A CONTRIBUTION TO THE METABOLISM OF CREATINE.

Thesis for the Degree of M.D.

by

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FORENOTE. The research whereof records are contained in this Thesis was conducted in the Physiology Department of the University while the Candidate was working as Barbour Scholar under the direction of Professor Noël Paton.

The majority of the results contained in paragraph A of Section I. have been published in a joint paper (Cathcart & Orr Journal of Physiology May 1914.) Dr Cathcart however is not responsible for any of the work of which use is here made nor for the deductions which are here drawn from that work.

The Author acknowledges the assistance of Professor Noël Paton to whom progress was periodically reported, and of Dr.Cathcart from whom advice was frequently obtained. While the actual work and the deductions drawn from that work are due to the Candidate, without the assistance hereby acknowledged, the carrying out of the research would have been impossible .

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INTRODUCTION.

The Chemistry of Creatine and Creatinine are fully dealt with in Neubauer's Textbook, and more or less complete résumés of the work done on the Metabolism of these bodies have been given by several authors, e.g. Myers (1910), Riesser (1913). For the purposes of this thesis therefore, the briefest introductory statement will suffice.

The first work on the subject was done by Liebig, who in 1847 discovered Creatinine as a normal constituent of urine. Creatine had previously been isolated from muscle. As it was found that Creatine was easily convertible into Creatinine it was naturally concluded that Creatine was a catabolite of muscular activity and that Creatinine was its urinary representative.

Many of the earlier workers made no attempt to obtain separate estimations of these two bodies in their experiments, which under the old Neubauer gravimetric method of quantitative analysis, was a matter of considerable difficulty. In 1889 Mallet published figures which indicated that ingested Creatine was excreted quantitatively as Creatinine and appeared to confirm the view that Creatine was a mere waste product of muscular

muscular metabolism which was excreted in the urine as Creatinine. Under these circumstances it was natural that the older workers should occupy themselves chiefly with the question as to whether or not the Creatine content of muscle and the excretion of Creatinine in the urine were increased by work. Acutely divergent results were obtained by different workers to some of whom reference will be made later.

All the workers used the Naubauer gravimetric method of quantitative analysis, or Salkowski's, or some other modification of that method. In the light of the more modern method, the figures stated in these papers show that the error of estimation was in many cases fully 50%, so that the records are of doubtful value.

Among these workers Meissner is of special interest, inasmuch as he gives in rather vague outline results which have recently been confirmed. He experimented extensively on dogs, rabbits, and fowls. The details of his experiments are meagre in the extreme; the figures he states show his error of estimation to have been gross, and the toxic effects of Creatine described by him show that he was working with impure substances. In spite of these obvious fallacies his conclusions are remarkable in their accuracy. He found an excretion of Creatine on starvation and ascribed it to Catabolism of Muscle

Muscle Flesh. He recognised a double source of urinary nitrogen, and showed that the portion due to food is excreted within a few hours, and that a record of the tissue metabolism can be obtained by withholding food. Folin has recently emphasised the difference and coined the terms "endogenous" and "exogenous" for the two processes of metabolism. He has insisted on a more rigid demarcation however than the facts warrant. Urea, which he has taken as typical of "exogenous" metabolism has been shown by Noël Paton to be both "exogenous" and "endogenous", and Creatinine, which he held as the representative of the "endogenous" type will be shown later to be an end product of both tissue and intermediate metabolism, which is in accordance with Meissner's view. The publication of Folin's paper on the subject has caused Meissner's observation as to the effect of recently ingested food on the quantitative composition of the nitrogenous excretory products of the urine to be universally accepted.

In 1904 Folin described a colorimetric method for the estimation of Creatine and Creatinine. The comparative accuracy and rapidity of this method afforded new facilities for research, and following upon its introduction a large amount of work has been done by many investigators. The results of that work, much of which is discussed later, show that the question as to the significance of Creatin in the economy

economy is much more complex than was originally supposed and that definite knowledge as to the origin, function, and fate of Creatinine and its connection with Creatinine would throw much needed light on several problems of metabolism that await solution.

PRESENT RESEARCH.

PART I. ON THE USE OF THE FOLIN METHOD.

