THE CALCIUM AND PHOSPHORUS CONTENT OF THE BLOOD IN NORMAL AND RACHITIC CHILDREN

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A. CALCIUM

INTRODUCTION:

The subject of the calcium content of the blood and soft tissues, and the possible function of this element in health and disease is by no means a new one since references to its probable importance are to be found in the literature of last century. (1), (2), (42). The more recent recognition of the importance of the part played by the inorganic salts in the general metabolism, together with the fact that numerous methods have been elaborated which permit of the accurate measurement of the Ca in reasonably small amounts of blood, have lent new interest to the study of the calcium content of the blood in human beings as well as in animals, and it is of considerable interest to compare the normal figures given by the earlier investigators with those more recently published. In this connection one is struck, in the first instance, by the fact that the earlier normal figures show a much wider range of variation than is now believed to be compatible with health, in spite of the fact that the available methods of estimating the calcium were much less numerous then than now. Thus Rumpf (1) found only 4.5 mg. CaO. per 100 ccs. in the blood of new born children, while Weiss (3) gives 20 mg. per 100 ccs. as the normal figure for infants just after birth. Cattaneo (4) who does not detail his method, gave as a normal amount for infants a quantity as high as 68.3 mg. CaO. per 100 ccs., and Longo (5) published results ranging between 14.06 and 21.1 mg. per cent., while Von Noorden in his Handbuch d. Pathol. d. Stoffwech~~h~~sels quotes Schmidt's normal adult figure for whole blood as 7.8 mg. per 100 ccs. and for serum as 16.5 mg. per cent. CaO. From the earlier figures in the literature one would therefore be led to believe that the calcium content of the blood shows a wide variation within normal limits. Neurath (6) however in 1910, using a method which gives comparative and not quantitative results, concluded from his investigations that the calcium normally present in the blood of infants is a fairly constant quantity. Katzenellenbogen (7) using the same method about three years later was able to confirm Neurath's results though her figures were all rather lower than his. Results published in 1914 by Aschenheim (8) further indicated that the normal average content of CaO in the blood of children between the ages of 2 months and 4 years varied only between 8 and 13 mg. per cent. The children he used as controls were not normal but were suffering from diseases not usually associated with any derangement of the calcium metabolism.

During and since 1917 published results representing the normal calcium content of the blood of human beings are fairly numerous and the table below is inserted for the purpose of comparing these results and the methods by which they were obtained.

Table I. overleaf:-

TABLE I.
RECENT RESULTS COLLECTED FROM THE LITERATURE

	Investigators	Method used	Amount of calcium as mg. of Ca. per 100 cc. Adults: Whole blood	Adults: Plasma	Adults: Serum	Children: Whole blood	Children: Plasma	Children: Serum
918	Howland & Marriott (12)	Howland & Mariott (13)	—	—	10	—	—	10.8
	Denis & Minot (15)	Lymans (11)	—	9.7	—	—	—	—
	Cowie & Calhoun (16)	Lymans	7.86	12.0	—	—	—	—
	Denis & Talbot (17)		—	—	—	—	10	—
917	Lyman (11)	Lymans	6.06	—	—	—	—	—
917	Halverson, Mohler & Bergheim (10)	Halverson & Bergheim's (41)	7.8	—	10.15	—	—	—
921	Jones & Nye (23)	Lymans (modified)	—	—	—	9.4	10.0	—
922	Von Meysenbug (28)	Lymans	—	—	—	—	11.2	—
920	Steeman & Arntzenius (18)	De Waard's (21)	—	—	—	—	—	8.66
921	Jones, Martha (22)	Lymans (23) (modified)	—	—	—	8.7	12.2	—
	Sharpe (24)	Sharpe's (24)	7.8	—	12.7	10.6	—	14.0
918	Jansen (14)	Jansens (27)	8.35	—	—	—	—	—
920	Brown, McLaughlan & Simpson (19)	Lymans	—	—	—	9.5	—	—
921	Richter-Quittner (20)	Jansens	—					
909	Loeper & Bechamp (9)	?	6.75	—	13.0	—	—	—
922	Ling & Bushel	Ashing method	8.1					
921	Kramer & Howland (26)	Kramer & Howlands (39)	—	—	9.95	—	—	10.6

In order to facilitate easy comparison all the calcium values in Table I are expressed as Ca. although in the original publications many of the figures were given in terms of CaO.

In studying the table, one is in the first place struck with the fact that the range of variation in the normal figures given by the different workers is decidedly smaller than that indicated by the earlier workers. It will also be noted, however, that the individual results quoted have been arrived at by very different methods, and the fact that some investigators have used whole blood, some serum, and others plasma, while, up till recently, very few have used all three, has rendered the literature on the subject not a little confusing. I propose, therefore, before proceeding further, firstly, to make a brief survey of the many methods available to the investigator and, secondly, to direct attention to certain physiological aspects of the problem which cannot be separated from any critical study of the pathological significance of variations in the calcium content of the blood.

METHODS:

Excluding the admittedly ingenious but now obsolete method of Blair Bell (29) who precipitated the Ca. from a drop of blood with oxalate and counted the calcium oxalate crystals so obtained, and giving only passing mention to the older gravimetric technique described in any textbook of chemistry, the available methods may be divided into four main groups as follows:-

(1) Those which depend (a) on the amount of oxalate required to prevent coagulation of the blood, or, (b) on the re-calcification of oxalated blood;

(2) Methods in which the Ca is precipitated by modifications of the McCrudden technique from solutions of the blood or serum ash;

(3) Methods in which the Ca. is precipitated by processes similar to those of group (2), after de-proteinisation of the blood by means of acids or picrates;

(4) Those in which the Ca is precipitated directly from fresh serum not previously subjected to ashing or de-proteinisation.

Methods falling in the first group include those of Wright (30) Voorhoeve (31), and Vines (32). The two last named investigators have modified their coagulation methods in such a way that they give quantitative results but Wright's technique gives only relative figures, while all three methods have the additional disadvantage of yielding information only regarding the so-called physiologically active Ca. which as shown by the recent work of Vines does not necessarily give any indication of the total Ca.

Voorhoeves method gives such undoubtedly high results even in his own hands that one must doubt its accuracy. The method of Vines seems to have given figures which agree more closely with those obtained by chemical methods though the technique appears to be an involved and laborious one.

In the second group are included all the old gravimetric methods such as those described by Pribram (33), Allers and Bondi (34), and Aron (35), as also the more recently published methods of Lamers (36), Jansen (27), Dienes (37), Sharpe (24), Kehrer (38), De Waard (21) and Kramer and Howland (39).

The third group comprises the methods of Marriott and Howland (13), who got rid of the protein by digesting with nitric acid, of Lyman (11) who de-proteinised with trichloroacetic acid, and of Greenwald (40), and of Halverson and Bergheim (41) who used picrate for the same purpose.

Lastly in the fourth group are placed the more recently published methods of Marriott and Howland (13), of Kramer and Tisdall (43), and of Clark (45), as also the later of the two de Waard (44) methods.

After ashing or de-proteinisation, the procedure in all the methods is with minor differences the same, and consists in (1), the precipitation of the Ca. as oxalate by the McCrudden (46) technique or some modification of it, from a solution of suitable hydrogen ion concentration, (2), the washing of the precipitate so obtained, and (3), the titration of the solution of washed precipitate with potassium permanganate.

Three exceptions to this procedure are to be found in the methods described by Howland and Marriott, by Lyman and by Jansen. Lyman's method appears to have been widely used particularly by American investigators though it has been adversely criticised by Clark and by Kramer and Howland, apparently chiefly on account of the fact that it has given a much wider normal range than any other method with the exception of that of Jansen.

The last named investigator removes the iron and phosphoric acid from his ash solution before precipitating the Ca. as oxalate, ignites the precipitate to CaO. and completes his estimation by an acidometric or iodometric titration. I have no experience of the use of the method but the amount of manipulation seems to be excessive when one takes into consideration the small quantity of blood used, and it has been criticised by de Toni (47) on this account. The technique of Haessler and Marriott is admittedly cumbersome, besides requiring both skill and practise before consistent results can be expected. In the few cases in which it has been tried in this laboratory the percentage error was much higher than that claimed for it by the originators, and, though this may have been largely due to lack of practice in the somewhat unusual technique, the method was very soon abandoned on account of its unwieldiness as well as the fact that it was not suited to the use of quantities of blood such as we were easily able to obtain.

The method used in the present series of estimations is that described by Sharpe (24) in an earlier series of estimations of which this investigation is a continuation. It differs from the usual type of ashing method only in respect of the fact that the iron is removed before precipitation of the Ca. as oxalate, and is as follows:-

> 5 ccs. of blood are evaporated to dryness, carefully ignited and completely ashed in a small platinum capsule. 2 ccs. of strong HCl. are then added, the capsule covered, gently heated, and allowed to stand about 10 mins. The contents are then diluted with 5 ccs. of water, and washed into a small glass beaker. Ammonia is added till the solution is alkaline when a yellowish flocculent precipitate appears, sufficient acetic acid is now added to make the solution distinctly acid and the iron phosphate is filtered off and thoroughly washed with hot water. To the filtrate 5 ccs. of a 2% soln. of ammonium oxalate are now added and the solution boiled, and set aside over night. Next day part of the clear supernatant fluid is poured off and the remainder washed with the precipitate into a specially pointed centrifuge tube. Centrifugalisation at high speed for not less than 5 mins. results in the complete precipitation of the calcium oxalate which is then washed three times with hot water. The precipitate from the last "swing" is dissolved in 5 ccs. of 20% sulphuric acid and titrated at a temperature of about 60° with N/100 potassium permanganate.

