is the case of the infant referred to previously who did not vomit. He was admitted to the ward at the age of four weeks with the diagnosis of mental deficiency and pyloric stenosis. Visible peristalsis was readily demonstrated and there was a tumour palpable in the region of the pylorus. This infant was in the ward for four weeks and finally succumbed to a secondary infection. At the post-mortem examination, typical hypertrophic pyloric stenosis was revealed. During his residence in hospital he took his feeds well but never once vomited. He was not constipated. He presented the typical clinical picture of a severe alkalosis and the total CO_2 content of the blood was 102 vol. per cent. The respiratory rate averaged about 12 per minute. Unfortunately the blood chlorides were not estimated. It is difficult to understand how the depletion of the chloride resulted in this particular instance. It is highly improbable that the chlorine lost to the body during the four weeks' stay in hospital could have been lost *via* the gastric secretions. We have no information regarding the renal efficiency in this case.

Another hypothesis which has been advanced is that the condition is in part due to renal inefficiency. The chief evidence in favour of this view is the presence of an increased non-protein nitrogen content of the blood. At first this increase was attributed to increased destruction of tissue proteins as a result of toxæmia (Cooke, Rodenbaugh and Whipple[12]). Brown, Eusterman, Hartmann and Rowntree[7] suggested that the increase in the blood urea is the result of inefficient renal activity and concluded that the tetany-like symptoms are really uræmic in nature. In their paper they give details of the macroscopic and microscopic appearances of toxic nephritis occurring in six cases of fatal pyloro-duodenal obstruction. Ellis supported this view by his finding of a

low percentage excretion of urea despite a high blood urea, and by the fact that an alkalosis can be produced by administration of alkali only when the renal function is impaired. In one of the two cases reported by him there was pyæmia involving the kidney and in both there was noted the excretion of an acid urine (pH=5·6) associated with an over-alkaline plasma.

Hartmann and Smyth[1] have given figures which lead them to believe that the increase in N.P.N. is for the purpose of maintaining the normal osmolar concentration. They calculated the molecular value of the excess N.P.N. on the assumption that it is all urea and that two molecules of urea constitute one milli-equivalent. In Table 1 we have attempted to do the same and the findings occasionally lend some support to their view. Especially is this the case in the patient W.A. whose blood was analysed on two separate occasions. On the second occasion there was a more marked diminution in chloride with an increase in the CO_2 which did not compensate for the extra chloride deficiency. On calculating the value for the excess N.P.N. it is found that the deficiency is almost exactly balanced by the rise in N.P.N. Many of the other results, however, fail to support the view. Thus in the blood of A.M. or W.G. the decrease in the combined CO_2 plus Cl value is not associated with any increase in the non-protein nitrogen. Two possible explanations of these discrepancies suggest themselves, first in the excessive formation of ketone bodies and second, an increase in the base binding power of the proteins. Atchley and Benedict[10] have shown that owing to anhydræmia, the serum protein is increased in amount. Its base-binding power depends in addition on the pH of the medium. The more alkaline the plasma the greater is the capacity of the protein to hold base.

A third view was put forward in a series of papers by Haden and Orr[5]. They suggested that the chlorine was fixed in the tissues by some toxin and supported this view by the beneficial results obtained from the administration of chlorides. This can be equally well explained on Gamble's theory which Haden and Orr[14] in a recent paper apparently accept. Drake and Tisdall[13] have obtained similar results to those found in experimental obstruction by the injection of histamine. They found that there was no relationship between the decreased Cl content and the degree of intoxication which was, however, closely related to the concentration of non-protein nitrogen. Further they concluded that the decreased Cl content was not due to loss in the gastric secretions since apomorphine which led to a greater loss of Cl in the vomitus did not induce any diminution in the plasma Cl. Our results do not throw any light on this point.

Conclusions.

1. Pyloric stenosis in infancy is associated with an alkalosis presumably produced by a loss of the acid radicule chlorine from the body. The loss of chlorine is roughly related to the severity of the vomiting. The total CO_2 content varies inversely with the blood chlorides. The fixed base as a rule is unaltered or at the most only slightly diminished.

2. The rise in CO_2 is evidenced by a depression of the breathing (Biot type of respiration), which affords a ready method of its clinical recognition.

3. Certain cases occur which do not permit of the explanation of loss of chlorine by vomiting. It would appear that in such cases another factor, possibly impairment of renal function is present.

4. The conclusion that the rise in the non-protein nitrogen occurs in order to restore the osmolar concentration of the blood is not always justified.

We desire to express our thanks to the Medical Research Council for their help in this work.

Analytical methods used:—Blood—Total CO_2—Haldane[15]: Chloride—Whitehorn[16]: Non-protein Nitrogen—Folin-Wu method: Fixed Base—Stadie and Ross[17]: Calcium—Kramer and Tisdall's method: Phosphorus—Tisdall's method: pH—Dale and Evans[18]. Urine chloride—Volhard's method.

1. Hartmann, A. F., & Smyth, F. S., *Amer. Jour. Dis. Child.*, Chicago, 1926, XXXII, 1.
2. McCann, W. S., *Jour. Biol. Chem.*, N.Y., 1918, XXXV, 553.
3. McCallum, W. G., Lintz. J., Vermilye, H. N., Leggett, T. H., & Boas, E., *Bull. J. Hopk. Hos.*, Baltimore, 1920, XXXI, 1.
4. Hastings, A.B., Murray, C. D., & Murray, A. A., *Jour. Biol. Chem.*, N. Y., 1918, XXXVI, 223.
5. Haden, R. L., & Orr, T. G., *Jour. Exper. Med.*, N.Y., 1923, XXXVII, 377.
6. Gamble, J. L., & Ross, S. G., *Jour. Clin. Inves.*, Baltimore, 1924–25, I, 403.
7. Brown, G. E., Eusterman, G. B., Hartmann, H. R. & Rowntree, L. G., *Arch. Inter. Med.*, Chicago, 1923, XXXII, 425.
8. Ellis, A., *Quart. Jour. Med.*, Oxford, 1923–24, XVII, 405.
9. Hardt, C. L., & Rivers, A. B., *Arch. Int. Med.*, Chicago, 1923, XXXI, 171.
10. Atchley, D. W. & Benedict, E. M., *Jour. Biol. Chem.*, N.Y., 1927, LXXV, 679.
11. Gamble, J. L., & McIver, M. A., *Jour. Clin. Invest.*, Baltimore, 1924–25, I, 531.
12. Cooke, J. V., Rodenbaugh, F. H.,& Whipple, G. H., *J. Exp. Med.*, N.Y., 1916, XXIII, 717.
13. Drake, T. G. H., & Tisdall, F. F., *Jour. Biol. Chem.*, N.Y., 1926, LXVII, 91.
14. Haden, R. L., & Orr, T. G., *Jour. Amer. Med. Assoc.*, Chicago, 1928, XCI, 1529.
15. Haldane, J. S., *Jour. Path & Bact.*, Edinburgh, 1920, XXIII, 443.
16. Whitehorn, J. C., *Jour. Biol. Chem.*, N.Y., 1921, XLV, 449.
17. Stadie, W. C., & Ross, E. C., *Jour. Biol. Chem.*, N.Y., 1925, XLV, 735.
18. Dale, H. H., & Evans, C. L., *Jour. Physiol.*, London, 1920, LIV, 167.

OBSERVATIONS ON THE CHLORIDE METABOLISM IN CONGENITAL PYLORIC STENOSIS

BY

NOAH MORRIS, M.D., and STANLEY GRAHAM, M.D.

(From the Department of Pædiatrics, Glasgow University, and the Biochemical Laboratory, Royal Hospital for Sick Children, Glasgow.)

A fall in the chlorine content of the blood in cases of high intestinal obstruction is a well-recognized biochemical finding. The opinion is generally held that the diminution in the level of the blood chlorine is due primarily to the loss of chlorine by the vomitus. Accompanying this decrease in chlorine there is an increase in the CO_2 content, resulting in a non-gaseous alkalosis with a diminution in the respiratory exchange. This increase in CO_2 would appear to be the result of the body's effort to maintain the ionic concentration of the blood at the normal level, and simultaneously, to compensate the deficiency in the acid radicle. Very strong experimental proof has been brought forward by Gamble[1] and others in favour of the truth of this hypothesis.

In a previous communication[2] we have shown that the blood chlorine tends to be low in congenital pyloric stenosis, the diminution being roughly related to the severity of the vomiting. But we have seen cases in which there was present a typical picture of alkalosis, as indicated by the raised blood CO_2 content and the depressed breathing, either during a period in which no vomiting occurred or, as in one instance, when there was a complete absence of vomiting. In Table 1 examples are given in which the depressed breathing was a feature despite the comparatively normal value for the blood chlorine. Incidentally, it shows that there is not necessarily a correlation between the

TABLE 1.

SHOWING THE BLOOD CHLORINE VALUES IN CASES OF PYLORIC STENOSIS WITH DEPRESSED BREATHING.

Name	Blood chlorides		Blood CO_2		Rate of breathing	Degree of vomiting	Urinary chloride
	mgrm. %	c.cm. N/10 %	Vol. %	c.cm. N/10 %			
T.C.	260	73·2	100·8	45·0	20 apn.	+++	nil
J.J.	260	73·2	98·8	44·1	24 ,,	occas.	haze
J.B.	320	90·1	—	—	25 ,,	++	+
R.S.	260	73·2	107·0	47·7	21 ,,	nil	nil
	320	90·1	102·4	45·7	20 ,,	v. occas.	nil

increase in CO_2 and the decrease in chlorine. Further, in our experience the raising of the blood chlorine to normal by the administration of saline, either intra-venously or otherwise, has never resulted in a definite fall in the CO_2 content or a restoration of the breathing to normal. The last case (R.S.) is an example of the statement made above that an increase of CO_2 and decrease of chlorine may occur during a prolonged absence of vomiting. Another point of interest is the absence of urinary chlorine despite a value for blood chlorine within normal limits. These instances, it must be admitted, are exceptions to the general findings of diminished chlorine accompanying symptoms. Nevertheless, several facts emerge which make one hesitate to assume that the findings in experimental intestinal obstruction hold good for pyloric stenosis in infancy.

Despite these findings of normal blood chlorine in a certain number of cases, we are convinced that the chlorine metabolism is definitely disturbed in congenital pyloric stenosis. We have published figures[2] indicating that a very marked retention of chlorine takes place in this condition after parenteral administration of NaCl, the retention greatly exceeding that which occurs in the normal infant as well as in infants who suffer from vomiting and depletion from any other cause. In general, it may be said that in pyloric stenosis never more than 20 per cent. of the amount injected leaves the body by the urine. It may be urged that this is due to the excessive excretion and loss of chlorine in the vomitus. In several instances, however, we have been fortunate enough to have had the opportunity of estimating the urinary chlorine excretion during periods when vomiting has been reduced to a minimum or even absent. These results are recorded in Table 2. It is remarkable that the greatest retention occurred when the vomiting was least (J.D. and R.M.).

TABLE 2.

SHOWING THE PERCENTAGE RETENTION OF CHLORINE DURING PERIODS OF MILD VOMITING OR ABSENCE OF VOMITING.

Name	Date	Amount of NaCl injected grm.	Volume of urine	NaCl excreted		Percentage retention Cl	Number of vomits
				Per cent.	Total		
J.D.1	24. 5.29		220	0·059	0·130		1
	25. 5.29	1·08	170	0·105	0·179	95·4	0
2	3. 9.29		800	0·012	0·096		0
	4. 9.29	0·99	536	0·023	0·123	97·3	0
J.R.	27. 4.29		265	0·048	0·128		2
	28. 4.29	0·54	335	0·070	0·232	80·7	1
C.C.	17. 3.29		232	0·0	0·0		0
	18. 3.29	0·99	350	0·047	0·164	83·4	2
R.M.	10.10.29	0·90	67	0·006	0·004		1
	11.10.29		129	0·059	0·076	92·0	0

In the cases of J.D. and R.M., the investigation took place after a period of four weeks during which there was comparatively little vomiting. It is also interesting to note that in the second observation on J.D., the blood

chlorine was 265 mgrm. per cent. (74·6 c.cm. N/10 per cent.) prior to the intravenous injection of saline. It is evident, therefore, that the body retains chlorine in amounts exceeding the normal whether as a result of fixation in and about the tissues or because of inability to excrete the chlorine in the normal way. Further, the presence of normal values for the blood chlorine in two of the cases make it clear that the retention of chlorine may take place even when the blood has its full complement. It has been suggested by some that the renal excretion is defective and the presence of the high blood non-protein nitrogen is adduced as evidence in favour of this view. In the first place a high blood non-protein nitrogen is by no means an invariable accompaniment of pyloric stenosis, although it is present in the most serious cases, whereas a disturbance in the chlorine metabolism, so far as we have observed, is a constant accompaniment of the condition. Also, we have on several occasions estimated the urea output and have found in all a normal percentage and total output of urea. In a few cases the retention of fixed base was determined after the injection of normal saline (Table 3).

TABLE 3.

COMPARING RETENTION OF CHLORINE AND FIXED BASE IN CASES WITH AND WITHOUT PYLORIC STENOSIS.

Name	NaCl injected (c.cm. N/10)	NaCl retained (c.cm. N/10)		Fixed base retained (c.cm. N/10)		Remarks
		Actual	% of extra intake	Actual	% of extra intake	
J.R.	92·3	74·3	80·7	45·6	49·4	Pyl. stenosis
C.C.	169·0	141·0	83·4	67·0	40·0	,, ,, (before op.)
,,	154·0	1·0	0·6	28·9	18·7	,, ,, (after op.)
B.W.	154·0	22·0	13·3	50·7	33·0	No pyl. stenosis

It is seen that the pyloric cases showed a much lower retention of the extra base than of the extra chlorine, whereas after operation and in the feeding case the reverse occurred. It would seem, therefore, that in pyloric stenosis there is a preferential retention of chlorine over fixed base, and that the reverse holds good in the non-pyloric cases.

Gamble and Ross[1] have shown that in dogs with experimental occlusion of the pylorus there is a deficiency both of fixed base and chlorine, the latter being the more marked because of the presence of HCl in addition to the BCl in the vomitus. This explanation is not wholly satisfactory as in the cases detailed vomiting was very slight. Further, we have never been able to demonstrate the presence of free HCl in the stomach contents. Neutral chlorides, however, were always present. We would therefore suggest that the chlorine is retained in the body, partially at any rate, in a form other than BCl.

Rominger and his co-workers[3] have shown that in the normal infant more sodium than chlorine is retained after the administration of NaCl. These

same observers have also pointed out that chlorine may be retained apart from water—in other words, a dry retention of chlorine. In view of these findings with urea and fixed base it appears improbable that defective renal excretion provides an adequate explanation for the high retention of chlorine after parenteral administration of NaCl.

These observations on the retention of chlorine led us to investigate the possibility of the existence of a partial chlorine vacuum in the tissues in the fatal cases. Considering the importance of chlorine to the body economy, remarkably few investigations appear in the literature as to the variation in the content of the tissues. Von Noorden[4] gives 0·188 per cent. Cl (52·9 c.cm. N/10) as the average of the bodies of new-born infants, the extreme values being 0·138 per cent. and 0·194 per cent. Analyses of individual tissues in the human subject are rare. Observations have, however, been made on adult tissues by Katz[5], Moraczewski[6] and Hutchison[7] who report figures varying from 0·070 per cent. (19·7 c.cm. N/10) in muscle to 0·219 per cent. (61·7 c.cm. N/10) chlorine in lung. Since no figures pertaining to individual tissues of infants could be found, it was considered advisable to obtain normal standards for the chlorine content of the various tissues as well as to determine the chlorine content of the tissues in the fatal cases of pyloric stenosis. One appreciates, of course, that actually no truly normal tissues can be had, but from the post-mortem material of those cases in which there was no reason to suspect any such change as has been observed in pyloric stenosis, we obtained samples of various tissues. In all, tissues from six cases were analysed (Table 4).

TABLE 4.

SHOWING THE CHLORINE CONTENT OF THE VARIOUS TISSUES OF THE INFANT, EXPRESSED IN C.CM. N/10.

Group	A			B			C		
Tissue	Normal cases (six)			Pylorics to whom no saline has been given (4 cases)			Pylorics to whom saline had been given (4 cases)		
	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Muscle	54·5	38·2	43·2	31·5	20·0	24·6	69·8	46·0	60·9
Liver ..	45·9	25·6	36·5	30·3	17·8	23·0	67·6	32·5	50·6
Lung ..	62·5	47·5	52·4	38·6	19·1	30·7	84·8	37·6	56·6
Heart ..	41·9	32·7	37·7	16·8	16·5	16·7	48·6	22·2	32·2
Kidney	44·8	39·8	42·1	29·2	15·9	23·0	73·2	29·0	49·2
Brain ..	58·5	35·9	48·9	41·5	8·2	24·9	62·8	39·7	48·9
Skin ..	47·7	32·5	39·2	25·2	19·1	24·5	65·4	39·4	56·3

The method used in estimating the chlorine content of the tissues was the one elaborated by Van Slyke[8]. Portions of the various organs were obtained at the time of the post-mortem examination, and in each case were minced, mixed and weighed as soon as possible. All determinations were done in

duplicate because of the possibility of error due to the unequal distribution of the tissue juices throughout the organ. Duplicates were consistent in all cases, the error never exceeding 10 per cent. and usually being much less. In Table 4, the results of the analysis of six such normal cases are shown (Group A) as well as the figures for the analysis of the tissues of eight fatal cases of pyloric stenosis in four of whom saline had been given (Group C) and four where no such treatment had been adopted (Group B). To conserve space, only the maximum, minimum and average figures have been quoted. One case, P.T. (Case 3), is not included in any group, since this infant, although not receiving any saline, had a very extensive broncho-pneumonia accompanied by a marked œdema.

The individual values of the various tissues in the cases of Groups B and C are given in Tables 5 and 6.

TABLE 5.

SHOWING THE CHLORINE CONTENT (NaCl C.CM. N/10%) OF THE VARIOUS TISSUES OF OF FOUR FATAL CASES OF PYLORIC STENOSIS TO WHOM NO SALINE HAD BEEN GIVEN.

Name	Muscle	Liver	Lung	Heart	Kidney	Brain	Skin
W.F. ..	21·6	22·6	—	16·5·	20·7	8·2	25·2
A.S. ..	25·4	21·2	34·4	—	29·2	—	19·1
J.L. ..	20·0	17·8	19·1	—	15·9	—	19·2
J.R. ..	31·5	30·3	38·6	16·8	26·3	41·5	—

TABLE 6.

SHOWING THE CHLORINE CONTENT (NaCl C.CM. N/10%) OF THE TISSUES OF FOUR FATAL CASES OF PYLORIC STENOSIS TO WHOM SALINE HAD BEEN GIVEN.

Name	Muscle	Liver	Lung	Heart	Kidney	Brain	Skin
T.C. ..	—	32·5	37·6	22·2	29·0	39·7	39·4
J.J. ..	46·0	42·2	47·5	25·7	40·5	42·4	50·2
J.B. ..	67·1	60·2	—	48·6	54·1	50·7	65·4
R.S. ..	69·8	67·6	84·8	—	73·2	62·8	60·0

It will be noted that in those cases not receiving saline the chlorine content is very greatly diminished, the maximal values of this group being usually below the minimal in the normals. These results point very convincingly to the existence of a diminished chlorine content of the tissues in cases of pyloric stenosis. Of the four cases of the last group, all of whom had been given saline, the route being intra-venous or intra-peritoneal or both, with the exception of one case in which the saline was given by mouth, high values were obtained, the average for this series exceeding the averages obtained for the control group. It must therefore be concluded that the administration of saline is capable of raising the diminished tissue chlorine content to normal or even to values above normal.

In three patients œdema occurred during the course of the disease; in two the œdema seemed to be related to the administration of the saline but in the third case, P.T. (Case 3), previously mentioned as not having been included in any group, no saline had been given. Administration of saline in similar amounts to healthy infants or to those suffering from gastro-enteritis rarely leads to the production of œdema, although a sudden rise in weight presumably due to a retention of water may occur. The anomaly of the presence in excess of a fluid rich in chlorine in the pericellular spaces during a condition which is characterized by chlorine impoverishment seems worthy of comment and investigation. The significant details of these three cases are therefore discussed.

Case 1.—J.B., a male infant aged six weeks had been vomiting since one week of age. He was a small emaciated infant weighing 4 lb. 13 oz. Visible gastric peristalsis was present and a pyloric tymour was palpable. The breathing was shallow with definite apnœic periods. The rate was 25 per minute.

The progress of the case and the associated blood findings were as follows :—

13.11.29.—Admitted to ward. The blood examination revealed the following :—Total CO_2 content : 83 vol. per cent. (28·0 c.cm. N/10 per cent.). Chlorides : 225 mgrm. per cent. (63·3 c.cm. N/10 per cent.). Non-protein nitrogen : 68 mgrm. per cent. The urine contained no chlorine. 100 c.cm. normal saline injected intra-venously.

14.11.29.—Feet puffy. 100 c.cm. normal saline injected intra-venously.

15.11.29.—Definite œdema present. 100 c.cm. normal saline injected intra-venously.

16.11.29.—Blood chlorine : 320 mgrm. per cent. (90·1 c.cm. N/10 per cent.). Œdema more marked. Breathing still shallow.

19.11.29.—Œdema still present but much less than on 16th.

20.11.29.—No œdema. Blood chlorine : 320 mgrm. per cent. (90·1 c.cm. N/10 per cent.). 100 c.cm. normal saline injected intra-venously. Infant died.

At the commencement of the treatment, it seems safe to assume on the strength of the low blood chlorine content that there was a depletion of the chlorine content of the tissues. The first injection of normal saline led to a retention of 90·9 per cent. of the amount injected and simultaneously to the development of œdema. After 24 hours there was puffiness of the feet and the next day after the second injection, definite pitting on pressure. The ensuing injections of saline led to retentions of 86·8 per cent. and 56·3 per cent. respectively. In all, 2·7 grm. were injected and only 0·54 grm. excreted, *i.e.*, a retention of 78 per cent. Yet this took place during the onset and definite increase of œdema. During this period there was an increase in weight of 206 grm., which approximates the weight of saline retained on the assumption that the NaCl was retained in a 0·9 per cent. solution. Thus, the weight of saline injected was roughly 300 grm., the weight of normal saline excreted was 66 grm., leaving 234 grm. retained. The urinary output of chlorine gradually increased to the period ending November 16th and was followed by a rapid decline accompanied by a diminution of the œdema. On 19th, the weight was 57 grm. above that on 12th, while the total amount of intra-venously administered saline not lost in the urine was 1·49 grm. ,corresponding to 167 grm. of 0·9 per cent. NaCl solution. Two possibilities as to the reason for this discrepancy offer themselves. Either the retention of NaCl might have been only apparent, the loss taking place through the vomitus

or the chlorine might have been stored apart from water. It was unfortunately impossible to collect the vomitus accurately, but on one day during which the vomiting was the most marked of the period an attempt was made and the collection yielded 200 mgrm. of NaCl. As it was believed that approximately only half the vomitus was obtained, this would mean a loss by the vomiting of 400 mgrm. daily and for the six day period, 2·4 grm. whereas the amount not excreted by the urine over the same period was 1·5 grm. It is possible therefore that the extra chlorine was lost in the vomiting. However, the tissues were found to have a very high value for chlorine, varying from 0·266 per cent. (67·1 c.cm. N/10 per cent.) in heart muscle to 0·172 per cent. (48·6 c.cm. N/10 per cent.) in lung. With the exception of the brain all were above the maximum values for the control series. The saline given prior to the death of the infant contained 0·9 grm. NaCl (15·4 c.cm. N/10 per cent.) which could not possibly have raised the chlorine content of the tissues to such an extent.

In any case, whatever the amount lost by the vomiting, this infant stored chlorine in the tissues to a degree far exceeding the normal. That the storage of chlorine had taken place prior to the last injection of saline is indicated by the high normal blood chloride value (320 mgrm. per cent., 90·1 c.cm. N/10 per cent.) previous to the last injection.

This case also raises the question of why the urinary output of chlorine was so low in the presence of œdema. Evidence of impairment of renal function could not be obtained. The defect in metabolism would appear to be in the tissues themselves. The picture presented resembles that seen in ' nephrosis ' where the chlorine appears in minimal quantities in the urine although the power of excreting nitrogenous substances seems quite unimpaired. The first injection of saline would, owing to the depletion of the tissue chlorine lead to an outpouring of chlorine from the blood to the tissue. This outpouring would, however, go beyond the equilibrium point, a state of affairs that is well known in in vitro experiments, and which would take some time to adjust itself. Next day, in spite of the now partially replete tissue chlorine, the intra-venous injection of more saline, by suddenly raising the blood chlorine would again lead to the passage of chlorine to the tissues beyond the equilibrium point. The third injection would still further increase the amount of chlorine in the tissues. Forty-eight hours after the last injection of saline, the œdema commenced to disappear but without the simultaneous excretion of chlorine by the urine. Some of the chlorine was lost in the vomitus and some was retained in the tissues as revealed by the post-mortem analysis. Whatever the proportion retained, one is forced to the conclusion that the chlorine was present either in the tissues or the blood, or both, in a form which can only be excreted with difficulty by the kidney. The fact that prior to the injection of saline the blood chlorine content was 320 mgrm. per cent. (90·1 c.cm. N/10 per cent.) while the urinary chlorine was fractional in amount would suggest that the fault is either in the blood or the kidney.

It should also be mentioned that although the chlorine content of the blood on November 16th was slightly above normal the shallow breathing persisted. Unfortunately the CO_2 was not estimated, but the presence of the

depressed breathing is sufficient to raise doubt regarding the diminution of the blood chlorine being the sole factor in the production of the alkalosis. One might suggest that part of the chlorine was not united to base, thereby leaving an excessive amount of the latter to unite with CO_2. It seems to us that only by assuming that a portion of the chlorine is not united to base can one explain, first, the depressed breathing accompanied by a high CO_2, and secondly, the fact that chlorine does not appear in the urine although the body seems to have its full quota. The urinary findings in this case are given in Table 7.

TABLE 7.

SHOWING THE URINARY FINDINGS IN CASE 1 (J.B.) AND THE PERCENTAGE RETENTION OF NaCl AFTER INTRA-VENOUS INJECTION OF NORMAL SALINE.

Date (Nov. 1929)	Volume of urine	Urinary NaCl %	Urinary NaCl total	NaCl injected intra-ven. grm.	Percentage retention of NaCl
12—13	82	nil	nil	—	—
13—14	100	0·0819	0·0819	0·9	90·9
14—15	91	0·1304	0·1187	0·9	86·8
15—16	105	0·3744	0·3931	0·9	56·3
16—17	93	0·3159	0·2937	—	—
17—18	71	0·2457	0·1743	—	—
18—19	60	0·2340	0·1404	—	—
19—20	39	0·3276	0·1278	—	—
20—20	59	0·5850	0·3452	0·9	75·8

Case 2.—R.M., a male infant came under observation at the age of six weeks with a history of expulsive vomiting since two weeks of age. Gastric peristalsis was visible and a pyloric tumour readily palpable. The breathing was depressed with apnœic periods.

On admission the blood findings were as follows:—

Total CO_2 content: 114·8 vol. per cent. (51·2 c.cm. N/10 per cent.). Chlorine content: 190 mgrm. per cent. (53·5 c.cm. N/10 per cent.). Non-protein nitrogen: 57·6 mgrm. per cent.

The injection of 50 c.cm. normal saline led to an increased output of chlorine in the urine equivalent, however, to only 2·7 per cent. of the extra intake. A week later, four daily intra-venous injections of saline were given. Just before the commencement of this treatment the blood examination yielded the following figures:—

Total CO_2 content: 121·6 vol. per cent. (54·3 c.cm. N/10 per cent.). Chlorine content: 230 mgrm. per cent. (64·8 c.cm. N/10 per cent.). Non-protein nitrogen: 57·6 mgrm. per cent., and between the third and fourth injections the findings were:—

Total CO_2 content: 69·6 vol. per cent. (31·0 c.cm. N/10 per cent.). Chlorine content: 270 mgrm. per cent. (76·0 c.cm. N/10 per cent.). Non-protein nitrogen: 32 mgrm. per cent.

The output of chlorine was very low, averaging only 0·048 grm. per day over a period of ten days, and there was a rapid rise in weight which was associated with the presence of considerable œdema (pitting on pressure). The vomiting was fairly severe but apparently not sufficient to get rid of the chlorine in the œdematous fluid. Despite the almost normal chlorine content of the blood on the second last day, the amount of NaCl excreted was only 0·062 grm. About a month later the blood chlorine was 305 mgrm. per cent. (86·0 c.cm. N/10 per cent.) and the daily output in the urine only amounted

to 0·004 grm. and given after the injection of 100 c.cm. of normal saline, this was only increased to 0·076 grm. although vomiting only occurred once. This infant made an uninterrupted recovery without operation and on dismissal was excreting as much as 1·0 grm. NaCl daily. It would therefore seem that during the active stage of pyloric stenosis the chlorine is present in some form that cannot be excreted by the kidney.

Case 3.—P.T., a male infant thirteen weeks of age came under observation because of vomiting which had begun at one month of age. Visible gastric peristalsis seen and a pyloric tumour palpable. The infant was acutely ill on admission with a broncho-pneumonia involving both lungs. On admission, Dec. 23rd, 1929, the blood analysis yielded the following figures :—

Total CO_2 content : 95·4 vol. per cent. (42·6 c.cm. N/10 per cent.). Chlorine content : 230 mgrm. per cent. (64·8 c.cm. N/10 per cent.). Non-protein nitrogen : 68·2 mgrm. per cent.

The vomiting was marked on the two following days but slight on Dec. 25th. On the 26th there was an increase in weight of 115 grm. and on the 27th of 145 grm. Simultaneously with this increase in weight, the vomiting ceased and did not recur. On the 27th slight œdema of the feet was noticed. This œdema gradually increased and before death there was well-marked œdema of both feet and lumbar region. Analysis of the tissues revealed the chlorine content of the tissues to be within normal limits.

This case illustrates the development of œdema without the parenteral introduction of saline. It is possible that the pneumonic condition as such led to the œdema. In lobar pneumonia, a retention of chlorine with increase in weight is a well-recognized phenomenon. The broncho-pneumonia involved all lobes of the right lung and the lower lobe of the left lung while the upper lobe was the seat of acute emphysema. It may be that the extensive consolidation rendered difficult the escape of CO_2 which accordingly would be retained and probably displace the chlorine. The chlorine would be either passed to the tissues or lost by the vomit in order to make room for the excess CO_2. It will, however, have been noted that the rise in weight dates from the cessation of the vomiting and in view of these facts it is suggested that the chlorine from the BCl of the blood is shunted to the tissue spaces. Once in the tissue spaces, despite the gradual repletion of the tissue chlorine, the base chlorine cannot get back into the blood stream in sufficient amount to be excreted by the kidney owing to the fact that the CO_2 is holding up the base. Here again it is necessary to postulate either a renal defect or the presence of the chlorine in a form which cannot be dealt with by the kidneys.