A. EFFECTS of PRESENCE of REDUCING BODIES in URINE:

The Folin Method is based on the Jaffe reaction which is the production of a red colour on the addition of Creatinine to a solution of Sodium Picramate got by the addition of Picric Acid to Sodium Hydrate. The colour is due to the dissociation of the Picramate with the liberation of a red coloured ion. (Thomson). The reaction is dependent upon the reducing power of Creatinine. (Chapman). It is of first importance therefore to ascertain to what extent any other reducing agents likely to be present in urine affect the colour saturation. Dextrose, Beta-oxybutyric Acid, Acetone, and Diacetic Acid, are the chief bodies concerned. The effects of Dextrose were investigated by Van Hoogenhuyze and Verploegh, (1905), Mellanby, (1908), Taylor, (1911), Greenwald, (1913), and Thomson, Wallace and Clotworthy, (1913), all of whom are agreed

N.B. The Folin Method depends on the fact that the colour saturation is in direct proportion to the amount of Creatinine present in the solution. To the fluid to be examined Sodium Hydrate and Picric Acid are added in fixed amounts and after a certain time allowed for the full development of the colour the fluid is diluted generally to 500 c.c.,and the colour saturation is read off against the colour of a standard solution of Potassium Bichromate by means of a colorimeter. The amount of Creatinine present varies inversely as the height of the column of fluid required to match the standard solution.

agreed that the presence of Dextrose in reasonable amounts, e.g to 5%., has no influence on the results.

Krause (1910) and Graham and Poulton (1914) found that Oxybutyric Acid had no adverse effects. With regard to Acetone and Diacetic Acid the published results are contradictory. Klercker (1907) found that Acetone caused a rapid fading of the colour. Van Hoogenhuyze and Verploegh (1908) found that the colour saturation was at first increased but the increased colour rapidly faded and later, correct readings were obtained, a result with which Krause (l.c.) agrees. Rose (1912) states that Acetone in all concentrations is without influence on the Creatinine readings. Greenwald (l.c.1913) found that the Acetone diminished the colour intensity, and Graham and Poulton (l.c.1913), found no effect if less than .2% were present.

AUTHOR'S EXPERIMENTS.

Normal human urine was taken, and to it Acetone was added in increasing amounts, and readings taken. Six minutes were allowed for the development of the colour. Dilution was to 500 c.c. It was found that in concentration of less than .3% no appreciable effects could be detected in the readings, but that, when the concentration of the Acetone surpassed

surpassed that figure, there resulted bleaching of the colour which became rapidly accentuated as the concentration increased up to 3.3%. TABLE I. summarises the results.

TABLE I. The Influence of Acetone on Readings.

Concentration of Acetone.	Nil.	.3%	.6%	1.6%	3.3%
Readings.	6.2	6.2	6.4	6.6	7.21

DIACETIC ACID: Krause (l.c.1910), Wolf and Osterberg, 1911, and Rose (l.c.1912), found the disturbing effects of Diacetic Acid to be negligible if in moderate amount, e.g. to .25%. Rose found that in stronger solutions the effect is to deepen the colour and increase the apparent amount of Creatinine. The results of the investigations of Greenwald (l.c.1913), and Graham and Poulton (l.c.) showed that the colour saturation is diminished, causing a decrease in the apparent amount of Creatinine present to a degree that vitiates the results obtained in urines containing Diacetic Acid.

In view of these diverse findings it was considered desirable to investigate the subject anew.

AUTHOR'S EXPERIMENTS:

Diacetic Acid was prepared, and its strength estimated by the Messenger Huppert Method[x]. To urine (human) was added

[x]The preparation of the Diacetic Acid was done by Dr.Cathcart, who also controlled in detail the estimation of the strength of the acid and its freedom from Acetone: a matter of first importance, as it is exceedingly difficult to obtain and preserve a pure preparation of Diacetic Acid on account of its instability.

varying amounts of the acid, and readings taken. Six minutes were allowed for development of the colour. Dilution was to 500 c.c.

The results, which are tabulated below, show that in even comparatively small quantities the presence of this body prevents the full development of the red colour, causing thereby the amount of Creatinine as found to be less than the amounts really present.

TABLE II. The Influence of Diacetic Acid on Readings.

Percentage of Diacetic Acid in Urine.	Nil.	.275%	.6875%	1.375%	2.75%	5.5%
Readings.	6.86	7.01	7.17	7.34	7.78	8.97
Amount of Creatin-ine found,	.118	.116	.1139	.110	.104	.090

Other series in which the readings were made by another worker who was in ignorance as to the nature of the solutions being read gave substantially the same results.