Methods of the above nature have been criticised by Howland

and Kramer (48) and by Clark (49) on the ground that complete precipitation of Ca. as oxalate by this technique is difficult without accompanying precipitation of magnesium, phosphorus and iron, thus making the results too high. Shohl (50) however has investigated the McCrudden technique in the light of the more recent knowledge of the influence of the hydrogen ion concentration on the precipitation of substances from solution. His results indicate that the range of hydrogen ion concentration compatible with complete precipitation of Ca. while magnesium and phosphorus are held in solution is a wide one (4 to 6.4), and several samples of the blood solutions brought to this stage, which I chose at random and tested were well within Shohl's safety range.

As already indicated the iron is removed and need not therefore be considered in this connection. The further difficulty of avoiding error due to loss of calcium during the process of washing has also been pointed out by Clark and by Howland and Kramer. The necessity for getting rid of excess of oxalate in the permanganate titration methods is admittedly of paramount importance, and the difficulty of accomplishing satisfactory washing without dissolving any of the precipitate is a well recognised one. It has been my experience, however, that titration readings are much more frequently too high on account of incomplete washing than too low on account of loss of precipitate during the process. If centrifuge tubes drawn out to a fine point are employed, the precipitation is accomplished rapidly by spinning at high speed, and hot water is used for the washing, the loss due to solubility of the calcium oxalate is negligible. I have never succeeded in recovering by re-precipitation from the washings of 16 lots an estimable quantity of Ca. The only other parts of the procedure at which error is liable to be appreciable are during ignition when sparking has to be avoided, and during titration when a variation in the end point may give different results in different hands. If the compared results have all been obtained by one investigator, however, the latter variation is negligible. Clark (49) estimates that an error of only 2% is involved in the process of titration. The experimental error involved in the use of Sharpe's method in the hands of the investigator has been gauged by a number of consecutive estimations on the same sample of blood and is found to be approximately 10%. Variations of 1 milligram are therefore not regarded as being of any significance except in dealing with the averages of large series of results.

THE RANGE OF VARIATION IN NORMAL CHILDREN

In order to establish a normal figure for comparison with results obtained in pathological conditions, a study of the blood calcium content in 31normal children of ages ranging between 2 months and 11 years was made in the first instance. The results are collected in table 2.

TABLE 2.

Sex	Age	Calcium content as milligrammes Ca. per 100 cc. whole blood	
Boy	2 months	9.5	
"	3 "	8.2	
"	3 "	7.7	
"	3 "	7.9	
"	3 "	7.9	
"	3 "	8.7	
"	3½ "	8.4	
"	4 "	8.8	
"	4 "	8.1	
"	4 "	6.4	
"	4 "	7.9	
"	4 "	9.5	
"	5 "	9.2	
"	5 "	7.6	
"	6 "	8.3	
"	6 "	7.7	
"	7 "	7.8	
"	8 "	7.1	
"	8 "	7.0	
"	8 "	7.7	
"	9 "	7.3	
"	14 "	7.1	
"	15 "	7.5	
"	15 "	9.1	
"	2½ years	8.2	Average = 8.0
Girl	7 "	7.3	
Boy	8 "	7.7	
"	10 "	7.0	
"	10 "	7.3	
"	11 "	6.5	
"	11 "	7.4	Average = 7.2

The results obtained indicate that the calcium content of the whole blood in children under 2½ years varies only between 6.4 and 9.5 mg. per cent., which figures compare favourably with those obtained by other workers. The younger children were all carefully examined clinically, and were accepted as normal only when no evidence or history of disease was obtainable. In some, though not in all, the presence of rickets was excluded by X-ray examination. Many were breast fed and the remainder were on artificial diets of whole milk, milk and sugar, or Glaxo, but, since no differences detected in the blood calcium could in any way be correlated with the method of feeding, whether breast or artificial, I have omitted details of this from the table. The older boys were orphan children from a home to which I was able to gain access through the help of Dr. Crockett, whose kindness I desire to acknowledge with gratitude. Unfortunately it has not been possible to obtain blood samples from normal children between the ages of three and ten years. From the figures at my disposal, however, I have been able to form the opinion that the normal amount of blood calcium in young children falls between 6.4 and 9.5 mg. per cent., the large majority of the figures lying between 7 and 9.

VARIATION WITH AGE:

It is further evident from the tabulated results that the average figure obtained for the older boys is slightly lower than that for the infants. The question of the possibility of the occurrence of a variation in the blood calcium with age therefore arises.

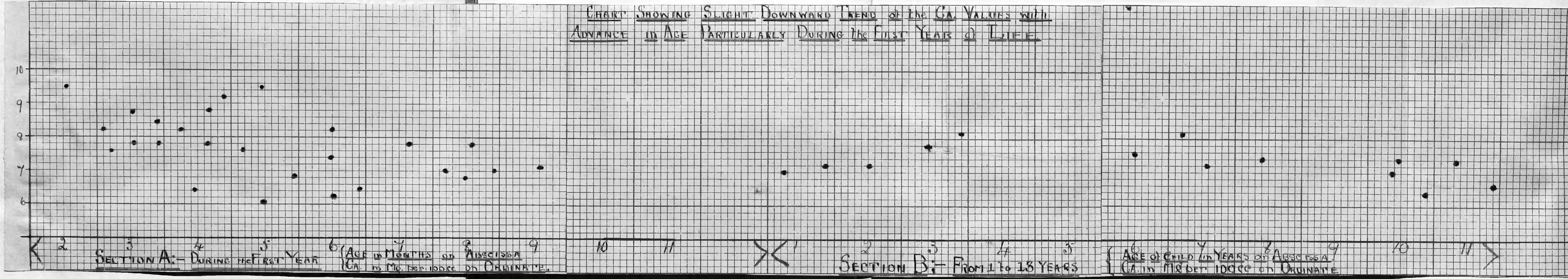
This is one of the physiological aspects of the blood calcium problem which increasing experience has taught me to regard as being of considerable importance, and the contradictory results to be found in the literature indicate the necessity for further investigation. Howland and Marriott (12), for instance, have found that the calcium content of the serum in the healthy human subject is singularly constant irrespective of age. Sharpe in the course of a previous investigation of the blood CaO conducted during one of the studies on experimental rickets by Findlay, Noël Paton, and Sharpe (24), found that in six individuals of varying ages a steady decrease which corresponded with advance in years could be demonstrated. A similar decrease was shown to occur in dogs of different ages. Jones and Nye (23) publish a series of figures which ~~indicating~~ fail to ~~that this~~ indicate ~~diminution~~ any ~~can~~ steady ~~be steadily~~ downward trend ~~traced~~ of Ca results from 4 weeks till the end of the first year of life, while Jansen (14), on the other hand, makes the statement that the infant at birth has the same blood calcium content as the mother but that during suckling the calcium content may rise to a figure as high as 20 mg. per cent., after which it falls steadily till adult life is reached.

This finding has not so far as I am aware been confirmed by any other investigator, and certainly the present series of figures give it no support. Meigs, Blatherwick and Carey's (51) results, with respect to animals, on the other hand, bear out Sharpe's observation on dogs of varying ages. It is clear that the only way to solve this problem satisfactorily is to get in touch with a series of normal children at birth and obtain blood samples for examination at monthly intervals during the first year of life. Such an investigation is manifestly well nigh impossible except in an institution for the care of healthy infants and I have not so far been able to carry out a complete study of this nature.

All the results available, however, have been taken from table 2 and graphically represented in diagram A which shows the calcium values plotted in a graph according to age.

A careful examination of this shows that there is a very slight downward trend in the Ca. figures with advance in age, and this is particularly evident during the first year of life. It is further evident that the widest variation in the normal range occurs between the ages of 4 and 6 months, the figures before and after this age being on the whole very constant.

FIGURE A.



Note A few results obtained after table 2 had been compiled are charted in figure above, thus bringing the number of available normal figures up to 40.

DISTRIBUTION OF THE CALCIUM OF THE BLOOD:

Since it seems reasonable to conclude from the first part of the investigation and from the results of other workers, that the normal calcium content of the whole blood in children falls within a very small range of variation, attention was next directed towards the establishing of a similar normal figure for the blood serum. This part of the investigation has been undertaken in view of the very conflicting opinions to be found in the literature regarding the presence or absence of calcium in the corpuscles. Howland and Marriott (12) state that, since they find in the serum approximately double the amount of calcium to be recovered from the whole blood, the corpuscles are Ca. free. This conclusion has been much quoted by investigators who advocate the use of serum for Ca. estimations and gains considerable support from the work of Richter-Quittner (20) who also fails to find calcium in the corpuscles. Meigs, Blatherwick and Carey (51) also appear to accept it as an established fact that the corpuscular Ca., if any, is negligible. Contradictory evidence has, however, been furnished by the more recent work of Hamburger, by Rona (52), by Jansen (14), Loeper and Bechamp (9), by Findlay Paton and Sharpe (24), by Cowie and Calhoun (16), and by Jones and Nye (23), while Parnas and V. Jasinski (54) state that they have failed to find Ca. free corpuscles. In the papers of several of those workers references are made to the opinions of the earlier investigators, and more particularly to that of Schmidt who stated that calcium is present in the corpuscles. Richter-Quittner (20) explains many of the above results by pointing out that in the separation of the plasma from the serum in oxalated or citrated blood a certain amount of Ca. is carried down with the deposit. Plasma results are therefore too low and corpuscle results too high. To get rid of this possible source of error he suggests the use of hirudin which he found did not tend to cause precipitation of the calcium with the corpuscles. His explanation, however, does not take into consideration those figures obtained for serum separated from the whole blood without the addition of any anti-coagulant, nor does he refer to the fact that serum and plasma results have, even in different hands, given closely similar results, although the use of plasma entails the employment of some agent for the prevention of coagulation.