The following case is briefly discussed because it lends additional support to the theory that the chlorine is present in a form other than BCl.

Case 4.—R.S., a female infant, came under observation at the age of 8 weeks. Vomiting had begun at the age of 3 weeks. Visible gastric peristalsis was present and a pyloric tumour palpable. The blood examination at this time was as follows :—

Total CO_2 content : 152·0 vol. per cent. (67·9 c.cm. N/10 per cent.). Chlorine content : 200 mgrm. per cent. (56·3 c.cm. N/10 per cent.). Non-protein nitrogen : 57·1 mgrm. per cent.

During the first fortnight in hospital the infant was given frequent intra-venous and intra-peritoneal injections of normal saline as well as saline by the mouth, and although vomiting was frequent no œdema was noted. The rise in weight following each injection was only temporary lasting usually not longer than one day. The blood chlorine, however, did increase and this was accompanied by a fall in the total CO_2 content. Three weeks after admission the blood was as follows :—

Total CO_2 content : 65·3 vol. per cent. (29·1 c.cm. N/10 per cent. Chlorine content : 305 mgrm. per cent. (86·0 c.cm. N/10 per cent.). Non-protein nitrogen : 65 mgrm. per cent.

Before the death of the infant the value for the blood chlorine was 380 mgrm. per cent. (107·0 c.cm. N/10 per cent.) an amount definitely in excess of normal. Although the chlorine content of the blood was more than replete, the breathing remained depressed. The analysis of the tissues for chlorine revealed very high values. It is difficult to understand how the great retention of chlorine took place without any signs of obvious œdema if the retention was in the form of inorganic chlorine. The urine was examined on several occasions and even when the blood chlorine content was high, it only contained a trace of chlorine. This finding by itself would lead to the conclusion that the chlorine must have been in great part present in a form other than BCl. The association of these various phenomena, namely the high chlorine content of the blood and tissues with no œdema, and the absence of more than a trace of chlorine in the urine seems to lead to no other conclusion.

Summary.

The observations recorded in the foregoing pages appear to justify the following conclusions :—

1. In untreated pyloric stenosis there is a partial chlorine vacuum in the tissues. This can be corrected by the administration of saline which, if continued, frequently leads to an excessive retention of chlorine and the production of œdema, even when the chlorine content of the blood is normal. In such cases the chlorine content of the tissues is higher than normal.

2. Restoration of the blood chlorine content to normal does not result in a correction of the alkalosis as is evidenced by the persistence of the high total CO_2 content and the depressed breathing.

3. A normal blood chlorine content may be accompanied by a fractional amount of chlorine in the urine.

4. To permit of a satisfactory explanation of these findings, it appears necessary to postulate either (1) the inability of the kidney to excrete chlorine : or (2) more probably, in view of the findings with urea and fixed base, the presence of chlorine in the tissues in a form other than BCl. Certain evidence is brought forward in support of the latter view.

We desire to express our thanks to the Medical Research Council by which the expenses of this investigation were defrayed.

REFERENCES,

1. Gamble, J. L., & Ross, S. G., *J. Clin. Invest.*, Baltimore, 1924–25, I, 403.
2. Graham, S., & Morris, N., *Arch. Dis. Childh.*, Lond., 1929, IV, 335.
3. Rominger, E., Berger, I., & Meyer, H., *Zeit. f. Kinderh.*, Berlin, 1929, XLVIII, 43.
4. Von Noorden, C., *Metabolism and Pract. Med.*, Lond., 1907, I, 416.
5. Katz, A., *Arch. f. d. ges. Physiol.*, Bonn, 1896, LXIII, 1.
6. Moraczewski, D., *Zeit. f. physiol. Chem.*, Strassburg, 1897, XXIII, 483.
7. Hutchison, R., *J. Path. Bact.*, Lond., 1898, V, 406.
8. Van Slyke, D. D., *J. Biol. Chem.*, Baltimore, 1923, LVIII, 523,

THE VALUE OF ALKALI IN SALICYLATE THERAPY

BY

NOAH MORRIS, M.D., and STANLEY GRAHAM, M.D.

(From the Department of Pædiatrics, University of Glasgow, and the Wards and Biochemical Laboratory, Royal Hospital for Sick Children, Glasgow.)

By virtue of their ability to control the arthritis of acute rheumatic fever, the salicylates have received a well-recognized place in the treatment of the rheumatic infection. Maclagan[1] of Dundee in 1874 commenced the use of salicin, a glucoside of salicylic acid. To Stricker[2], however, working in Traube's clinic in 1876, is given the credit of having first recognized the value of sodium salicylate. Broadbent[3] in the same year also wrote enthusiastically in favour of its use, and it is interesting to note that even at this time the value of the larger doses was appreciated, Broadbent recommending 7½ to 20 grains hourly.

In 1908 Lees[4] spoke of the salicylates as specific in the cure of rheumatism, believing that the failure to obtain good results was due to the smallness of the dose. He also emphasized the necessity of giving alkali in addition and, while recognizing that the tolerance of the patient was greatly enhanced by this procedure, stated that the alkali was of benefit because it neutralized the toxins elaborated by the infecting agent and because the bicarbonate per se tended to lessen the degree of cardiac dilatation. This idea of salicylate being 'as specific to rheumatism as quinine is to malaria or mercury to syphilis' was not wholly accepted even at that time, and at present the general view of the value of the salicylates in rheumatism would appear to be that they are specific only for the arthritis but have no influence on the other manifestations such as carditis, chorea, etc.

Signs and symptoms of intolerance to salicylates are not infrequently observed, especially where the larger doses are given unaccompanied by alkali, and hence the use of alkali in equal or greater amounts than that of the salicylate employed has achieved a certain amount of popularity in the prevention of the toxic manifestations. In the writers' opinion, the value of the combination of salicylate and alkali would seem to have been definitely proved by routine clinical work. Nevertheless certain observers still deny the necessity of the use of alkali. In a comprehensive monograph on the salicylates written in 1924, Hanzlik[5] categorically asserts that alkalis do not prevent or modify the appearance of toxic symptoms, and that the absence of toxic symptoms means absence of therapeutic efficiency. He quotes Meara[6] as stating that the use of alkali 'is directed more by tradition than rationale,' but Meara was discussing the alkali treatment of rheumatism rather than the beneficial effect of the addition of alkali to sodium salicylate.

At the Royal Hospital for Sick Children, Glasgow, it has been the custom to give sodium salicylate combined with twice the amount of sodium bicarbonate to every rheumatic patient during residence in hospital. The dosage of salicylate usually employed is 15 grains four-hourly, or 90 grains daily; occasionally 20 grains four-hourly (120 grains daily) were given. With these doses signs of intolerance are seldom observed. Vomiting is the only one which may be said

TABLE 1.

SHOWING TOLERANCE OF CHILDREN TO SODIUM SALICYLATE WITHOUT SODIUM BICARBONATE.

Name	Age Years	Daily dose of Sod. Salicyl. Grains	Duration of treatment. Days	Vomiting	Other signs of intolerance	Acetonuria
B.G.	7	50	40	nil	nil	nil
M.N.	12	50	23	nil	nil	nil
F.K.	10	60	35	1	nil	nil
I.J.	12	60	14	9*	nil	nil
J.McL.	9	60	48	1	nil	± When vomiting occurred.
J.W.	8	60	30	4	nil	± on 2 occasions.
K.C.	9	60	16	5	nil	± occasionally.
A.S.	6	90	3	3	Tremor, nervousness, acidosis.	± last day.
J.S.	7	90	4	2	Nervousness, headache, depression.	+ last day.
W.P.	7	90	2	2	Tremor, nervousness, headache.	nil

*This patient vomited twice before commencement of sodium salicylate administration.

to occur with any frequency and if Langmead's[7] advice is remembered and the bowels kept freely open, it is rare that any intermission of the drug therapy is necessary. There are, of course, cases in which there does seem to be a special idiosyncrasy to salicylate just as occurs with many other drugs, e.g., arsenic and morphine. In these circumstances it is advisable to commence with smaller doses which can be gradually increased till the usual amounts are

reached. Aspirin is thought by some to be tolerated better than salicylate of sodium, but actually the tolerance would appear to be related to dosage rather than to the particular form of salicylate used.

Clinical observations of the value of the added alkali.—A logical method of testing the value of the added sodium bicarbonate seemed to be to give certain patients salicylate alone and observe the ill-effects, if any, produced; then to add bicarbonate and note any diminution or alleviation of the toxic manifestations. It was soon found that 60 grains daily was the maximum amount which, without the addition of alkali, could be tolerated by the average child without toxic signs appearing. 50 grains daily in no case produced any ill effects, but 60 grains not infrequently produced slight vomiting which was not always attributable to constipation (Table 1).

If the daily amount was increased to 90 grains, signs of intolerance were invariably produced in 3 to 5 days, and in some cases these were of a severe and alarming nature. Vomiting was a constant and early sign and usually preceded all other manifestations of salicylism. Drowsiness and confused mental states or mental torpor occurred in some. Many became apathetic and disinterested, the speech in one instance was thick and slurring. Air-hunger of greater or less severity, but not necessarily in proportion to the other signs, occurred in all cases and sometimes to a marked degree; this was the typical acyanotic dyspnœa which is known to be associated with an acidosis of the acid-poisoning type. Acetonuria was present in the majority of the cases, but was never extreme and in a few cases was entirely absent. Tinnitus, strangely enough, was not troublesome and in the present investigation was not complained of by any of the patients. This manifestation, however, has occasionally proved troublesome in some patients receiving both sodium salicylate and sodium bicarbonate in the routine way. All the above signs and symptoms disappeared rapidly on discontinuing the salicylate, or on the addition of twice the amount of bicarbonate.

It is an interesting and practical observation that in those cases to whom salicylate and bicarbonate had been previously given, the signs of poisoning on omitting the alkali either did not develop, or if they did were not as severe as in those to whom no alkali had previously been given. From Table 2 it will be seen that of the 9 patients to whom sodium salicylate and sodium bicarbonate had previously been given, 4 showed no toxic symptoms, in 5 vomiting occurred and in only 1 were there headache and depression. The last child, it is interesting to note, had only had the alkali combined with the salicylate for a short period of 7 days.

It seems indisputable, then, that whatever else the alkali may do, it is extraordinarily valuable in preventing the development of the signs and symptoms of salicylate poisoning. So striking is this that, in our opinion, it is unjustifiable to prescribe large doses of salicylate without giving in addition sodium bicarbonate.

The nature of the toxic manifestations.—There is considerable difference of opinion regarding the nature of the toxic manifestations caused by the salicylates. Certainly the clinical picture of a moderately severe case is that

of a non-gaseous acidosis. There is definite air-hunger, the deep and frequent respirations being in some instances readily heard on entering the room. The beneficial effect of alkali would lend considerable support to this view, but so far no unequivocal evidence has been adduced to substantiate this. Hanzlik[5] does not consider the reaction to be of the nature of an acidosis, but none of his objections are incompatible with such a disturbance. He draws a comparison between salicylate poisoning and the condition seen in diabetic acidosis

TABLE 2.

SHOWING BENEFICIAL INFLUENCE OF PREVIOUS ADMINISTRATION OF SODIUM BICARBONATE ON TOLERANCE OF CHILDREN TO SODIUM SALICYLATE.

Name	Age : Years	Duration of administration of Sod. Sal. gr. 90+ Sod. Bic. gr. 180. Days	Vomiting	Duration of administration of Sod. Sal. gr. 90. Days	Vomiting	Other signs of intolerance
A.McG.	11	80	nil	28	nil	nil
J.W.	9	78	nil	18	nil	nil
M.J.	11	68	nil	33	Once on 6th day.	nil
M.F.	9	67	nil	20	nil	nil
M.J.	8	34	nil	11	nil	nil
R.McI.	9	28	nil	12	Once on 2nd day.	nil
D.W.	8	27	nil	15	Once on 8th day.	nil
R.S.	8	24	nil	23	Twice.	nil
A.McC.	11	7	nil	6	Twice on 5th day. Twice on 6th day.	Headache, depression.

which he puts down to the action of certain enol acids. He finds air-hunger and acetonuria, but because there is no shift in the hydrogen ion-concentration, concludes that there is no acidosis.

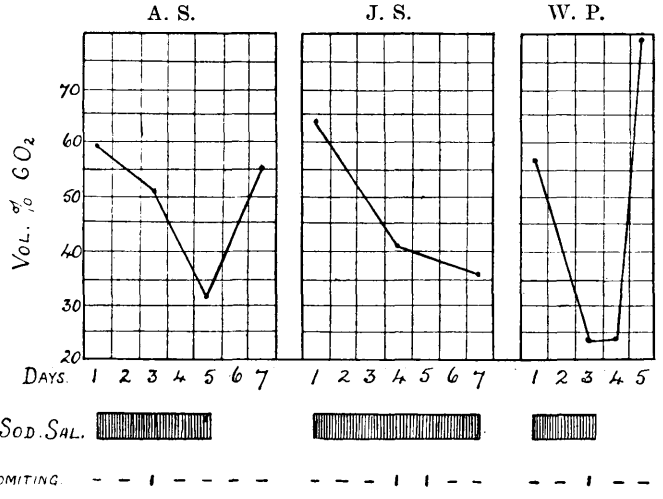
The behaviour of the CO_2 content of the blood.—The oral administration of sodium salicylate alone in doses amounting to 90 grains daily produced a marked fall in the CO_2 content of the blood in every instance. The extent of this diminution is shown in Fig. 1. In all cases in which the blood was examined the fall was to a figure below 40 vol. per cent. With values between this and

35 vol. per cent. symptoms such as vomiting and mild air-hunger began to appear. At 30 vol. per cent. symptoms were invariably present and frequently severe. On discontinuing the drug the blood fairly quickly returned to normal.

A fall in the CO_2 content of this extent must mean either the development of a non-gaseous acidosis or a gaseous alkalosis. If the air-hunger be secondary, and we believe it is, then the condition must be a non-gaseous acidosis. Changes in the pH of the blood do not necessarily have to be demonstrated in order to diagnose a state of acidosis. The differentiation of compensated and uncompensated states depends on the sensitivity of the method of determining the pH more than anything else.

Changes observed in the urine.—It has long been known that albuminuria occasionally appears during salicylate administration. This naturally leads to the assumption that salicylates damage the renal cells. Hanzlik and his

FIGURE 1.



EFFECT OF SODIUM SALICYLATE ON CO_2 CONTENT OF BLOOD.

co-workers have published results which indicate a diminution in renal functional efficiency. We studied in three cases the changes which occurred in the urine as the result of salicylate administration with and without the addition of alkali.

The urine was collected from each patient for 3 periods of 6 days each. The first was a pre-period, during which time no drugs were given. During period II sodium salicylate (six doses daily of 15 grn. in one, and 10 grn. in the other two cases) was given ; and during period III, the same amount of salicylate with twice the amount of sodium bicarbonate. During all three periods the diet, consisting of measured amounts of milk and sugar, was kept constant. In no case was there any loss by vomiting. These results are tabulated in Table 3.

From our investigations many interesting facts emerge, the most striking being the fall in the ammonia coefficient of the urine during period II. As is

well known a rise in this figure would be expected if the condition were an acidosis of the acid-poisoning type, and the fact that in each of the three cases there was a slight but distinct fall would appear to point to impaired renal function. It is of interest to mention here that one of us (N.M.) has recently had an opportunity of investigating a patient with cyclical vomiting during the course of an attack, and contrary to what was expected a distinct fall in the ammonia coefficient was observed at the height of the vomiting, although the CO_2 content of the blood was very low. During period III, as expected, the fall was pronounced. Further investigation strengthens the suspicion of impaired renal efficiency. In two of the subjects there was roughly a 20 per cent. reduction in the volume of urine excreted during period II ; in the third

TABLE 3.

CHANGES OBSERVED IN THE URINE AS A RESULT OF ADMINISTRATION OF SODIUM SALICYLATE WITH AND WITHOUT SODIUM BICARBONATE.

Name	Period	Volume in c.cm.	Titratable acidity in c.cm. N/10	Ammonia in grm.	Total Nitrogen. grm.	$\frac{NH_3N \times 100}{\text{Total N.}}$	Urea grm.	NcCl grm.
B	I	1,171	167·2	0·378	9·31	4·0	18·81	2·780
	II	954	255·3	0·311	8·22	3·8	13·24	1·906
	III	1,257	32·9	0·100	8·081	1·2	15·66	2·425
M	I	1,126	155·2	0·291	8·49	3·4	15·85	2·093
	II	1,125	222·3	0·231	7·68	3·0	15·43	1·545
	III	1,299	34·2	0·062	8·18	0·77	15·97	2·398
S	I	970	132·5	0·344	7·92	4·34	15·99	1·727
	II	721	176·9	0·189	7·41	2·70	13·11	1·061
	III	1,103	11·7	0·052	8·03	0·65	16·59	1·776

Period I =No medication.
Period II =Sod. salicylate.
Period III=Sod. salicylate+ sod. bicarbonate.

case there was no change. It is worthy of note that in all three instances the addition of the alkali resulted in a marked increase of urinary volume. The urea output also was diminished in the second period and increased by the addition of the alkali. The total nitrogen of the urine was similarly affected, while evidence of chloride retention on salicylate alone is very definite. It may well be that the fall in CO_2 of the blood and tissues is responsible for this retention of Cl in order to compensate for lost CO_2, but the other observations lend more support to diminished renal activity being the causal factor. The urea-concentration test (15 grm. urea given) also gave corroborative evidence (Table 4). Here again the alkali restored the urea-concentrating power of the kidney to a normal level. Blood examination did not reveal any positive indication of kidney damage. The non-protein nitrogen and chlorine content were as far as we found always within normal limits. Hanzlik states that while the non-protein nitrogen of the blood is somewhat diminished the urea

TABLE 4.

SHOWING EFFECT OF ADMINISTRATION OF SODIUM SALICYLATE WITH AND WITHOUT SODIUM BICARBONATE ON UREA-CONCENTRATING POWERS OF KIDNEY. (UREA CONCENTRATION TEST).

Name	B		M		S	
Period	II	III	II	III	II	III
% of urinary urea before administration of urea	1·56	1·08	1·86	0·90	1·86	1·20
% of urinary urea one hour after administration of urea 15 grm.	1·74	1·74	1·44	1·86	1·26	2·28
% of urinary urea two hours after administration of urea 15 grm.	1·44	2·86	1·22	2·58	1·24	3·01

nitrogen is increased. He also maintains that the impairment of kidney function occurs equally with and without the use of alkalis. Our results with the urea-concentration test (Table 4), as well as the improved urinary output of those patients receiving sodium bicarbonate as shown in Table 3, would appear to warrant the statement that sodium bicarbonate protects renal function. Albuminuria to us seemed of much more common occurrence when the patients were on salicylate alone.

Gastric secretion.—In view of the retention of chlorine resulting from administration of the salicylate alone, the effect on the gastric secretion of hydrochloric acid was studied. Leichentritt[8] found that after the administration of sodium salicylate to dogs there occurred a definite increase in the volume of the gastric secretion. Veil and Graubner[9], on the other hand, report cases in which one dose of a salicyl compound inhibited the formation of hydrochloric acid in the gastric juice. Our own results (Table 5) are inconclusive. It seems clear, however, that the secretion of acid is not necessarily prevented by salicylate, and that the diminished chlorine output in the urine cannot be explained by excessive secretion of gastric juice.

TABLE 5.

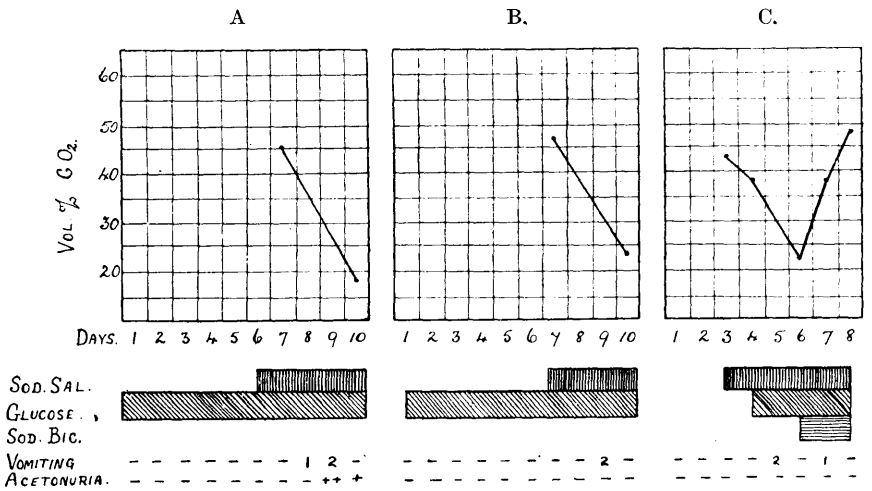
SHOWING EFFECT OF ADMINISTRATION OF SODIUM SALICYLATE WITH AND WITHOUT SODIUM BICARBONATE ON THE GASTRIC SECRETION OF HCl FOLLOWING EWALD TEST MEAL.

Name	M		S		M	
Period	II	III	II	III	II	III
c.cm. N/10 HCl in 100 c.cm. gastric juice	20	12	0	22	14	0

Response to glucose and alkali.—It has been stated by Hanzlik[5] that the symptoms of salicyl poisoning resemble those occurring in diabetic acidosis. He quoted references to states of ketosis occurring in poisoning from methyl salicylate and mentioned the observation of Hurtley and Trevan[10] on the similarity of the symptoms produced by intravenous administration of salicylate and sodium aceto-acetate. He brought forward the fact that salicyl gives a similar colour reaction with iron as does aceto-acetate as suggestive of the common cause of both types of intoxication.

It is true that occasionally acetone appears in the urine during administration of salicylate, but even in severe cases of poisoning it is by no means a constant constituent of the urine, and when present, it is rarely in greater amount than is indicated by a mild Rothera reaction. It may be urged that the impaired function is the cause of its non-appearance in quantity in the urine. The work of Myers and Ferguson[11] indicates quite clearly that both

FIGURE 2. (A. B. & C.)



EFFECT OF GLUCOSE AND $NaHCO_3$ IN PREVENTION OF FALL IN CO_2 CONTENT OF BLOOD.

in rabbits and man administration of sodium salicylate leads to little change in the percentage content of acetone bodies in the blood, although some of the rabbits received fatal doses of salicylate. The fact that acetone was said to play a part in the production of symptoms led us to investigate the effect of salicylate administration on the blood sugar as well as the influence of glucose ingestion in the prevention and amelioration of symptoms.

The fasting blood sugar was invariably higher during the period of salicylate ingestion, by about 15 per cent. It would seem, therefore, that there is available in the blood-stream a sufficiency of glucose. It is possible that owing to the action of salicylate the tissues are unable to make use of this glucose. This, however, is improbable and a much more likely explanation would appear to be a toxic action on the liver, by which that organ is rendered less capable of storing food material. This would explain not only the rise of fasting blood

sugar but also the slight excretion of acetone bodies, since the liver also acts as a reservoir of fatty acids. It is also interesting in this connection to draw attention to the diminution in the formation of urea.

If there is any basis for the suggestion that ketone bodies play a part in the production of the symptoms of salicyl intoxication one would expect that the administration of glucose would have an inhibitory effect. In Fig. 2 is shown the effect of this on the blood CO_2 content. Each patient was given 50 grammes of glucose daily for a period of about 6 days; thereafter sodium salicylate was administered in 15 grain doses six times daily while the glucose was continued. In each instance symptoms developed and the total CO_2 content of the blood fell just as quickly in those cases where no glucose or bicarbonate had been given previously or simultaneously. This chart also illustrates the variability of the acetonuria which was absent in two of the three cases. As the giving of glucose exerts not the slightest beneficial effect, it seems justifiable to infer that the symptoms are not necessarily associated with incomplete fat oxidation. In the mineral acid poisoning type of acidosis, such as one sees after the administration of HCl or acid-producing salts ($CaCl_2$ and NH_4Cl), glucose has no influence in preventing or lessening the symptoms. One is inclined to deduce from the results of these investigations a similarity between the nature of salicylate acidosis and that caused by such a substance as calcium chloride.

It has already been shown that the combination of sodium bicarbonate with the salicylate is effectual in lessening the impairment of renal function and in the great majority of patients preventing the onset of symptoms. Fig. 2 (C) shows that the addition of sodium bicarbonate in 30 grain (2 gramme) doses to salicylate on the sixth day resulted in a rapid return of the blood CO_2 to normal and a disappearance of symptoms. This also is very similar to what occurs in the alkali treatment of $CaCl_2$ over-dosage.

The excretion of salicylate.—In searching for the reason why the sodium bicarbonate is effectual in the prevention of the signs and symptoms of salicylate poisoning, the question of the rate of excretion of the drug with and without the addition of sodium bicarbonate naturally arises. The urine from periods II and III of the three children on metabolism was analysed quantitatively for salicylic acid. The fæces were also examined in one case but a negligible amount was found. These results are given in Table 6. The giving of the alkali increased the excretion of salicylic acid by the urine two or four fold, and it seems impossible to avoid the conclusion that this increased rate of excretion is a potent factor in the beneficial effects of the alkali.

The amount of sodium salicylate in the blood of these three cases at the end of a night's fasting was estimated once during each period (Table 6). Free salicylic acid was never found except in traces, the drug always being present as the salt. The addition of the alkali also increased the salicylate content of the blood two to four times. The increase in the blood and the increased rate of excretion, however, do not necessarily go parallel. In the case of S. the blood salicylate content was increased seven fold in the third period, but the rate of excretion was only doubled. This observation, however, is not of great

significance since the blood estimations were not done more than once and consequently the variations are not known, but it might be taken as suggesting that there are other factors, for example, kidney damage, as well as the ' head ' of salicylate influencing the rate of excretion. Hanzlik's[12,13] findings are contrary to ours. He was unable to demonstrate any difference in the duration or rate of excretion by the addition of alkali. Fleischer[14], however, stated that sodium bicarbonate shortens the period of elimination from 36 to 14 hours, and Ehrmann[15] obtained similar results.

TABLE 6.

INFLUENCE OF SODIUM BICARBONATE ON THE URINARY EXCRETION AND BLOOD-CONTENT SODIUM SALICYLATE.

Name	B		M		S	
Period	II	III	II	III	II	III
Total amount of sod. sal. given in grm.	23·33	23·33	23·33	23·33	34·99	34·99
Total amount of sod. bic. given in grm.	—	46·66	—	46·66	—	69·98
Total amount of sod. sal. excreted in grm.	6·069	20·25	6·57	21·33	13·17	27·91
% of sod. sal. excreted	26·0	86·8	28·1	91·4	37·6	80·0
Salicylate content of blood in mgrm. per 100 c.cm.	9·3	29·0	6·9	13·0	13·0	94·0

Discussion.

The question of the production of an acidosis in the poisoning by salicylate can, we believe, be answered in the affirmative. The clinical picture, the behaviour of the CO_2 content, and the response to alkali, point very convincingly to this. The fact that renal function is impaired seems also quite definite and this is, in our opinion, the explanation of the reduced ammonia coefficient, since it is well recognized that the kidneys are the site of the formation of ammonia.

If there is an acidosis the question arises as to the nature of the excess acid. Although in this investigation the salicylate was always given as a neutral salt one naturally turns to the salicylic ion as a possible cause. The fact that this acid is present in the blood in greater abundance in the alkali period, in spite of the absence of symptoms, is itself strong evidence against it being the excess acid. Further, the amount found to be present during the low CO_2 period is quite insufficient to compensate as a base-holding substance for the deficit in CO_2. The maximum amount of salicylic acid which we found in the blood during the presence of toxic symptoms amounted to 13 mgrm.

per cent., which is equivalent to 0·00094 mol. per litre. This amount would only account for a diminution in the CO_2 of 2·1 vol. per cent., whereas the fall in CO_2 amounted to more than 20 vol. per cent. It is clear therefore that the fall in CO_2 is not merely the result of its replacement by salicylic acid.

The other alternative is the presence of an excess of acid produced as a result of disordered katabolism. Moderate increase of lactic acid has been observed in cats and rabbits after intravenous administration of salicylate preparations. Johnston[16] who noted this could not correlate the lactate increase with the change in the alkaline reserve. There is strong evidence, however, of abnormal protein katabolism with the resultant production of acid nitrogenous substances. Unfortunately the partition of nitrogen in the urine was not studied, but it is clear from the figures presented that urea forms in two of the three subjects a much smaller proportion of the urinary nitrogen during the period when salicylate alone was given than in either of the other two periods. This cannot be explained by an increased formation of ammonia which was decreased in amount, absolutely as well as relatively. One possible explanation would be that owing to the action of the salicylic ion the process of de-aminization is inhibited, with a resultant increase of amino-acids in the blood. If to this is added the effects of impaired renal function, it might explain the excess acid accumulation leading to dyspnœa and a low CO_2 content of the blood.

It is more than likely, too, that all the toxic symptoms and signs are not due to the presence of an acidosis. The delirium, speech disturbances, apathy and lassitude might quite well be the direct result of the salicylate itself or some of the compounds formed with it. Indeed, it seems more than probable that salicylate disturbs the metabolism of all the tissues just as has been demonstrated in the case of liver and kidney. Accordingly the nervous phenomena described can be more justifiably attributed to a direct action on nervous tissue than to the effects of acidosis. Both the acidosis and the manifestations of nervous involvement are concomitant phenomena, and it would appear that the extent to which the CO_2 is lowered is a very good index of the degree of general disturbance of the tissues.

Whatever may be the immediate cause of the toxic symptoms it is beyond dispute that administration of alkali effectively prevents in the vast majority of individuals the onset of clinical symptoms or chemical changes of poisoning. The alkali undoubtedly accelerates the excretion of salicylate and so prevents its accumulation in the tissues. This would to a great extent explain its action in preventing signs of toxæmia. It would appear from the results shown that the functional activity of the kidney is restored to normal by the addition of sodium bicarbonate to the salicylate. Whether this is the sole action or not, one cannot say. It may in some way protect the tissues but conclusive evidence of this is lacking. At any rate the administration of sodium bicarbonate along with the salicylate is essential. Toxic manifestations are avoided and large doses of salicylate can be given with safety. Further, the alkali permits of a much greater concentration of salicylate in the blood, which presumably is of advantage in promoting the therapeutic effect.

Conclusions.

1. The oral administration of sodium salicylate alone in doses of over 60 grains daily to children results in the production of a non-gaseous acidosis. The acidosis is not due to the presence of the acid salicyl ion per se.

2. In such patients there is also definite evidence of impaired renal function.

3. The development of the acidosis and the impairment of renal function can be prevented by the addition of sodium bicarbonate but not by glucose.

4. One effect of the alkali would appear to be the prevention of renal damage, thus permitting more rapid excretion of salicylate.