It was next determined to what extent the readings in urine containing the Acid were affected by the time allowed for the development of the colour. In normal urine the colour continues to develop for about 6 minutes and then remains unaltered up till 10 minutes, as the following Table shows.

TABLE

TABLE III. Showing effect on reading of various lengths of time allowed for the development of colour.

Length of time, in Minutes, allowed for the development of the colour	1	2	3	4	5	6	7	8	9	10
Readings.	13.5	10.7	10.2	9.9	9.7	9.6	9.6	9.6	9.6	9.6

To Urine was added .5 c.c. of 25% (approx.) Diacetic Acid, and the colour allowed to develop for varying periods. Table IV. gives the results. It is seen that at no point of time is the true maximum colour reached.

TABLE IV. Showing the effect on readings of varying lengths of time allowed for development of colour in Urine with diacetic Acid.

A. Intervals of 3 Minutes.

Time allowed.	1 min.	3 mins.	6 mins.	9 mins.	11½ mins.	True reading
Readings.	8.3	7	7.3	7.8	8	6.05

B. Intervals of 2 Minutes.

Time allowed.	2 mins.	4 mins.	6 mins.	8 mins.	13 mins.
Readings.	8.4	7.3	7.7	7.93	8.0	6.2

C. Intervals of 1 Minute.

Time allowed.	1 min.	3 mins.	4 mins.	5 mins.	7 mins.
Readings.	11.4	8.8	8.6	9.0	9.2	7.1

It appears therefore that the maximum colour is developed in 4 minutes, after which fading occurs. Evidently two factors are present, viz. the production of the red coloured ion by the Creatinine and the further dissolution of that ion

ion by the acid. The former is most active and the colour develops up till 4 minutes, whereafter the destructive factor prevails, and the colour fades.

Even in the absence of the Diacetic Acid the red colour produced by the Creatinine tends to fade. Thus, in the same urine if the colour be allowed to develop for A. 6 minutes, B. 45 minutes, C. 90 minutes the respective readings are A. 9.6, B. 9.8, C. 10.9. It is probable that the reducing action of the Creatinine continues, so that the Picramate suffers further reduction to a colourless solution. Chapman (l.c.) has suggested that the reduction of the Picramate is to Amino-di-nitro-phenol and then to di-amino-nitro-phenol and if carried further which can be done by excess of Creatinine, to tri-amino-phenol, a colourless substance. The Diacetic Acid may itself perform this final reduction, or it may act as a catalytic agent activating a natural tendency of Creatinine to the final reduction of the Picramate to that colourless substance.

A.3. On the Method of getting rid of the Diacetic Acid.

Graham and Poulton (l.c.) have suggested that the Diacetic Acid may be got rid of by aspirating air for 40 minutes at reduced pressure through a 10 c.c. sample of urine to which 1 c.c. of 10% Phosphoric Acid has been added, the temperature

temperature being maintained at 65 to 70 degrees C. The accuracy of this method was investigated. Readings were taken of normal urine.

10 c.c. samples of the same urine were taken, to some of which no acid, to others .25 c.c. of 25% (approx.) Diacetic Acid was added, and treated as described. In both cases there resulted a destruction of Creatinine which was most marked in the sample containing the acid. The driving off of the acid was complete as was shown by the set of controls. Table V. gives the results.

TABLE V. Showing the effect on readings of driving off Diacetic Acid.

Reading obtained before treatment.	Reading obtained in urine free from acid treated as described.	Reading obtained from urine to which Acid was added and then driven off.
6.62	6.75	(a) 6.81 (b) 6.95
6.65	6.6	(a) 6.75 (b) 6.75

Further tests of the accuracy of the method were carried out on a series of urines from an experiment in which very small amounts of Diacetic Acid were obtained. Table VI. shows the same tendency to destruction of Creatinine.

TABLE

TABLE VI. Showing effect on readings of driving off Diacetic Acid.