Abderhalden (42) however, failed to find Ca in the corpuscles of any animal with the exception of the goose, and one finds in the literature numerous references to the work of Gryns, Koppe, Hamburger, Erykman, Overton and Hedin who produced evidence to show that the blood cells are impermeable to calcium. In the face of such contradictory evidence it is desirable that more work should be done on this subject, particularly since ~~even~~ those investigators who believe in the presence of Ca. in the corpuscles give vastly different values for the amounts obtained. Thus Cowie and Calhoun (16) found 4.26 to 4.98 mg. per cent. in unwashed and slightly less in washed corpuscles, while Jones and Nye (23) give~~s~~ figures varying between 5.2 and 11 mg. per cent., and Rona (53) found the corpuscles of cat's blood to contain anything from 0 to 6 mg. per cent.

If this problem is first studied from the standpoint of a critical survey of all the figures available for comparison in the literature it is found that plasma and serum give the same calcium values within the limits of experimental error. This would seem to justify the conclusion either that the fibrin and the serum contain exactly equal amounts of calcium or that, if the fibrin is calcium free, its bulk is sufficiently small to make no demonstrable diminution in the plasma values. Gram (55) has recently given 0.27 to 0.36 as the average percentage of fibrin in the plasma of man, Berggrum (56) found 0.29 to 0.42 per cent. in the plasma of children and Whipple (57) found about 0.4 per cent. in the plasma of men. If the average taken from these three figures, namely, 0.34 per cent. is accepted as an index of the normal fibrin content, the difference between the serum and plasma values, provided the fibrin contains no calcium, would be only 0.04 per cent., an amount which is undoubtedly well within the range of experimental error of any of the available methods. The fibrin may therefore be left out of account in dealing with the differences between serum and whole blood, and the figures for these two may now be compared.

The average of the available figures for whole blood, including adults and children, gives a calcium value of 8.68 mg. per cent., and a similar average of 10.9 may be obtained for the serum (see Table 1.). The bulk of the corpuscles may be taken as roughly 50 per cent. of the whole blood and from the above figures a theoretical value for the corpuscles of 6.46mg. per 100 cc. is calculated.

In order to find out whether actual results tally with the theoretical values, I have carried out in each of 12 normal children estimations of the calcium in the whole blood and in the serum, at the same time taking readings of the approximate percentage volume of serum in each sample of whole blood.

METHOD:

For this purpose 15 ccs. of blood were withdrawn from the longitudinal sinus into a clean dry syringe. 5 ccs. of this were used for the whole blood estimations and the remaining 10 were quickly transferred to an ordinary pointed centrifuge tube and centrifuged at high speed for 20 minutes. Any small strands of clot then found adhering to the upper part of the tube were gently separated from the glass by passing a fine platinum wire round the inside of the container and the specimen further centrifugalised for 10 minutes. The level of the clot was then marked off accurately on the outside of the tube, the serum poured off, the clot washed out, and its volume measured by filling the emptied and dried tube up to the mark with water from a finely graduated burette. The results obtained by this method are admittedly not as accurate as haematocrit readings, but, since the object was, not so much the establishment of an accurate figure for the calcium value of the corpuscles, as to find out whether this element is present in the corpuscles in sufficient amount to justify the habitual use of whole blood in preference to serum, the approximate readings are considered sufficiently accurate.

The results obtained from the series of 12 normal cases are collected in Table 3.

TABLE 3

Sex	Age	% of serum	Ca of whole blood in mg. per 100 cc.	Ca of serum in mg. %.	Ca of corp. in mg. %.
Male	2 months	51%	8.2	10.8	5.5
"	3 "	45%	7.7	10.6	5.3
"	3 "	47%	7.9	11.4	4.7
"	3 "	46%	7.9	11.0	5.2
"	3 "	55%	8.0	11.0	4.4
"	4 "	49%	7.8	11.1	4.6
"	6 "	60.1%	8.6	10.9	5.2
"	7 "	53.9%	7.7	10.7	4.1
"	10 years	51.1%	7.0	10.0	3.9
"	10 "	53.4%	7.3	10.5	3.6
"	11 "	51.2%	6.6	10.0	3.1
"	11 "	47.3%	7.3	10.6	4.2
Averages			7.66	10.7	4.2

The series of results indicate that the average figure of 4.2 though lower than the theoretical values calculated from table 1, is clearly an amount which must be taken into consideration.

Whole blood has therefore been used throughout the investigation in spite of the facts, that considerably more time is required, since all the rapid methods are applicable to serum only, and that the normal range may be slightly wider than that obtained for serum owing to difference in the bulk of the corpuscles.

SEASONAL VARIATION

I am not aware that there is any reference in the literature to the possibility of the occurrence of a seasonal variation in the calcium content of the blood of normal infants, though such a possibility has been indicated in connection with the blood phosphorus by Hess (58) in America. Some recent results obtained in very young babies this spring have suggested to me that calcium values may be somewhat lower in the early months of the year than at any other time. The samples were obtained from a series of children in whom I hope to be able to make monthly examinations of the blood Ca. during the first year of life. However, as neither rickets nor tetany has so far been definitely excluded in the patients in question, the Ca figures have not been included in the present series of normals.

All the other blood samples were obtained either in the late summer or early winter of 1921 and can therefore give no indication of the presence or absence of variation due to the time of year.

The subject of seasonal variation must therefore be left open until next year when it is hoped that a series of estimations may have been completed in the group of young children mentioned above.

THE EFFECT OF AN INCREASED OR DIMINISHED INTAKE OF CALCIUM ON THE NORMAL BLOOD CALCIUM CONTENT

The last of the physiological problems which have presented themselves for consideration is the important question as to whether in the normal child the amount of calcium in the diet has any influence on the calcium content of the blood.

It was pointed out in the earlier part of the paper that variations in the normal calcium content could in no way be correlated with variations in the diet, nor was it evident that the breast-fed infants had a higher normal figure than those on whole milk or other artificial food. Katzenellenbogen's (7) figures have also indicated that no difference can be detected between the blood calcium of children on natural and those on artificial diets.

Dr.Telfer kindly gave me the opportunity of making blood calcium estimations in the case of two normal children of six months old whose diets were modified to contain varying amounts of lime for the purpose of a series of metabolic studies, the results of which have since been published (68). The figures obtained during the study are recorded below.

A. G. (Case 4 in Dr. Telfer's series)

Nature of diet	No. of days for which diet was given	Average daily intake of CaO in grammes	Calcium of blood as mg. of Ca per 100 cc. at end of period of dieting
Cow's milk 1000 cc. per diem	7	1.62	7.4
Diluted cow's milk. Caloric value made up with sugar	3))6	0.81))0.55	7.8
Diluted whey	3)	0.3)	

G.K. (Case 5 in Dr. Telfer's series)

Nature of Diet	No. of days for which diet was given	Average daily intake of CaO in grammes	Calcium of blood as mg. of Ca per 100 cc. at end of period of dieting
Cow's milk 1000 ccs. per diem	3	1.66	8.3
Diluted whey	3	0.21	8.5
Diluted whey plus 1.8 gms. CaO as calcium lactate	3	2.01	9.2

The above experiments indicate that the calcium content of the blood cannot be readily influenced by varying the calcium intake over short periods.

Most of the investigations carried out along these lines have concerned themselves with finding out whether the calcium of the blood is influenced (a) by feeding with excessive doses of lime such as are seldom to be found in any ordinary diet, or, (b) by depriving the organism of all or most of this element in the food, while metabolism experiments indicating the actual lime retention have seldom been accompanied by systematic estimations of the blood calcium. Findlay, Noël Paton and Sharpe (24), however have shown that the calcium retention is roughly proportionate to the intake and increased and diminished supply in the diet may therefore be presumed to produce some variation in the absorption under normal conditions.

Handovsky (59) stated that in rabbits he found no relationship between the calcium balance and the calcium values of the blood, and Steeman (60) states that over-consumption of lime does not lead to increased absorption beyond the required amount, any excess being merely excreted, and that, furthermore, he failed to find any co-relation between the food calcium and the blood calcium. Denis and Minot (15) were unable to increase the blood CaO above the normal figure in men, cats, and rabbits by the administration of even larger doses of calcium. Voorhoeve (61), on the other hand, reports an enormous increase in the calcium above the normal figure after administration of large doses of lime salts to adults. His control figures are, however, so high that they lead one to accept his results with reservation. Richter-Quittner (20) also records a definite increase in the blood after administration of large quantities of Ca by the mouth and even finds Ca in the corpuscles under these circumstances though he believes that blood cells are normally lime free. Where the blood has been examined in dogs after feeding experiments conducted in this Institute by Prof. Noël Paton and co-workers no evidence has been obtained to show that a normal blood calcium content can be augmented by the feeding of a diet rich in lime, even over long periods. Of other investigators who have also found no increase in the normal blood calcium due to feeding with lime salts, Howland and Kramer (62) and Loeper and Béchamp (9) may be quoted.