We desire to thank Dr. Leonard Findlay for his valuable suggestions and criticisms throughout the course of this investigation. We also tender our thanks to the Medical Research Council by whom the expenses of this work were defrayed.

REFERENCES.

1. Maclagan, T., *Lancet*, London, 1876, i, 349.
2. Stricker, Dr., *Berlin Klin. Woch.*, Berlin, 1876, I, 15 & 99.
3. Broadbent, W. H., *Lancet*, London, 1876, i, 530.
4. Lees, D. B., *Proc. Roy. Soc. Med.*, London, 1908–9, II, (*Pharmac. Sect.*), 34; *Brit. Med. J.*, London, 1912, ii, 930.
5. Hanzlik, P. J., *Medicine*, Baltimore, 1926, V, 197.
6. Meara, F. S., *Amer. J. Med. Sci.*, Philad., 1910, CXXXIX, 328.
7. Langmead, F., *Lancet*, London, 1906, i, 1822.
8. Leichentritt, B., *Ztschr. f. physiol. Chem.*, Berlin, 1919, CIV, 154.
9. Veil, W. H., & Graubner, W., *Arch. f. exp. Path. u. Pharmak.*, Leipzig, 1926, CXVII, 208.
10. Hurtley, W. H., & Trevan, J. W., *J. Physiol.*, Cambridge, 1915–16, Proc., 49.
11. Myers, H. B., & Ferguson, C., *J. Pharmac. & Exper. Therap.*, Baltimore, 1929, XXXV, 313.
12. Hanzlik, P. J., Scott, R. W., & Thorburn, T. W., *Pharmac. & Exper. Therap.*, Baltimore, 1917, IX, 247.
13. Hanzlik, P. J., Scott, R. W., & Reycraft, J. L., *Arch. Int. Med.*, Chicago, 1917, XX, 329.
14. Fleischer, R., *Berl. Klin. Woch.*, Berlin, 1875, XII, 529 & 547.
15. Ehrmann, R., *Münch. Med. Woch.*, München, 1907, LIV, 2595.
16. Johnston, C. C., *J. Am. Med. Ass.*, Chicago, 1930, XCIV, 784.

METABOLISM STUDIES IN CŒLIAC DISEASE

BY

OLIVE MACRAE, M.D., and NOAH MORRIS, M.D., B.Sc.

(From the Department of Pædiatrics, Glasgow University, and the Biochemical Laboratory, Royal Hospital for Sick Children, Glasgow.)

Since the publication of Cheadle's paper[1] in 1903, the presence of excess of fat in the fæces has attracted much notice as the most characteristic sign of the metabolic disturbance in cœliac disease. It has been recognized that associated with this disturbance of fat absorption, there occurs also a defective retention of minerals and an excessive loss of nitrogen in the fæces. In consequence some attention has been paid to the effect of varying the composition of the dietary, especially from the therapeutic standpoint. The interrelationship of the disturbances in the absorption of the various elements of the diet is a matter of importance both from the practical point of view, and because of the possible value in the explanation of other metabolic disorders. Parsons[2] has already indicated the importance of vitamin D in the prevention of cœliac rickets, showing that the continued absence of this vitamin from the fat-poor diets used in treatment is the cause of the rickets of convalescence. The series of observations detailed in this paper are published in the hope that some further light may be thrown on the underlying chemical pathology of the cœliac state. A more complete understanding of the disorder should ultimately prove of value in the prevention and cure of the disease.

Metabolic studies in cœliac disease are subject to the great disadvantage that sudden changes occur in the condition of the patient although the diet is constant and the environment unaltered. Accordingly it is difficult to determine whether differences in the metabolic findings are to be attributed to changes in food or other treatment, or to natural aggravation or amelioration of the condition. In these investigations as much care as possible was taken to rule out the idiopathic changes in the severity of the disease. A brief description of the salient features of the case-histories of the patients is appended at the end of the paper (see Appendix). It is proposed in the first place to summarize the metabolic findings with regard to the various forms of foodstuffs and thereafter to discuss the bearing of these results on the chemical pathogenesis of cœliac disease.

Utilization of fat (see Table 1).—A characteristic feature of cœliac disease is the presence of a large amount of fat in the fæces which are bulkier than normal. Frequently they are whitish and fatty in appearance, but this depends to a large extent on the intake of fat. It has been stated that there may take place a re-excretion of fat through the intestinal epithelium and

TABLE 1.

SHOWING PERCENTAGE AND ABSOLUTE DAILY CONTENT OF FÆCAL FAT WITH PERCENTAGE AND ACTUAL DAILY UTILIZATION.

Case No.	Days	Diet*	Dried fæces grm.	% fat in dried fæces	% of fæcal fat			Daily output				Utilization			
					N.F.	F.F.A.	C.F.A.	N.F. grm.	F.F.A. grm.	C.F.A. grm.	Total fat grm.	Intake grm.	Absorption Total grm.	%	per kgrm. body wt.
1 (i)	6	N	11·49	32·70	11·6	28·8	59·2	0·43	1·09	2·23	3·77	29·4	25·61	87·2	3·5
(ii)	6	H	11·99	50·00	13·4	24·8	61·6	0·80	1·48	3·68	5·98	99·2	93·22	94·2	12·1
(iii)	7	N	15·70	50·94	20·9	40·4	38·6	1·67	3·22	3·09	7·99	24·2	16·21	67·0	2·2
(iv)	7	H	32·20	57·51	17·0	35·1	47·7	3·17	6·49	8·87	18·53	97·0	78·47	80·9	10·7
(v)	7	H(a)	16·81	55·74	11·1	31·4	57·4	1·04	2·96	5·40	9·40	97·0	87·6	88·8	10·4
(vi)	6	N	6·62	44·50	5·6	69·9	24·4	0·15	2·07	0·72	2·94	24·0	21·1	87·6	2·4
(vii)	6	H	14·79	81·20	2·5	41·6	55·7	0·31	5·05	6·75	12·11	104·3	92·2	88·4	10·6
(viii)	5	H(b)	11·50	70·20	11·4	35·5	52·4	0·97	2·90	4·27	8·14	104·3	96·2	92·2	10·1
2 (i)	6	N	12·55	35·60	22·3	54·1	24·0	0·99	2·38	1·08	4·45	34·1	29·6	87·2	3·4
(ii)	6	H	11·95	38·35	18·2	47·5	36·6	0·83	2·17	1·56	4·56	99·9	95·3	95·4	10·8
(iii)	7	N	7·99	47·60	11·9	39·0	50·0	0·45	1·46	1·90	3·81	27·1	23·8	86·0	2·8
(iv)	7	N	12·42	48·00	18·1	65·8	16·0	1·09	3·92	0·96	5·97	35·0	29·0	83·1	3·3
(v)	7	N	20·10	53·00	13·3	51·4	35·3	1·41	6·91	3·76	12·08	38·4	26·4	68·7	2·5
(vi)	7	H	17·03	66·80	15·2	41·7	43·1	1·74	4·73	4·90	11·37	75·1	63·7	84·8	6·0
(vii)	7	H(b)	18·57	64·98	18·2	46·0	35·6	2·20	5·57	4·30	12·07	75·1	63·0	83·9	5·6
3 (i)	6	N	18·45	40·45	20·9	48·8	30·2	1·58	3·63	2·25	7·46	28·2	20·7	73·6	2·5
(ii)	6	H	29·77	74·58	20·5	48·6	30·9	3·93	9·37	8·90	22·20	114·7	92·7	80·8	11·6
(iii)	6	H	39·73	73·95	22·5	53·1	24·4	6·60	15·58	7·17	29·35	126·8	96·4	76·0	12·1
(iv)	6	H(a)	33·25	69·40	21·6	45·4	32·9	5·00	10·50	7·60	23·10	126·8	103·7	81·8	12·3
(v)	7	L	19·33	25·98	17·0	49·9	32·6	0·87	2·50	1·64	5·01	8·1	3·1	38·2	0·3
(vi)	7	N	23·13	49·10	7·2	26·8	65·8	0·83	3·05	7·48	11·36	25·5	14·1	55·3	1·5
4 (i)	6	N	8·23	26·82	20·3	37·3	42·6	0·45	0·82	0·93	2·21	26·6	24·4	91·0	4·1
(ii)	7	N	16·73	39·02	15·7	50·3	34·0	1·03	3·30	2·21	6·54	20·5	14·0	68·1	2·1
(iii)	7	N(c)	13·57	49·01	5·1	19·9	74·9	0·34	1·32	4·99	6·65	17·9	11·3	62·8	2·0
5 (i)	7	N	28·31	35·03	13·2	72·4	14·4	1·32	7·17	1·42	9·91	34·6	24·7	71·3	3·7
(ii)	7	N	37·17	44·55	39·4	40·9	19·6	6·50	6·86	3·25	16·61	21·0	4·4	20·9	0·8
6 (i)	7	N	16·90	58·70	13·6	48·3	38·3	1·33	4·78	3·80	9·91	38·8	28·9	74·4	2·6
(ii)	6	H	13·14	62·75	13·8	26·5	59·8	1·32	2·55	5·75	9·62	115·0	105·4	91·6	9·4

*L. Low fat intake.
N. Normal fat intake.
H. High fat intake.

(a) NaH_2PO_4 added.
(b) Sodium glycocholate added.
(c) Radiostol added.

Fanconi[3] gives figures showing an excretion of 28·55 grm. of fat although the intake only amounted to 20·66 grm., thus indicating a re-excretion of almost 8 grm. of fat in the twenty-four hours. Schick and Wagner[4] found a greatly increased percentage of neutral fat, but it is generally agreed the fat-splitting is quite normal. Fanconi describes two types as regards the disturbance in fat absorption, first a group in which the absorption is very poor and where most of the fat is unsplit, and secondly a group in which, despite poor absorption, the splitting is normal.

In our series the neutral fat formed less than one-quarter of the total fæcal fat except in one period in one Case 5 (ii), when the child was obviously going down-hill rapidly. In that period 39·4 per cent. of the total fat was unsplit which could not have been accounted for by increased peristalsis as the daily number of motions was only one or at most two. Otherwise the results of the fæcal analyses indicated excellent fat splitting in most of the cases, and that although the percentage absorption was below normal.

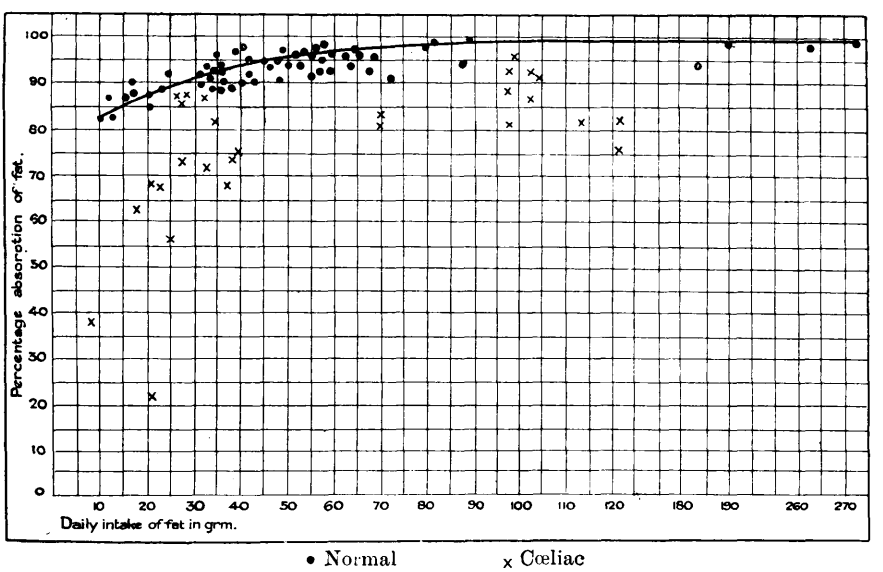


FIG. 1. Showing percentage absorption of fat during different daily intake of fat in health and cœliac disease.

The percentage absorption of fat varied in the individual cases and at different times. Generally it bore a rough relationship to the clinical condition of the patient, provided one kept in mind the actual amount of fat ingested. Harrison and Sheldon[5] state that the finding of a high percentage of total fat in the fæces does not necessarily indicate cœliac disease, but that a normal result in an untreated patient is almost definitely against the diagnosis of cœliac disease. In Case 1 (ii), however, the fat formed half the weight of the dried fæces, whereas in Case 5 (i) only a third, i.e., within normal limits, although the former patient was convalescing rapidly and steadily and the latter was in an acute stage. The percentage absorption was in Case 1 (ii) 94·2 per cent. and in Case 5 (i) 71·3 per cent. It is therefore impossible to

judge of the absorption of fat from a knowledge alone of the percentage composition of the fæces. It is essential to have figures for the fat intake and the weight of the dried fæces so that the percentage absorption of the intake can be calculated.

It has been shown by Hutchison[6] and others that in health the fat forms about one-third of the weight of the dried fæces. Accordingly an increase in the intake of fat will lead to both an absolute and relative increase in its absorption provided the fæces are not excessive in amount. The results charted on Fig. 1 indicate the percentage absorption of fat with varying daily intakes in a series of children, either healthy, or at any rate without gastro-intestinal disorder. If one takes into consideration the varying norm in different individuals the gradual rise in the curve as the intake of fat is increased is all the more striking. When the percentage absorption of fat on varying intakes is estimated in the same individual it is invariably found to increase with the rise in the absolute amount of fat ingested. This held good with a daily intake of some 290 grm. of fat. It is therefore justifiable to conclude that in health the absorptive power of the intestine for fat is practically unlimited, provided, of course that the excess of fat does not lead to intestinal upset.

When the results of the cœliac analyses are examined it is clear that here too the percentage absorption of fat rises with the increase in the daily intake. This can obviously only hold good in the one individual at any particular phase of the cœliac condition owing to the different grades of severity of the disease in different patients and in the same patient at different times. It might be suggested that the lower percentage absorption on the lower fat diet was due to an aggravation of the cœliac state. In all cases, however, the high fat diet followed immediately on the moderate, and everyone is agreed that high fat diet is not beneficial to the cœliac patient. One point of difference between the behaviour of the normal and the cœliac to the increased ingestion of fat is the much greater increase in the weight of the dried fæces in the cœliac patient. Despite this increase in fæcal weight, both the absolute and percentage amounts of fat absorbed were greatly raised. The percentage of total fat in the dried fæces was invariably raised as a result of a higher fat intake. The ratio of neutral to total fæcal fat remained practically unchanged, tending if anything, to be rather lower on the high fat diet. The combined fatty acids (calcium soaps) became appreciably greater in amount both absolutely and relatively to fæcal weight and total fat.

Effect of acid-sodium phosphate.—Administration of di-hydrogen sodium phosphate during a high fat period caused a definite increase in the ratio of insoluble soaps to total fæcal fat and fæcal weight. The percentage absorption was increased quite markedly in both cases. It would seem therefore that this substance promotes conditions favouring fat absorption.

Effect of sodium glycocholate.—The effect was to increase the relative and actual amounts of neutral fat with corresponding diminution in those of the insoluble soaps. The percentage absorption of fat showed a marked increase in one and a slight reduction in the other case. It would appear that glycocholate promotes the absorption of the soaps.

EFFECT OF RADIOSTOL.—The percentage absorption of fat was slightly diminished. The absolute amount of combined fatty acids in the fæces was greatly increased as was the ratio of combined fatty acids to total fat A similar effect of vitamin D preparations has been noted in metabolism studies on rickets (MacRae[7]).

The ability of the cœliac patient to deal with fat in the intestine is undoubtedly impaired. During the active stage the percentage absorption is below normal while as a general rule the fat-splitting properties of the intestinal juices are quite up to standard. The defect in fat absorption is much more marked when the intake is low. During a period of high-fat intake the percentage absorption rises usually to a very marked degree. Accordingly the actual amount of fat absorbed during such a period usually exceeds the normal requirements of the child although the percentage absorption is still below the normal figure for that particular daily intake of fat.

It is generally held that administration of fat in large amount immediately produces a recurrence of active symptoms. By this is meant the re-appearance of bulky fatty motions. Two patients, Cases 1 and 2, were given high fat diets for a period of 14 days during a quiescent stage when the fat absorption was about 90 per cent. The fat absorption on the increased fat intake became greater both relatively and absolutely, being quite within normal limits. The motions were practically unchanged in weight and did not present any abnormal appearance. Shortly after this high fat period the patients were discharged without symptoms on a low-fat high-protein diet and remained well for several months only to return with a recurrence of the manifestations of the active disease. It seems justifiable to conclude from this experience that administration of large amounts of fat in a stage of true convalescence does not, for short periods at any rate, lead to recrudescence of symptoms. If, however, the patient is only apparently convalescent, the addition of fat to the diet will lead to the appearance of typical cœliac motions. It is questionable how far the appearance of these fatty stools is indicative of an aggravation of the condition. Even if the percentage absorption of fat were much below normal, fatty stools could not occur when the fat intake was low. Accordingly when such a patient, who has been receiving a minimal amount of fat, ingests more fat, there will appear in the fæces a much greater amount of fatty products than normal, leading to the formation of typical cœliac motions. This gives rise to the impression that the condition has been aggravated although the presence of fatty motions is merely an indication that the intestine is at the time unable to deal properly with fat. Naturally this was not evident on the minimal fat diet as the amount of fat in the food was insufficient to give bulk to the fæces, although the intestinal condition was no better than during the period when the stools were large and fatty.

The varying effects of the administration of acid phosphate, high-fat diet, vitamin D, and sodium glycocholate on the distribution of fat in the fæces are of interest. The first three lead to an increased amount, relatively at any rate, of calcium soaps, whereas glycocholate produces a decrease in the soaps and

presumably an increased absorption of fat in that form. Verzàr and Kúthy[8] maintain that absorption of fats as soaps is impossible unless at a pH of 9 when the soaps are soluble. One of the main functions of the bile salts is to make the soaps soluble at an acid pH which is the normal for the intestine. Klinke[9] also states that calcium soaps are soluble in the presence of bile salts. Adler[27] showed that withdrawal of the bile secretion led to an appearance of calcium soaps which greatly diminished in amount on administration of bile-salts. Our results show that increasing the acidity of the gut by the presence of excess fatty acids, or administration of acid salt or by ingestion of vitamin D raises the proportion of fat found as insoluble soaps. It would therefore seem justifiable to conclude that in these cases the reaction of the intestine is alkaline. The question remains how far this reaction is due simply to the associated vitamin D deficiency which has become secondarily superimposed on the cœliac condition. Sodium glycocholate decreases the absolute and relative amounts of the insoluble soaps. Accordingly it would appear that there is a deficiency in bile salt or at any rate that addition of bile salt is an aid to the absorption of soaps in the cœliac state.

Lime and phosphorus (see Table 2).—The daily retention of lime and phosphorus on a moderate fat-diet, when calculated on the basis of kilogramme of actual weight is either normal or slightly subnormal. The total amount retained, however, is much below that found in the healthy child of the corresponding age, owing to the dwarfism and greatly reduced weight of the cœliac child. Linder and Harris[10] state that the calcium utilization has a rough relationship to the total fat output, being high only when the fat excretion is low. Generally this may be so, but there are numerous exceptions, and it would be unwise to deduce a low calcium retention because of high fat content of the fæces. Thus in Case 2 (vi) there was an average daily excretion of 11·4 grm. fat while the calcium retention was quite good (47 mgrm./kgrm./day), whereas in the previous period when the fat output was practically the same (12·1 grm./days) there was a marked negative retention.

Further evidence that the bulk of fæcal fat cannot be taken as the cause of low calcium retention is obtained from a consideration of the mode of mineral excretion in jaundice. In this condition there is a faulty absorption of fats, the splitting of which is quite normal. The mineral retention is low but while the bulk of the calcium is found in the fæces by far the greater part of the phosphorus is excreted in the urine. The $CaO : P_2O_5$ ratio of the fæces and the urinary P_2O_5 : fæcal P_2O_5 ratio are both abnormally high. This is attributed to the large amount of fatty acid fixing the calcium in the intestine and so liberating much phosphorus to be absorbed and subsequently excreted in the urine.

The results in cœliac disease bear a strong resemblance as far as the mode of mineral excretion is concerned to those seen in rickets where the bulk of the phosphorus is found in the fæces. In rickets, the fat content of the fæces is not raised ; indeed it is during healing that the amount of soaps is relatively and absolutely increased.

EFFECT OF HIGH FAT DIET.—In two experiments this produced a very marked increase in the amount of fæcal calcium. In the third, the fæcal calcium was quite markedly diminished with a consequent rise of the retention of lime. An increased amount of phosphorus was found in the urine in all three cases. This increase in urinary phosphorus cannot be taken as evidence of increased absorption of phosphorus since in the first two experiments (Case 1, iv and vii) the amount found in the fæces was also increased. Only in the third (Case 2, vi), which showed a decrease in fæcal calcium was the phosphorus

TABLE 2.

CALCIUM AND PHOSPHORUS METABOLISM: DAILY INTAKE, OUTPUT AND RETENTION.

Case No.	Days	Diet*	CaO (grm.)					P_2O_5 (grm.)				Urine P_2O_5 / Fæcal P_2O_5 × 100	Fæcal CaO / Fæcal P_2O_5 × 100	Retention grm. per kgrm.	
			Intake	Fæcal output	Fæcal output as soaps	Urinary output	Total retention	Intake	Fæcal output	Urinary output	Total retention			CaO	P_2O_5
1 (iii)	7	N	1·223	1·043	·31	·053	·165	1·791	·972	·421	·399	43·2	103	·022	·055
(iv)	7	H	·973	1·577	·887	·065	−·669	1·495	·998	·562	−·064	56·3	158	−·093	−·009
(v)	7	H(a)	·976	·823	·54	·126	·026	3·007	·756	·967	1·284	128·0	109	·004	·178
(vi)	6	N	1·21	·734	·072	·023	·457	1·81	·913	·361	·542	40·0	80·4	·051	·061
(vii)	6	H	1·15	1·14	·67	·025	−·008	1·79	1·005	·63	·159	62·6	113	−·001	+·018
(viii)	5	H(b)	1·205	·747	·426	·025	·432	1·835	·540	·676	·618	125	138	·045	·066
2 (v)	7	N	1·80	2·31	·375	·037	−·548	2·64	2·090	·368	·181	18	110	−·053	+·017
(vi)	7	H	1·825	1·311	·490	·021	·493	2·25	1·158	1·031	·061	8·9	113	·047	·006
(vii)	7	H(b)	1·775	1·69	·43	·026	·059	2·287	1·43	1·042	−·185	72·8	118	+·005	−·016
4 (i)	6	N	1·287	·650	·093	·031	·606	1·618	·535	·363	·721	68	121	·10	·118
(ii)	7	N	1·48	1·372	·221	·020	·087	2·406	1·606	·311	·489	19	85	·013	·075
(iii)	7	N(c)	1·497	1·085	·499	·026	·385	2·391	·597	1·204	·589	202	181	·060	·094
6 (i)	7	N	1·987	1·673	·378	·022	·292	2·687	1·538	·624	·525	40·6	109	·028	·050
(ii)	6	H	1·890	1·211	·575	·013	·649	2·849	·904	1·10	·845	121	66	·053	·069

*L. Low fat intake.
N. Normal fat intake.
H. High fat intake.
(a) NaH_2PO_4 added.
(b) Sodium glycocholate added.
(c) Radiostol added.

in the fæces diminished. In all three instances the total amount of fæcal fat was increased as was that of insoluble soaps. Accordingly since the increased retention of lime and phosphorus occurring in Case 2 (vi) took place simultaneously with an increase in fæcal fat and soaps, it seems justifiable to argue that the amount of fat in the fæces cannot be more than a secondary factor in hindering the absorption of minerals.

Further, although in Case 1 (iv) and (vii), we find that about 4 grm. extra CaO was fixed as soaps there was no apparent liberation of phosphorus, since

that element was actually increased in amount in the fæces. This is in contrast to the picture met with in jaundice where there is an increased fæcal amount of calcium soaps associated with a diminished fæcal content of phosphorus and an increased excretion of phosphorus in the urine. As already pointed out the increase in urinary phosphorus in these cases has been attributed to an increased intestinal absorption of phosphorus liberated as a result of the increased fixation of calcium as soaps. In cases of cœliac disease on the other hand, there is no evidence of increased absorption of phosphorus as with the increased formation of calcium soaps there is also an increased fæcal phosphorus. It seems fair to conclude, therefore, that the extra urinary phosphorus was not the result of increased absorption.

As regards Case 2 (vi) it might be argued that the increased mineral retention took place during a period of general improvement. That this was not so is indicated by the low percentage absorption of fat for the amount ingested. We have evidence that during this period the carbohydrate metabolism also showed signs of definite impairment. Accordingly it must be admitted that during this period there occurred a marked improvement in the retention of lime and phosphorus, although all the other metabolic findings showed no signs of betterment.

There remains one hypothesis, namely, that the poor retention of minerals is the result of deficiency in vitamin D. Parsons[2] states that the development of rickets in cœliac infantilism is due primarily to the deficient absorption of fat and therefore of vitamin D, calcium and phosphorus. The 'rachitic' mode of mineral excretion is in this connection very suggestive. Further, in agreement with Parsons and other workers, we have found the values for serum calcium and inorganic phosphorus to be low (Table 6). We have studied the effect of vitamin D administration in one case and find that there is a very marked increase of lime and phosphorus retention, and that the main excretory route of phosphorus is shifted from fæces to urine. Further, the percentage absorption of fat is slightly reduced and the weight of fæcal fat is increased, thus affording still more proof that the metabolism of fat and of minerals in cœliac disease are only secondarily connected. The increased retention of lime during the high-fat period in Case 2 (vi) would on this line of reasoning be explained by the increased absorption of vitamin D resulting from the increased intake of fat. The change in the distribution of calcium between phosphorus and soaps and the divergence of the phosphorus from fæces to urine during the high fat period bear a close resemblance to the observations in healing rickets.

In the other two periods, Case 1 (v) and (viii) with high fat the changes in distribution of lime and phosphoric acid, as indicated by the ratios fæcal CaO to fæcal P_2O_5 and urinary P_2O_5 to fæcal P_2O_5 are very similar to those seen in Case 2. The retention of these minerals was, however, greatly reduced, and this despite the fact that a much larger actual amount of fat was absorbed by the patient in these two periods than in the case of Case 2 (vi). Linder and Harris[10] put forward the suggestion that most of the sterols remain with the unabsorbed fat in the gut. Even if this is correct, it would not explain

the marked difference in the results since it would be expected that the greater amount of fat absorbed would carry with it a larger moiety of the sterols. It seems to us that the explanation lies in the larger amount of fat absorbed by Case 1 leading to the production of a ketosis the effects of which overshadow the influence of increased vitamin D absorption. We have shown[11] that the osseous tissues play an important part in getting rid of excess of acid substances, and as a consequence during the administration of an acid-producing substance such as ammonium chloride there occurs a great increase in the excretion of calcium both by the urine and fæces. A similar occurrence takes place in conditions of ketosis. Of the presence of ketosis during the high-fat periods of W.B., further proof is given by the rise in excretion of the titratable acid and ammonia in the urine. In addition, the renal excretion of lime is slightly increased during these periods although the retention is diminished. Admittedly the increase in urinary output is very slight but it must be remembered that the store of lime is much below normal as is indicated by the radiographic evidence of marked osteoporosis. In the high-fat period in Case 2, on the other hand, where a great retention of lime took place the urinary output of this substance was slightly diminished.

If this line of reasoning be correct then it would appear that the low retention of minerals during the high-fat periods of Case 1 must be attributed to the effect of ketosis more than neutralizing the influence of the extra vitamin D made available by the increased absorption of fat. This naturally involves the assumption of a re-excretion of lime by the gut. This view however, is supported by the strong experimental evidence of Percival and Stewart[28] and other workers. Indeed in two of the series recorded here there was much more calcium excreted in the fæces than was ingested :—0·51 grm. in Case 2 (v) and 0·60 grm. in Case 1 (iv).

Effect of NaH_2PO_4.—The effect of NaH_2PO_4 was to decrease the amounts of fæcal calcium and phosphorus although there was a greatly increased ingestion of the latter substance. This must be due either to an increased absorption or diminished re-excretion into the gut. Simultaneously there was a decrease in the amount of fæcal fat although the relative amount of soaps was increased from 47·7 to 57·4 per cent. of the total fæcal fat. The ketogenic action of the increased fat absorption must have been present : therefore it seems safe to assume that the mineral excretion must have played as great a part as in the previous period in combating the acidosis. It would therefore appear that the action of the NaH_2PO_4 was to increase the absorption of lime and phosphorus, so that the increased output resulting from ketosis was rather more than neutralized by the increased absorption. It is impossible to decide whether calcium or phosphorus was primarily affected. It seems reasonable, however, to suggest that the excess of phosphorus in the gut attracted some of the calcium from the fat, and that the combination of phosphorus and calcium was rendered more easily absorbed by the presence of an acid reaction in the gut due to the presence of excess fatty acids and acid sodium phosphate.

EFFECT OF SODIUM GLYCOCHOLATE.—In one case there was a marked increase in the retention of lime and phosphorus while in the other there was a definite decrease. On the whole this was in conformity with the effect on the absorption of fat since in the second there was an increase in the amount of fæcal fat. With the data at our disposal it is not possible to explain this variability in the action of sodium glycocholate.

CONCLUSIONS.—On summing up the findings on mineral metabolism the following conclusions seem justified :—

1. The retention of lime and phosphorus is low while the patient is on a normal diet. The loss of these elements is mainly by the gut. While the retention of calcium is, generally, inversely proportional to the amount of fæcal fat, this is not invariable as an increased retention may occur in spite of a rise in the amount of fat in the motions. The retention of calcium can therefore only be secondarily related to the utilization of fat.

2. Administration of vitamin D raises the retention of lime although the absorption of fat is certainly not increased.

3. Increase in the absorption of fat leads to an increased absorption of lime. If the increased absorption produces a ketogenic effect, the rise in the amount of lime absorbed is masked by an increased re-excretion. The increased absorption of lime is probably the result of an increase in the absorption of vitamin D and an increase in the acidity of the intestinal contents.

4. The effect of rendering the intestinal contents more acid is exemplified by the increased absorption of lime during administration of an acid salt (NaH_2PO_4).

From these findings it would appear that the poor calcium absorption in cœliac disease is due to a lack of vitamin D and inadequate acidity of the intestinal juices. Any factor which leads to an increased utilization of fat containing vitamin D without undue ketogenic effect will produce a rise in vitamin D absorption and consequently an increase in calcium retention as in the sodium glycocholate period of Case 1.

Utilization of carbohydrate (see Tables 3 and 4).—It is a well recognized clinical fact that patients with cœliac disease do not tolerate carbohydrate well. Starchy foods undergo fermentation, and as indicated by the frequent presence of starch granules in the fæces are not well digested. This might, of course, be due to the large bulk of the intestinal contents, which prevents the amylases reaching the starch as quickly as usual, and so allowing fermentation to take place.