Percentage of Acid present.	.001	.001	.004	.004	.003
Reading before driv-ing off Acid.	6.48	6.57	7.17	6.52	6.50
Reading after driv-ing off Acid.	6.8	6.88	7.59	6.82	6.85

The destruction is probably due in part to the heating under reduced pressure. Van Hoogenhuyze (l.c.) Dorner and Gino Frontali all observed a slight destruction of Creatinine on heating with acid in the process of converting the Creatine to Creatinine, an observation which has been amply confirmed in the experiments of the author. The choice of acid may be unfortunate, as Thomson, Wallace and Clotworthy found on heating with Phosphoric Acid instead of Hydrochloric Acid in converting Creatine to Creatinine that a very substantial destruction of Creatinine resulted if acid of strength over 3% was used. This method therefore, while successful in disposing the the Diacetic Acid, is of doubtful utility.

The Effects of Diacetic Acid on the Estimation of Creatine

The presence of this acid as shown by the above experiments diminishes the colour reaction and causes the apparent amount of Creatinine to be less than the real amount. Creatine is estimated indirectly by first finding the amount of Creatinine present, then converting the Creatine to Creatinine by heating with acid. The total Creatine plus Creatinine

Creatinine is then estimated, and the difference in the two results is taken to represent Creatine. If however, Diacetic Acid be present, the first result will be depressed by the acid which is dissipated in the process of heating, and the second result, freed from the disturbing factor will show an increase independent of the presence of Creatine, an increase which has been interpreted as indicating the amount of Creatine. The effects of this in certain results obtained in cases where Diacetic Acid is present, viz. diabetes and inanition, will be discussed later.

At the present time, the only method of proving the presence of small amounts of Creatine in urines containing Acetone bodies is by the direct Diacetyl Method described by Walpole (1911), a method which is used in the later experiments herein recorded.

B. The Effect on Readings of time allowed for development of Colour.

Folin allowed 5 minutes for the development of the colour before dilution, a period which has been adopted by most workers. Dorner (l.c.) found no difference between 5 and 15 minutes. Mellanby (l.c.) gave 3 to 9 minutes, Benedict and Myers gave 3 to 5 minutes, Mendel and Rose 10 minutes. Gino

Gino Frontali (l.c.) found a diminution in the colour if time allowed for development exceeded 10 minutes., Chapman (l.c.) states that the colour up to a certain time deepens and then fades. Thomson, Wallace, and Clotworthy (l.c.) gave 7 minutes as the optimum time. These last workers give specific results in support of their statements - readings at 5 minutes and at 7 minutes are given.

In view of these conflicting statements and the absence of experimental data in their support, it was considered desirable to obtain definite results based on recorded experiments.

AUTHOR'S EXPERIMENTS.

Using the same urine the length of time allowed for the development of the colour was varied from 1 to 90 minutes. Dilution was made to 500 c.c. Each reading recorded is the average of 10 readings. Table VII. shows the results.

TABLE VII. Showing the effect on readings of various times allowed for development of colour.

Time, in Minutes, allowed for development of colour.	1	2	3	4	5	6	7	8	9
Readings A.	13.5	10.7	10.15	9.91	9.7	9.61	9.63	9.58	9.57
Readings B.	7.45	5.78	5.02	5.0	4.7	4.62	4.66	-	-

Time, in Minutes, allowed for development of colour.	10	12½	15	30	45	90
Readings A.	9.65	9.65	9.7	9.62	9.81	10.9
Readings B.	4.52	4.65	4.65	-	-	-

The following abridged table gives the figures obtained in a series of 10 urines in which the readings were taken after the colour had been allowed to develop for 5, 6, and 10 minutes.

TABLE VIII. Showing variation in readings obtained by allowing colour to develop for periods of 5, 6, and 10 minutes.

Time allowed for development of Colour.	5 minutes.	6 minutes.	10 minutes.
Urine No.1	4.70	4.62	4.55
do. 2.	5.57	5.45	5.44
do. 3.	6.04	6.00	6.01
do. 4.	6.20	6.02	6.01
do. 5.	6.50	6.30	6.33
do. 6.	6.70	6.35	6.35
do. 7.	6.80	6.50	6.45
do. 8.	8.42	8.30	8.31
do. 9.	9.70	9.61	9.65
do. 10.	10.33	10.19	10.14

The average reading for 5 minutes is 7.10, for 6 minutes 6.93, and for 10 minutes 6.92. At 5 minutes, the period stated by Folin and adopted by most workers, the colour is not fully developed, the development at that period varies with the temperature. It was found that on hot days the reading at

at 5 minutes most nearly approximated the readings at 6 minutes, and on cold days the difference was accentuated. On no occasion during the period of investigation in which there occurred considerable variation of temperature, could any difference be found between the readings at 6 minutes and those at 10 minutes.