The balance of the experimental evidence seems therefore to be in favour of the view that no increase in the normal blood CaO results from increase in the lime of the diet, and, on reading over the recent literature, one gathers that this is now a ~~widely~~ generally accepted fact. It is, however, conceivable that, during some period of digestion, an increase in the blood CaO does occur, if the tissues are to receive their lime requirements by absorption from the food supply. The amount absorbed at any one time must therefore be small or deposition or excretion of the excess over the normal accomplished so rapidly that the increase occurring during the process of digestion is not detectable by the methods now in use.

Whether the deprivation of lime in the diet leads to a diminished blood content is a subject of considerably more controversy. Of the earlier workers, Weiske (63) could produce no decrease in the calcium of the dried blood by calcium deprivation alone, while Forster (64), Voit (65), and Aron and Sebauer (66) indicated that such a diminution did occur on prolonged Ca starvation, and Quest (67) found a definitely lower blood Ca in dogs on a Ca low diet than in those supplied with an ample lime intake.

In the two experiments on Dr. Telfer's cases already quoted (68) no diminution in the blood Ca was evident after 6 and 3 day periods on a Ca low diet, and in dogs kept for long periods on a lime poor diet in connection with Prof. Noël Paton's feeding experiments, the blood calcium values have not on an average been lower than those of the controls. The fact that the lime supply can be very materially varied without causing changes in the calcium content of the blood seems to indicate that the maintenance of the blood calcium content at a definite constant level is of considerable importance for the health of the infant, and that some very efficient mechanism is normally at work for the purpose of regulating the lime in the body fluids.

11. R I C K E T S

Reference to the prominence given to investigations of the behaviour of the calcium salts in all studies of the etiology of rickets need hardly be made, nor is it necessary to trace the advance in knowledge along these lines since full historical surveys of the work done have already been published by investigators in the Glasgow school (~~78~~ 69) (24) which has long had, and continues to have, a very active interest in the subject.

In the course of a former study of the calcium metabolism in rachitic and normal dogs, the results of which were published in 1921 (24), Sharpe failed to find any significant difference between the CaO blood content of the animals which developed the disease and those kept as controls. A study of the amount of lime to be found in the blood in cases of human rickets was not, however, possible at that time and the present investigation in children has consequently been undertaken.

The experimental evidence quoted in the first section of the paper indicates the extreme difficulty of causing any deviation of the blood calcium in either direction by the variation of the lime supply. One would not therefore be surprised if faulty absorption which is equivalent to defective supply on the one hand, and faulty fixation or excessive decalcification, both of which are equivalent to increased supply, on the other, failed to lead to any alteration in the blood Ca values. A failure to find abnormal calcium values in rachitic subjects need not therefore be interpreted as an indication of the absence of even profound disturbance of the calcium metabolism, but rather points to the fact that the mechanism which regulates the constancy of the lime in the blood has not suffered during the general disturbance.

Furthermore, it is not expected that a positive result, i.e., the finding of low or high Ca values, will give any solution of the very important problem as to whether the calcium regulating mechanism has broken down as a result of the prolonged or sudden strain of dealing with abnormal lime supplies or from some entirely different cause, such as alteration in the pH of the blood, the action of some toxin, or the lack of some essential substance other than lime in the diet. The strict limitations of the information to be gained from a study of the blood calcium values alone are fully realised in the interpretation of the following results.

Published accounts of the investigations of the calcium content of the blood in rickets are by no means in agreement. Both Neurath (6) and Katzenellenbogen (7), using Wright's method, found no diminution in the so called physiologically active blood Ca in rachitic children, while Szenes (70) reports that such a diminution does occur. Aschenheim (8) found that the amount in rachitic children varied, in some cases being lower than the normal and in some higher, and calls particular attention to the importance of this variation. Howland and Marriott's (12) figures indicate an occasional reduction, though some of their low results are explained by the presence of anodal hyperexcitability and therefore presumably latent tetany in the children from whom the Ca. low blood samples were obtained. Denis and Talbot (17) also found a low calcium content in the blood of rachitic infants, though the very wide variation found by them in other diseases and the fact that some additional disturbance was present in most of the cases of rickets renders their results of doubtful significance. Howland and Kramer have found an occasional reduction of the Ca content in rickets and in a recent series of five cases (71) in whom the disease was apparent at an early age the values given for the serum were all decidedly low. Shipley, Park, McCollum and Simmonds (91) advance the theory that there may be two kinds of rickets, one characterised by a normal or nearly normal blood Ca and a low blood phosphorus, and the other by a normal or nearly normal blood phosphorus and a low blood calcium, while figures published by György (73) indicate a slight reduction of the lime in the serum of rachitic children.

The following table shows the results of estimations of the blood Ca in 45 cases of rickets. The signs representing the degree of rickets require some explanation which may be given as follows:-

Degree of rickets estimated clinically.

−+ = showing definite evidence of rickets (rosary, epiphyseal enlargement, and undue patency of the fontanelle), but able to stand and walk alone.

\+ = showing definite evidence of rickets but able to stand and walk with support.

++ = showing definite evidence of rickets and able to stand with support but will make no attempt to walk.

+++ = showing definite evidence of rickets and unable even to stand with support.

In the very young children the above signs represent the degree of epiphyseal enlargment, rosary etc.

In order to explain the signs used to represent the severity of the rickets as estimated radiographically, I have affixed typical examples of each of the degrees as they are represented by X-ray photographs of the wrists.

Rickets +

Rickets ++

Rickets +++

TABLE 4

	Case Number	Sex	Age	Degree of rickets: Estimated clinically	Degree of rickets: Estimated by X-ray examination of wrists	Ca. of whole blood in millegrammes per 100 cc.
(1)	40	Male	4 mos.	-+	+	9.2
(2)	39	"	5 "	-	+	6.9
(3)	38	"	5 "	-	+	6.0
(4)	37	"	5 "	?+	+	9.4
(5)	44	"	5 "	?+	+	8.0
(6)	45	"	6 "	+	++	6.2
(7)	41	"	6 "	+	+	7.4
(8)	43	"	7 "	+	+	6.7
(9)	42	"	7 "	+	+	8.2
(10)	1	"	9 "	?+	?+	7.3
(11)	2	"	11 "	+++	++	6.8
(12)	3	"	11 "	-	+	6.2
(13)	4	"	16 "	++	+	7.7
(14)	5	"	18 "	++	+++	8.1
(15)	6	"	19 "	++	+++	7.9
(16)	7	"	21 "	-+	+++	7.4
(17)	27	"	20 "	++	+	6.0
(18)	32	Female	21 "	+++	+++	6.5
(19)	36	"	21 "	++	++	8.0
(20)	31	"	22 "	++	+++	7.2
(21)	29	Male	16 "	++	?-+	6.9
(22)	8	"	21 "	+	+++	8.5
(23)	9	"	22 "	+++	+++	7.2
(24)	10	"	22 "	+++	++	9.9
(25)	11	Female	" "	+++	+++	7.0
(26)	12	Male	22 "	+	+++	7.6
(27)	13	Female	22 "	-+	+	8.7
(28)	14	"	23 "	++	+++	7.8
(29)	15	"	2 yrs.	+	+++	8.4
(30)	16	Male	2 "	+	+	7.8
(31)	17	"	2 "	-+	+	7.0
(32)	18	"	2 "	+	+	6.6
(33)	19	"	2½ "	++	++	8.1
(34)	20	"	2½ "	+++	+++	6.5
(35)	21	Female	2½ "	++	+++	6.4
(36)	34	Male	2 "	++	+++	6.8
(37)	33	"	2½ "	+++	+++	7.8
(38)	30	Female	2½ "	+	+++	6.4
(39)	26	"	2 "	-+	++	7.4
(40)	22	Male	3 "	+++	+++	7.5
(41)	23	"	3½ "	+++	+++	8.3
(42)	24	"	3½ "	+++	+++	7.4
(43)	35	Female	3 "	-	?+	8.1
(44)	25	"	4½ "	++	++	7.4
(45)	28	Male	4½ "	+++	+++	7.2