The ability of the intestine to absorb simple monosaccharides is very difficult to test. Schaap[12] found values of fæcal carbohydrate higher in cœliac disease than in any other condition. McCrudden [13] on the other hand states that the lower fatty acids which are formed from carbohydrates are if anything less in cœliac stools than normal. Poynton and Cole[14] have reported one case of cœliac disease showing glycosuria : this might have been due to low renal threshold independent of the cœliac condition or possibly was the result of previous carbohydrate starvation. The frequent existence of acetonuria is undoubtedly the result of defective supply of carbohydrate in the diet.

TABLE 3.

BLOOD-SUGAR CURVE IN CŒLIAC DISEASE

Case No.	Wt. in kgrm.	Blood-sugar (mgrm. per 100 c.cm.) after glucose (1 grm. per kgrm. body wt.)					Remarks
		Fasting	30 min.	60 min.	90 min.	120 min.	
1	8·60	77	83	87	79	81	Typical cœliac motions.
	8·60	79	67	73	75	67	,, ,, ,,
	8·60	100	85	79	85	85	,, ,, ,,
2	9·40	77	73	83	77	84	,, ,, ,,
	9·40	95	79	77	73	67	,, ,, ,,
	10·17	58	66	85	66	60	,, ,, ,,
	10·63	67	67	67	67	—	,, ,, ,,
	10·30	104	100	104	100	—	,, ,, ,,
	11·25	63	104	100	122	113	After 14 days' of sod. glycocholate
5	6·54	81	72	72	81	—	Absorp. of fat = 71·3%
6	10·33	106	104	102	98	112	,, ,, ,, = 74·4%
	11·62	82	89	136	109	97	,, ,, ,, = 91·0%
4	5·54	85	106	104	102	100	,, ,, ,, = 91·0%
	5·80	83	87	106	102	100	,, ,, ,, = 68·1%
7	15·5	89	109	109	109	100	Large fatty motions.
	17·0	115	139	170	147	98	Motions apparently quite normal.

TABLE 4.

BLOOD-SUGAR CURVE IN NON-CŒLIAC STEATORRHŒA.

Name	Age	Wt. in kgrm.	Blood-sugar (mgrm. per 100 c.cm.) after glucose (1 grm. per kgrm. body wt.)					Condition of patient
			Fasting	30 min.	60 min.	90 min.	120 min.	
J.T.	10 yr.	17·90	82	134	206	159	109	Chronic intestinal indigestion.
I.W.	1½ ,,	9·80	94	94	122	152	139	Chronic intestinal indigestion.
A.O'B.	7 ,,	22·45	104	143	152	122	—	Tub. peritonitis: absorpt. of fat = 72%
H.D.	3 ,,	14·10	113	146	120	113	100	Chronic intestinal indigestion.
G.L.	6 wk.	5·00	84	106	137	104	102	Biliary atresia.
M.B.	5 ,,	3·10	95	177	134	120	118	Biliary atresia.
M.M.	9 yr.	24·85	72	152	199	50	50	Catarrhal jaundice.

The fasting blood sugar is reported as being very variable but frequently low. The behaviour of the blood sugar after administration of glucose has been investigated by several authors. Fanconi[3] reports varied results. Generally the rise of blood sugar after ingestion of glucose was very slight or even nil, but occasionally a normal or prolonged rise was noted. McLean and Sullivan[15] obtained flat blood-sugar curves after 1·75 grm. glucose per kgrm. body weight, and in some patients even after as much as 9 grm. per kgrm. Administration of lævulose or galactose led also to no rise of blood sugar, although in two cases galactosuria was found. Thaysen[16], and Thaysen and Norgaard[17] report a subnormal rise of blood sugar after the ingestion of 1 grm. of glucose per kgrm. bodyweight.

In our series the fasting blood sugar was normal or subnormal, thus falling into line with the observations of other investigators. During the active stage of the disease, as indicated either by clinical manifestations or metabolic results, the administration of either glucose or lævulose led to an insignificant rise of blood-sugar. During a period of convalescence, resulting from dietetic treatment or otherwise, the blood-sugar curve becomes more normal in type. The low blood-sugar curve seems to be pathognomonic of the active stage of true cœliac disease. In Table 4 are noted the values of the blood sugar curves found in six cases of non-cœliac steatorrhœa : in all the curve is normal in height. The abnormality in the curve therefore cannot be due to the excessive bulk of the intestinal contents.

The flat blood-sugar curve in cœliac disease might be due either to an increased glycogenic function of the liver or to a greatly delayed intestinal absorption. It is unlikely that there is any increased glycogenic function since the low blood-sugar curve still persisted even when the patient was on a high fat diet. It is well recognized that such a diet raises the height and prolongs the fall of the blood-sugar curve. Accordingly the increase in glycogenic function should have been impaired ; instead of this the blood-sugar curve was just as flat as when the subject was on a normal fat-intake. The rise of the blood sugar after subcutaneous injection of adrenalin was quite within normal limits, thus affording further evidence that as far as carbohydrate metabolism is concerned hepatic function is normal.

Thaysen[16] has argued against defective absorption being the cause of the low blood-sugar curve on the following grounds. (1) The blood sugar is also lower than normal after intra-venous injection of glucose. (2) The R.Q. rises to about unity after ingestion of glucose. (3) The R.Q. is higher on a carbohydrate diet than on an ordinary mixed diet. He believes that the cause is some toxic effect on the endocrine glands. None of the objections advanced carry much conviction. No mention is made of change in blood volume after intra-venous injection of glucose and without information on this point it is impossible to determine whether the difference in blood sugar is due to disturbance in carbohydrate metabolism or to alterations in the concentration of the blood. It is possible that the diminished blood sugar is a result of the passage of a greater volume of fluid than normal from the blood stream. The evidence from the R.Q. results is also equivocal. First, it has been shown by Cathcart and Markowitz[18] that the value of the R.Q. being really the resultant of all

the metabolic processes does not necessarily indicate the metabolism of any particular food material. Secondly, it is not denied that absorption of carbohydrate does take place. It is merely a delay in absorption that is postulated. Accordingly the fact that the R.Q. is raised during a high carbohydrate intake is evidence solely that more glucose is oxidized and not that it is absorbed as quickly as normal.

Thaysen and Norgaard[17] hold the view that the abnormality in carbohydrate metabolism is due to some toxic effect on the endocrine glands. The evidence in support of this is a slightly hypernormal rise of blood sugar after the subcutaneous injection of epinephrine. In the few instances in which we have investigated the action of epinephrine we have found that the rise in blood sugar is quite normal when compared with the results obtained in normal children.

It has already been pointed out that the abnormality in glucose metabolism is not due to the bulk of the fæces mechanically impeding absorption. In this connection it is interesting to note the normal blood-sugar curve in a child suffering from tuberculous peritonitis in whom the percentage absorption of fat was much below normal. The defective utilization of fat was due to the blockage of the lacteals, leaving the intestinal epithelium unimpaired and thus allowing normal absorption of all blood-borne foodstuffs. In cœliac disease on the other hand it would seem that the intestinal epithelium or its immediate environment is at fault, so accounting for defective absorption of both fat and glucose.

Utilization of protein (see Table 5).—Herter[19] concluded that although absorption of protein in cœliac disease is better than that of fat it is still not

TABLE 5.

SHOWING OUTPUT OF NITROGEN IN THE FÆCES.

Case No.	Intake of N. per day grm.	Nitrogen in fæces		
		Output per day grm.	As % of dried fæces	As % of intake
1 (iii)	3·521	0·305	2·0	8·7
(iv)	3·111	0·414	1·3	12·9
(v)	2·991	0·302	2·6	10·1
(vi)	3·939	0·154	2·6	3·9
(vii)	4·239	0·271	1·8	6·4
(viii)	4·135	0·230	2·0	5·6
2 (v)	5·998	0·235	1·2	3·9
(vi)	5·058	0·209	1·2	4·1
(vii)	5·617	0·271	1·5	4·8
3 (i)	3·760	0·711	4·5	19·0
(ii)	3·431	0·464	1·9	13·5
(iii)	3·617	0·550	1·7	15·2
(iv)	3·601	0·520	1·9	14·4
5 (i)	5·14	1·05	3·7	20·4
(ii)	3·81	2·04	5·5	53·5

as good as in health. Schaap[12] found that the percentage of nitrogen in the fæces was practically the same as normal: owing to the excess in the amount of fæces passed there was naturally a much greater loss of nitrogen than in health. McCrudden and Fales[20] maintain that the nitrogen in cœliac fæces is derived from the same source as in health, i.e., chiefly from the intestinal secretion. In one case a five-fold increase of nitrogen intake was actually accompanied by a reduction in the nitrogen content of the fæces. They conclude that the high fæcal output of nitrogen in cœliac disease is due not to defective absorption but to re-excretion. Fanconi[3], on the other hand, states that with rich protein intake relatively more nitrogen appears in the fæces both in normals and cœliacs. He gives figures which show that in mild cœliac disease about 16 to 20 per cent. of the nitrogen intake appears in the fæces, while in severe cases as much as 48·7 to 63·6 per cent. This is shown even more

TABLE 6.

CHEMICAL ANALYSIS OF BLOOD.

Case No.	Serum		Plasma	Blood		
	Calcium mgrm. %	Phos. mgrm. %	Fatty ac. as grm. tripalmitin per 100 c.cm.	Chlorine mgrm. %	CO_2 vol. %	NPN mgrm. %
Normal			330–500	280–340	40–60	20–40
1	7·8	4·7	376	310	47·2	35·1
	—	—	405	—	—	—
2	7·7	3·0	354	340	57·6	—
	7·1	2·7	410	290	—	29·6
3	—	—	387	325	51·2	37·4
4	7·0	3·0	—	—	—	—
	6·3	2·0	—	—	—	—

clearly when the fæcal nitrogen is computed as a percentage of the total excreted: in the normal this is 13 to 20 per cent. in the mild cœliac 19 to 27 per cent.: in the severe 43·7 to 52·1 per cent.

Our results show a much smaller percentage of nitrogen in the fæces than do those of Fanconi, probably because of the easily absorbable nature of the proteins (milk) given to our patients. The nitrogen percentage of the dried fæces seems to be quite within normal limits so that any excess in nitrogen loss must be the result of the great fæcal weight. In three instances in our series a high percentage of nitrogen was found in the dried fæces: in these periods 19·4, 20·4, and 53·5 per cent. of the total intake of nitrogen was found in the motions. These results occurred simultaneously with a defective absorption of fat. It would appear, therefore, that in an acute stage of the disease the absorption of protein is hindered both relatively and absolutely, but not to the same extent as fat.

In two instances the ability of the patient to absorb urea from the intestine was tested : in both the excretion was normal both as regards time and amount. It seems, therefore, that whatever fault is in the intestinal utilization of nitrogen, it is not concerned with simple nitrogenous substances but with complex molecules.

Discussion.

The most striking feature in the chemical pathology of cœliac disease is undoubtedly the abnormality in the utilization of fat. All attempts to formulate a conception of the pathogenesis of the condition have been based on this abnormality. Generally speaking it has been held that the defect is one of absorption, but Moncrieff and Payne[21] have tentatively suggested that the abnormality is one of defective intermediate metabolism, possibly a result of the impaired action of blood or tissue lipase. In a preliminary communication they have given figures for blood fat in cœliac disease which are in excess of those obtained from normal individuals. It is exceedingly difficult to obtain blood in different individuals when the intermediate metabolism of fat is at the same stage, as the various conditions affecting the migration of fat to and from the tissues are in great part unknown. We have estimated the blood fat in a few cases (Table 6), and have found all the values within the normal range which admittedly in a wide one. Fanconi[3] reports low, normal, or even subnormal values for the blood fat in the fasting condition. He further states that in cœliac disease the blood fat curve after oral administration of olive oil or butter is flatter than normal. These results indicate that whatever the state of intermediate fat metabolism the defective utilization is not the result of a high blood fat hindering absorption or promoting re-excretion into the gut.

Further evidence has been brought forward to show that carbohydrate is absorbed with more difficulty than normal. Another substance showing a defective absorption-curve is acid sodium phosphate (Fig. 2). On the other hand, oral administration of sodium chloride or urea led to the output of these substances quantitatively in the urine as speedily as in the normal individual. Methylene blue appeared in the urine as soon after ingestion as it did in the normal subject. It is possible that the apparently normal absorption of urea, chloride, and methylene blue may be due to the fact that these substances pass through the intestinal epithelium by the simple physical process of diffusion. Fat, glucose and protein probably require a more complex set of conditions such as narrow limits of pH, presence of bile-salts and so forth. It must be emphasized again that the defect in carbohydrate absorption cannot be attributed, unless in a minor degree, to the mechanical interference of the large amount of fat, since in the case of steatorrhœa due to tuberculous peritonitis the absorption of carbohydrate and protein was quite normal.

There is no evidence of any structural change in the intestine which could account for the grave defect in absorption. Lehndorff and Mautner[22] sum up the post-mortem findings by saying that in general, the atrophy of the organs produced in cœliac disease is a result of the hunger condition. In two of our patients who died the pathological reports (for which we are indebted

to Dr. J. W. S. Blacklock), indicate that no abnormalities either macro- or micro-scopic were noted in the gastro-intestinal tract, while in only one was there atrophy of liver and spleen.

Freise and Jahr[23] attributed the defective fat absorption to an over-rapid passage of the chyme through the intestine. They have been able to increase the percentage utilization of fat by slowing the movement of food through administration of opium or atropine. Meyer[24] points out that fat-splitting also suffers as a result of increased rapidity of the passage of food through the bowel. In cœliac disease, however, fat-splitting is usually quite normal. In two cases we have followed the passage of a barium meal radiographically, but no abnormality was noted in the times taken to complete the various stages.

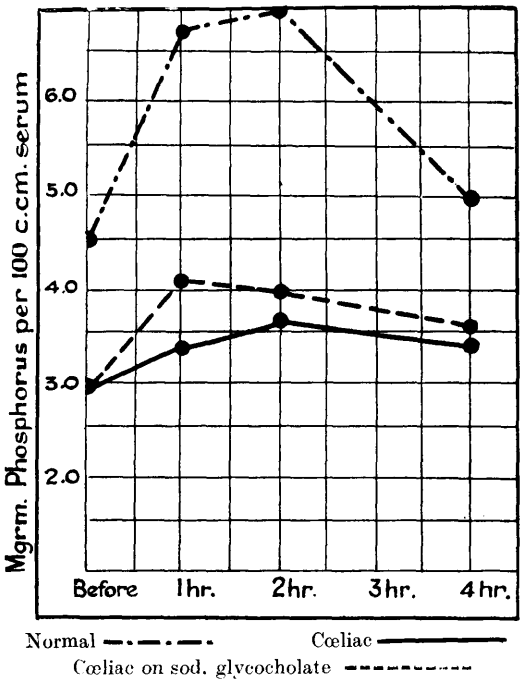


FIG. 2. Inorganic phosphorus content of serum after oral administration of NaH_2PO_4

Further, carmine or charcoal ingested orally did not appear any more rapidly in the fæces of the cœliac patient than in the normal. The improvement resulting from the administration of opium was probably due to slowing down of the passage of the chyme, thus allowing longer contact between food material and intestinal epithelium. The defect in cœliac disease is after all not an absolute one. The state of affairs might be compared to a catalytic reaction taking place in the presence of an insufficiency of catalyst.

It has been clearly shown that the abnormality is not present in the intermediate metabolism, nor can it be detected either in the intestinal epithelium or in the rate at which the intestinal contents pass. It would seem therefore that the fault lies in some physico-chemical abnormality of the intestinal contents.

The absence or paucity of bile in the intestine has long been suggested as the cause of the mal-absorption of fats in the cœliac state. The whitish colour of cœliac motions has been attributed to absence of bile products. These, however, can be demonstrated both in the fæces and the duodenum. It is generally held that the normal fæcal colour is masked by the excess amount of fat. Nevertheless it is quite possible that although the bile pigments are present, there may be a defective supply of bile-salts which, after all, for purposes of absorption form the all-important constituent of the bile. Miller, Webster, and Perkins[25] in 1920 published results of three cases treated by bile-salts. Unfortunately in only one case was a complete metabolic determination performed, and in it improvement was not marked as the child during the control period had commenced to improve spontaneously. The percentage absorption of fat was only increased from 85·8 to 87·0. In the other two

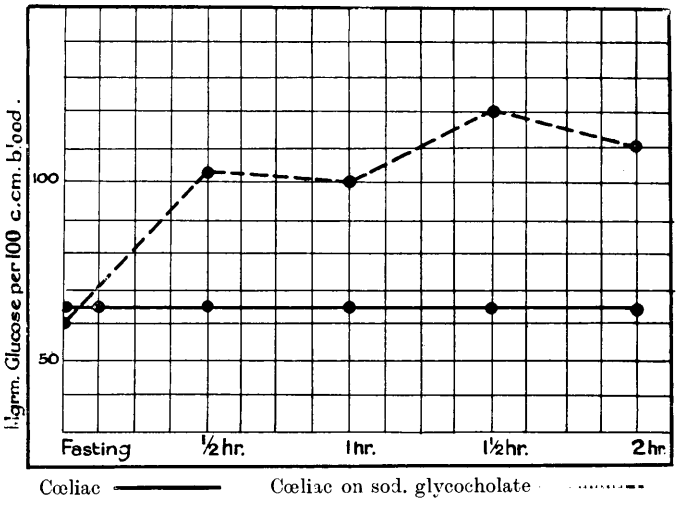


FIG. 3. Blood sugar curve in cœliac disease.

cases only the fæcal content of fat was estimated and this was certainly reduced with bile-salt treatment. The work, therefore, while strongly suggestive of the beneficial action of bile-salts on the absorption of fat is not conclusive. Fanconi[3] could get no definite improvement by the use of bile-salt preparation (decholin). Bischoff[26], however, found that this substance led to an increased retention of fat and calcium : he could arrive at no conclusion as to the ætiological relationship between cœliac disease and bile-salt absence. Our own results are conflicting. In one there was definite improvement in fat and mineral absorption, but in the other there was quite a marked decrease in the retention of these substances. In one case (Fig. 3) the blood-sugar curve gave evidence of more normal carbohydrate absorption during the administration of bile-salts. Against the view that the absence of bile-salts is the important factor in the chemical pathogenesis of cœliac disease is the

great difference of the metabolic picture in states such as atresia of the bile duct where there is complete absence of bile. In such a condition the bulk of the phosphorus appears in the urine while the blood-sugar curve is quite normal in type. Administration of bile-salt in biliary atresia does not lead to increased retention of minerals (Table 7).

One other point brought out in the course of this series of observations seems to be the beneficial effect of acid sodium phosphate both on fat and mineral metabolism. It is difficult to believe that this benefit is the result of phosphorus as such, and it would seem probable that its action is due to its acid nature. It is now largely accepted that increasing the acid reaction of the intestinal contents leads to a better absorption of lime and phosphorus. Further, the production of bile is stimulated by secretin which is also the result of the action of acid on the intestinal epithelium. The administration of an

TABLE 7.

SHOWING EFFECT OF BILE-SALT ADMINISTRATION ON MINERAL METABOLISM IN A CASE OF BILIARY ATRESIA: DAILY INTAKE, OUTPUT AND RETENTION.

Diet	Days	CaO (grm.)					P_2O_5 (grm.)				$\frac{\text{Urine } P_2O_5}{\text{Fæcal } P_2O_5} \times 100$	$\frac{\text{Fæcal CaO}}{\text{Fæcal } P_2O_5} \times 100$	Retention grm. per kgrm. body weight	
		Intake	Fæcal output	Fæcal output as soaps	Urinary output	Total retention	Intake	Fæcal output	Urinary output	Total retention			CaO	P_2O_5
Cow's Milk 540 c.cm. + sugar grm. 24	5	0·837	0·8105	0·4290	0·0186	·0079	1·161	0·4711	0·560	0·130	118·8	172·0	·002	·033
Cow's milk 540 c.cm. + sugar grm. 24 +sod. glycocholate	6	0·837	0·8061	0·6507	0·0128	·0181	1·161	0·5303	0·5973	0·0337	112·6	152·0	·004	·008

acid salt should therefore favour both the absorption of minerals and the production of bile-salts. Verzàr and Kúthy[8] have shown that the presence of bile-salts permit the absorption of soaps at an acid pH. In the absence of these salts the absorption of fats as soaps is impossible unless at a pH of 9·0. If accordingly the reaction of the cœliac intestine were alkaline, but not, of course, with as high a pH as 9·0, there would be a defective absorption of minerals and a defective flow of bile. Shift of the reaction to the acid side would thus facilitate the absorption of lime and phosphorus and simultaneously increase the amount of bile in the intestine thus raising the utilization of fat. If with the added amount of fat there is also absorbed more vitamin D, the fixation of the calcium and phosphorus in the bone would naturally follow.

Summary.

Summing up, it appears that the results obtained in metabolic investigations on cœliac disease may be at present best explained on the following assumptions :—

(a) The defect is one of absorption and is due to change or changes in the physico-chemical constitution of the intestinal contents.

(b) These changes probably include a shift of reaction to the alkaline side and a deficiency in the bile-salts.

(c) The poor retention of minerals is probably the result of the alkaline reaction of the contents of the gut together with a difficulty in vitamin D. Improvement in the retention of minerals may occur without synchronous improvement in fat absorption.

We desire to convey out thanks to the Medical Research Council for the defrayment of the expenses connected with the investigation and for a personal grant to one of us (N. M.).

REFERENCES.

1. Cheadle, W. B., *Lancet*, London, 1903, i, 1497
2. Parsons, L. G., *Arch. Dis. Childh.*, London, 1927, II, 198.
3. Fanconi, G., *Beiheft. z. Jahrb. f. Kinderheilk.*, Berlin, 1928, XXI.
4. Schick, B., & Wagner, R., *Ztschr. f. Kinderh.*, Berlin, 1921, XXX, 223, and 1923, XXXV, 263.
5. Harrison, G. A., & Sheldon, W. P. H., *Arch. Dis. in Childh.*, London, 1927, II, 338.
6. Hutchison, H. S., *Quart. J. Med.*, Oxford, 1919-20, XIII, 277.
7. MacRae, O., *Arch. Dis. Childh.*, London, 1930, V, 431.
8. Verzár, F., & Kúthy, A., *Bioch. Ztschr.*, Berlin, 1929, CCV, 369.
9. Klinke, K., *Ergebn. d. Physiologie*, München, 1928, XXVI, 235.
10. Linder, G., & Harris, C. F., *Quart. J. Med.*, Oxford, 1929-30, XXIII, 195.
11. Morris, N., & MacRae, O., *Arch. Dis. Childh.*, London, 1930, V, 207.
12. Schaap, quoted by Lehndorff and Mautner.
13. McCrudden, quoted by Lehndorff and Mautner.
14. Poynton, F. J., & Cole, L. B., *Brit. J. Child. Dis.*, London, 1925, XXIII, 30.
15. McLean, A. B., & Sullivan, R. C., *Amer. J. Dis. Child.*, Chicago, 1929, XXXVIII, 16.

16. Thaysen, T. E. H., *Lancet*, London, 1929, i, 1086.
17. Thaysen, T. E. H., & Norgaard, A., *Arch. Int. Med.*, Chicago, 1929, XLIV, 17, 477.
18. Cathcart, E. P., & Markowitz, J., *J. Physiol.*, London, 1927, LXIII, 309.
19. Herter, quoted by Lehndorff and Mautner.
20. McCrudden, F. C., & Fales, H., *J. Exp. Med.*, N.Y., 1913, XVII, 199.
21. Moncrieff, A., & Payne, W. W., *Arch. Dis. Child.*, London, 1928, III, 257.
22. Lehndorff, H., & Mautner, H., *Ergebn. d. inn. Med. u. Kinderheilk*, Berlin, 1927, XXXI, 456.
23. Freise, R., & Jahr, J. M., *Jahrb. f. Kinderh.*, Berlin, 1925, CX, 205.
24. Meyer, L. F., quoted by Lehndorff and Mautner.
25. Miller, R., Webster, J., & Perkins, H., *Lancet*, London, 1920, ii, 894.
26. Bischoff, G., *Arch. f. Kinderh.*, Stuttgart, 1930, XC, 73.
27. Adler, E., *Arch. f. Verdauungskr.*, Berlin, 1927, XL, 174.
28. Percival, G. H., & Stewart, C. P., *Biochem. J.*, London, 1927, XXI, 301.

APPENDIX.

Case 1.—W. B., male, was admitted to hospital on May 25th, 1927, at the age of 2 years and 10 months, with a history of diarrhœa and vomiting since he was 11 months old. He was small—wt.=8·68 kgrm.—ht.=77 cm. (normal 87 cm.) with a prominent abdomen. The stools were large, pale and offensive. During residence in hospital diarrhœa continued with short periods of improvement. His weight on dismissal 10 months later (March 28th, 1928) was 7·36 kgrm. Symptoms continued after dismissal and on May 14th, 1928, he was re-admitted. Weight was then 7·8 kgrm. On Sept. 10th, he took diphtheria and later scarlet fever. He was removed to a fever hospital. On March 11th, 1929, one year and ten months after his first admission, he was re-admitted because of persistence of the diarrhœa. His weight was 8·61 kgrm. He was dismissed 5 months later at the age of 5 years with a weight of 7·43 kgrm.

Case 2.—E. A., female, was admitted to hospital when 4 years old with the following history: Healthy at birth but at 3½ months weighed only 6¼ lb. and motions were bulky and pale. This continued with occasional intervals of improvement of from 2 to 3 weeks. She was small and spare—wt.=8·48 kgrm.—ht.=78 cm. (normal 96·7 cm.) with prominent abdomen. She was kept in hospital for 3½ months and stools continued pale and offensive with periods of improvement of from 1 to 3 weeks. On dismissal weight was 10·0 kgrm. Five months later she was re-admitted because of return of diarrhœa and loss in weight. Weight on re-admission was 9·4 kgrm. The attacks of diarrhœa became gradually less frequent, and on discharge one year later, when she was 5½ years old, the weight had increased to 14·18 kgrm.

Case 3.—M. R., female, aged 4 years and 10 months, was admitted with a history of prominence of the abdomen and attacks of diarrhœa for 2 years. She was small—wt.=8·8 kgrm.—ht.=78 cm. (normal 105·3 cm.) with a prominent abdomen. The stools were pale and bulky. She was kept in hospital for 6 months during which time diarrhœa continued with occasional periods of improvement. The weight on dismissal was 9·0 kgrm.

Case 4.—J. S., male, was admitted on August 21st, 1929, when 2 years and 2 months of age. Except for a convulsion when 11 months old he throve well until 1 year and 10 months, when attacks of vomiting and diarrhœa appeared and the weight began to fall. He was emaciated and dehydrated—wt.=5·95 kgrm.—ht.=75 cm. (normal 82·8 cm.). The stools were frequent, and green with numerous curds and much mucus. During residence in hospital there was but little gain in weight and the stools were frequently large, pale and offensive. Weight on dismissal 3 months later with measles was 6·65 kgrm. On Feb. 12th, 1930, he was re-admitted. He had made a good recovery from measles and had remained well until 2 weeks before re-admission when diarrhœa and vomiting returned, the stools being bulky and pale. He was emaciated—wt.=6·8 kgrm.—and Chvostek's sign was positive. Two weeks later carpo-pedal spasm appeared and was relieved with large doses of calcium chloride (30 grm. six times daily). Chvostek's sign reappeared on March 18th, and from April 2nd 2 pellets of radiostol were given twice daily.

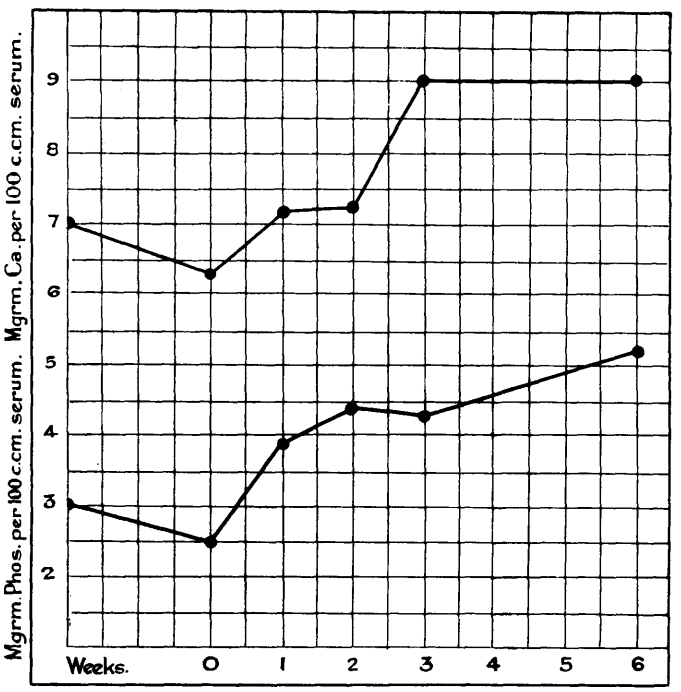


FIG. 4. Case 4. Change in calcium and inorganic phosphorus content of serum during treatment with radiostol.

The behaviour of the calcium and inorganic phosphorus content of the blood serum during this period of treatment is shown in Fig. 4. It is of interest to note that both the calcium content and inorganic phosphorus content of the serum were low, a finding not uncommon in cases of rickets complicated by tetany, and that during treatment with radiostol there occurred a gradual increase in both. All clinical evidences of tetany disappeared in 2 weeks. Radiological examination of the bones showed no evidence of rickets.

The child continued to have large pasty motions, and on June 26th he took diphtheria and was dismissed, weighing 6·36 kgrm. He was re-admitted on August 6th and died suddenly within 24 hours. The post-mortem examination revealed atrophy of the spleen, liver and kidneys. here was no abnormality detected in any part of the stomach or intestines.

Case 5.—M. McC., female, was admitted on January 8th, 1930, aged 1¼ years. She had thriven well until 1 year old, when motions became pale and bulky, and she ceased to gain in weight. Weight on admission was 6·8 kgrm. and height 73 cm. (normal 74 cm.). Stools continued to be frequent, pale and bulky, and her weight fell steadily. On February 12th, 5 weeks after admission, when the weight had fallen to 5·3 kgrm. she died. Post-mortem examination revealed a few patches of broncho-pneumonic consolidation throughout the lungs. There was no atrophy of any of the organs. Irregular areas of congestion of the mucous membrane occurred throughout both large and small bowel.

Case 6.—A. F., male, aged 3 years. For 6 months previous to admission he had been losing weight, had vomited and had frequent loose motions. He was small and pale—wt.=11·14 kgrm. —ht.=78 cm. (normal 89·1 cm.). Stools were frequent, large and pale. He was kept in hospital for 5 months and attacks of diarrhœa continued with occasional intervals of improvement lasting 2 to 4 weeks. Weight on dismissal was 11·17 kgrm.

Case 7.—M. L., a private patient under the care of Dr. L. Findlay. The child was aged 3½ and 4 years at the time of the two blood-sugar tests (Table 3).