It may be stated therefore that for ordinary room temperature the time allowed for development of colour before dilution may be varied from 6 to 10 minutes without affecting the results.

C. ON THE INCONSTANCY OF THE COLOUR AFTER DILUTION.

No data could be obtained as to the constancy or inconstancy of the colour after dilution. The subject was investigated as follows. A urine was taken and the time allowed for the development of the colour varied from 1 to 10 minutes. It was them diluted to 500 c.c. and readings taken at intervals of 0, 15, 30, 45, and 60 minutes. The results are recorded below.

TABLE IX.

TABLE IX.

Effect of Standing after Dilution.

Interval allowed between dilution & reading.	Readings. Length of time colour allowed to develop (in Minutes).										
	1	2	3	4	5	6	7	8	9	10	
0 mins.	13.5	10.7	10.2	9.9	9.6	9.6	9.6	9.6	9.6	9.7	
15 mins.	13.5	10.7	10.3	9.8	9.7	9.7	9.6	9.6	9.6	9.8	
30 mins.	13.5	10.7	10.8	10.1	9.9	9.9	9.9	9.8	9.8	9.9	
45 mins.	13.5	10.9	11.0	10.3	10.0	10.2	9.9	10.1	9.9	10.0	
60 mins.	13.5	10.9	11.0	10.5	10.2	10.4	10.1	10.2	10.0	10.1	

In all cases, where full development of the colour had been reached, there is a progressive fading.

It will be observed that in those cases where dilution occurred before the maximum colour was attained, the effect of the dilution is to prevent almost, if not quite entirely, further development of the colour. This observation is of interest inasmuch as it points to the concentration of the Creatinine being a factor in determining the production of the colour.

D. THE ERROR PRODUCED BY FAILURE TO MAINTAIN A FIXED STANDARD OF DILUTION IN A SERIES OF ANALYSIS.

Folin directs that in dilute urines 20. c.c. and in concentrated

concentrated urines, 5 c.c. may be taken instead of 20 c.c. If the above observation be correct, this procedure must give erroneous results. The following experiments demonstrate the error.

10 c.c. of urine were taken and readings obtained. 10 c.c. of the same urine plus 10 c.c. of water were treated in exactly the same method: another set of readings was taken for 10 c.c. of urine plus 30 c.c. of water. In each, the amount of Creatinine is identical. The results tabulated below show that in the diluted urine a less amount of Creatinine is found.

TABLE X. Showing effect of varying the quantity of urine taken - the Creatinine in each case being identica

	Readings.			
10 c.c. urine.	8.0	6.58	10.26	8.3
do. and 10 c.c. Water.	8.2	6.9	10.8	8.52
do. and 30 c.c. Water.	10.36	8.5	12.9	10.5

Folin alsc directs that in cases where the amount of Creatinine is small and high readings are expected, dilution may be made to 250 c.c. instead of to 500 c.c. Readings were taken in a urine (10 c.c. being taken) in which dilution was carried to 500 c.c. The same urine was diluted with equal parts

parts of water. The conditions were maintained otherwise identical except that in this case dilution was carried only to 250 c.c. Theoretically and in accordance with Folin's direction similar readings should be obtained. Here also in the diluted urine was found higher reading indicating a less total amount of Creatinine.

TABLE XI. Showing variation in readings given by the same quantity of Creatinine in different dilutions.

Urine.	Readings.			
Whole urine, dilution to 500 c.c.	6.30	7.49	7.90	10.36
Equal parts of urine and water, dilution to 250 c.c.	6.38	7.50	8.08	10.48

A further double series of readings were taken in which the urine was not diluted. 10 c.c. were taken in each case. In one series dilution was carried to 500 c.c., and in the other to 250 c.c. Urines were chosen so that the readings in both instances would fall between 5 and 12 m.m., the limits of accuracy defined by Folin. In the case of dilution to 250 c.c. the reading should be exactly half that found on dilution to 500 c.c. In each case the reading on dilution to 250 c.c. was more than half, indicating again a less amount of Total Creatinine.

TABLE XII.

TABLE XII. Showing relative value of reading at dilution to 500 c.c. and 250 c.c.

Urine	Readings.		
10 c.c. whole urine diluted to 500 c.c.	10.33	10.19	10.14
10 c.c. whole urine diluted to 250 c.c.	5.57	5.43	5.44

Tests identical with those recorded in the above table were carried on on an extensive scale by Thomson and Wallace, and Clotworthy (l.c.) with whose findings these are in entire accord.