TABLE 5

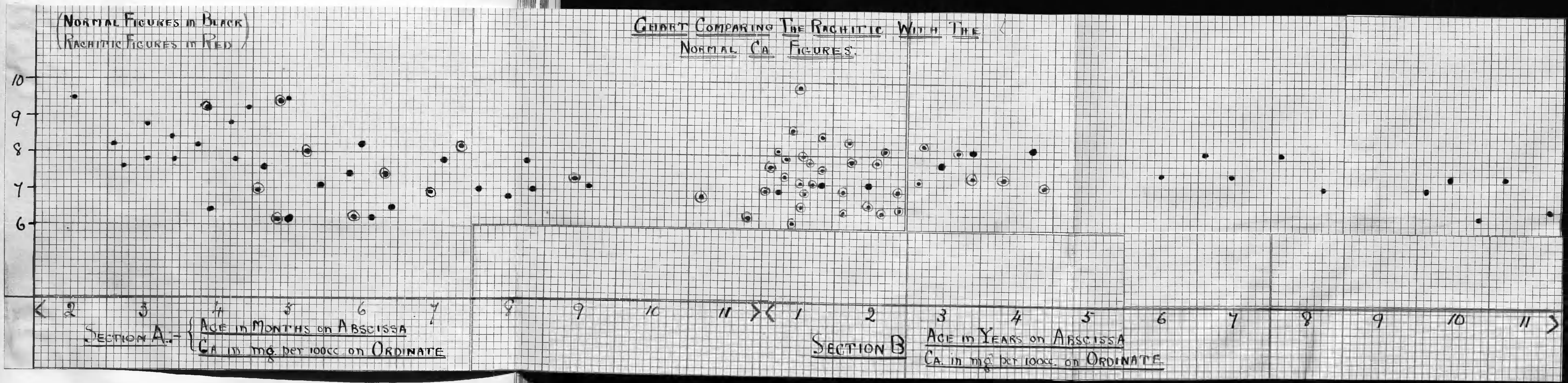
Age	Degree of rickets Clinical	X-ray	Serum as percentage of whole blood	Ca of whole blood	Ca of serum
9 mos.	Doubtful	Very slight	49	7.3	10.4
18 "	Severe	Severe	43	8.1	10.5
19 "	Very severe	Severe ? some healing	45	7.9	11.1
22 "	Severe	Very severe	48	7.2	10.4
22 "	Severe	Severe	60	9.9	11.1
22 "	Severe	Severe ? some healing	50.8	7.6	9.6
22 "			-	7.2	10.4
22 "	Moderately severe	Definite not marked	48	8.7	12.0
2 yrs.	Severe	Very severe	52.6	7.8	10.0
$2\frac{1}{2}$ "	Moderately severe	Definite not marked	56.5	7.1	9.7
$2\frac{1}{2}$ "	Moderately severe	Definite not marked	55	6.4	9.1
$3\frac{1}{2}$ "	Severe	Severe	50	7.6	10.7
Averages			50.7	7.78	10.4

Average value for the corpuscles = 5.0 mg. per 100 ccs.

It is very evident from a glance at Tables 1 and 4 that the children of the normal series are for the most part either older or younger than those of the rachitic group. While being fully alive to the difficulty of interpreting the results in the light of this difference, I have been unable to obtain blood samples from normal infants of the desired age. Even taking into consideration the slight diminution of the blood calcium with advancing age, however, an average of all the normal figures can be taken as a fair one with which to compare the rachitic average.

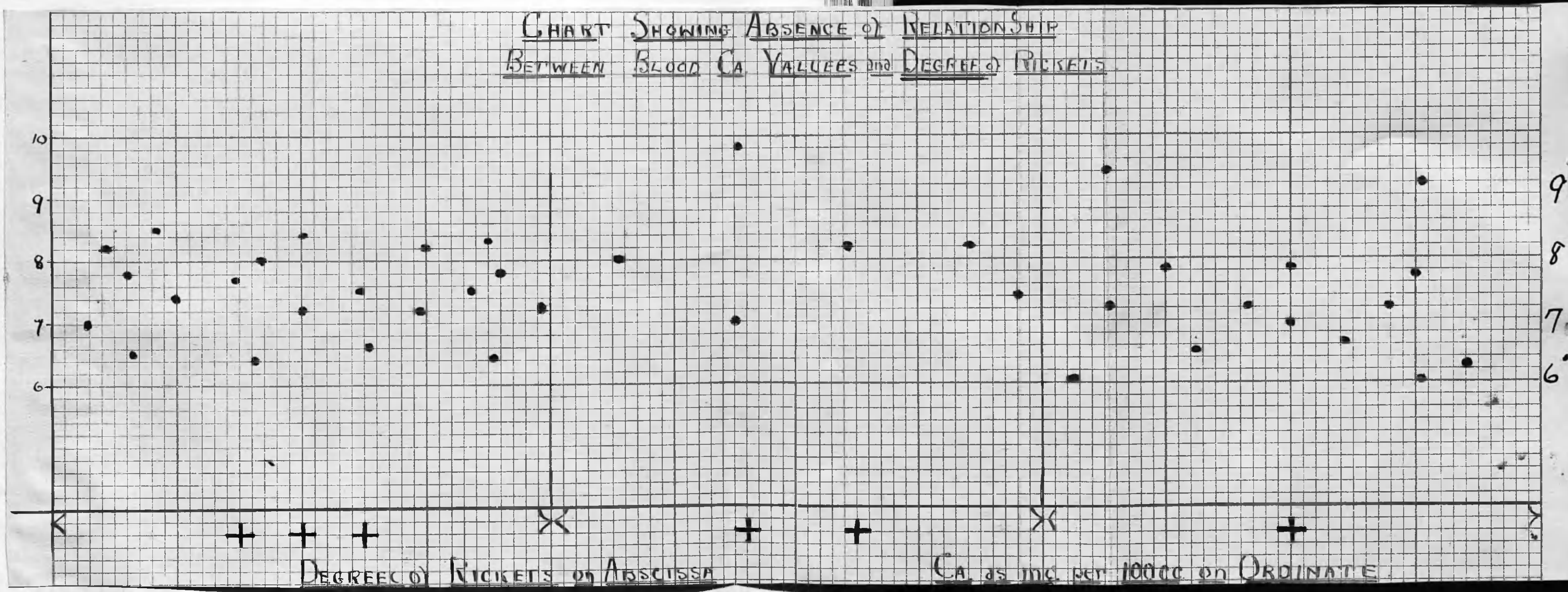
It is evident from such a comparison that no evidence has been obtained from this investigation which warrants the conclusion that there is any significant difference between the blood calcium of normal children and that of the rachitic group as a whole, while a further comparison of Tables 3 and 5 shows that the Ca. distribution is the same in both groups. The charting of the rachitic along with the normal figures as in Diagram B (overleaf), does, however, indicate that, at an age where the normal values appear fairly constant, the values obtained for rachitic children show a much wider variation. This confirms the statement of Aschenheim (8) that the blood Ca in rickets is sometimes higher and sometimes lower than normally. A more detailed examination of the results must therefore be made with a view to finding out whether these variations bear any relationship (a) to the degree of severity of the disease, and (b) to the stage of the disease.

FIGURE B.



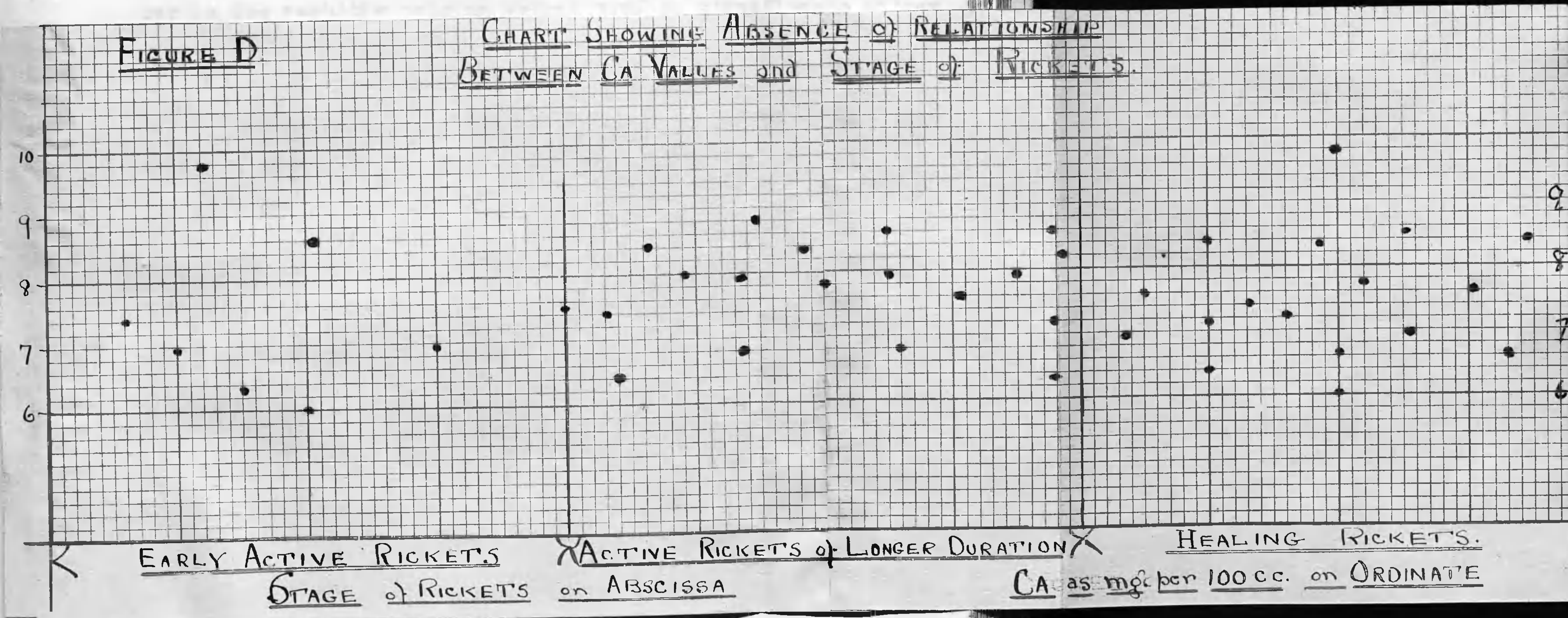
The calcium figures have therefore been plotted on the accompanying graph (Diagram C) along with a representation of the degree of rickets as estimated radiographically. It is evident from this that such variations as do occur in the figures can in no way be taken as an indication of the severity of the disease.