[From THE BIOCHEMICAL JOURNAL, Vol. XXIV, No. 6, pp. 1716–1722, 1930]

CXC. ON THE PRESENCE OF A VOLATILE ORGANIC CHLORINE COMPOUND IN BLOOD.

By NOAH MORRIS and SAMUEL MORRIS.

From the Department of Paediatrics, Glasgow University, and the Biochemical Department, Royal Hospital for Sick Children.

(*Received October 20th, 1930.*)

It is generally assumed that chlorine exists in the blood solely as inorganic chloride. While this hypothesis is supported by a great deal of the classical work of L. J. Henderson, Van Slyke and others on the interchange of ions during disturbance of acid-base equilibrium, it is nevertheless true that some problems are not solved by such a conception. During a prolonged investigation into changes in the chemistry of the blood in pyloric stenosis of infancy, it has become evident that the loss of chlorine from the blood is not necessarily balanced by the gain in carbonic acid or other known acid [Graham and Morris, 1929]. Further as Hanke and Donovan [1927] state "The current theories (of the formation of gastric hydrochloric acid) involve the unreasonable assumption of the separation of the acid locally from an equivalent of alkali." These authors maintain that 10–50 % of the total chlorine is present in organic form in the blood and tissues. They suggest that it is by the action of a special chloro-esterase on this organic chlorine compound that the free HCl of the gastric juice is produced.

The work reported in this paper was commenced at the suggestion of Prof. Leonard Findlay in an endeavour to throw some light on the nature of the chlorine combination in the blood. The method of chlorine analysis employed was that of Van Slyke [1923]. The subjects from whom the blood was obtained were healthy adults or children apparently completely recovered from such conditions as rheumatism. In all cases the subject had ingested nothing for a period of at least 12 hours prior to the withdrawal of the blood.

Effect of drying on the chlorine content of blood-serum.

After a preliminary drying of serum it was found that the amount of chlorine recovered was less than when no drying was employed. In each case to obtain the effect of drying the serum was heated in an Erlenmeyer flask in an oven at 105° for 45 minutes. Table I shows the results obtained from the sera of normal adults.

Table I. *Effect of drying on the chlorine content of serum.*

Serum No.	Chlorine milli-eq./litre	
	No drying	Preliminary drying
1	102·11	97·65
2	100·00	95·10
3	102·39	98·24
4	104·76	100·32

The loss of chlorine on drying might be due to one of four causes: (1) the presence of free chlorine in the blood which was liberated by some complex action similar to that of porous clay on NaCl; (2) the formation and volatilisation of HCl; (3) the loss of inorganic chloride by volatilisation; (4) the presence of volatile organic chlorine.

The presence of free volatile Cl or HCl is excluded by the following experiments. The gases from 100 cc. of blood heated to 105° when led into a solution containing KI and starch failed to give a blue colour. The amount of Cl which is lost during the drying of 100 cc. of blood is sufficient immediately to produce a blue colour with starch iodide. The absence of this reaction together with the fact that no precipitate was found on distilling into silver nitrate solution indicates that only mere traces of free Cl or HCl could have been present.

Table II. *Effect of drying on the chlorine and fixed base content of serum.*

Serum No.	Chlorine milli-eq./litre		Fixed base milli-eq./litre	
	No drying	Preliminary drying	No drying	Preliminary drying
5	106·5	102·2	147·7	147·5
6	110·3	106·7	153·1	153·4
7	103·2	100·0	149·9	149·8
8	102·9	98·3	150·1	150·2
9	99·4	95·7	151·9	152·0

In order to determine the possibility of loss of inorganic chlorides the fixed base content of the serum was determined before and after drying by the method of Stadie and Ross [1925]. Table II gives the results. It is evident that the fixed base is unaltered by preliminary drying while definite loss of chlorine results from this treatment. Accordingly, inorganic chloride could not have been lost during the drying. One is therefore forced to the conclusion that the chlorine lost by drying is in the form of a volatile organic chlorine compound.

The effect of drying on the chlorine content of whole blood and serum was then estimated in order to determine the distribution of the volatile organic chlorine between serum and corpuscles. Blood was collected under oil and defibrinated; samples of whole blood, serum and cells were then analysed. The percentage cell content was determined by the haematocrit. Table III gives the results, from which it is evident that the cells contain a much greater amount of volatile organic chlorine than does the serum.

Table III.

Blood No.	Red blood corpuscles %	Chlorine milli-eq./litre: Whole blood, No drying	Whole blood, Drying	Serum, No drying	Serum, Drying	Cells, No drying	Cells, Drying	Organic volatile chlorine milli-eq./litre: Blood	Serum	Cells
10	45	78·57	65·47	101·56	96·82	49·77	24·58	13·10	4·74	25·19
11	46	80·11	65·88	100·48	96·04	55·58	25·10	14·23	4·44	30·48
12	46	79·95	66·15	102·15	98·10	54·06	24·82	13·80	4·05	29·24
13	44	78·99	66·55	102·76	97·85	48·43	25·77	12·44	4·91	22·66
14	45	79·15	66·90	101·09	97·14	52·44	28·77	13·25	3·95	23·67

Effect of varying the duration of the drying process on the organic volatile chlorine.

It is important that the drying process should proceed for at least 400 minutes in order to ensure the volatilisation of the chlorine to a maximum. The following results give proof of this.

Duration of drying in minutes	Volatile organic chlorine in milli-eq./litre
3	3·20
15	3·25
30	4·25
45	6·25
60	7·85
75	8·55
90	9·24
105	9·50
210	11·15
400	12·60
600	12·60

The isolation of the volatile organic complex of chlorine has so far been found impossible owing to the volatilisation of various substances at the same time. On distillation acetic acid and ammonia appear in very large amounts. Distillation at 110° accompanied by the passage of an air current through the blood led to the collection of but minimal amounts of chlorine in the receiving flask. By the usual distillation procedure, however, it was possible to recover and estimate by the Carius or Parr method the amount of Cl volatilised; Table IV gives the amounts so recovered. The amounts collected in the distillates compare very satisfactorily with those lost from the blood samples especially when one remembers the practical difficulties which involve a large experimental error such as the possibility of some of the volatile chlorine being held back by the material as it dries.

Table IV. *Chlorine lost from the blood on distillation.*

Blood No.	Chlorine milli-eq./litre: Lost from blood	Collected in distillate: Parr method	Collected in distillate: Carius tube
15	172·4	168·0	165·1
16	146·5	142·1	144·3
17	141·8	140·1	140·7
18	138·7	132·4	136·2

Effect of addition of various substances on the volatile organic chlorine content of blood.

Various compounds containing the NH_2 group were used, including urea, histamine, glycine and a few of the simpler primary and secondary amines. In all cases the materials employed were purified before using. In Fig. 1 is

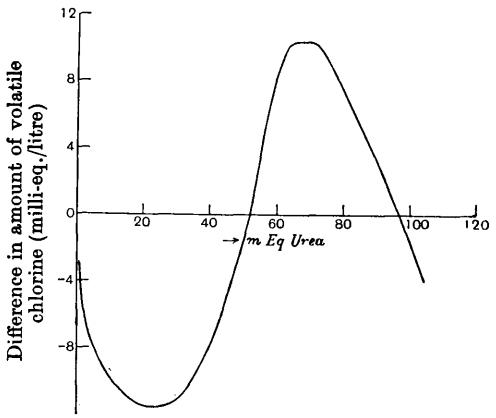


Fig. 1. Effect of urea-concentration on volatile chlorine.

given a graphic representation of the results obtained when urea was added to the blood in progressively increasing amounts.

To a sample of blood a known amount of urea was added: the two portions were then analysed (one with and the other without previous drying) to obtain the value for the volatile chlorine. All analyses were done in duplicate. It will be seen that this concentration curve corresponds with the adsorption formula $y/m = kc^{1/p}$, where y = amount adsorbed by a quantity m of adsorbent, c = concentration at equilibrium and k and p are constants. In the present case y represents the increase in urea concentration, m the original amount of volatile chlorine and c the amount of volatile chlorine after the addition of the urea. Between the concentrations of urea 2·14–24·10 milli-eq./litre the values of $1/p$ and k are $-3{\cdot}4$ and $-3{\cdot}16$ respectively. There is a marked resemblance between the curve and the coagulator concentration curves. In this case the coagulator is urea and the reaction consists of a coagulation of the volatile chlorine complex up to a range of concentration of urea of 24 milli-eq., when reversal occurs with consequent peptisation.

When an ammonium salt is added, *e.g.* $(NH_4)_2SO_4$, a curve similar to that of the NH_2 series is obtained (Fig. 2). The maximum and minimum values (*i.e.* those concentrations at which reversal takes place) are lower than those found with urea. The curve again corresponds with the adsorption equation. Between concentrations of $(NH_4)_2SO_4$ of 0·9672–4·8360 milli-eq./litre $1/p = 2{\cdot}4$ and $k = -12{\cdot}13$.

When a chloride is added, the results obtained are the inverse of those

obtained with NH_2 or NH_4 groups (Fig. 3). This indicates that the addition of the chlorine ion produces an increase in the amount of volatile chlorine, possibly because the Cl having the opposite electric charge to that of NH_2 breaks the union of NH_2 and volatile chlorine. At a certain concentration reversal sets in with the consequent diminution of volatile chlorine.

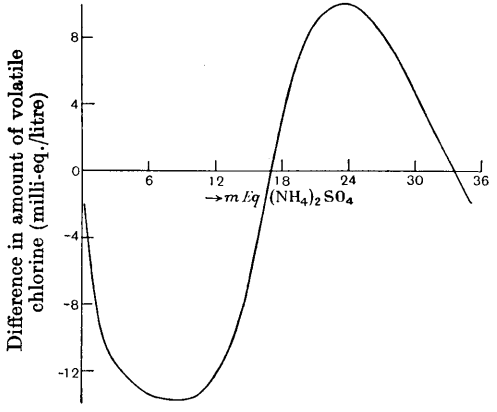


Fig. 2. Effect of NH_4 content on volatile chlorine.

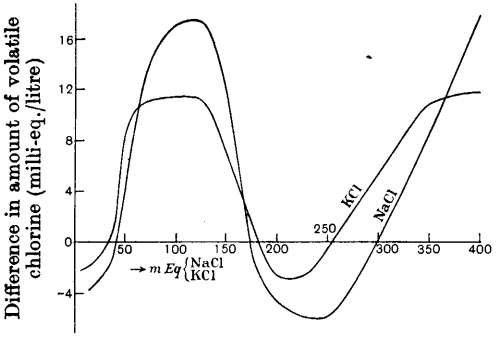


Fig. 3. Effect of KCl and NaCl content on volatile chlorine.

Addition of a very dilute acid produces the same effect on volatile chlorine as does NaCl. As the strength of acid is increased, the amount of volatile chlorine is at first raised and then lowered till with higher concentrations free HCl is formed.

Effect of prolonging the time during which blood was allowed to stand at room-temperature.

The mixtures of blood and coagulator substances (urea or NaCl) were allowed to stand at room-temperature before drying. The mixtures had urea 1·605 and NaCl 50 milli-eq./litre respectively. The results are given in Table V. The general equation for both curves is $y = \alpha c^n$. For the urea mixture $\alpha = 0{\cdot}54$, $n = 1{\cdot}7$ and for the NaCl mixture $\alpha = 44$, $n = -0{\cdot}15$. The effect on

Table V. *Effect of time-factor on volatile organic chlorine of blood to which urea or NaCl has been added.*

Time in minutes	Difference in amount of volatile organic chlorine in milli-eq./litre Blood urea	Difference in amount of volatile organic chlorine in milli-eq./litre Blood NaCl
5	−4·1	−5·7
10	−4·2	−5·46
15	−4·88	−4·62
20	−5·76	−3·58
25	−7·08	−2·56
30	−8·05	−1·56
40	−8·90	−0·46

the volatile organic chlorine of allowing untreated blood to stand was to cause a gradual decrease to a value which remained steady about 5 milli-eq. below the original. Thereafter prolonged standing did not appreciably alter the amount of volatile chlorine. This is interesting in view of the results of Havard and Kerridge [1929] on the change of c_H in blood allowed to stand. They found that the c_H increased to a point and there remained steady. It has already been pointed out that the addition of acid in small amount decreases the content of volatile chlorine so that the effect of standing on the volatile chlorine is associated with the increase in c_H. It seems feasible to assume that the liberation of volatile chlorine produces an increase in the c_H. It is possible however that the relationship of cause and effect is reversed or that loss of volatile chlorine and increase in c_H are both due to some other underlying factor.

From these data we may conclude that the amount of volatile chlorine may be altered by the addition of various substances to the blood but that for each blood there is a certain maximum and minimum beyond which the content does not pass. The simplest hypothesis on which to explain these results is to assume that the chlorine in normal blood exists in three forms (*a*) inorganic, (*b*) organic non-volatile, and (*c*) organic volatile. The relative amounts of non-volatile and volatile organic chlorine seem to depend on the concentration of urea, amino-acid etc. Thus in blood I (Fig. 1) the maximum amount of volatile chlorine is 24·79 milli-eq. per litre. Varying amounts of urea lead to the production of an organic non-volatile chlorine compound in amounts that satisfy the adsorption formula. This indicates the presence of a complex organic colloidal system in which there is present a loose union of amino-compounds with chlorine forming a non-volatile organic compound. As the amino-compounds are increased in amount there occurs an increased formation of this non-volatile compound up to the point where reversal of charge sets in when peptisation occurs. The addition of an inorganic chloride breaks this complex up to a certain concentration of the salt beyond which the chlorine once again commences to unite with the amino-group. This is probably also an effect of the electric charge.

We wish to express our thanks to the Medical Research Council for defraying the expenses of the investigation and for a personal grant to one of us (N. M.).

REFERENCES.

Graham and Morris (1929). *Arch. Dis. Child.* **4**, 335.
Hanke and Donovan (1927). *J. Biol. Chem.* **74**, xxiv.
Havard and Kerridge (1929). *Biochem. J.* **23**, 600.
Stadie and Ross (1925). *J. Biol. Chem.* **65**, 735.
Van Slyke (1923). *J. Biol. Chem.* **58**, 523.

[From THE BIOCHEMICAL JOURNAL, Vol. XXVI, No. 6, pp. 2015–2020, 1932]

CCXL. FURTHER OBSERVATIONS ON THE VOLATILE CHLORINE OF BLOOD.

By SAMUEL MORRIS and NOAH MORRIS.

From the Hannah Dairy Research Institute, Ayr, and the Biochemical Laboratory, Department of Paediatrics, Hospital for Sick Children, University of Glasgow.

(*Received October 20th, 1932.*)

In a previous paper [Morris and Morris, 1930] it was shown that drying of blood at 105° for a period of 4 hours led to a diminution in the chlorine content and it was suggested that the chlorine lost by drying was probably contained in a volatile organic compound. Sunderman and Williams [1931] published figures indicating that while a loss of chlorine occurred on drying blood and tissues it could be prevented by adding water to the dried blood and allowing the mixture to remain for 3 hours prior to the estimation of the chlorine. They further showed that the addition of olive oil to a solution of sodium chloride with subsequent drying led to the loss of a certain amount of chlorine which also could be prevented by the addition of water after drying and prior to the estimation, and concluded that in the case of blood the loss of chlorine was only an apparent one, being probably due to the effect of occlusion of the chlorine by fat. Quagliariello and Mazza [1931], on the other hand, state that the loss of chlorine on the heating of blood is due to drying and loss of HCl and is therefore no indication of the presence of organic chlorine compounds. Hogartz [1931] found no evidence of organic compounds of chlorine by extraction methods involving the use of alcohol and ether.

In our previous paper the results of experiments were given showing that the chlorine lost from the dried blood could be recovered almost quantitatively although not in the form of free chlorine or hydrochloric acid. In view of the results of Sunderman and Williams with olive oil, it was thought necessary to confirm our previous results and study the variations in the amounts of chlorine volatilised under different conditions.

Effect of drying blood at 105°.

Each sample of blood was divided into three equal parts, and the Cl content of the first was determined immediately by the Van Slyke method. The remaining two were dried for 4 hours at 105°; thereafter one was analysed by

the Van Slyke method and the other was completely ashed and its Cl content estimated by the same method. Table I indicates that practically the same diminution was obtained by drying whether the Van Slyke method was used directly or preliminary ashing was employed. It seems clear therefore that the decrease in the Cl value was not the result of occlusion.

Table I.

Chlorine milliequiv./litre.

Blood no.	No preliminary drying	Preliminary drying	Preliminary drying and ashing
35	89·00	71·90	68·50
36	89·60	71·90	72·30
37	87·10	77·00	76·00

Effect of oxygen on the volatilisation of the chlorine from blood.

To study the effect of oxygen, samples of the same blood were dried in ordinary air, air deprived of oxygen and in an atmosphere of pure nitrogen. The air was deprived of oxygen by passing it through two flasks of pyrogallol prior to its entry into the drying flask. The atmosphere of nitrogen was supplied by heating NH_4NO_2. Table II shows that in the absence of oxygen no loss of chlorine is produced by drying at 105°. Furthermore, in the presence of air, addition to the blood of a strong oxidising agent such as potassium persulphate leads to an increased loss of chlorine on drying (Table III). This suggests that the presence of active oxygen facilitates the formation of the volatile chlorine compound.

Table II.

	No drying	Preliminary drying			
Blood no.		Ordinary air	Dry air	Dry air deprived of oxygen	Nitrogen
46	86·2	72·4	72·8	86·2	86·0
47	79·8	70·1	70·0	78·6	79·1
48	84·5	69·9	70·4	82·9	84·8
49	88·9	74·5	73·9	87·9	88·3
50	79·8	64·8	64·8	78·7	79·6
51	86·5	75·6	75·5	86·0	86·1

Table III. *Effect of addition of potassium persulphate to blood on the chlorine content after drying.*

	No drying		Preliminary drying	
Blood no.	Blood	Blood + $K_2S_2O_8$	Blood	Blood + $K_2S_2O_8$
51	85·66	85·60	73·00	62·30
52	85·20	85·25	69·95	59·24
53	87·68	87·72	65·36	57·71
54 (serum)	115·85	115·81	114·32	103·61

Effect of drying blood in a desiccator (Table IV).

Sunderman and Williams showed that drying blood over P_2O_5 also involved a loss of Cl and concluded that the essential factor was the loss of moisture. We studied the effect of drying blood for 48 hours over H_2SO_4: from some flasks oxygen was removed, others were filled with nitrogen, while others contained sodium cyanide solution. The effect of drying over H_2SO_4 was to produce a

Table IV. *Effect of drying blood in desiccator at room temperature (18°) in atmospheres of* (a) *air*, (b) *air deprived of oxygen*, (c) *nitrogen*, (d) *air, with NaCN added to the blood and* (e) *air, with $K_2S_2O_8$ added to the blood.*

Blood no.	No drying	Preliminary drying in oven at 105°	Air	Air deprived of oxygen	Nitrogen	Ordinary air; NaCN added to blood	Ordinary air; $K_2S_2O_8$ added to blood
(*a*) 46	86·2	72·4	78·8	86·2	86·0	85·5	71·1
(*b*) 47	79·8	70·1	74·9	79·5	79·8	79·8	71·9
(*c*) 48	84·5	69·9	74·9	84·0	84·1	84·0	67·3
(*d*) 49	88·9	74·5	80·6	89·0	87·9	87·8	76·0
(*e*) 50	79·8	64·8	71·4	78·8	78·9	78·4	60·6

diminution in the amount of chlorine recovered, although the loss was not so great as when the blood was dried at 105°. The addition of potassium persulphate increased the loss to at least that found on drying at 105°. The absence of oxygen from the flask or the presence of cyanide, which inhibits oxidation, prevented any loss of chlorine. It is clear therefore that the withdrawal of moisture in itself is not responsible for the diminution in chlorine content. It should here be stated that if the cyanide-blood mixtures are dried for over 48 hours the cyanide is converted into HCN and completely volatilised and there may occur some oxidative process with consequent loss of chlorine (Table V).

Table V. *Effect of drying blood with NaCN in desiccator for varying periods.*

		Preliminary drying		
Blood no.	No drying	Duration of drying in hours	Blood alone	Blood + NaCN
46	86·2	24	82·4	85·9
—	—	48	80·6	86·0
—	—	72	72·4	85·5
—	—	120	72·9	80·6
—	—	168	72·1	72·8

Table VI. *Effect of drying tissues* (a) *at 105°*, (b) *in desiccator and* (c) *with acetone and ether.*

Tissue (rabbit)	No drying	In oven at 105°	In desiccator	By acetone and ether
Heart	35·4	27·5	32·6	35·8
,,	33·8	26·2	30·1	35·0
Kidney	49·2	31·8	40·4	48·8
,,	54·1	36·5	46·2	54·0
Muscle	12·2	8·1	10·4	12·2
,,	16·4	11·9	14·0	16·0
,,	10·5	7·0	8·8	10·5

Effect of drying tissues under various conditions.

Prior to determination of their Cl content tissues were dried either in the oven at 105° or over concentrated H_2SO_4 or by Best and McHenry's acetone and ether method [1930]. The results (Table VI) fully corroborate the blood analyses, indicating that oxygen is essential for the volatilisation of the chlorine and that more chlorine is lost at a temperature of 105° than as a result of drying in a desiccator.

Effect of addition of olive oil to NaCl solution.

It seemed to us possible that the loss of chlorine on drying a mixture of olive oil and NaCl [Sunderman and Williams, 1931] might be due to reaction between the NaCl and unsaturated fatty acids.

This view was supported by the fact that heat is necessary to produce a loss of Cl from the olive oil-NaCl mixture. Further, the use of a fully saturated acid such as palmitic instead of olive oil should lead to no diminution of Cl content on drying the fatty acid-NaCl mixture at 105°. Experiments show that heating of palmitic acid-NaCl mixtures at 105° leads to no decrease in the Cl.

The only difference between blood and the olive oil-NaCl mixture as far as the loss of chlorine is concerned is the fact that the chlorine is lost from the latter mixture only after heating to over 100° whereas it can be driven off from the blood on drying in the desiccator without the aid of heat. This, however, is insufficient evidence that chlorine is present as an organic compound in blood. In order to demonstrate the presence of such a compound in blood it is necessary to extract it.

Organic solvents such as ether, benzene and toluene were found to be useless for the extraction of any chlorine. It was thought that a mixture of alcohol and ether might be efficient. Alcohol, however, mixed with the water present in blood and carried into solution some of the inorganic chlorine. It was therefore necessary to remove water from the blood by some method other than heat. For this purpose a modification of Best and McHenry's method for drying tissues was used. This consisted in the addition of acetone to blood, filtering, and washing four times with acetone in excess until no chlorine appeared in the acetone filtrate and then three or four times with ether. Air was then drawn over the residue by a suction pump, until a dry powder was obtained. The powder was removed to a flask and a mixture of equal parts of absolute alcohol and anhydrous ether was added. The powder was shaken with the alcohol-ether mixture for three periods of 15 minutes and then allowed to stand overnight. The alcohol-ether extract was then filtered off into a pyrex tube, and the alcohol and ether were evaporated off at a low temperature. Chlorine was invariably found to be present in the extract when this was treated with silver nitrate and nitric acid. So far we have been unable to recover more than 80 % of the amount lost on heating blood. With tissues the same method was employed after they were finely minced.

The alcohol-ether mixture does not extract chlorine from inorganic chlorides unless water is present. This we have repeatedly confirmed for the alcohol-ether mixtures we have been using.

It seemed possible, however, that the blood might have been incompletely de-hydrated by the acetone-ether treatment, and that the chlorine extracted was due simply to the inorganic chlorides being dissolved in the alcohol-ether mixture owing to the presence of moisture. To test this possibility a series of experiments was undertaken in which a synthetic mixture analogous to blood was used, containing 16 g. of protein (egg-albumin, serum-albumin or caseinogen), 0·5 g. of olive oil, 0·5 g. of NaCl and 0·1 g. of glucose per 100 cc. water. The mixture was well stirred and allowed to stand for about 4 hours prior to extraction, by the method used for the blood. In no case was chlorine found in the alcohol-ether extract.

Further, it was shown in a previous paper [Morris and Morris, 1930] that the volatile chlorine of blood diminishes on adding acids to the blood, the diminution being dependent on the strength of the acid. The addition of a strong acid should therefore diminish the amount of organic chlorine extracted from blood by means of alcohol and ether. From the same sample of blood two lots of 100 cc. were taken to one of which was added 10 cc. concentrated HCl. Both were allowed to stand for 4 hours and then extracted by acetone and finally by alcohol and ether. In the blood-HCl sample only minimum traces of chlorine (1–2 milliequiv.) could be detected in the alcohol-ether extract. This experiment which was repeated on four occasions affords further confirmation of the fact that inorganic chlorine is not extracted by the method described.

In view of these facts it seems justifiable to conclude that there exists in blood an organic chlorine compound.

Summary.

1. The presence of oxygen is essential for the loss of chlorine to take place.

2. The heating of an olive oil-NaCl mixture at a temperature over 100° leads to loss of chlorine.

3. Evidence is brought to show that there is close similarity between the two systems, blood and olive oil-NaCl, as far as the loss of Cl is concerned, except that in the latter mixture heat is required whereas with blood drying in a desiccator is sufficient to cause volatilisation of the Cl complex.

4. A method is described whereby organic chlorine can be extracted from blood. The method depends on removal of water by acetone and ether and subsequent extraction of the chlorine by an alcohol-ether mixture.

The authors regret that owing to an unfortunate oversight there was omitted from their previous paper an acknowledgment that the work therein described was commenced by one of them (S. M.) while in the Chemical Department of the Pennsylvania Hospital, Philadelphia.

We wish to express our thanks to Prof. G. G. Henderson, Prof. G. B. Fleming and Dr N. C. Wright for their helpful criticism and to the Medical Research Council for defraying the expenses of the investigation and for a personal grant to one of us (N. M.).

REFERENCES.

Best and McHenry (1930). *J. Physiol.* **70**, 349.
Hogartz (1931). *Z. physiol. Chem.* **200**, 119.
Morris and Morris (1930). *Biochem. J.* **24**, 1716.
Quagliariello and Mazza (1931). *Boll. Soc. Ital. Biol. Sper.* **6**, 703.
Sunderman and Williams (1931). *J. Biol. Chem.* **92**, 99.

Reprinted from the "Glasgow Medical Journal," Nov., 1932, vol. cxviii, p. 321.]

DIABETES IN CHILDHOOD.

II. TREATMENT.*

By NOAH MORRIS, M.D., F.R.F.P.S.,
Lecturer in Pathological Biochemistry, Glasgow University;
Biochemist, Royal Hospital for Sick Children, Glasgow.

In many diseases it is becoming more and more recognized that prolonged after-care is essential for successful return to and maintenance of health. The one condition that specially exemplifies this is diabetes. Even after diet and insulin have been arranged it is important that the progress of the diabetic be carefully supervised. When the patient is a child, after-care becomes even more important, as the treatment of diabetes in childhood, apart from general difficulties, presents special problems of its own, such as the extra strain of growth and the increased liability to infection. In addition, the child is said to be less capable of understanding the gravity of the condition and the importance of careful and accurate dietary regulation. In our experience, however, patients over the age of ten seem to understand what is wanted of them and co-operate intelligently.

Any scheme for the treatment of diabetes in childhood must be devised with a view to two ends, (*a*) the arrangement of the diet and insulin administration, and (*b*) the guidance of the parents in the maintenance of the patient's well-being during ordinary life and such mischances as infection. This guidance involves not only a knowledge of caloric values and insulin therapy, but also a proper appreciation of the social and economic conditions of the patient's home and the psychological make-up of the parents.

In some instances it is possible to determine the best diet and insulin dosage in the patient's own home, but in most cases residence in hospital or nursing home is beneficial, if not essential, because of the more efficient supervision which is

* A paper communicated to the Royal Medico-Chirurgical Society of Glasgow, 22nd January, 1932.

possible and of opportunities of education. The older children are taught to weigh their food and even to measure out and inject insulin. After-care is best carried out at home by the family doctor or in a special clinic : at anyrate, the child should be seen at least once a month when he is doing well and oftener when glycosuria or any complication appears.

As soon as a child is found to have glycosuria, steps should be taken either in hospital or at home to determine whether the condition is one of diabetes mellitus. Once the diagnosis of diabetes is made there is the choice of two methods of initiating treatment. (1) A low intake of food is prescribed and continued until the urine is sugar free. Thereafter the diet is gradually brought up to the required caloric value and glycosuria, if and when it occurs, is rectified by the administration of insulin. (2) The optimum requirement of food is given from the beginning and sufficient insulin is injected to deal with the carbohydrate. In both methods it is helpful to estimate the amount of sugar excreted in the urine and to allow one unit of insulin for every 2 grams of urinary sugar as a first approximation.

When there is marked ketonuria the second is the preferable method, because it is very unwise to restrict the food of a child when this is present since children are very susceptible to acidotic conditions and coma may easily be precipitated. Otherwise the first or " crescendo " method of treatment is to be preferred, and for the following reasons :—(1) It enables one to determine the carbohydrate tolerance before giving insulin. (2) It permits the gradual storage of glycogen in the liver, thereby lessening the chance of hypoglycæmia. This occurrence should be avoided if possible, particularly in the early stages of insulin treatment, lest it irreparably undermine the patient's and parents' confidence in insulin. With small initial doses the parents are gradually introduced to insulin treatment. (3) By beginning with large doses of insulin there may be a tendency to overfeeding.

It is sometimes urged even in medical circles that insulin should be withheld if at all possible on the ground that with prolonged administration its efficiency diminishes. There is absolutely no evidence in support of this view. It is unwise to spend too long a time in determining the sugar tolerance

without insulin if glycosuria is present, as there is some danger that the previous poor tolerance may be rendered still poorer. Indeed, the statement is made by several authorities that the shorter the duration of diabetes the more amenable it is to insulin therapy. Our records, however, do not bear this out.

Caloric Requirements.