In experimental work on metabolism or nutrition the output of urine on successive days varies in quantity within very wide limits. Folin's recommendation to alter the quantity of urine taken for analysis or to alter the dilution has been shown to be productive of error.

The above somewhat laborious investigation has been undertaken because as will be shown later, it affords an explanation of somewhat anomalous results which have been published.

The only method which secures accuracy is to fix at the beginning of the experiment, a quantity which is not likely to be exceeded on any day and to dilute each day's urine to that fixed standard. Then the same amount, viz. 10 c.c., should be taken. The time for development of the colour

colour should be fixed at a point within the limits of 6 and 10 minutes, and dilution carried to the same extent preferably 500 c.c. In every series of experiments the value of the results is decreased to the extent that the conditions of analysis are not accurately defined and rigidly adhered to.

In view of the large amount of work being done on the Metabolism of Creatine and creatinine by various workers who, as shown above, adopt varying conditions, it has become urgent that definite conditions of analysis should be universally adopted and adhered to.

PART II.

METABOLISM of CREATINE.

FORENOTE:

In dealing with the experimental work that finds place in this part, at the close of each series of experiments the conclusions arrived at, as proved by the results, are merely stated: the bearing of these conclusions on the general problem of the metabolism of Creatine is reserved for discussion until all the experimental data has been recorded.

To avoid obscuring the main issue, which is the determination of the function of Creatine in the economy, all results which have not a direct bearing upon the metabolism of Creatine are ignored in this thesis.

SECTION I.

THE EFFECTS OF PRIVATION OF CARBOHYDRATES ON THE EXCRETION OF CREATINE.

A. PRIVATION OF CARBOHYDRATES BY INJECTIONS OF SODIUM SELENITE.

It has been known for a considerable time that Selenium salts exert a poisonous influence on all forms of life, but the ultimate cause of the poisonous effect has been

been a matter of speculation.

Several workers, in histological examination of the tissues of animals poisoned by these salts have observed a dark brown deposit within the cells which Schleurlen found to be reduced Selenium.

In 1909, Charles O.Jones undertook an exhaustive investigation of the physiological effects of Selenium compounds. He injected Sodium Selenite hypodermically, and after the death of the animal extracted from the tissues the granular deposit obeserved by previous workers. This deposit he showed to be identical in every respect with the amorphous form of Selenium got on reducing Sodium Selenite in the test tube.

Investing the cause of the reduction, he found that it took place if a solution of Sodium Selenite were allowed to act upon animal tissue in which enzyme action and all metabolic processes had been destroyed by heat, and concluded therefore, that the reduction was a purely chemical action.

He next sought the constituent of the tissue that affected the reduction, and, as a result of extensive observation, came to the conclusion that, of substabces of animal origin, reduction could only be performed by glucose and a few allied sugars or bodies yielding these, and could not be produced by organic matter from which these are absent.

To

To prove this he treated a portion of some minced liver with yeast to remove the carbohydrates, and then killed the yeast by heat. A second portion was subjected to exactly the same treatment, except that no yeast was added. The second portion, which retained its carbohydrates, reduced the Selenite, while the first portion, deprived of its carbohydrates, was unable to perform the reduction.

To show that in the body the reduction is effected by carbohydrates, he administered hypodermically several sublethal doses of selenite of Sodium to an animal which was then killed and its liver examined. No glycogen was found. In a control animal the glycogen was abundant. The glycogen in the injected animal had evidently been drawn upon to supply glucose for the reduction of the Selenite.

As a result of these experiments Jones concluded from apparently quite ample and well founded data, that Sodium Selenite injected hypodermically is carried by the blood stream to the tissues and there reduced to Selenium by glucose.

Assuming the validity of Jones' work, the injection of Sodium Selenite which utilises the glucose of the body for its reduction, affords a means of rapidly withdrawing carbohydrates from the metabolic processes in the tissues and of studying the effects on metabolism of the resulting carbohydrate

carbohydrate privation.

AUTHOR'S EXPERIMENTS.

Some preliminary experiments were conducted upon rabbits to obtain practical knowledge as to the effects of the poison and to ascertain in a general way, whether the previous carbohydrate feeding would modify the poisonous influence of the drug.