FIGURE C.



The question of the stage of the rachitic changes is a much more difficult one since it is at best doubtful whether we are ever able to diagnose the condition in its very earliest stage. Most of the patients come under observation only when obvious disability has led to alarm on the part of the parents and by that time the rachitic changes are generally so definitely established as to be unmistakable. It is therefore in very young children attending hospital regularly for other causes that we must seek to trace the development of rickets from its earliest stages and even here considerable difficulty is encountered in diagnosing the condition with certainty before definite radiographic evidence of bone changes has appeared. I find it difficult to believe that these bone changes can occur simultaneously with the onset of the disease since such a supposition would indicate the occurrence of a sudden decalcification at the epiphyseal ends of the long bones. Rickets in the healing stage is certainly much easier to recognise though radiographic evidence of improvement is not always consistent with the clinical state of the patient. For instance, one encounters cases which, under treatment, show marked signs of healing in the skeleton and still remain completely helpless from extreme malaise and inability to stand or walk. Cases on the other hand who have shown very striking clinical improvement, have, not infrequently, shown little or no sign of increasing calcification at the epiphyses.

In view of these difficulties, advantage has been taken of the seasonal incidence of rickets in attempting to correlate the calcium results with the stage of the disease, and the cases have been grouped in the various classes as indicated below.



during the summer months have been included in the "healing rickets" group.

In the present state of our knowledge this method of grouping may be considered suitable for the purpose since the activity of rickets during the late winter and spring and its marked tendency to spontaneous healing during the summer are well recognised.

A consideration of the figures charted in this way leads undoubtedly to the conclusion that such variations as do occur in the rachitic calcium values have no significance either as an indication of the severity of the disease or as an indication of activity on the one hand and healing on the other.

It therefore seems justifiable to conclude that though the blood calcium is slightly more variable in rachitic than in normal children, this variability has no prognostic nor diagnostic value and a study of calcium values alone is unlikely to prove of much assistance as an indication of cause or treatment.

B. PHOSPHORUS

Estimations of the total phosphorus content of the whole blood were undertaken early in the investigation but as it was found after a fair number of figures had been obtained that the range of variation in normal infants was a wide one, further research along these lines was abandoned.

Howland & Kramer (74) in 1921 drew attention to the fact that there is an appreciable difference in the inorganic phosphorus content of the serum of rachitic infants as compared with that of healthy children, and, in reporting their results, refer to the earlier work of Iversen and Lenstrup(75) whose conclusions their investigation confirmed. A number of estimations in which the Marriott & Haessler (76) method was used were carried out by the present investigator whenever the total phosphorus estimations were found to be of doubtful value, and though the method was found to be difficult and tedious, the results obtained appeared to confirm those of previous workers.

Since the elaboration of a simple and rapid method of estimating the inorganic phosphorus of the blood by Bell & Doisey (77) numerous results referring to the blood in rickets have been published. It is perhaps unfortunate that so much significance has been attached to these results before we had learned anything about the behaviour of this element in the blood under varying physiological conditions in childhood. Hess (58), for instance, has only recently called attention to the possibility of the occurrence of a definite seasonal variation, while but little is known concerning the immediate effect of the ingestion of phosphorus in the ordinary diet, or of possible variations related to age. In this section of the investigation, therefore, as in the case of calcium, an attempt has been made to ~~deal~~ consider first of all ~~with~~ such physiological aspects of the blood phosphorus problem as were likely to be of importance in dealing with rachitic figures, and a preliminary communication on this subject has already been published.* Since the beginning of this year, however, the normal figures have been very considerably added to and the conclusions arrived at in the following discussions are based on the consideration of a much larger series of results.

A. THE RANGE OF VARIATION IN NORMAL CHILDREN:

The normal figures so far published are for the most part in close agreement, and the table of collected results already published can now be extended as follows:-

* The paper has just appeared in the Biochemical Journal, 1923, Vol XVII p 43
(Reprint enclosed for reference)

TABLE 6.

Investigators	Inorganic Phosphorus in mg. per 100 cc. A. Adults. Whole blood	Serum	Plasma	B. Children Whole blood	Serum	Plasma
Howland & Kramer* (74)	-	-	-	-	4.5 to 6.8	-
Tisdall (83)*	-	-	-	-	4.6 to 6.4	-
György (84)	-	-	-	-	5.2	
Hess & Unger (78)	-	-	-	4 to 4.8	-	-
Von Meysenbug (79)	-	-	-	-	4.1 to 5.8	-
McKelleps de Young* & Bloor (80)	-	-	-	-	-	1.2 to 4.4
Hess & Lundagen (58)	-	-	-	3.6 to 4.3	-	-
Denis & Hobson (81)	-	2.6	-	-	-	-
Edward Tolstoi (82)	-	2.5 to 3.4	2.4 to 3.3			
Jones & Nye* (23)	-	-	-	5.8 to 16.3	-	5.0 to 15.0

All of the investigators with the exception of those marked with an asterisk used the Bell-Doisey method, and the results, with the exception of Jones and Nye's, are in such close agreement that they require no further comment. The figures available from the present investigation are collected below for comparison with those of Table 6.

TABLE 7

No.	Sex	Age	Inorganic P. of whole blood in mg. per 100 cc.
1	Male	3 mos.	4.8
2	"	3 "	5.5
3	"	4 "	5.5
4	"	4 "	5.0
5	"	5 "	5.8
6	"	5 "	5.4
7	Female	5 "	5.2
8	Male	5 "	5.1
9	"	5 "	4.4
10	"	5 "	4.3
11	Female	6 "	5.2
12	"	6 "	5.3
13	"	6 "	5.0
14	Male	6 "	4.8
15	"	6 "	3.8
16	"	6 "	3.9
17	"	7 "	4.0
18	"	7 "	3.7
19	"	8 "	3.9
20	Female	8 "	5.0
21	Male	8 "	3.7
22	"	8 "	5.0
23	"	8 "	5.8
24	"	9 "	5.2
25	"	9 "	4.6
26	"	10 "	4.6
27	"	11 "	4.6
28	"	11 "	4.6
29	"	12 "	4.2
		Average	4.8
30	"	2 yrs.	5.6
31	"	2 "	4.8
32	Female	2 "	4.7
33	Male	2 "	4.2
34	Female	3 "	4.5
35	"	5 "	4.6
36	Male	6 "	4.0
37	"	6 "	4.5
38	"	6 "	4.8
39	"	7 "	4.2
40	"	7 "	5.1
41	"	8 "	4.0
42	"	8 "	5.2
43	"	9 "	5.2
44	"	9 "	5.2
45	"	10 "	4.8
46	"	10 "	4.8
47	"	11 "	5.2
48	"	12 "	5.4
49	"	13 "	4.8
		Average	4.77
		Total range	3.7 to 6.6

The lowest figure obtained for a normal child was 3.7 and the highest 6.8. It is evident, however, that the very large proportion of the figures lie between 4 and 5.5 values over or under these figures being extremely rare.

On the other hand, if the present series of figures for whole blood are compared with those of Hess & Unger (78) and Hess & Lundagen (58) it is found that the range of variation in the former group is much greater than that of the latter two groups, though I have used the same method as the American investigators.

The greater variation in the author's results suggests one of three possibilities, namely, variation with age, variation with diet, or seasonal variation, each of which must be considered separately.

A. VARIATION WITH AGE:

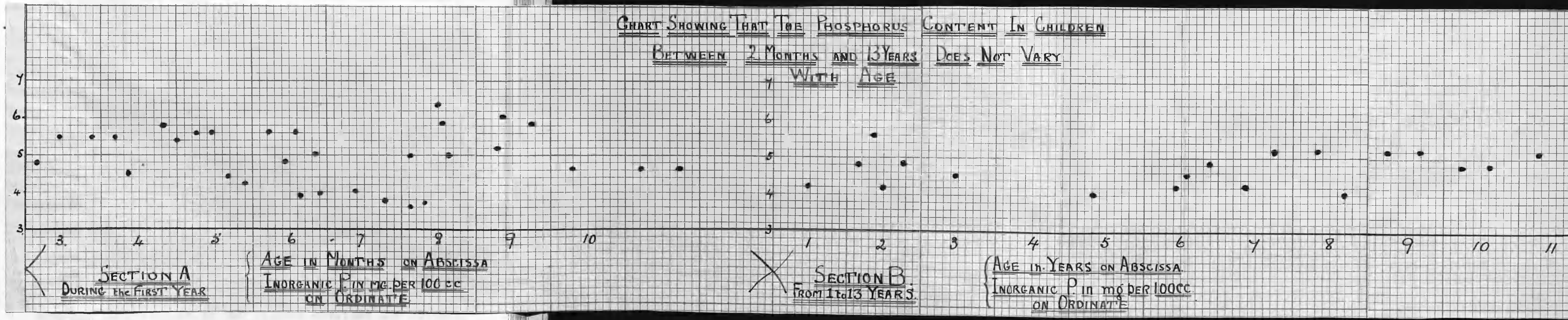
The figures in Table 7 have been arranged according to age and a comparison of the average figure for children under 1 year with that for children over this age shows the absence of any significant difference between the two age groups. This is a conclusion justified only by the consideration of a large group of normal figures since the smaller number of results published in the earlier communication seemed to indicate a slightly lower average for the older group of cases.