It is generally accepted that the diabetic should be rather below average weight. Accordingly the caloric value of the

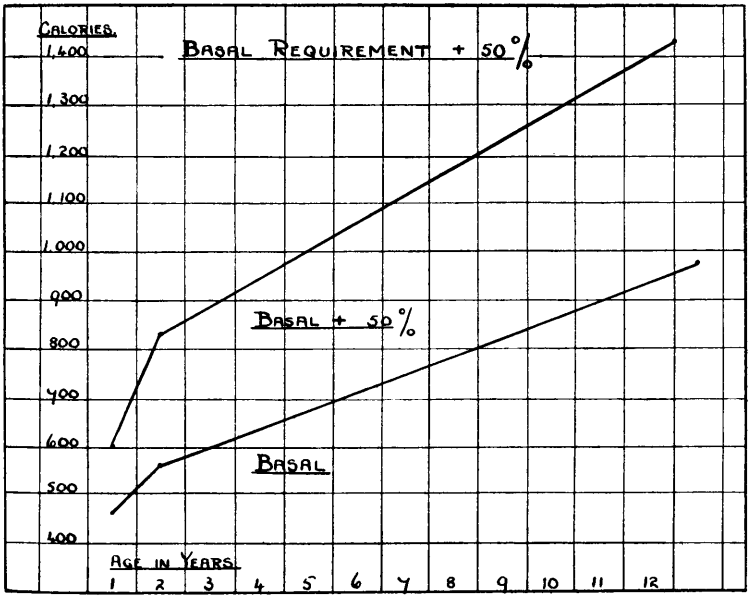


Chart 1.

food supplied should be less than that normally required by the child of the same age. For a time we supplied a diet of a rather high caloric value, but this was stopped because it was found very difficult to keep the children free from glycosuria even with very large doses of insulin. At the same time it should be remembered that the provision of too low a dietary will lead to the breaking of dietetic restrictions with consequent glycosuria which often proves very intractable. A scheme for

caloric requirements has been drawn up on the standard of 50 per cent above the basal values (Chart I). This has proved most satisfactory as a guide to the diet. When weight increases too quickly the diet is reduced, but it should be emphasized that therapeutic loss of weight must be very gradual, since excessive underfeeding may easily precipitate coma. On the other hand, it is occasionally necessary to increase the diet if weight is lost.

Partition of Diet among Protein, Carbohydrate and Fat.

Recently it has been urged from many quarters that the carbohydrate should not be stinted, since its utilization can always be ensured by a liberal supply of insulin. While this seems theoretically sound and apparently has been followed with good effect in adults, it is our experience that it is better to restrict the carbohydrate to not more than one quarter of the total caloric requirements. It is well to remember the possibility of infection and the consequent necessity for extra insulin. In one case the insulin dosage was increased fourfold during respiratory infection although the food intake was reduced. If ordinarily a large daily dose is being given the carbohydrate values of a unit of insulin will be relatively small, so that an enormous dose will be required during any condition of toxæmia. It is wise, therefore, to arrange the diet so that, though adequate, the minimum amount of insulin is required to keep the urine free of sugar and acetone.

As regards the fat intake, it seems inadvisable from our experience to increase the fat in the child's diet to more than 60 per cent of the total caloric requirements. Joslin also notes that a high-fat diet is tolerated well by the adult but not by the child or adolescent. Lawrence in his scheme has the fat intake as high as three-quarters of the caloric intake, but the frequency of ketonuria led us to reduce the fat of the Lawrence diet by 50 per cent.

With this modification the Lawrence diet has proved to be well suited for the treatment of children. The diet is ordered as lines, of which there are two types, black and red. Each black line corresponds to 5 grm. carbohydrate with a caloric

value of 20, and each red one to 7·5 grm. protein plus 7·5 grm. fat with a caloric value of 100. It is advisable in arranging the diet to balance every red line by at least one black, the object being to prevent over-production of ketone bodies. It is quite permissible, however, to give more black than red lines if carbohydrate tolerance is not greatly impaired, or if the extra carbohydrate can be utilized with the help of not too large a dose of insulin. The advantages of this scheme are twofold. (1) The adjustment of the diet merely entails the division of the total number of calories required by 120 and ordering the appropriate number of black and red lines. If relatively more carbohydrate is desirable it is easy to make the necessary adjustment. (2) The parents are able to vary the diet from day to day and so prevent monotony. Since this scheme has been adopted we have found the mothers much better pleased and more able to cope with the desires of the children. The scheme is easy to understand and is generally carried out correctly within the first few weeks, usually days, of out-patient life.

Distribution of Diet throughout the Day.

This is obviously bound up with the administration of insulin. In none of our cases did a single injection of insulin suffice even when the bulk of the diet was given at the corresponding meal. We find it better even in mild cases to give the insulin in two doses equally distant in time. This allows a better distribution of food and also prevents the rise of blood-sugar which tends to occur in some patients after too long a time without insulin, even although no food has been taken. The ideal method would be to distribute food and insulin equally throughout the twenty-four hours, but this is inconvenient for domestic reasons and also because it necessitates too many injections. In some cases, however, it is found that three injections must be given. The actual spacing of diet and insulin is so arranged that there is no glycosuria throughout the twenty-four hours; this is done by testing the urine before each meal and at 12 midnight. In this way the time of the leakage of sugar is detected and appropriate changes in diet and insulin are made.

Table I.

Date - October, 1929.	17th.		18th.		19th.		20th.		21st.		22nd.		23rd.		24th.		25th.		26th.		27th.		28th.		29th.		30th.	
	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.
Reddish, ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	—	...	...	...	...	...	...	...
Green, ...	...	...	...	...	...	...	...	...	...	...	...	...	—	...	—	...	—	...	—	...	...	...	...	...	...	...	—	...
Slight Green, . . .	...	...	...	...	—	...	...	...	—	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
No Change, . . .	—	—	—	—	...	—	—	—	...	—	—	—	...	—	...	—	...	—	...	—	...	—	—	—		—	...	—

Date - October—November.	31st.		Nov. 1st		2nd.		3rd.		4th.		5th.		6th.		7th.		8th.		9th.		10th.		11th.		12th.		13th.	
	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.	M.	E.
Reddish, ...	—	...	...	...	...	...	...	...	—	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Green, ...	...	...	...	...	...	...	...	...	...	...	—	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Slight Green, . . .	...	...	—	...	...	...	—	...	...	...	...	...	—	...	...	...	...	...	...	...	...	...	...	...	...	...	..	...
No Change, . . .	...	—	...	—	—	—	...	—	...	—	...	—	...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Date.	Breakfast.		Dinner.		Tea.		Supper.	
	Insulin.	Diet.	Insulin.	Diet.	Insulin.	Diet.	Insulin.	Diet.
17/10/29	6	3	...	2	6	3	...	2
31/10/29	7	3	...	2	7	3	...	2
7/11/29	8	3	...	2	8	3	...	2

TABLE II.

Date.	Wt. Kilos.	Ht. Cms.	Calories.					Urine.		Diet.				Insulin.				Fasting Blood Sugar.	Remarks.
			Reqd.	Supplied.				Sugar.	Acetone										
				C.	P.	F.	K.			B.	D.	T.	S.	B.	D.	T.	S.		
5/11/28	15·0	94	970	30	45	45	720	+	–	2	1	1	2	5	...	...	5	216	Glycosuria during past week.
12/11/28	15·5	94	...	...	...	...	...	...	...	2	1	1	2	8	...	...	8	...	No glycosuria. Well.
17/12/28	16·47	94	...	...	...	...	...	–	–	2	1	1	2	8	...	...	8	70	,, ,,
14/1/29	16·3	95	...	...	..	..	...	–	–	2	1	1	2	8	...	...	8	93	,, ,,
21/2/29	16·66	96	...	...	...	...	...	–	–	2	1	1	2	8	...	...	8	109	,, ,,
21/3/29	16·82	97	1000	...	...	...	...	–	– +	2	1	1	2	8	...	...	8	77	,, ,,
18/4/29	16·6	97	...	...	...	...	...	–	–	2	1	1	2	8	...	...	8	...	,, ,,
16/5/29	16·9	97	...	35	52·5	52·5	840	–	–	3	1	1	2	8	...	...	8	75	,, ,,
13/6/29	17·6	98	...	...	...	...	...	+	–	3	1	1	2	10	...	...	8	...	Occasional glyc. Hypo. attack $\frac{2}{52}$ ago.
11/7/29	17·2	99	...	...	..	...	...	–	–	3	1	1	2	10	...	...	8	...	No glycosuria. Well.
5/8/29	17·0	101	...	...	...	...	...	–	–	3	1	1	2	10	...	...	8	...	,, ,,
3/10/29	17·6	101	...	45	67·5	67·5	1080	–	–	3	2	1	3	10	...	...	8	...	,, ,,
31/10/29	17·6	101	1040	50	75	75	1200	+ +	–	3	2	2	3	10	...	...	8	311	"Cold" for past $\frac{1}{52}$. To be admitted.
2/1/30	18·1	103	...	40	60	60	960	–	–	3	1	1	3	10	...	...	6	...	Out of Hosp. $\frac{1}{52}$. No glyc.
16/1/30	17·5	103	...	...	...	...	...	–	–	3	1	1	3	10	...	...	6	...	No glycosuria.
13/2/30	17·5	103	...	...	...	...	...	+	–	3	1	1	3	12	...	...	8	139	Slight glycosuria.
20/2/30	17·6	103	1070	...	...	...	...	+ +	– +	3	1	1	3	12	...	...	8	225	Glyc. + + during past $\frac{1}{52}$.
27/2/30	17·6	103	...	...	...	...	...	+ +	– +	3	2	1	2	14	...	...	12	...	Glycos. + +. Syringe defective.
6/3/30	17·4	103	...	...	...	...	...	–	–	3	2	1	2	14	...	...	12	...	No glycosuria. Well.
20/3/30	17·5	103	...	...	...	...	...	–	–	3	2	1	2	14	...	...	12	...	,, ,,
17/4/30	17·5	103	..	...	...	...	...	–	–	3	2	1	2	14	...	...	12	...	.. ..

Out-Patient Treatment.

Almost invariably the patient is sent out of hospital with the urine sugar-free on a specified line diet. The mother is shown by the ward-sister how to arrange the diet, and the importance of accurate weighing of food is impressed upon her. She is also taught how to use a hypodermic syringe and how to test the urine for sugar. The older patients are also taught these things, and indeed the girls often supervise their own treatment at home. Typewritten instructions for testing the urine are supplied, together with some Benedict's qualitative reagent, a chart for noting the results of the tests (Table I), and a line diet scheme. The patient is asked to attend at the Diabetic Clinic within two weeks of dismissal from hospital. The Almoner meanwhile has obtained information of home conditions, income and general management, with special reference to the capability and general intelligence of the mother. This is of the utmost importance to the well-running of the clinic, as it enables one to judge whether persistent glycosuria may be due to lack of means in providing the necessary food, lack of intelligence or carelessness. Very often a talk with the Almoner helps the mother considerably in the arrangement of the prescribed diet, especially with reference to the best foods to buy at different seasons. The Almoner is also of great help in investigating the home conditions in cases of persistent glycosuria which were dismissed from hospital with the urine sugar-free. It one case it was found that the scales were faulty. It may be said that the interest of the Almoner is welcomed by the parents, and, further, that since the clinic has been inaugurated we have received encouragement and assistance from the patients' family doctors.

Table II is an example of the record kept of the patients' progress. The weight gives an indication of the sufficiency of the diet, while the presence or absence of glycosuria provides a first test of the adequacy of the insulin dosage. The general health and development of the diabetic child under insulin therapy is quite as good as normal. Both weight and height are usually maintained at about 90 to 95 per cent of normal, and the incidence of illness is not greater than in the non-

diabetic child. The weight, of course, is intentionally kept below the normal average.

Glycosuria.

It is important to keep the urine sugar-free if at all possible. In some cases, however, it seems impossible to do so without simultaneously producing hypoglycæmic attacks. In one instance the mother definitely states that the child is not well if there is not morning glycosuria. As a general rule, however, it is advisable to impress on parents the fact that a sugar-free urine is just as necessary in insulin-treated patients as in those not requiring injections. If glycosuria is present it is exceedingly difficult to determine without frequent blood-sugar estimations the status of the patient. When parents realize this, their supervision of the diabetic regime becomes even more marked and the obtaining of negative tests encourages both patient and parents to persevere.

It is unwise, however, to depend entirely on urinary tests, at anyrate in children, because of the great variation in renal threshold which has been shown by Dr. Gilchrist to exist even in normal children. In diabetes the threshold for sugar is often considerably higher than in normal, probably because the renal cells become impermeable to the high blood-sugar so that the concentrations of 250 mgm. per cent and more have been met with although no glycosuria has been noted. Rabinowitsch has shown that the renal threshold rises considerably under insulin treatment, so that a blood-sugar of 300 mgm. per cent may be present without glycosuria and this without any evidence of impaired renal efficiency. Occasionally the fasting blood-sugar has risen without the appearance of sugar in the urine, and this has invariably been the forerunner of persistent glycosuria, entailing a long process of dietary and insulin readjustment. As a general routine, therefore, we estimate the fasting blood-sugar in every patient three times yearly, oftener if it is above 140 mgm. per cent. If the fasting blood-sugar is repeatedly above this value it is taken as an indication of an inadequate supply of insulin, and steps are taken to remedy this defect. Joslin considers a normal blood-sugar desirable in every case provided it can be attained with a reasonable restriction of diet and moderate dosage of insulin, but he does not believe it a necessity for improvement.

In determining the fasting blood-sugar in diabetes it is well to remember that too long a fast may lead to a high sugar value owing to the absence of sufficient insulin. On the other hand, prolonging the fast in the normal person is followed by steady decline in the level of the blood-sugar (Chart II). Another disturbing factor is the effect of excitement. In

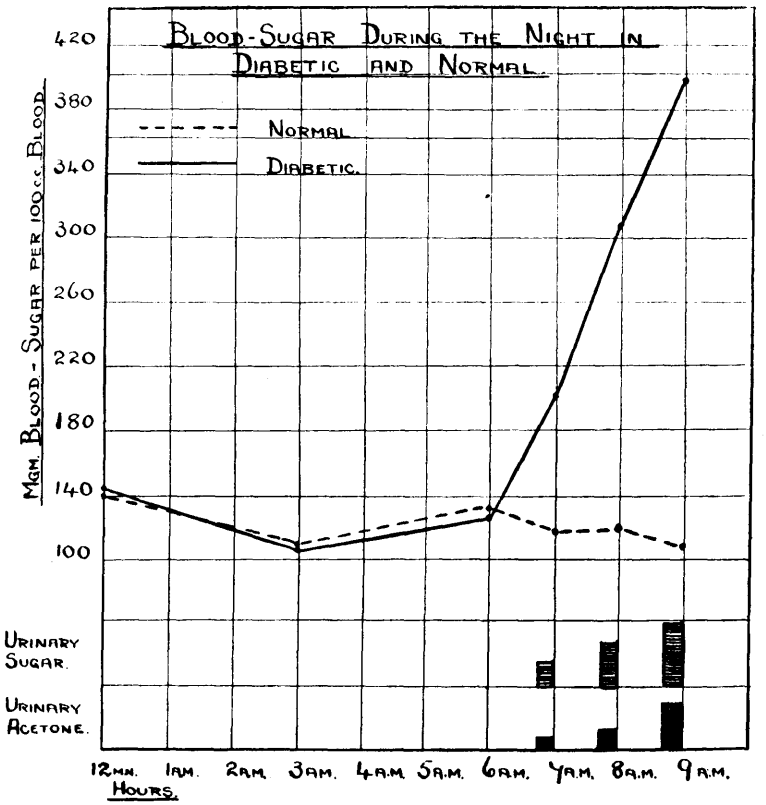


Chart 2.

one case the taking of blood-pressure for the first time raised the blood-sugar to 210 mgm. per cent, while the following week, when the blood-pressure was again taken, the blood-sugar was 105 mgm. per cent.

The severity of the diabetic condition cannot, however, be measured by the amount of sugar lost in the urine and by the value of the fasting blood-sugar. It depends on the residuum

of carbohydrate tolerance of which the patient is capable under the best conditions. Thus a high fasting blood-sugar during the course of an infection, while clearly indicating the necessity for more insulin, provides no index of the severity of the diabetes *per se*. Generally speaking, the level of the fasting blood-sugar depends upon the following factors :—

1. Severity of the disease.
2. Type of the disease. It is high where there is arterio-sclerosis. In none of our patients, however, was there evidence of a rise in blood-pressure.
3. Complicating conditions, *e.g.*, infection.
4. Diet of the preceding day—a previous high fat diet tends to lower the fasting blood-sugar both in the normal and diabetic.

Difficulties in Treatment.

Breaking dietetic rules.—This is one of the chief troubles in the treatment of children, although it is not met with as often as one would expect. It is usually very difficult to elicit the truth as regards the extra diet, but with frankness and the avoidance of undue indignation as a rule one is ultimately successful. Very often an increase of the official diet and insulin will satisfy the child without extras. It is clearly of no avail to put the child on a low carbohydrate diet if he on his own makes up for its deficiencies. Occasionally the diet-breaking may be involuntary, as in the case of one patient sucking barley grains for use in a pea-shooter.

Emotional excitement.—The diabetic child in our experience is not more neurotic or emotional than the non-diabetic. Instances of glycosuria have, however, been met with which can only be attributed to excitement. Thus one child had a marked glycosuria whenever she was allowed to use a tricycle.

Infections.—Undoubtedly any septic focus will lower the tolerance for sugar and necessitate an extra supply of insulin. Catarrhs of the upper respiratory passages also have a deleterious effect on carbohydrate tolerance, and it is advisable to remove any offending cause, such as enlarged tonsils, now that surgical procedure has been rendered safe by the use of insulin. Glycosuria is much more common during the winter months, and this is undoubtedly due to the prevalence of catarrhal infections. This has been noted by the mothers,

who now, of their own accord, promptly increase the insulin dosage at the onset of a cold. The loss of carbohydrate tolerance during infection is only temporary, and, unless the mother is careful, a hypoglycæmic reaction may occur after the acute stage has passed. One is inclined to infer from this that the effect of a toxin is to inhibit the action of insulin and not to prevent its elaboration by the pancreas.

Hypoglycæmia.

At the outset it may be said that hypoglycæmia, if promptly treated, is not a dangerous condition. It is much more dangerous to supply too little insulin than too much. The premonitory symptoms and signs of hypoglycæmia are soon recognized by the mothers and in some cases by the children themselves. A small amount of sugar (lump-sugar) or sweetened orange-juice given at the onset of a turn usually averts the condition. In a severe case the injection of adrenalin, minims 5, will produce a return to complete consciousness within a few minutes.

The time of occurrence of insulin hypoglycæmia is of some practical importance. Generally, the maximum effect is observed about four hours after the injection of the insulin. Frequent exceptions occur in which " the turn " takes place half an hour to two hours after the insulin. The piercing of a small vessel by the needle and consequent direct entry of insulin into the blood-stream is sometimes the cause. Certain patients, however, prove liable to insulin hypoglycæmia quite apart from the technique of the injection. It is difficult to explain this, and one can only point out how small a difference in the insulin dosage may precipitate either hyper- or hypoglycæmia. In one case the cessation of insulin for two days led to coma, although during the previous week the patient had been having hypoglycæmic attacks daily, and even twice daily.

Muscular exercise *per se* leads to a reduction in blood-sugar, and a hypoglycæmic turn becomes more likely if the child has been doing extra muscular exercise. One child had turns only on Friday nights, and it was found that only on this evening was he allowed to indulge in football. This probably explains the occurrence of hypoglycæmic turns after the

patient has been allowed out of bed and especially out of hospital. In the provision of insulin, therefore, the performance of violent exercise must be allowed for, since only very severe diabetics fail to react to exercise by the lowering of blood-sugar.

The occurrence of digestive disorders is also a cause of hypoglycæmic attacks. Especially is this the case with diarrhœa. On several occasions we have noted severe hypoglycæmic reactions following the onset of loose watery motions. But even dyspepsia has produced hypoglycæmic attacks following insulin, and this despite the fact that the patient took her proper amount of food. It can only be concluded that absorption had been greatly delayed.

Other Insulin Reactions.

Of other effects produced by prolonged insulin administration we have noted in one patient the occurrence of raised erythematous patches (papules) which were very irritable and painful, in two others fairly generalized urticaria, and in several patients the appearance of limited areas of fat atrophy even in sites not directly subjected to insulin injection.

Summing up, I would emphasize again that the chief danger in insulin treatment is its omission or inadequacy rather than an overdose. Furthermore, the sufficiency of the dosage must be continually supervised, as the insulin requirements are liable to undergo very rapid alteration. Apart altogether from the occurrence of catarrhal and other infections, there occasionally sets in an increased demand for insulin, with no apparent cause, and I have the impression that changes connected with puberty have an important bearing on this, although diminished parental control must also be suspected. There are some patients, however, whose truthfulness seems above suspicion and in whom it is tempting to attribute the sudden increased demand for insulin to metabolic changes associated with puberty.

At the introduction of insulin therapy it was hoped that by providing rest for the islets of Langerhans regeneration of the impaired cells would take place. As far as I am aware, there is no unequivocal evidence of regeneration of islet tissue in experimental diabetes. Boyd has made the statement that

betterment and presumably restoration, partial at anyrate, of islet tissue takes place in children under insulin therapy. Lawrence recently noted definite recovery in certain cases of very young children. Our results give little support to the view that improvement is common even when treatment is commenced early in the disease. Although there are three patients in whom the carbohydrate value of the insulin has increased, these changes are not of great account.

German statistics indicate clearly that the death-rate from uncomplicated diabetes is much greater in the poorer than in the better-off classes, whereas death due to complications are somewhat greater in the latter group. This has been attributed to two causes—(1) the ability of richer families to provide a greater variety of suitable food-stuffs and (2) the fact that the better-off group has more constant medical attention. These facts demonstrate the extreme importance of after-care. Joslin, whose results with diabetes are probably the best, undoubtedly owes a large part of his success to the great efficiency of the out-patient department, which takes within its purview not merely the purely medical but the economic, psychological, and social factors as well. In diabetes it is not solely a question of ordering grams of protein, carbohydrate and fat, and adding up calories. It is equally important to see that the patient and patient's parents have a clear understanding of what is being attempted and thus obtain their co-operation. It is in this way that an Almoner's department is so necessary in the conduct of a diabetic clinic. Much closer contact is attained between Almoner and patient than between patient and doctor. The mother is given advice—indeed, very soon comes to ask for it—on the types of food that should be bought and on methods of cooking. Another benefit derived from the diabetic clinic is the meeting of patients' mothers with one another and the development of a very healthy rivalry in being able to produce urine charts with no evidence of glycosuria. Mutual encouragement is thus afforded.

In conclusion, it may be said that the successful treatment of diabetes in childhood depends upon a correct dietary, continuous after-care associated with the intelligent co-operation of the parents and child, and, above all, adequate insulin.


ALEX·MACDOUGALL
GLASGOW.

Reprinted from the BRITISH MEDICAL JOURNAL,
April 7th, 1934

DIABETES AND ULTRA-VIOLET IRRADIATION THERAPY

BY

NOAH MORRIS, M.D., B.Sc., F.R.F.P.S.G., D.P.H.

AND

D. CAMPBELL SUTTIE, *M.C.*, M.B.,
F.R.C.S.ED., D.P.H.

(From the Department of Paediatrics, Glasgow University, and the Biochemical Laboratory, Royal Hospital for Sick Children, Glasgow)

During the past twelve years reports have appeared in the literature regarding the beneficial effects of ultra-violet light irradiation on sugar tolerance and hyperglycaemia in diabetes.

Pincussen[1] found that irradiation produced definite improvement in a series of diabetic patients with regard to level of blood sugar and excretion of glucose and acetone. He gave no details of the clinical condition of the patients apart from the diagnosis of diabetes mellitus, but stated that those diabetics who had marked polyuria (hypophyseal diabetes) showed no improvement. Andersen[2] recorded a beneficial result in the treatment of one diabetic patient whose sugar tolerance increased from 24 to 96 grams of carbohydrate, and whose fasting blood sugar fell from 100 mg. per cent. to 70 mg. per cent. immediately after irradiation. Saidman[3] has reported benefit from the use of ultra-violet irradiation in five patients, in two of whom a mild glycosuria completely disappeared. He considered that ultra-violet treatment was indicated in glycosuria associated with cutaneous manifestations, dental lesions, and malnutrition, and also in severe diabetes where insulin was necessary. Rothmann[4] has given records of two diabetic patients treated with ultra-violet light. One with acne rosacea responded favourably, with disappearance of acetonuria and glycosuria (5.3 grams glucose daily) and a fall of fasting blood sugar from 183 to 120 mg. per cent. The other, who had generalized eczema, showed no improvement after ninety-five days, both blood sugar and glycosuria tending to increase. A patient of Rollier (quoted by Furniss[5]), aged 45 years, with benign diabetes mellitus of many years' standing and easily controlled by dieting, was able, after natural sunlight treatment, to live on ordinary hotel diet without glycosuria: after twenty-four hours without a sun-bath all the symptoms

reappeared. J. Wilson,[6] after a brief résumé of the literature, stated that all workers found decrease of glycosuria and acetonia after ultra-violet irradiation of diabetic patients: he considered this treatment specially suitable for diabetes complicated by neuritis, carbuncles, and vasomotor and trophic disturbances. Rothmann[7] also obtained good results in a case of diabetic xanthomatosis: in another diabetic with pruritus the skin irritation disappeared after ultra-violet irradiation, although the fasting blood sugar rose from 140 to 210 mg. per cent.

All the reports in the literature that we have obtained have dealt with adults, and on the whole have been favourable. It is noticeable, however, that many of the patients successfully treated have had pathological conditions of the skin. In view of the well-known fact that sunlight has a marked effect on the development of children it seemed advisable to determine whether ultra-violet light influenced their sugar tolerance and insulin requirements. As diabetes in childhood is not usually complicated by other diseased conditions, such as arterio-sclerosis or tuberculosis, it was hoped to decide whether the improvement observed in adults was the result of direct action on the carbohydrate-regulating system, or of a secondary effect due to alleviation of some other pathological condition such as skin lesions.

Scope of Investigation

The immediate effect of irradiation on blood sugar was investigated in a few normal and diabetic children. Blood was withdrawn prior to, and immediately after, irradiation lasting two minutes. The children were in the post-absorptive state, having refrained from food for at least six hours previously. Table I shows some of the results obtained.

Ultra-violet irradiation of five non-diabetic subjects produced in four an immediate fall of blood sugar and in one a rise, while irradiation of six diabetic patients on ten occasions led to an immediate fall in blood sugar on five, a rise on four, and no change on one. It is evident from these figures that irradiation may cause an immediate increase or decrease of the blood sugar both in non-diabetics and in diabetics. Furthermore, different results were obtained on different days with the same patient. These findings differ somewhat from those of Lucca and Reviglio,[8] who reported that in children after irradiation with infra-red or ultra-violet rays the sugar content of the blood was always diminished, though there were individual variations in the decrease.

TABLE I

Name	Age in Years	Condition	Blood Sugar	
			Before Irradiation	After Irradiation
			mg. per cent.	mg. per cent.
A. R.	8	Non-diabetic	66.0	58.0
J. H.	10½	,,	83.0	53.0
E. G.	11	,,	72.0	91.0
E. B.	6½	,,	90.0	82.0
W. A.	10	,,	68.0	59.0
M. H.	14½	Diabetic	126.5 138.8 98.0 100.0	133.5 133.5 85.1 100.4
S. N.	7½	,,	80.9	66.6
W. D.	8½	,,	149.2	161.2
P. McK. ...	10½	,,	205.0 157.5	222.7 148.1
J. P.	11½	,,	160.0	153.8
J. L.	8	,,	108.0	117.6

Despite these conflicting results it was determined to study the effect of a course of ultra-violet irradiation on carbohydrate tolerance in a series of diabetic patients.

MATERIAL TESTED

The subjects of the test were regular attendants at the diabetic clinic of the Royal Hospital for Sick Children, Glasgow. All had been attending for at least six months, and some for five or six years. Their dietary and insulin requirements were fairly accurately known. The mothers of the children were all intelligent, and had been co-operating successfully with us. The possibility of improving sugar tolerance by ultra-violet therapy was explained to the mothers of all the children attending the clinic, and those that desired to try out the effect of this treatment did so enthusiastically, in the hope that the treatment might permit a decrease in the dose of insulin. The children of the more sceptical mothers acted as controls.

In all ten children (ages varying from 7½ to 14½ years) received ultra-violet therapy, and four (aged 2½ to 13 years) did not. The treatment was given twice weekly ; at first one-minute exposures were given to the whole thoracico-abdominal region, dorsal aspect of the body, and limbs (small slips being worn and goggles). Gradually

the exposures were increased by thirty seconds every week until three-minute exposures were given. The source of light was a mercury vapour vacuum burner (new) 250 volts D.C. Five minutes was allowed after lighting to permit the output to settle. The distance of the exposed area from the source of light was 36 inches. When pigmentation was observed the therapy was stopped for an interval, as most workers are agreed that pigmentation lessens the therapeutic effect. (Rothmann[4] states that when pigmentation occurs the blood sugar begins to rise.)

Results Obtained

The following tables give a brief résumé of the results after ultra-violet irradiation had been given. The carbohydrate tolerance was estimated from the amount of total carbohydrate—that is, carbohydrate plus half the protein—utilized per unit of insulin. The weight and height were calculated as percentages of the expected weight and height respectively.

Table II

Name	Age in Years at July, 1932	Carbohydrate Tolerance		Percentage Expected Weight		Percentage Expected Height	
		Before	After	Before	After	Before	After
M. D. ...	11	4.1	8.0	86	87	95	95
A. McC. ...	7½	6.0	10.3	72	77	88	88
J. R. ...	12½	2.1	2.2	94	95	99	98
M. H. ...	14½	1.5	1.2	—	—	—	—
W. D. ...	8½	1.8	2.2	90	90	92	91
J. L. ...	8	15.0	10.5	83	80	96	95
A. G. ...	11	4 0	3.4	82	76	97	94
P. McK. ...	10½	2.8	1.7	99	94	105	103
S. N. ...	7½	3.0	2.6	100	95	97	96
S. U. ...	11½	2.4	1.7	82	86	92	90
C. F.* ...	2½	4.2	9.9	106	106	105	101
J. B.* ...	13	3.8	3.2	94	92	95	95
J. McR.*...	1½	7.6	4.1	100	110	—	—
J. P.* ...	11½	2.6	2.4	97	89	98	97

* These patients did not receive ultra-violet irradiation therapy.

The carbohydrate tolerance improved in four out of the ten patients given ultra-violet therapy. The same proportion was found when the estimation was based on

the absolute amount of insulin required, four patients requiring less insulin at the end of the year. In six patients, however, the tolerance diminished. Although the proportion with decreased tolerance was greater in those not receiving ultra-violet irradiation the numbers are much too small to make this difference significant.

The incidence of infection in the two groups was also noted with special reference to the occurrence of glycosuria. In the ultra-violet irradiation group three of the patients had mild infections—for example, nasal catarrh with or without mild pyrexia—and four had moderately severe attacks—influenza, adenitis, scarlet fever. Two of the latter four had to be admitted into hospital because of diabetic acidosis. Of the control group one patient had a mild enteritis and two had moderately severe attacks of adenitis necessitating their admission to hospital because of impending coma.

TABLE III*

	Total No.	Carbohydrate Tolerance			Percentage Expected Weight			Percentage Expected Height		
		Improved	No Change	Decreased	Improved	No Change	Decreased	Improved	No Change	Decreased
Receiving U.V.R. ...	10	4	—	6	4	1	4	—	2	7
Not receiving U.V.R.	4	1	—	3	1	1	2	—	1	2

*Cases receiving U.V.R.—data of height and weight from only nine cases. Cases not receiving U.V.R.—data of height from only three cases.