EXPERIMENTS OF RABBITS: A moderately well nourished rabbit weighing about 2 kilos. was fed for several days on carrot which is rich in carbohydrates. At 11 a.m. 2.5 mgrams. of Sodium Selenite was injected subcutaneously. During the day of the injection it appeared somewhat lethargic and ate only 11.4 grams of carrot. Next day it appeared normal and ate 60 grams of carrot. On the third day it ate 75 grams., and at 5 p.m. had 2.5 mgrams. injected as before. At 8 a.m. next morning it was found dead.

A lean rabbit which had previously received a mixed diet of bran, corn, and carrots, was fasted for 36 hours so that its store of carbohydrates might be somewhat depleted. At 5 p.m. it received an injection of 1 mgrm. of the salt. Next morning at 8 a.m. it was found dead.

An exceedingly well nourished rabbit weighing 1.9 kilos. was taken. It had received a mixed diet up to the time of

of the injection, when food was withdrawn. The amounts injected were .5 mgrms. 16 hours later, 2 mgrm., 5 hours later, 10 mgrm., 3 hours later, 12.5 mgrm. Half an hour after the last injection the animal appeared moribund and was killed. The liver was immediately extracted and examined for glycogen. Only a trace was found. Post mortem examination in these three cases showed no macroscopical change in any of the organs.

Histological examination of the liver revealed an amorphous dark coloured deposit within the cells, corresponding to that found by Jones, and proved by him, by chemical examination to be identical with the amorphous form of selenium, got on reducing selenite in the test tube.

From these, it would appear that the lethal dose is determined not so much by the weight of the animal, as by the amount of carbohydrates available for the reduction of the noxious Selenite to the inert Selenium. Thus, the lean fasted animal is killed by 1 mgrm., while the average nourished animal fed on carrot required a second injection of 2.5 mgrms. In the metabolic experiments that follow it will be seen that the disturbance of metabolism caused by the Selenite is inversely proportional to the amount of carbohydrates previously fed.

COLLOIDAL

COLLOIDAL SELENIUM: To ascertain whether the Selenium "per se" exercised any poisonous effect, a subcutaneous injection of 20 c.c. of a solution of colloidal selenium was given to a dog, the animal having been for several days previously on a fixed diet, and having reached a condition of nitrogenous equilibrium.

No harmful effects could be detected. The urinary analysis showed no evidence of an altered metabolism.

TABLE XIII.

Shewing effects of Colloidal Selenium.

Amount of Urine. c.c.	Total Nitrogen. grms.	Ammonia. grms.	Creatinine. grms.	Creatine. grms.	Remarks.
800	2.68	.152	.381	.029	
490	2.60	.155	.370	.027	
387	2.72	.157	.370	.048	
437	2.52	.193	.393	. 042	Injection of 20 c.c. colloidal Selenium.
300	2.53	.173	.390	.042	
448	2.58	.202	.393	.039	

From these preliminary experiments it may reasonably be concluded that Sodium Selenite injected into the body utilises the glucose of the tissues for its reduction and thereby

thereby withdraws the carbohydrates from the metabolic field. The further experiments therefore constitute a research into the effects on metabolism of the withdrawal of carbohydrates from the metabolic processes.

METHODS of EXPERIMENTS: A bitch of 12 kilos. in weight was placed in a cage so that the urine could be collected. At 10 a.m. each day the bladder was emptied by catheter so that the 24 hours' collection of urine was from 10 a.m. one day till 10 a.m. the following day. The urine of the preceding 24 hours was examined each day as follows:- The Total Nitrogen was estimated by Kjeldahl's Method: Creatinine by the Folin Colorimetric Method using a Duboscq Colorimeter. 10 c.c. of urine was used for analysis; 6 minutes was allowed for development of colour, and dilution was to 250 c.c. The Creatin was converted to Creatinine by heating 10 c.c. of urine with 7 c.c. of 1/10 HCl on the steam bath for four hours. The Total Creatinine thus obtained, less the Creatinine estimated as above, was reckoned as Creatine.

Throughout each individual experiment the dog was kept upon a fixed diet and fed at 12.30 p.m. each day. After the diet had been given for from 5 to 7 days until nitrogenous equilibrium had been reached and maintained for 2 or 3 days, a sublethal

sublethal does, viz. 2 c.c. of a 1% solution of Sodium Selenite was injected subcutaneously into the flank of the animal which was kept under observation on the day of the injection and notes made of its condition. Thereafter the fixed diet was continued for 5 or 6 days until examination of the urine showed that the various nitrogenous constituents of the urine were being excreted in nearly the same amounts as during the period of nitrogenous equilibrium preceding the injection.