On reading over the literature on the subject of the inorganic phosphorus of the blood one gathers that there is some doubt as to whether any variation with advancing age occurs in growing children though there is no doubt about the fact that adult figures are very definitely lower than even the lowest figures obtained in the case of the child, a fact which has been confirmed by the few results obtained in the case of adults during the course of the present investigation.

The difficulty of obtaining a sufficient number of normal children representing each of the stages of childhood to justify the expression of a definite opinion regarding age variation is a very obvious one.

All the results available for the present investigation have, however, been graphed in figure 6, which shows at a glance that as far as growing children of between three months and 13 years are concerned there is no co-relation between age and the blood phosphorus content, nor can any diminution corresponding with advance in years be demonstrated.

FIGURE E.



B. VARIATION WITH DIET:

Very little work is so far recorded, regarding the immediate influence of the diet on the normal inorganic phosphorus of the blood in human beings. Hess & Lundagen (58) however, indicated that they found no differences in the figures obtained from infants fed on breast milk, raw certified milk, or dried milk.

The children used for the present series of phosphorus estimations were, as in the case of the calcium group, fed on very varied diets, and the blood samples were obtained at varying intervals after feeding. It therefore seemed possible that the fairly wide variation in these figures as compared with Hess & Unger's (78) might be due to the more varied diet or to the fact that some alteration in the blood phosphorus level takes place during the process of digestion.

An attempt to correlate the results with the method of feeding has, however, been quite unsuccessful. Breast fed babies do not appear to give higher values than those on cow's milk or dried milk preparations and no significant difference appeared to exist between the blood phosphorus of milk fed children and those on a general mixed diet.. Furthermore, during one stage of the investigation careful enquiry was made as to the time and the amount of the last feed, but it soon became evident that the stage of digestion had no influence on the figures obtained and latterly details regarding this point were omitted from the reports. It was strikingly shown in the course of an experiment already recorded* that in the dog no demonstrable variation in the blood phosphorus could be discovered, (a) during a period of fasting which lasted 24 hours, and (b) during the 4 hours following the ingestion of a large quantity of lean meat containing more phosphorus than is ever likely to be consumed by a child at any one time.

As a result of the investigation it was concluded that neither the nature of the diet nor the interval of time which

* Biochem. Jour., 1923 Vol XVII P 43

had elapsed since the last feed could account for the variations occurring in the blood P. values of the different children.

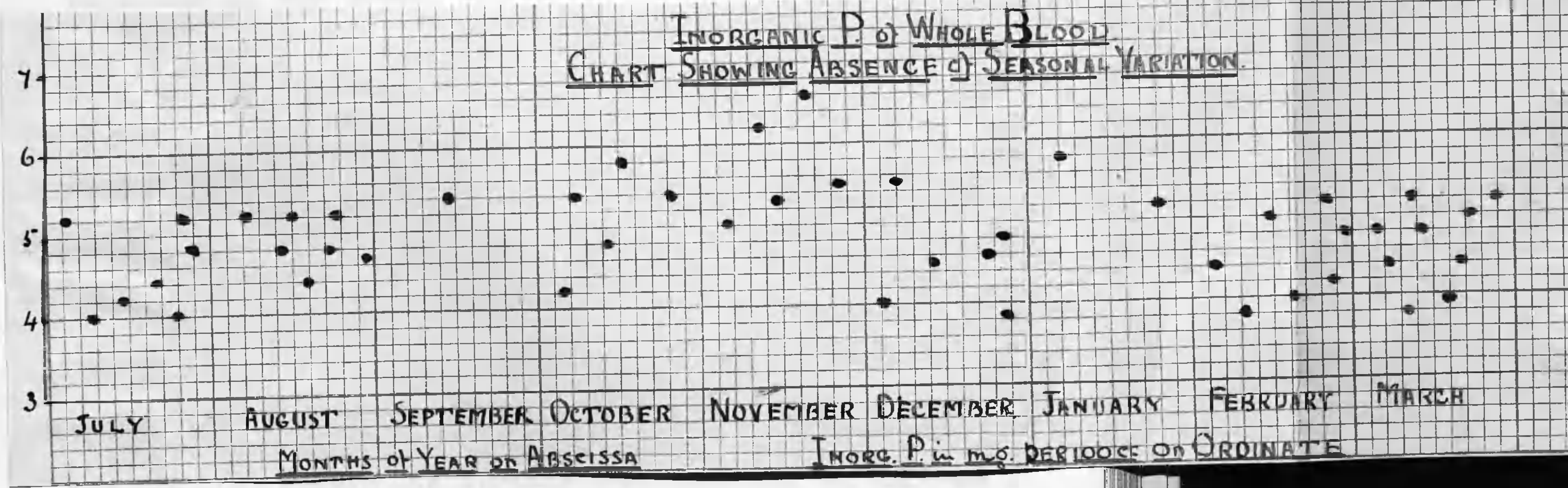
C. SEASONAL VARIATION:

Hess & Lundagen (58) have published figures indicating that the inorganic phosphorus content of the blood in children undergoes a slight seasonal variation which they appear to regard as a physiological phenomenon. Such a discovery is not only interesting but of very considerable importance, since it appears to suggest that the sunlight has some influence on the blood phosphorus level in the healthy infant. This suggestion appearing as it does at a time when the subject of the prevention and treatment of rickets by sunlight and violet rays is a subject of so much controversy has aroused very special interest. Hess & Lundagen's figures showed that the highest level of the blood phosphorus tide was reached in the months of June and July after which it diminished steadily to reach the lowest level in March. It must, however, be pointed out that the actual range of variation in Hess's figures was exceedingly small (3.6 to 4.3) and no indication is given as to what is his experimental error in the use of the Bell-Doisey method. This is, I believe, a matter of some importance since the number of figures from which the average for each month was struck is not given. Sixty children appear to have been used for the investigation, but whether each of these was subjected to monthly examination is not clear. It is therefore somewhat difficult to interpret the exact significance of the results of Hess & Lundagen.

The present series of investigations ~~were~~ was not carried out for the specific purpose of demonstrating a seasonal variation, but since an attempt was made to obtain blood samples from a small group of normal infants at or about the same time as each group of rachitic cases, a fair number of results are available for each month from July 1922 till March 1923. Each result, of course, refers to a different child, but since it has been shown that variation with age and with diet are negligible, the figures appear to be well worth recording.

Figure E has therefore been inserted below for the purpose of showing all the available results charted according to season.

FIGURE F.



It is, I think, evident that Hess & Lundagen's work in this connection has not been confirmed by the present results since the figures for July are exactly the same as those obtained for February and March, while those obtained during the autumn months were much more variable, thus causing the seasonal curve to present a slight apparent rise at this period. It must, however, be admitted that the failure to demonstrate a seasonal variation in the present instance, might conceivably be due to the fact that last summer was a poor one with very little sunshine and that the autumn and winter were perhaps milder than is usually the case in this part of the world. One must therefore conclude that the variation in the amount of sunlight available at the different seasons has not been as striking here as in the climate in which Hess & Lundagen's series of cases were living.

Park (85) has pointed out that the downward tendency of the inorganic phosphorus curve in March which Hess demonstrated may simply be an indication of the increased frequency, at this period of the year, of rachitic changes not sufficiently advanced to be demonstrable by ordinary clinical methods. The difficulty of diagnosing rickets in its earliest stages has already been referred to, but the group of children whose blood p. values have been graphed in Fig. E were all subjected to careful X-ray examination and only those who presented no evidence of rachitic changes in the bones were included in the group.

Further work on this subject in different districts and countries would be interesting and is, I think, necessary before definite conclusions can be reached regarding a physiological seasonal variation.

D. DISTRIBUTION OF THE INORGANIC P. OF THE BLOOD:

Before leaving the study of the physiological aspects of the blood phosphorus problem it is necessary to deal shortly with the distribution of this element in the serum and corpuscles.

It is evident from Table 6 that serum and plasma have been much more frequently used than whole blood.

It has been necessary during the present investigation to complete each estimation as rapidly as possible after the withdrawal of the sample of blood, and since this object, as well as uniformity of calcium and phosphorus results, was apparently to be achieved by the use of whole blood, the following preliminary experiments were carried out.*

1. In a series of eight children 15 ccs. of blood were collected, 5 ccs. were immediately lysed in 15 ccs. of water by vigorous shaking and the protein precipitated without delay. The serum from the remaining 10 ccs. was allowed to separate out but was left standing in contact with the clot for periods varying between 1½ and 3 hours. In this group the serum results were in all instances higher than those obtained for the whole blood, the average figures being: whole blood 4.86 mg. per cent., serum, 6.1 mg. per cent.