As regards other effects of the treatment, the mother of one patient stated that about one hour after irradiation the boy felt very limp, and that sugar had occasionally to be given because of the threat of hypoglycaemia. This particular patient was one whose carbohydrate tolerance per unit of insulin was reduced at the end of the year. Another patient (a boy of 13 years) stated that he felt better after ultra-violet irradiation treatment was commenced: in this case it had been noticed in previous years that during long spells of bright weather in the summer the insulin had to be reduced owing to a tendency to hypoglycaemic attacks.

SUMMARY

The effect of ultra-violet therapy on sugar tolerance, insulin requirements, growth, and general health was

determined in ten diabetic children, aged $7\frac{1}{2}$ to $14\frac{1}{2}$ years, over a period of one year. The results, compared with those of a control series of four children who did not receive ultra-violet therapy, showed no significant improvement that could be attributed to the irradiation.

We should like to express our thanks to Professor G. B. Fleming for his interest in this investigation, and to the Medical Research Council for a personal grant to one of us (N. M.).

References

[1] Pincussen, L.: *Biochem. Zeit.*, 1922, xxvi, 127.
[2] Andersen, A.: *Münch. med. Woch.*, 1923, lxx, 1484.
[3] Saidman, J.: *Les Rayons Ultra-violets et Associés en Thérapeutique*, 1925, Doin, Paris, p. 736.
[4] Rothmann, S.: *Zeit. f. d. ges. Exp. Med.*, 1923, xxxvi, 398.
[5] Furniss, A.: *Brit. Journ. Actinotherap. and Physiotherap.*, 1931, v, 252.
[6] Wilson, J.: *Clin. Journ.*, 1928, lvii, 97.
[7] Rothmann, S.: *Strahlentherapie*, 1927, xxiv, 465.
[8] Lucca, A., and Reviglio, G. M.: *Actinoterapia*, 1929, viii, 153.

GLYCOSURIA IN CHILDHOOD.†

By NOAH MORRIS, M.D.

(*From the Department of Pædiatrics, University of Glasgow and Biochemical Laboratory, Royal Hospital for Sick Children, Glasgow.*)

Glycosuria although not in itself serious is of the utmost importance as a diagnostic clue to a metabolic defect which may or may not be of grave import. In the majority of cases the onset of diabetes mellitus in childhood is heralded by the symptoms of thirst and polyuria: thus in 30 cases of diabetes at the Royal Hospital for Sick Children, Glasgow, thirst was a first manifestation in 27 and polyuria in 23. Nevertheless, there exists a certain number in whom the onset of glycosuria is silent so that in the routine examination of children it is of importance not to neglect the urinary tests which in children are so apt to be overlooked. It must, however, be emphasized that although glycosuria associated with polyuria and thirst is almost invariably due to diabetes, silent glycosuria is frequently not of the same grave significance. Holst has followed the subsequent histories of 150 persons who were refused life insurance because of glycosuria: he found that over periods varying from 5 to 16 years only 30 exhibited the clinical picture of true diabetes mellitus. It is clear, therefore, that the correct interpretation of "silent" glycosuria presents a problem of great practical importance to the patient.

The common tests for glycosuria are Fehling's and Benedict's. The latter is rather the more delicate of the two but not sufficiently so to detect the small amount of sugar which is present in normal urine. It may be as well to point out that Benedict's test as performed with the qualitative reagent is definitely positive only when the precipitate is yellow or red: a white or whitish grey precipitate is due to phosphates. Even with this precaution it is important to remember that there are substances other than glucose which give a positive test with Benedict's or Fehling's reagents. If a preservative has to be used chloroform and formaline should be avoided since both are reducing agents. Uric acid and creatinine in excess, glycuronates derived from antipyrine, camphor, chloral, morphia, etc., salicyluric acid from salicylates, and homogentisic acid (alkaptonuria) all may give a positive test. Of the sugars glucose, lævulose, galactose, lactose and pentose reduce Fehling's and Benedict's reagents. In a case of doubt the yeast fermentation test is the best; it is positive only in the presence of glucose and lævulose: these two sugars may be differentiated by their action on polarised light. Galactose which ferments slowly with yeast is a very rare constituent of urine.

Incidentally the presence in the urine of sugars other than glucose is not of serious import. It has been stated that lævulosuria is indicative of hepatic inefficiency but the evidence for such a view is not very strong. Chronic lævulosuria can be cured by withdrawing cane-sugar and honey from the diet. Lactosuria which is not uncommon in nursing mothers may appear in breast-fed infants with digestive disorders. Pentosuria arising apart from the ingestion of fruits, is a familial "inborn error" of metabolism and is not known to have any pathological significance.

†A Lecture delivered to the Glasgow Post-Graduate Association, November 28th, 1933.

When true glycosuria has been detected it is of the greatest importance to determine its cause as soon as possible. Ketonuria as indicated by a positive Rothera Test is suggestive of diabetes mellitus but the association of ketosis with non-diabetic glycosurias is not uncommon. A single blood-sugar estimation is of value especially if the sample is taken in the post-absorptive state. If the blood-sugar of the fasting specimen is above 140 mg. per cent., or that of the sample taken in the course of the day some hours after a meal is above 200 mg. per cent., it is wise provisionally to presume the case to be one of true diabetes mellitus. If, however, the above figures are not reached it is necessary to estimate the course of the blood-sugar after the ingestion of glucose.

The Blood-Sugar Curve.

In children it is usual to determine this immediately before and after the ingestion of one gm. of glucose per kilo. body weight. The child should be in the post-absorptive state so that the test should be carried out in the morning before any food has been taken. Three points are to be noted in the curve (*a*) the fasting level, (*b*) the height of the peak, and (*c*) the time taken to reach the fasting level. Normally the fasting level should fall between 80 and 120 mg. per cent. depending on whether capillary or venous blood is used and on the method of analysis. As a general rule it may be said that the fasting blood-sugar is lowest in the early months of life, rises with advancing age until at three years it is on the same level as that of the adult. The peak of the curve, which depends on the rates of absorption and storage, is usually reached at 30 minutes and should not exceed 200 mg. per cent. Within 2 hours the blood-sugar should have returned to the fasting level.

Factors Affecting Blood-Sugar Curve.

(*a*) Dosage of glucose. In a child an increase in the amount of glucose ingested raises the height of the curve and causes a delay in the return to normal.

(*b*) Exercise before the ingestion of glucose frequently produces a slight increase in the fasting value while if there is any muscular exertion after the ingestion of glucose the peak is lowered and the fall quickened.

(*c*) Time of day. It has been shown that in over 60 per cent. of subjects the same dose of glucose gives a higher peak when given in the afternoon than in the morning.

(*d*) Nausea. If the ingestion of glucose leads to nausea, the rise of blood sugar is considerably lessened and possibly prevented owing probably to inhibition of peristalsis and diminished absorption.

(*e*) Emotional disturbances are specially liable in childhood to produce a rise in the blood-sugar curve.

Renal Threshold.

During a blood-sugar tolerance test the urine should be examined for glucose before and one and two hours after the ingestion of glucose. The occurrence of glycosuria depends on whether the concentration of blood-sugar rises beyond that point at which the kidney excretes sugar in significant amounts: this is known as the renal threshold. It varies in different individuals but is said to remain

constant in the healthy subject throughout life. In an investigation in children Dr. Gilchrist found the renal threshold to vary between 180 and 310 with an average of about 230 mg. per cent. Much higher values have, however, been recorded in the literature. It is of interest to note that Faber and Hansen have found a lower threshold with a falling than with a rising blood-sugar.

Classification of Glycosurias.

Glycosuria may be divided into three groups.

I. With low renal threshold—renal glycosuria.
II. With normal renal threshold—non-diabetic.
III. With normal renal threshold—diabetic.

I.—Renal Glycosuria.

This may be defined as a condition in which glycosuria occurs in spite of the fact that the blood-sugar even after high carbohydrate intake remains within normal limits. Several authors have pointed out its tendency to occur in families. It is not often seen in childhood probably because there are no symptoms which lead to the examination of urine for sugar. Graham has divided renal glycosuria into two groups, (*a*) intermittent, cyclic or continuous glycosuria with a low sugar output associated with a normal or flattened blood-sugar curve showing a quick return to the fasting level, (*b*) transient or continuous glycosuria with a much larger sugar output and associated with a curve which may be higher than normal and may show some delay in returning to the fasting level.

Everyone is agreed that the first group can be considered quite up to standard so far as carbohydrate metabolism is concerned: prognosis is good and treatment is not necessary. In order to establish the diagnosis it is essential to carry out a blood-sugar tolerance test. The presence or absence of ketonuria is no criterion, since this has been reported as occurring in association with renal glycosuria.

As regards the second group opinion is divided. Graham considers that there is diminished carbohydrate tolerance and Joslin also holds this conservative view. Reports are, however, appearing in the literature which seem to indicate that this type is not one of true diabetes. Thus Faber reports Holst's detection of 27 cases of this type among 163 so-called diabetics: these 27 have been under observation for periods of 1 to 25 years without any other sign of true diabetes mellitus appearing although the majority quickly reverted to ordinary diet. Because of the serious results of missing true diabetes, it is probably wisest to adopt Joslin's view and treat the condition as one of diabetes mellitus until the course of events shows that the glycosuria is innocent.

II.—Non-Diabetic Glycosurias with Normal Renal Threshold.

Alimentary Glycosuria. This is a rather unsatisfactory term applied to the transient glycosuria occurring in some individuals after a meal containing a large amount of carbohydrate. It is due ultimately to the blood-sugar rising above the renal threshold. The height of the blood-sugar depends upon the rates of absorption, storage and consumption so that the appearance of glycosuria may result from very rapid absorption with delayed storage. It was at one time common to estimate sugar tolerance by determining the amount of sugar that could be ingested without the appearance of glycosuria. As a general rule it may be said that the child can take glucose up to the point of nausea without glycosuria.

There are, however, a few apparently healthy subjects who show transient glycosuria even with moderate doses of glucose. To these may be applied the diagnosis of alimentary glycosuria but this should always be confirmed by a blood-sugar tolerance test. In a series of 28 children investigated at the Royal Hospital for Sick Children with regard to their ability to deal with glucose, Dr. Gilchrist found that 4 showed glycosuria, one with one gram of glucose per kilo. body weight, one with four, one with five and one with six grams.

Emotional Glycosuria. It has long been known that emotional disturbances are likely to give rise to glycosuria. This is attributed to the increased secretion of epinephrine with a consequent rise in the rate of glycogen breakdown in the liver. In one of our cases the prospect of irradiation with ultra-violet light for the first time was sufficient to raise the fasting blood-sugar level from 98 mg. per cent. to 210 mg. per cent. In some children and adolescents the playing of a game of football is accompanied with sufficient emotional disturbance to produce a marked rise in blood-sugar with glycosuria. Edwards and his co-workers have demonstrated that a definite hyperglycæmia occurred about the middle of a game of football but only in those players showing emotional excitement.

Cerebral Glycosuria. Probably allied to the last group are those cases associated with intra-cranial disturbances in which hyperglycæmia and glycosuria originate from over-stimulation of the splanchnic sympathetic with rise in the output of epinephrine. Glycosuria of this nature is comparable to that produced by "Bernard's diabetic puncture". Cerebral hæmorrhage, sinus thrombosis, embolism, concussion, meningitis, cerebral tumour and increased intra-cranial pressure have all been found to be accompanied at times with glycosuria. Occasionally this has led to mistakes in diagnosis. A child may be admitted in coma and the routine examination of the urine reveal abundant glucose and acetone. This naturally leads to the suspicion of diabetic coma. In the absence of a history of thirst and polyuria it is advisable in such a case to obtain cerebrospinal fluid by lumbar puncture when an increase of cells and a positive Pandy test will frequently demonstrate the true cause of the glycosuria, and in the case of cerebral tumour or injury signs of involvement of the central nervous system will be elicited. The estimation of the blood-sugar is of little value in the differential diagnosis since it will be high unless the cranial condition happens to have occurred in a patient with renal glycosuria. Hyperglycæmia and glycosuria have also been reported as a sequel of encephalitis.

Glycosuria and Hyperthyroidism. Glycosuria is frequently met with in conditions of hyperthyroidism due to disturbance of carbohydrate metabolism. Unfortunately there is no blood-sugar curve typical of this condition. In the few cases observed at the Royal Hospital for Sick Children, Glasgow, the peak of the curve was high and the return to fasting level delayed. John, however, has pointed out that apparently normal curves are sometimes found in severe cases of hyperthyroidism and concluded that the renal threshold may be lowered. The association of hyperthyroidism with true diabetes mellitus is a matter of some importance. At present it is the opinion of competent observers that the combination of true diabetes and hyperthyroidism is rare. In a series of 1,800 cases of Graves' disease at the Mayo Clinic, Fitz found only 9 with true diabetes. As a general rule it has been found that the hyperthyroidism preceded the onset of diabetes and that the incidence of the latter was greatest in cases of toxic adenoma. The possibility is therefore not excluded that prolonged severe hyperthyroidism may tend to induce diabetes mellitus.

Pituitary Glycosuria. Owing to the fact that the functions of the pituitary hormones have not been completely worked out, much confusion still exists as to the relationship between the pituitary and carbohydrate metabolism. There is undoubtedly an antagonism between the pituitary secretion and insulin whether directly or as a result of opposite actions on the liver. It would appear that in the earlier or hyperpituitary stages of acromegaly there is intolerance for carbohydrate which may be so marked as to produce continuous glycosuria with hyperglycæmia almost indistinguishable from diabetes. Ketosis and coma may be features of tumours of the pituitary region and Fleming has reported a case in which marked glycosuria and ketonuria with coma led to the diagnosis of diabetic coma but which ultimately turned out to be one of supra-pituitary adamantinoma. Generally the blood-sugar curve is high in acromegaly although both the fasting blood-sugar and the value at two hours may fall within normal limits. In Fröhlich's syndrome (hypopituitarism) the curve that has been generally obtained by Dr. Badenoch* in this hospital is one characterised by a delayed return to the fasting level and not accompanied by glycosuria. In the literature, however, a flattened curve indicating an increased tolerance for carbohydrate is not an uncommon finding. It is probable that most of the cases which have been investigated by Dr. Badenoch have been in the early stages of the disease. Pituitary hyperglycæmia and glycosuria appear to respond to insulin just as does true diabetes. It is stated, however, that glycosuria resulting from overaction of the pituitary frequently undergoes spontaneous cure in contrast to what occurs in diabetes mellitus. This is probably due to the temporary nature of the hyperpituitarism which may rapidly change into hypopituitarism.

Glycosuria Associated with Infections. Hyperglycæmia and glycosuria of all grades are known to occur in the course of infections. It is probable, too, that a latent diabetes mellitus may be increased in severity and thus brought to light. The degree of disturbance of carbohydrate metabolism appears to depend less on the severity than on the site of infection. Boils, carbuncles and other pyogenic infections of the skin are specially liable to produce glycosuria. The disturbance in carbohydrate metabolism is frequently characterised by marked ketonuria as well as hyperglycæmia and glycosuria and may prove very refractory and even simulate true diabetes. It is indeed not certain whether the condition should not be considered as one of transient diabetes. Certainly dietetic and insulin treatment appear to be strongly indicated both for the disturbance of carbohydrate metabolism and for the beneficial effects on the infection. In infants with gastro-enteritis, especially when associated with marked dehydration, glycosuria is frequently found and in the treatment of this condition with parenteral administration of glucose it is advisable to add insulin. Glycosuria has also been reported in many other forms of bacterial and protozoal infection.

Acidosis. Patients with acidosis occasionally show glycosuria. In 9 subjects prolonged administration of ammonium chloride induced glycosuria in 2. The blood-sugar curve during the same period showed a somewhat higher peak than normal and a delayed return to the original fasting level which was within normal limits. When ketosis is produced by the ingestion of high fat diet the change in the blood-sugar curve is much more marked than that produced by ammonium chloride although the glycosuria is not more frequent. Gilchrist concludes that the hyperglycæmia found with ketosis is only in small part due to the ketosis but is chiefly the result of increased accumulation of fats in the liver and the consequent

*Personal communication.

impairment of its glycogenic function. In clinical acidosis without ketosis glycosuria is not frequent but in acidotic conditions associated with ketosis, such as starvation acidosis and cyclical vomiting, glycosuria is occasionally detected. This may lead to the suspicion of true diabetes but in the non-diabetic acidosis the glycosuria usually occurs during treatment with high carbohydrate intake and does not appear after the acidotic condition has been relieved.

Post-Anæsthetic Glycosuria. The glycosuria which sometimes occurs after general anæsthesia appears to have some relation to the duration of the anæsthesia. It is associated with hyperglycæmia and is probably due to an increased rate of glycogenolysis. Frequently glycosuria is associated with ketonuria and actual coma may supervene. The absence of any previous symptoms and the fact that only a short time has elapsed since the administration of an anæsthetic renders its differentiation from true diabetes an easy matter. Although the general condition is one of the utmost gravity, the glycosuria *per se* is not of any serious significance. Insulin may be given chiefly to facilitate the combustion of carbohydrate but it is doubtful whether it is of any value. It certainly should always be accompanied with abundant glucose.

III.—Diabetic Glycosuria.

The diagnosis of true diabetic glycosuria is generally not difficult in childhood since in the majority of cases it is heralded by the onset of thirst and polyuria. In the absence of symptoms other than glycosuria it is advisable to perform a blood-sugar tolerance test. Hale-White and Payne have pointed out that only one type of blood-sugar curve is pathognomonic of true diabetic mellitus, viz.:—one in which the fasting level and peak are both high and the fall very prolonged. Diabetes mellitus may, nevertheless, be present with many other types of curve but conversely other forms of abnormal curve do not necessarily indicate diabetes. Frequently it is possible by a consideration of the joint clinical and laboratory findings—and incidentally laboratory findings must never be used without taking into account the clinical picture—to conclude that a case is not one of true diabetes mellitus. If, however, this is impossible it is wise to look on the condition as a true diabetic one until the future events declare whether or not the suspicion is well-grounded.

In view of the proneness of untreated juvenile diabetes to run a very rapid downhill course it is advisable to commence active treatment with as little delay as possible. Diabetic therapy in childhood is in essence no different from that in adult life. It depends for its success firstly on the administration of sufficient insulin to allow the carbohydrate metabolism to be carried on normally and thus to permit a reasonable diet being taken and secondly on careful and continuous after-care. It seems unnecessary at the present state of knowledge to insist on the fact that there is no virtue in reducing the insulin dosage at the expense of glycosuria and dietary restriction. But experience with parents and doctors has shown one that there is a strong tendency to keep the insulin dosage low even when the patient is excreting abundant glucose in the urine. The result almost invariably follows that it is much more difficult to restore carbohydrate metabolism to normal and occasionally disaster ensues before the necessary steps can be taken. After-care, of advantage for patients of all ages and with all types of disease, is of vital importance for diabetic children. The child presents special problems of his own, such as growth and the increased liability to infection both of which throw an extra strain on the metabolic processes.

As soon as the diagnosis of diabetes is made it is essential to initiate treatment and this can be done in one of two ways. In the first the diet is reduced until glycosuria has disappeared and thereafter it is gradually increased up to the required intake the glycosuria being controlled by the administration of insulin as and when required. In the second method the optimum requirement of diet is given at once and sufficient insulin administered to prevent glycosuria. The amount of insulin required can be gauged approximately by estimating the amount of glucose excreted per 24 hours in the urine, and allowing one unit of insulin for every two grams of urinary sugar. In the choice of methods it is to be remembered that children are much more liable to acidotic conditions than are adults. Accordingly, if acetonuria is marked, it is advisable not to restrict carbohydrate unduly but to give a fairly liberal supply along with insulin since sudden restriction may increase the ketosis and precipitate coma. If, however, the Rothera's test gives but a mildly positive result, the first or crescendo method is probably the one of choice since it enables one to determine the carbohydrate tolerance before insulin administration is commenced. Furthermore, less strain is thrown on the organs concerned with carbohydrate metabolism and glycogen storage in the liver is a more gradual process. With gradual increase in the insulin dosage there is less likelihood of hypoglycæmia occurring with consequent undermining of the patients' and parents' confidence before that has been fully established. Lastly, there is a tendency to overfeeding when too little attention is paid to diet and too much stress is laid on insulin therapy. Although the gradual increase of diet to the required amount is recommended, it is wise not to spend too long a time in adjusting a fine balance between the insulin requirements and a diet of low caloric value. The patient becomes wearied and is liable to develop the invalid outlook whereas it is important that he should realise that apart from requiring insulin and dietary regulation he should, and with care will, live an ordinary life with no other restrictions.

Dietary requirements must satisfy the caloric needs of the child. It is generally accepted that the diabetic patient is better when below average weight and since the overweight child is specially liable to show abnormalities of fat metabolism and to coma we have found that children get along better when the caloric value of the food supplied is less than that generally recommended for healthy children of the same age. Overactive children will naturally require more than lethargic: in Joslin's clinic the caloric intake is increased by ten per cent. for very active patients. Insistence on too low a dietary defeats itself by leading to illicit breaking of the dietary regimen. There is no reason why the child should not satisfy his appetite provided the amount of food taken is weighed. The safe rule is to ensure that the child gets sufficient but no more than sufficient to allow of normal development. For this reason diabetic children should be weighed at regular intervals and the diet adjusted accordingly. In actual practice we have found that a caloric intake exceeding the basal requirements by 50 per cent. is a very useful standard and that only rarely does the child's appetite or state of nutrition demand more. As regards the partition of the diet among the various proximate principles, opinion has now veered to a more liberal supply of carbohydrate as far as the adult patient is concerned. Apparently with a large carbohydrate intake the insulin required increases little if at all. In the child, however, we feel that on the whole it is wiser to restrict the supply of carbohydrate to a quarter of the total caloric intake. This is because of the greater liability of the child to infection and consequent reduction of the potency of insulin. Probably the best guiding principle is to arrange the diet so that, though adequate, the minimum amount of insulin

is required to keep the urine free of sugar and acetone. It is still an open question, however, whether the restriction of the food value of carbohydrate to 25 per cent. of the total calories is the best way to achieve this desideratum. As regards fat there is no doubt that the intake should not in the child represent more than 60 per cent. of the total calories, since a high fat diet is not tolerated well by the child or adolescent. The quota of protein will depend on the age and weight of the child, but 20 per cent. of the total caloric requirements should in all cases suffice.

In actual practice we have found that a modification of the Lawrence line diet provides a very suitable scheme for regulating the diet. The red line has been modified so that each contains 7.5 gm. of protein plus 7.5 gram. of fat with a caloric value of 100: the black line corresponds to 5 gm. carbohydrate with a caloric value of 20. In prescribing a diet it is easiest first to allot sufficient black lines to make up 25 per cent. of the total calories: this is easily done by dividing the carbohydrate calories (one quarter of the total) by 20. The number of red lines are obtained by dividing the non-carbohydrate calories (three quarters of the total) by 100. An example will make this clear.

A child aged 8 years requires approximately 1,200 calories.

Carbohydrate Calories ... ... = 300

Number of black lines ... ... $= \frac{300}{20} = 15$

Non-carbohydrate Calories ... = 900

Number of red lines ... ... $= \frac{900}{100} = 9$

This ensures a sufficiency of protein. It may be desired to give less carbohydrate and the diet may be arranged accordingly but it is necessary that for every red line there is at least one black line.

Two important requirements must be borne in mind when instituting any scheme of treatment. It must be easily understood and managed. Furthermore, it must be possible to vary the diet readily and so prevent monotony. The line dietary satisfies these requirements: since its adoption some years ago in the diabetic clinic of the Royal Hospital for Sick Children, Glasgow, it has proved a boon to the mothers of the patients, since they can understand it and are better able to cope with the desires of the children.

There next falls to be considered the distribution of the diet throughout the day: this is clearly bound up with the administration of insulin. The ideal method would be to distribute food and insulin equally throughout the 24 hours but this is inconvenient for domestic reasons and also because it necessitates too many injections. Probably the best method is to give two large meals at breakfast and supper and thus limit the injections of insulin to two. A single injection of insulin may suffice in the very mild cases and in this circumstance it should be given before the largest meal of the day. Occasionally when the insulin effect wears off quickly it may be necessary to give three injections. The actual spacing of diet and insulin should be so arranged that there is no glycosuria. This can be most readily accomplished by testing the urine before each meal. Thus the time of sugar-leakage is detected, and to prevent this, appropriate changes in diet and insulin can be made.

For the satisfactory carrying out of the treatment it is necessary to instruct the child (or if too young, the mother) in the use of a syringe and the testing of urine. For the latter purpose Benedict's qualitative reagent is used. Six drops of urine are added to half an inch depth of reagent in a test tube which is immersed in a pan of boiling water for 5 minutes when the presence or absence of reduction is noted. It is advisable that records of the results of urine tests should be kept. Both patient and parents should be impressed with the necessity of keeping the urine sugar-free. If glycosuria is present it is exceedingly difficult to determine the state of the patient without frequent blood analysis. When parents realise this their supervision becomes more careful and the obtaining of negative tests encourages both patients and parents. If, however, occasional glycosuria is treated too lightly, carelessness is engendered and gradual worsening of the condition ensues which often terminates in coma. As a general rule the aglycosuric state is attained, but occasionally it seems impracticable to achieve this without producing severe hypoglycæmic attacks. Such cases are fortunately not common and are probably due to an abnormally low renal threshold. Indeed, there have been published the records of some patients with true diabetes mellitus associated with renal glycosuria. The opposite condition of high renal threshold is more frequently encountered: this will mask any deterioration in the sugar tolerance so that it is advisable, even in the absence of glycosuria, occasionally, perhaps once a year, to estimate the blood-sugar. The presence of a septic focus lowers the tolerance for sugar and necessitates an extra supply of insulin. Catarrhs of the upper respiratory passages also have a deleterious effect. Now that insulin has rendered surgical procedures safe in diabetes it is advisable to remove any offending cause, such as enlarged tonsils or adenoids. Glycosuria is much more common during the winter months: this is almost certainly due to the greater prevalence of catarrhal infections. In all these circumstances the insulin dosage must be temporarily increased so as to prevent glycosuria, if possible. The former state of carbohydrate tolerance is generally regained as soon as the acute stage of the infection has passed, so that it is necessary to be on guard and reduce the insulin as the infection subsides since otherwise a hypoglycæmic reaction may occur.

The only risk of insulin therapy is the liability to attacks of hypoglycæmia. It may be stated at once that hypoglycæmia, if promptly treated, is not a dangerous condition. The fear of hypoglycæmia should not lead to under-administration of insulin. The risk is much greater when too little insulin is given than when too much. The maximum effect of insulin is usually observed about four hours after the injection but occasionally a hypoglycæmic turn may occur as soon as half an hour after the insulin has been given. Muscular exercise by its call on the carbohydrate stores of the body leads to a reduction in blood-sugar which may fall below the critical level. This is more likely to occur in the child than the adult whose glycogen stores are very much greater. In one patient hypoglycæmic attacks occurred regularly on Friday evenings during the summer months: these remained inexplicable until it was discovered that he indulged in football only on this evening of the week. The increase in muscular exercise on leaving hospital probably explains the occurrence of hypoglycæmic turns after the patient returns to his home. Since only very severe diabetics fail to react to exercise by reduction of blood-sugar it is wise to allow for this in the event of any unusual exercise being performed. Another occasional cause of hypoglycæmia is gastro-intestinal disturbance with interference with intestinal absorption: in these circumstances insulin shock is prone to occur.

little value in the differential diagnosis. The finding of glycosuria and acetonuria usually points to the true cause of the condition. Cases are on record in which acetone has been absent from the urine. Two other findings are not uncommonly present, viz.:—the presence of albuminuria and the occurrence of casts in the urine. As regards the other clinical phenomena little need be said. The respirations are laboured and increased in frequency, the pulse frequent and feeble, the blood pressure low and the temperature generally subnormal. The tension of the eyeball is said to be invariably low. The reflexes are normal but an extensor type of plantar response has been reported, thus minimising the importance of this finding as an index of hypoglycæmic coma.

The treatment of coma is based on a rational understanding of the disturbed physiology. This may be summarised under the following headings: (1) insufficient oxidation of glucose, (2) presence of acidosis due to accumulation of ketones, (3) loss of water (dehydration) and salts especially sodium chloride. The last of these is of considerable importance because the unsuccessful treatment of coma is not infrequently due to neglect of this factor. The general lines of therapy are therefore administration of insulin and carbohydrate and the plentiful supply of fluid with salt. It must be emphasized that diabetic coma is an emergency of as great urgency as an acute abdominal condition and must be treated actively without delay.

The first aim is to abolish the acidosis. This can be done by ensuring the effective oxidation of glucose for by this means the formation of ketones is prevented and the oxidation of those already present is accelerated. To achieve this, large doses of insulin are injected at frequent intervals: simultaneously large quantities of glucose are given *per os, per rectum,* subcutaneously or intravenously. The administration of glucose is advisable in all cases (and especially so when laboratory facilities for blood analysis are not available) since in many the store of carbohydrate is very depleted. When glucose is given with insulin the risk of converting coma to hypoglycæmia is negligible. The urine should be examined frequently for glucose and acetone, but the clinical condition of the patient affords the best guide as to the necessity for increasing or decreasing the dosage of insulin and glucose. The amounts to be given naturally vary with the age and weight of the patient and the severity of the diabetes and coma.

The second aim, which is as important as the first, is to replenish the depleted stores of water and salt. This may be best accomplished by supplying the glucose in the form of a dextro-saline solution (10% glucose in normal saline). Should it be desired for any reason to diminish the intake of glucose it is imperative to continue with the administration of saline which can be given either by the mouth, rectum, or parenterally. In cases where the acidosis is marked and recovery slow, alkali as sodium bicarbonate may be given orally, but vigorous insulin-glucose-saline therapy is usually sufficient. The other important points in the treatment are application of warmth, induction of free action of the bowels and, if necessary, gastric lavage with an alkaline solution to decrease vomiting.

Diabetic coma has now almost reached the status of an avoidable accident. The parents of diabetic children must therefore be educated as to the significance of the various danger signals and the special liability of the occurrence of coma in the presence of an infection. They should also be given definite instructions regarding the immediate treatment when symptoms suggestive of impending coma

become manifest: the doctor should be immediately sent for and meanwhile the child should be kept warm in bed and be given hot drinks, containing carbohydrate, if possible with saline, and an increased dose of insulin.

The discovery of insulin has entirely changed the prognosis of diabetes in childhood. Whereas in pre-insulin days the outlook was almost hopeless, the patient seldom lived longer than two years, the prognosis now appears to be very favourable. Growth is perfectly normal as are physical and mental activity, and there does not appear to be any increased susceptibility to infections although the onset of an infective process makes the treatment temporarily more difficult and anxious. Growth and mental development proceed quite normally. Some of Joslin's diabetic patients have become mothers and all lead perfectly normal lives apart from the necessity of diet regulation and insulin administration. The secret seems to be the education of parents, and of the children when old enough, in the principles and details of treatment. In addition, the importance of supervision by the family doctor or in a properly constituted diabetic clinic cannot be over-emphasized since this goes far to prevent the occurrence of the serious complications and ensures the correct adjustment of insulin dosage and diet to the increasing needs of the growing child.