2. In a further series of eight cases the procedure adopted was exactly the same except that the serum was separated from the clot and treated with trichloroacetic acid within 40 minutes of venipuncture. In this case the serum results were almost identical with those obtained for the

* Actual figures in enclosed preliminary communication p45.

whole blood; the averages being as follows:- Whole blood, 4.6 mg. per cent., Serum 4.7 mg. per cent. Howland and Kramer (74) had already pointed out that a definite increase in the phosphorus of the serum results if the latter is allowed to stand in contact with the clot at room temperature for any length of time, while the record of a recent investigation by Tolstoi (82) shows that figures obtained from serum which had been kept were quite out of line with the rest of his results. This may give rise to a serious possibility of error particularly since we have no evidence to indicate the nature of the changes taking place in the distribution of inorganic phosphorus during the process of clotting, though the closely similar results obtained for whole blood and serum when the latter was separated as quickly as possible, seem~~s~~ to indicate that corpuscles and plasma contain an equal proportion of the element under discussion. Bloor found that the corpuscles were richer in all forms of phosphorus than plasma and the work of Jones & Nye has confirmed this finding, while they state that the inorganic P. content of the corpuscles varied considerably. In view of the increase which undoubtedly occurs on standing, the question of the distribution of the inorganic phosphorus is therefore likely to be a very difficult one; and since every sample of whole blood can be subjected to exactly the same treatment immedlately it is withdrawn from the vein ~~in each case~~, it has seemed not only more convenient but also probably more accurate for habitual use where large series of figures are to be compared.

THE INORGANIC PHOSPHORUS OF THE WHOLE BLOOD IN RICKETS

Evidence has been published by Sherman & Pappenheimer (86), and by Shipley, Park, McCollum and Simmonds (87) to show that rickets-like changes can be produced in animals by means of a deficiency of phosphorus in the diet. The results which Howland & Kramer (88), and later, Hess and collaborators (89), György (84), Von Meysenbug (79), and Kramer, Casparis and Howland (90) obtained during the examination of the phosphorus content of the blood in rachitic infants, seemed to fit in admirably with the experimental evidence cited above. It therefore appeared that here at last, from amidst the vast tangle of contradictory theories regarding the causation of rickets had emerged one clinical phenomenon which experimental evidence had led us to expect. Certainly all the investigators who undertook blood examination in the course of the study of human rickets have been agreed as to the diminution in the inorganic phosphorus content which takes place.

The results obtained from the present investigation have for the most part fallen into line with those of other workers, as shown in Table 8.

TABLE 8

Date	No.	Age	Degree of Rickets Clinical	X-ray	Inorg. P. of whole blood in mg. per 100 ccs.			
Dec. 1922	1	21 mos.	+++	+++	4.8	mg.	%	x
" "	2	18 "	+++	+++	3.8	"	"	
" "	3	2½ yrs.	+++	+++	3.3	"	"	
" "	4	9 mos.	+++	++	3.1	"	"	
" "	5	1 yr.	+++	+++	2.4	"	"	
" "	6	2½ yrs.	+++	+++	2.9	"	"	
Jan. 1923	7	2 "	+++	+++	3.9	"	"	*
" "	8	1 "	+++	+++	4.5	"	"	x
Feb. "	9	2½ "	+++	+++	2.9	"	"	
" "	10	2½ "	+++	+++	2.5	"	"	
" "	11	1½ "	+++	+++	4.6	"	"	x
" "	12	1½ "	+++	+++	5.3	"	"	x
" "	13	1½ "	+++	+++	3.6	"	"	
" "	14	1½ "	++	++	4.2	"	"	x
" "	15	3 "	+++	+++	3.2	"	"	
" "	16	3 "	+++	+++	3.3	"	"	
" "	17	3 "	+	healed	3.6	"	"	
" "	18	1½ "	++	+++	2.4	"	"	
Mar. 1923	19	4 mos.	—	+	3.0	"	"	
" "	20	5 "	—	+	3.3	"	"	
" "	21	2 "	—	?+	2.8	"	"	
" "	22	2 yrs.	+++	+++	2.8	"	"	
" "	23	2½ "	++	+++	2.5	"	"	
" "	24	2½ "	+++	+++	2.8	"	"	
" "	25	1½ "	+++	+++	3.4	"	"	
" "	26	1½ "	++	+++	3.0	"	"	
" "	27	3 "	+++	+++	2.5	"	"	
" "	28	7 mos.	+	++	2.7	"	"	
" "	29	7 "	+	++	2.7	"	"	

While one can say quite definitely that the inorganic phosphorus content of the whole blood of the rachitic group of children is lower than that of normal children, the interpretation of some of the results presents no little difficulty. For example in the case of those figures in Table 8 which have been marked with an asterisk, the presence of marked rickets was beyond doubt and evidence of healing was entirely lacking, yet the inorganic phosphorus of the blood is apparently normal. In each of these cases the presence of tetany, in which disease we have invariably found the blood phosphorus to be normal or high, has been carefully excluded. A possible explanation of these high results which suggested itself was that here we were dealing with the calcium low type of rickets described as one of two forms by Park and collaborators (91). On examining the calcium of the blood in these cases, however, I found it to be well up to the normal standard, and, indeed, none of the results obtained during blood examinations in cases of rickets in this city has lent any support to the theory of these American investigators regarding the possible existence of two types of rickets. Hess (92) has also called attention to the fact that in several cases of undoubted rickets he obtained normal phosphorus figures.

A further difficulty in interpreting the significance of a low blood phosphorus in rachitic children presents itself in dealing with those cases which show undoubted signs of healing. I have been able to trace several children through various stages of the disease and have entirely failed to demonstrate any rise in the blood phosphorus corresponding with the advance in healing. This is well brought out in the typical case whose X-ray plates are shown in Figure 6 (overleaf). Clinical improvement in this case was just as marked as the very evident signs of healing in the bones would have led one to expect. Yet the blood phosphorus remained at the same level throughout as did also the calcium.

FIGURE 6. (Case J.R. aged 2 yrs)

DATE

30 : XII : 22

No. 1

Inorg. P. of Whole Blood = 2.9 mg per 100cc
Total Ca of " " = 7.85 " "

6 : III : 23.

No. 2.

Inorg. P. of Whole Blood = 2.5 mg. per 100cc.
Total Ca " " = 7.64 " " "

17 : III : 23

No. 3.

Inorg. P. of Whole Blood = 2.5 mg per 100cc
Total Ca. " " " = 7.7 " " "

25 : III : 23.

No. 4.

Inorg. P. of Whole Blood = 2.5 mg per 100cc
Ca not done at this date.

It will be noted that in the above case, one of several who have been carefully followed, the phosphorus content has remained constant at a low level, in spite of definite evidence of healing.

The American literature so far published has indicated that the blood phosphorus content shows a definite increase corresponding with advance in healing and it has perhaps been this fact more than any other which caused so much significance to be attached to the importance of the blood-phosphorus in the rachitic child. (90) (78) (93).

It is therefore somewhat disappointing that I have been entirely unable to confirm these results by demonstrating in any case of healing rickets a corresponding improvement in the blood phosphorus. Indeed, so very striking has been the failure to obtain such straightforward results that I have come to regard examinations of the blood phosphorus in rickets as being of very little clinical assistance and this for reasons which may be summarised as follows:-

(1) Several cases of manifest rickets have a normal and others a doubtfully low blood phosphorus content.

(2) The blood phosphorus has never been found to be low before other signs of rickets were in evidence, i.e., examination of the blood is not of any help in the <u>early</u> diagnosis of rickets.

(3) The blood phosphorus content does not necessarily give any indication of the relative success of the various kinds of treatment since manifest improvement in the bones and in the general condition has been shown to take place without any improvement in the level of the blood phosphorus.

SUMMARY AND CONCLUSIONS

The calcium content of the blood in 31 normal children of ages ranging from 2 months to 11 years was found to vary between 6.4 and 9.5 mg. per 100 cc. (p. 12)

A very slight diminution in the blood calcium which corresponds with advance in age can be demonstrated during this period of life. (pp. 12 + 16)

The corpuscles were found to contain an appreciable quantity of calcium. (p. 22)

Varying the calcium intake over short periods in 2 normal infants, and over much longer periods in dogs, failed to produce any demonstrable variation in the blood calcium. (pp 25 & 28)

In 45 cases of rickets ~~of varying severity~~ the blood calcium content was found to vary between 6.0 and 9.9 mg. per 100 cc. The variation is therefore slightly greater than in the case of healthy children though most of the figures are within the normal range. (pp. 33 + 36)

There appeared to be no correspondence between either the stage of the disease (p.39) or the severity of the rachitic changes and the level of the blood CaO. (p.37)

In the case of the blood phosphorus the variation in 49 normal infants between 3 months and 13 years was found to be 3.7 to 5.8 mg. per 100 cc. (p.44)

No diminution corresponding with age during this period of life could be demonstrated. (p.47)

Serum and whole blood give exactly the same results provided the former is used at once and the latter as soon as it can be separated. (pp. 53 & 54, see also preliminary communication on Inorganic P. enclosed)

Neither the nature of the diet nor the recent ingestion of phosphorus rich food appeared to be responsible for the variations occurring in normal infants. (pp. 47 & 48, also enclosed paper p 47)

Hess's results indicating that a seasonal variation of the blood phosphorus occurs in healthy infants were not confirmed by the present series of cases. (p. 51)

In the 29 cases of rickets in whom blood examinations were carried out the blood phosphorus was for the most part

(p.56)

found to be lower than normal~~ly~~. Several children, in whom I found undoubted signs of active rickets, were, however, found to have a normal blood phosphorus content. (p.56) These cases were not of the calcium low type described in the recent American literature since careful estimation failed to reveal any diminution in the CaO. (p.57)

Definite signs of healing have not so far been accompanied by a corresponding rise in the level of the blood phosphorus, and in no case has a low inorganic P. content given an indication of the presence of rickets before other definite signs of the disease had been recognised. (pp.58, 59 & 60)

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