REFERENCES.

Edwards, H. T., Richards, T. K. and Dill, D. B. *Amer. J. Physiol.*, 1931, *98*, 352.
Faber, K. Lectures in Internal Medicine, 1923, quoted by Cameron, A. T. and Gilmour, C. R. The Biochemistry of Medicine, London, 1933.
Faber, K. and Hansen, K. M. *Acta med. Scand.*, 1923, *58*, 372.
Fitz, R. *Arch. Int. Med.*, 1921, *27*, 305.
Fleming, G. B. *Glasgow Med. Jour.*, 1932, *118*, 314.
Gilchrist, M. L. *Arch. Dis. Child.*, 1929, *4*, 129 : 1932, *7*, 169.
Graham, G. *Quart. J. Med.*, 1922—23, *16*, 236.
Hale-White, R. and Payne, W. W. *Quart. J. Med.*, 1925—26, *19*, 393.
Holst, J. E. *Acta med. Scand.*, 1925—26, *63*, 47.
John, H. J. *Endocrinology*, 1927, *11*, 497.
Joslin, E. P. Treatment of Diabetes Mellitus, London, 1928.
Lawrence, R. D. The Diabetic Life : Its Control by Diet and Insulin. London, 1931.
White, P. Diabetes in Childhood and Adolescence, London, 1933.

Reprinted from THE LANCET, *Dec. 9th*, 1933, *p.* 1314.

NUTRITIVE VALUE OF BOILED AND RAW MILK IN INFANT FEEDING

BY NOAH MORRIS, M.D., B.Sc., F.R.F.P.S. Glasg.
BIOCHEMIST TO THE ROYAL HOSPITAL FOR SICK CHILDREN, GLASGOW; UNIVERSITY LECTURER IN BIOCHEMISTRY IN RELATION TO DISEASES OF CHILDREN; AND

STANLEY GRAHAM, M.D. Toronto, F.R.F.P.S. Glasg.
VISITING PHYSICIAN TO THE HOSPITAL; UNIVERSITY LECTURER IN THE MEDICAL DISEASES OF CHILDHOOD

THE relative nutritive values of raw and sterilised milk have received much consideration of late years. In the literature which has recently been summarised by Stirling and Blackwood [1] and by Savage [2] it is clear that although definite statements are made about the absorption of the various food constituents, there are but few exact data regarding the influence of sterilisation on the nutritive value of milk for the infant. It therefore seems worth while to put the following results on record.

Two apparently healthy infants, aged 8 months and 7 months, were fed on an adequate measured amount of boiled cow's milk for 10 to 14 days, on the last seven of which the excreta were collected. Thereafter the same amounts of raw milk obtained from the same source were given for a fortnight, the excreta as before being collected on the last seven days. Aliquot samples of the milk were collected for analysis each day during the metabolism periods. The intake and output of nitrogen, fat, calcium, and phosphorus in each of the seven-day periods were then determined and the retention of each calculated.

The results recorded in the accompanying Table show lower retentions of nitrogen, calcium, and phosphorus during the raw milk period than during the period when boiled milk was given. It is of interest that while Case 1 was retaining a large amount of mineral during both periods the retention in Case 2 was practically nil. Nevertheless even in Case 2 the substitution of raw for boiled milk over a period of a fortnight did not improve the mineral retention as one might have expected, if it is true that raw milk possesses a superior nutritive value. In passing, one might remark that this metabolic picture is that presented by the early stages of rickets in infants.

The retention of fat was almost the same in the two types of feeding.

We do not wish to infer too much from these results, since the scope of these experiments is too limited to allow one to affirm that sterilised milk is always superior to raw milk as far as retention of nitrogen and minerals is concerned. It is also to be remembered that the experiments recorded here were of short duration, and do not reflect the state of affairs which might exist on the continued administration of boiled or raw milk. Nevertheless they afford no evidence that the absorption of nitrogenous substances, fat, and minerals is diminished by the boiling of milk.

—	Diet.*	Retention per kg. per day.				Gain in weight.
		Nitrogen.	CaO	P_2O_5	Fat.	
Case 1.		(g.)	(g.)	(g.)	(g.)	(g.)
Period 1.	(Boiled).	0·162	0·060	0·0575	3·8	150
Period 2.	(Unboiled).	0·071	0·041	0·0410	3·85	140
Case 2.						
Period 1.	(Boiled).	0·194	0·0003	−0·0098	3·75	100
Period 2.	(Unboiled).	0·125	−0·009	−0·090	3·70	250

* In all the experiments the diet consisted of 200 c.cm. milk, plus 4 g. cane sugar, five times daily.

Stirling and Blackwood [1] come to the conclusion that there is no decisive evidence that pasteurisation destroys the vitamin content of milk to any appreciable extent, with the exception of the vitamin C. This is not a serious drawback, since it is almost universally admitted that raw milk does not provide a sufficient supply of vitamins, and that these must be obtained in other ways.

As far as these results go there is no evidence to support the idea that the boiling of milk interferes with its usefulness as a food for infants.

We desire to express our thanks to the Medical Research Council, by whom the expenses of this work were defrayed.

REFERENCES

1. Stirling, J. D., and Blackwood, J. H.: Nutritive Properties of Milk in Relation to Pasteurisation. Hannah Dairy Research Institute, Bull. No. 5, 1933.
2. Savage, W. G.: THE LANCET, 1933, i., 429 and 485.

The Lancet Office,
7, Adam Street, Adelphi, W.C.2.

Reprinted from THE LANCET, *May 14th*, 1927, *p.* 1020.

THE EFFECT OF THE ADMINISTRATION OF SODIUM BETA-HYDROXY-BUTYRATE ON THE GLYCOSURIA OF PHLORIDZIN DIABETES.

BY NOAH MORRIS, M.D. GLASG.,

AND

STANLEY GRAHAM, M.B. TOR.

(*From the Medical Department, Royal Hospital for Sick Children, and the Institute of Physiology, Glasgow.*)

THE question of the conversion of fat into carbohydrate by the animal organism has been one of great controversy for many years. Most authorities agree that so far as the vegetable kingdom is concerned this change can occur, but in animals experimental proof is still lacking and opinion is divided. Lusk,[1] for instance, stoutly denies that such a transformation takes place, and certainly many of the experimental results justify such a conclusion. Geelmuyden,[2] on the other hand, believes that sugar can be formed from fat by the animal organism, and he quotes experiments in which he has found a distinct rise in the sugar output as a result of feeding acetone bodies to rabbits. In a series of injections of sodium aceto-acetate and oxybutyrate to phloridzinised dogs, Marriott,[3] however, failed to find any increase in the output of glucose.

In the resting state it has been shown that, on the basis of oxidation of fats to CO_2 and water, it is possible for the tissues to make use of the energy liberated in this way. Murlin and Riche[4] showed that the intravenous injection of an emulsion of fat led to an increased heat production together with a fall in the respiratory quotient. In the case of external work, however, there is no evidence that muscles can use

fat directly, although it has been clearly shown that they utilise glycogen. Benedict and Cathcart [5] have demonstrated that during muscular exercise fat and carbohydrate are apparently equally well utilised, and the work of Krogh and Lindhard [6] indicates that muscular work can be performed at the expense of fat in the diet.

Recently Hill, Long, and Lupton [7] have shown that the respiratory quotient of excess metabolism during short periods of exercise is unity, irrespective of diet. This indicates that, although fat in the diet may be used up during muscular exercise, the muscles themselves can only utilise carbohydrate.

If carbohydrate is the sole source of energy for contracting muscle, and if fat is utilised in the performance of muscular work, the conclusion appears inevitable that fat must undergo a preliminary conversion into carbohydrate previous to its use by the muscles. Geelmuyden [2] also suggests that fats are transformed into carbohydrate via a ketone body stage, the transformation taking place in the liver. This hypothesis seems reasonable when one considers that in all conditions where energy is obtained mainly from fat, as in starvation and during the administration of ketogenic diets, acetone bodies appear in excess in the blood and urine.

If this be true the administration of acetone bodies to a dog rendered diabetic by means of phloridzin might be expected to cause an increase in the output of sugar. This could be readily detected by a rise of the dextrose-nitrogen ratio in the urine, since in a completely phloridzinised animal all the glucose is presumably derived from the protein.

Two Experiments.

The following experiments were performed in order to find out whether any support could be adduced for this hypothesis. Sodium beta-hydroxy-butyrate, prepared by neutralising the racemic beta-hydroxy-butyric acid with sodium hydroxide was used. It was unfortunately impossible to obtain the lævo-rotatory acid. The same dog was used for both experiments, and on each occasion received a constant diet of 500 g. of minced meat twice daily. Phloridzin in olive oil was given subcutaneously in one gramme doses twice daily on each day of the experiment. The butyrate was given by stomach-tube at the end of the third day. In the second experiment a small amount

was vomited shortly after administration so that probably not more than 15 g. was retained. The animal was catheterised at the end of each 24-hour period to ensure accuracy of the 24-hour sample of urine.

EXPERIMENT 1.—*Female Dog; weight 8·8 kilos.*

Day.	Total nitrogen.	Sugar output.	D : N ratio.
1st ..	14·3	43·1	3·01
2nd ..	15·0	47·1	3·14
3rd ..	16·1	52·6	3·26
25 g. sod. beta-hydroxy-butyrate given.			
4th ..	17·9	51·0	2·84
5th ..	17·5	55·5	3·17
6th ..	16·8	53·7	3·20
7th ..	14·7	45·4	3·08

EXPERIMENT 2.—*Female Dog; weight 9·3 kilos.*

Day.	Total nitrogen.	Sugar output.	D : N ratio.
1st ..	29·2 *	82·0	2·80
2nd..	17·1	87·7	5·12
3rd ..	18·5	93·4	5·05
20 g. sod. beta-hydroxy-butyrate given. Slight amount vomited.			
4th ..	17·4	95·2	5·47
5th ..	16·5	96·1	5·82
6th ..	19·7	109·8	5·57

* Done in triplicate.

In neither of the above experiments did the butyrate cause an appreciable rise in the D : N. ratio, thus agreeing with the results of Marriott.[3] Unfortunately we used only the racemic acid, and we feel that the results might have been more conclusive if we had also given the lævo-rotatory variety. Marriott suggests that, because the acid present in the urine is the lævo-rotatory form the body can only use the dextro-rotatory type, a conclusion which is scarcely warranted.

Whether the butyrate can be changed into sugar or not still remains an open question. Certainly no evidence of its conversion into sugar is afforded by the two experiments above. The experiments of Magnus-Levy [6] suggest that because of the phloridzinisation the animal might be incapable of burning the ingested beta-hydroxy-butyrate.

Also, it is quite possible that the extra sugar might have been stored in the muscles as glycogen, to be used during muscular exercise later on. As blood-sugar and presumably urinary sugar cannot be derived from the muscle glycogen there would be no change in the D : N ratio.

A further point of interest is that in the two experiments in the same animal under identical conditions, the D : N ratio should show such a marked difference.

We wish to acknowledge our thanks to Prof. D. Noël Paton for performing the operation of perineotomy and for his helpful advice, and to Prof. Leonard Findlay for his kindly criticism.

The expenses of this work were defrayed by the Medical Research Council, for which we wish to express our gratitude.

References.

1. Lusk, G.: The Science of Nutrition, London and Philadelphia, W. B. Saunders Co., 1919.
2. Geelmuyden, H. C.: Ergeb. d. Physiol., Wiesbaden, 1923, xxi., 274.
3. Marriott, W. McK.: Jour. Biol. Chem., Baltimore, 1914, xviii., 241.
4. Murlin, J. R., and Riche, J. A.: Proc. Soc. Exp. Biol. and Med., New York, 1915, xiii., 7.
5. Benedict, F. G., and Cathcart, E. P.: Carnegie Inst. Publications, No. 187, Washington, 1913.
6. Krogh, A., and Lindhard, J.: London, Biochem. Jour., 1920, xiv., 290.
7. Hill, A. V., Long, C. N. H., and Lupton, H.: London, Proc. Royal Soc., 1924, 96B, 438: 1924, 97B, 84, and 155.
8. Magnus-Levy, A.: Ergeb. d. Inn. Med. und Kinderheilk., Berlin, 1908, i., 352.

The Lancet Office,
1, Bedford Street, Strand, W.C. 2.

OBSERVATIONS ON THE ACTION OF ACETONE.

BY

NOAH MORRIS, M.D., and STANLEY GRAHAM, M.D.

From the Institute of Physiology, University of Glasgow, and the Medical Department, Royal Hospital for Sick Children, Glasgow.

Ever since it has been shown that diabetic coma is associated with a marked acetonuria and acetonæmia, it has been suggested that the presence of acetone is the *fons et origo* of the symptoms of coma. Kussmaul[1] first investigated the pharmacological action of acetone experimentally on man, but with oral doses amounting to six grams he obtained no results. Using rabbits he found that the subcutaneous injections of acetone led to symptoms of intoxication and stupor, the action being less than that of chloroform or ether but stronger than alcohol. The symptoms were even more marked if the acetone was inhaled, and resembled clinically the coma of diabetes. Kussmaul accordingly concluded with the earlier workers that acetone was the direct cause of diabetic coma. Frerichs[2] gave large doses to men and dogs but without any special results and decided that acetone *per se* was not the cause of the coma. Penzoldt[3], however, as the result of his experiments on rabbits maintained that the acetone played an important role though it was not the only cause. Tappeiner[4] studied the effect of acetone inhalations on dogs and rabbits, and described two stages of acetone poisoning: first, a stage in which the blood pressure is raised with an increase in the pulse and respiration rates, and secondly, a stage of depression associated with a lowering of blood pressure, a decrease in the pulse and respiration rates and a gradual abolition of reflex activity. The second stage was marked by a steady fall in the temperature of the body until death ensued from respiratory failure. Prolonged inhalation of large amounts of acetone was required to produce the second stage. Albertoni [5] in a long series of experiments on dogs found that oral administration of acetone in doses amounting to 5 grams per kilo produced at most a slight fall in the systolic blood pressure, while on man the only effect to be observed was a slight narcosis. He also produced albuminuria in dogs with these large doses. Rhamy[6] stated that subcutaneous injections of acetone in guinea-pigs were more toxic than those of methyl alcohol. Hewlett[7], on the other hand, maintained that the toxicity of acetone was very low. Salant and Kleitman[8], as a result of their experimental work concluded that acetone possesses a double action causing both depression and stimulation, but chiefly the former. They drew attention to the fact that although the amount of acetone present in the blood in acidosis is comparatively small, yet the tissues and the organs are exposed to its action for a relatively long period, and they inclined to the view that acetone *per se* is the cause of the symptoms in diabetic coma.

Allen and Wishart[9] have shown that young fasting animals and animals that have been phlorhizinised or depancreatised (in other words, suffering from

carbohydrate starvation) are rendered more susceptible to the injection of acetone bodies. They also found that a hyperglycæmia resulted from the injection of acetone and the neutral salts of keto-acids.

In view of the varying experimental results the effect of acetone was considered worthy of further investigation.

Effect of Acetone on the Nervous System.

This effect was studied on rabbits and some of the results are recorded in Table 1. A 20% solution of acetone was injected intravenously, into the veins of the ear. In each case there was an interval of 24 hours between the injections to prevent cumulative action. Doses below 0.8 c.cm. pure acetone per kilo of body weight had no apparent effect on the animal. When, however, this dose was exceeded, it was found that the animals become stuporous and lay unconscious for a short period, usually 1 to 3 minutes. Recovery was fairly rapid and usually complete within three minutes. With very large doses convulsions were observed. These were clonic in type and lasted for much less than half the total period of unconsciousness. Reflexes were invariably abolished during the period of complete unconsciousness. The respirations during the period of coma were usually more rapid. The severity and duration of the symptoms were increased by increasing the dose of acetone, but not always proportionately. The rate of injection within limits (1 to 5 minutes) had no effect on the duration or severity of the symptoms produced.

TABLE I.

Number.	Weight of Rabbit in Kilos.	Acetone per Kilo. cc.	Reaction.
1	1·5	0·4	Nil.
	1·5	0·6	Nil.
22	1·3	0·8	Nil.
1	1·5	1·0	Unconscious for 30 sec. Recovery period 10 sec.
18	1·9	1·0	Unconscious for 75 sec. Recovery rapid.
4	1·1	1·2	Unconscious for 30 sec. Recovery period 60 sec.
	1·1	1·2	Same result. This dose given 10 min. after previous one.
1	1·5	1·3	Unconscious for 60 sec. Recovery period 10 sec.
4	1·1	2·4	Convulsions for 2½ min. Respiration rapid. Nystagmus. Regained balance in 8 min. Still dazed at end of 17 min.

The effects of Intravenous Injections of 20% Acetone Solution.

Hirschfeld[10], in 1895, pointed out that the presence of carbohydrates prevented the formation of acetone bodies and Shaffer[11], in 1921, showed that the oxidation of acetone was greatly accelerated by the presence of glucose. Geelmuyden[12] has also suggested that a combination of acetone bodies and

carbohydrate was necessary before the former could be oxidised. In order to test if glucose was able to prevent or minimise the toxic symptoms, a 10% solution of glucose was injected simultaneously with the acetone. In every case the period of unconsciousness was shortened and in two cases the occurrence of convulsions was prevented. In some experiments glucose was also injected 5 minutes before the acetone and invariably there resulted not only shortening of the duration of the reaction but a diminished intensity of the toxic effects on the nervous system.

The value of alkali in the treatment of ketosis has long been known and until recently occupied a prominent place in the treatment of diabetic coma. In a series of experiments in which the acetone was injected along with 6% sodium bicarbonate solution, the effect observed was similar to that of the simultaneous injection of glucose. When, however, the sodium bicarbonate solution was administered before the acetone, its preventive action was much stronger than that of glucose.

These results are tabulated in Tables II and III. In one case, the same amount of saline was injected previously instead of glucose or bicarbonate solution in order to rule out the possible effect of the fluid *per se*.

TABLE II.

Number of Animal.	Weight in Kilos.	Acetone per Kilo. cc.	Remarks.	Reaction.
1	1·5	1·3		Unconscious 30 sec. Recovery period 10 secs.
		1·3	8 cc. 20% glucose soln. simultaneously.	Nil.
2	2·0	1·2		Unconscious 3 mins. Recovery period 2 mins.
		1·2	10 cc. 20% glucose soln. simultaneously.	Dazed 10 sec.
		1·2	10 cc. 40% glucose soln. simultaneously.	Dazed 10 sec.
		1·2	10 cc. 6% $NaHCO_3$ soln. simultaneously.	Unconscious 30 sec. Recovery period 2 min.
3	1·2	1·2		Convulsion. Unconscious for 3 min. Recovery period 3 min.
		1·2	6 cc. 20% glucose simultaneously.	Unconscious 30 sec. Recovery period 2½ mins.
		1·2	6 cc. 6% $NaHCO_3$ simultaneously.	Unconscious 20 sec. Recovery 40 sec.

The effect of Injections of Acetone modified by simultaneous injections of Glucose or Sodium Bicarbonate.

Effect of Acetone on the Respiration.

As a general rule the effect of acetone on the breathing was an increase in both rate and depth. Occasionally, this increase in minute volume was preceded by a short period of apnœa. In rabbits, the respiratory rate and depth were always increased, leading in one case to an increase in minute volume of 340%. In cats, there was occasionally observed a very slight depression of the respiratory rate together with a decrease in the depth of the individual

respirations. Repeated doses produced no increase of effect in rabbits but in cats the period of apnœa was greatly prolonged. Both in cats and rabbits, the effect very soon passed off although the blood contained a very large amount of acetone (303 mgm. % in one case).

TABLE III.

Number of Rabbit.	Weight in Kilos.	Acetone per Kilo cc.	Remarks.	Reaction.
18	1·9	1·0		Unconscious 75 secs.
		1·0	10 cc. 10% glucose 5 min. previously.	,, 15 ,,
		1·0	10 cc. 6% $NaHCO_3$ 5 mins. previously.	,, 5 ,,
20	1·8	1·0		Unconscious 90 secs.
		1·0	10 cc. 10% glucose 5 min. previously.	,, 20 ,,
		1·0	10 cc. 6% $NaHCO_3$ 5 min. previously.	,, 5 ,,
21	1·4	1·0		Unconscious 75 sec.
		1·0	10 cc. 10% glucose 5 mins. previously.	,, 50 ,,
		1·0	10 cc. 6% $NaHCO_3$ 5 mins. previously.	,, 30 ,,
		1·0	10 cc. 0·9% NaCl 5 mins. previously.	,, 70 ,,
23	1·4	1·0		Unconscious 70 sec.
		1·0	10 cc. 6% $NaHCO_3$ 5 mins. previously.	,, 25 ,,
		1·0	10 cc. 10% glucose 5 min. previously.	,, 50 ,,

The effect of injections of Acetone modified by previous injections of Glucose or Sodium Bicarbonate.

Injection of either glucose or bicarbonate along with the acetone did not alter the effect of the acetone on the respiration, nor was the previous injection of these substances more efficient. With lethal doses, respiratory failure took place some minutes before the heart ceased to beat, thus confirming the observation of Salant and Kleitman[8].

Effect of Acetone on the Circulation.

In all cases acetone injections led to a fall in the blood pressure unless the injection was very slow when practically no effect was observed. On one or two occasions a slight rise preceded the fall. Previous injections of glucose seemed occasionally to lessen the fall. The rate of recovery depended for the most part on the period of apnœa. The recommencement of the respiratory movements was usually accompanied by a fairly sharp rise in the blood pressure. Administration of suprarenal extract was more than sufficient to counteract the fall of blood pressure due to the acetone.

Excretion of Acetone.

In all cases, irrespective of the dose, acetone was excreted within twenty hours of its administration, the complete excretion being determined by its disappearance from the urine. One fact of interest may here be noted, viz., that within half an hour of the administration of a large dose of acetone, the blood-acetone bodies were not nearly as high as might have been expected. For example, in one case where 14·5 cc. of 20% acetone were injected, the blood should have contained 2·9 cc. acetone if none had been excreted. The total acetone bodies were determined on a sample of blood taken approximately 5 minutes after the injection and were found to be 0·303%. Taking the blood-volume as one-thirteenth of the body weight, this represents only 0·56 grms.—roughly one-fifth of the amount injected. The urine at this time was free of acetone and as there was no perceptible odour of acetone in the breath, it seems unlikely that the other 4/5ths, or 2·3 grms., were excreted by the lungs. This suggests that the acetone was dealt with by the tissues in some way.

Effect of Acetone on the Blood Sugar.

Some observations were also made on the effect of acetone on the blood sugar. The animals were in all cases starved for twenty-four hours and the fasting blood sugar estimated. Results were neglected unless the fasting blood sugar value was between 0·100 and 0·125%. The acetone was injected into the ear veins and the blood was taken from these veins at intervals after the injection. The results are given in Table IV.

TABLE IV.

Number.	Weight of Animal.	Acetone per kilo cc.	Fasting Blood Sugar.	Blood Sugar at end of—				
				5 min.	15 min.	30 min.	45 min.	60 min.
4	1·1	1·2	·115	·154	·166	—	—	—
5	1·05	1·2	·119	·113	·118	·113	—	—
8	2·15	1·0	·107	·110	·139	·106	·108	·100
9	1.4	0·5	·125	·111	·169	·164	·149	—
10	1.2	0·3	·117	·128	·156	—	·119	—
11	2.15	water	·117	·127	·117	·117	·109	—

The effect of Injections of Acetone on the fasting Blood Sugar.

In every case except one there is a slight rise in the blood sugar following the injection of acetone. The rise in the blood sugar is not directly proportionate to the dose of acetone—in fact the smaller doses gave the greater rise. It is quite conceivable that if the dose of acetone is large enough to produce convulsions, no rise in the blood sugar would occur, because it has been definitely shown that convulsions themselves will cause a fall in the blood sugar.

In no case did the rise in blood sugar lead to a glycosuria. The presence of glycosuria following the injections of acetone has been recorded by von Jaksch [13],

and others. Von Noorden[14] also states that hyperglycæmia and glycosuria occur after the administration of acetone and seems to be quite definite in his contention that this condition is the result of deficiency of oxygen. In none of our experiments was there ever a hyperglycæmia great enough to cause a suspicion of a glycosuria even when taken at the end of an apnœic period. It seems to us that the slight rise cannot be attributed to defective oxidation of the blood, although the possibility still remains that acetone inhibits the oxidation processes in the cells.

Effect of Acetone on the CO_2-Content and the CO_2-Combining Power of the Blood.

In two cases the total CO_2-content of the arterial blood was determined as well as three points on the CO_2-dissociation curve. For ready comparison Table V shows the volumes per cent. of CO_2 taken up by the blood when exposed to tensions of CO_2 of 20, 40 and 60 mm. before and after the intravenous administration of 10 cc. of 20% acetone to cats.

TABLE V.

	Volumes of CO_2% taken up at—			CO_2-content of Arterial Blood.	*p*H.
	20 mm.	40 mm.	60 mm.		
Cat. 1.—Before ...	33·5	47·0	54·5	54·6	7·20
After	31·8	45·8	52·3	53·8	7·15
Cat. 2.—Before ...	36·5	48·5	56·0	44·6	7·38
After	31·5	44·5	51·2	45·0	7·31

It will be seen that in each case there is a slight decrease in the CO_2-combining power, that is, the curve is shifted slightly to the right, although in the first case the difference is probably almost within the limits of experimental error. The *p*H values before and after the injections were worked out from the information obtained above by means of the arterial point on the dissociation curve and the application of Hasselbalch's equation. These figures are also given in Table V. In each case there is a definite fall in the *p*H indicating an acid-poisoning effect of the acetone. Apropos of the low initial *p*H of the blood of Cat 1, it should be mentioned that in each case the animal was under the influence of urethane, which may have accounted for the low figure recorded before the injection.

Discussion.

The results of acetone injection show a close resemblance to those produced by acid poisoning. The same effect on respiration and circulation have been described as occurring after injections of acids such as phosphoric acid or hydrochloric acid by Mathison[15]. It is an old observation that both oral and intravenous administration of acids leads to glycosuria[16]. This close similarity in effects leads us to think that the symptoms of acetone poisoning may be due, in part at least, to acid-poisoning of the blood. It may be argued

that this change in acid-base equilibrium is secondary to the effect on respiration as produced by the action on the respiratory centre. In some of the experiments, however, the effect on the blood sugar occurred without any change in the nervous activity. As a matter of fact, the most pronounced rise of blood sugar occurred with the small doses and when the rabbit showed no nervous symptoms. This conclusion is supported by the beneficial effects resulting from preceding injections of sodium bicarbonate. The results with glucose, although not so striking as those with bicarbonate, are of interest in that they are in accord with what has been long known clinically, viz. : that administration of glucose is one of the best methods of treating a ketosis.

At the same time it is very clear from our results that the effect of acetone is not limited merely to an alteration of the acid base balance. It has also a direct action on the central nervous system not unlike that of alcohol, particularly methyl alcohol, the toxic action of which is frequently manifested by convulsions. This narcotic action is apparently influenced by the presence of alkali and glucose, and in order to produce it large doses of acetone must be injected.

It would seem that this double action of acetone is frequently lost sight of in assessing the influence of various factors in the production of coma. As has been pointed out by many investigators[8] [17], it is hardly fair to compare the results of an acute intoxication with acetone on the cells of an animal that is previously normal, with the gradual and continuous action of small percentages of acetone over long periods. In the latter case the acetone is having a continuous effect on the acid-base equilibrium and the organism is in a condition of stress in the constant effort to maintain a normal reaction. It may be that in this way the cells become more liable to the narcotic action of acetone so that at a certain stage in the condition with the gradual rise in the acetonæmia, coma sets in, although the actual percentage of acetone is much below that found to be necessary for the experimental production of coma.

Conclusions.

1. Experiments were done on cats and rabbits to demonstrate the effects of acetone administered intravenously.

2. Doses of acetone exceeding 0·8 cc. per kilo body weight produced unconsciousness, occasionally with clonic convulsions. The respiratory minute volume was increased. The blood pressure was lowered, with a preliminary slight rise, on a few occasions.

3. In each case the urine was free of ketone bodies within twenty hours of the administration of acetone, irrespective of the dose.

4. In all cases but one there was a slight but definite rise in blood-sugar following intravenous injection of acetone.

5. In two cases the CO_2-dissociation curve of the arterial blood was determined, and the pH of the "arterial" point calculated. In both cases there was a slight shift to the acid side.

6. Previous or simultaneous injection of glucose or sodium bicarbonate always lessened the intensity of the acetone effect. Sodium bicarbonate seemed slightly the more efficacious.

7. The suggestion is made that acetone has a double action : (1) a toxic effect similar to that of methyl alcohol and (2) an acid effect on the acid-base equilibrium.

We desire to thank Professor L. Findlay for his helpful advice and criticism throughout the course of the research. Our thanks are also due to the Medical Research Council with whose aid the work has been done.

REFERENCES.

1. Kussmaul, A., *Deut. Arch. f. klin. Med.*, Leipzig, 1874, XIV, 1.
2. Frerichs, F. T., *Ztsch. f. klin. Med.*, Berlin, 1883, VI, 1.
3. Penzoldt, F., *Deut. Arch. f. klin. Med.*, Leipzig, 1884, XXXIV, 127.
4. Tappeiner, H., *Ibid*, Leipzig, 1884, XXXIV, 450.
5. Albertoni, P., *Arch. f. exp. Path. u. Pharm.*, Leipzig, 1884, XVIII, 218.
6. Rhamy, B. W., *J. Amer. Med. Ass.*, Chic., 1912, LVIII, 628.
7. Hewlett, A. W., *The Pathological Physiology of Internal Diseases*, N.Y. and Lond., 1923.
8. Salant, W., and Kleitman, N., *J. Pharm. and Exp. Therap.*, Balt., 1922, XIX, 293.
9. Allen, F. M. and Wishart, M. B., *J. Metab. Res.*, N. Jersey, 1923, IV, 613.
10. Hirschfield, F., *Ztschr. f. klin. Med.*, Berlin, 1895, XVIII, 176.
11. Shaffer, P. A., *J. Biol. Chem.*, Baltimore, 1921, XLIX, 143.
12. Geelmuyden, H. C., *Ztschr. physiol. Chem.*, Berlin, 1904, XLI, 135.
13. Von Jaksch, R., *Ztschr. f. klin. Med.*, Berlin, 1885, X, 362.
14. Von Noorden, C., *Metabolism and Practical Medicine*, London, 1907, III, 1099.
15. Mathison, G. C., *J. Physiol*, Camb., 1910-11, XLI, 416.
16. Pavy, F. W., *Diabetes Mellitus*, Lond., 1869, 154.
17. Wells, H. G., *Chemical Pathology*, Philad., 1920.