Throughout the series of experiments the protein content of the food was little altered but the amount of carbohydrates was varied from 33 to 204 grams. per diem, while the fat content was varied inversely to maintain the caloric value a constant.

RECORDS.

EXPERIMENT I. December 1912.

DIET:

Oatmeal.	40 grams.		
Dried skimmed Milk.	30 grams.	Protein	28 grams.
Margarine.	40 grams.	Fat.	44 grams.
Tapioca.	80 grams.	Carbohydrates.	161 grams.
NaCl.	2 grams.		
Water ad lib.			

SYMPTOMS PRODUCED: Twenty minutes after the injection the animal vomited and continued to do so at intervals for about an

an hour and a half. The vomited matter consisted of saliva and mucous. Beyond this temporary sickness no ill effects were recorded. Next day, only one eighth of the diet was eaten. On the two succeeding days one half of the diet was taken, and thereafter the full specified diet was eaten each day.

URINARY ANALYSIS:

TABLE XIV. Showing excretion of Creatine on privation of Carbohydrates produced by Sodium Selenite.

Day.	Urine. c.c.	Specific Gravity. grams.	Total Nitrogen. grams.	Creatinine. grams.	Creatinine. grams.	Remarks
3.	970	1008	2.63	.362	.032	Full diet
4.	1099	1007	2.71	.367	.012	"
5.	1100	1007	2.67	.356		"
7.		Urine lost				Injection: no food.
8.	240	1020	4.02	.379	.153	One-eighth diet.
9.	349	1013	2.93	.315	.148	half diet.
10.	560	1007	2.57	.334	.045	"
11.	460	1009	2.12	.343	-	full diet.
12.	700	1007	1.94	.357	-	"

EXPERIMENT

EXPERIMENT II. January, 1913.

DIET:

Oatmeal	70 grams.		
Margarine.	80 grams.	Protein.	25 grams.
Dried skimmed milk.	30 grams.	Carbohydrates	64 grams.
NaCl.	2 grams.	Fat.	74 grams.
Water ad lib.			

As the animal had refused all food on the day of the injection in Experiment I. it was on this occasion fasted for a day after nitrogenous equilibrium had been attained so that the effects of the lack of food might be determined and considered in estimating the effect of the injection. It was fasted on the sixth day and injected on the twelfth after the commencement of the fiet.

SYMPTOMS PRODUCED: Saliva was observed to be dripping from the corners of the mouth a few minutes after the injection. The animal soon appeared limp and listless. In 20 minutes it vomited. As before, the vomit consisted of mucous and saliva. It appeared sick and lethargic all day and several times vomited small mouthfuls. Next morning it was found that it had vomited during the night. On the following day it continued listless and refused its food. On the third day the lethargy was largely passed off, and with coaxing, it managed to eat the full prescribed diet. On the fourth day it was quite normal.

URINARY

URINARY ANALYSIS:

TABLE XV. Showing excretion of Creatine on privation of Carbohydrates produced by Sodium Selenite.

Day.	Urine. c.c.	Specific Gravity.	Total Nitrogen. grams.	Creatinine. grams.	Creatine. grams.	Remarks.
1.	540	1013	2.89	.308	-	full diet.
2.	340	1016	2.60	.318	.006	"
3.	602	1015	2.98	.386	.028	"
4.	518	1017	2.54	.346	.025	"
5.	390	1018	2.24	.283	.061	"
6.	144	1028	2.10	.368	.044	fast.
7.	520	1015	2.55	.360	.053	full diet.
8.	525	1013	2.55	.343	.054	"
9.	420	1018	2.24	.291	.044	"
10.	340	1038	4.27	.369	.157	Injection, no food.
11.	687	1011	5.36	.302	.282	no food.
12.	430	1020	7.06	.352	.358	very little food.
13.	200	1027	3.37	.296	.036	full diet.
14.	330	1019	2.32	.290	.016	"

EXPERIMENT III. February, 1913.

DIET:

Oatmeal.	100 grams.		
Tapioca.	80 grams.	Protein	25.9 grams.
Cane Sugar.	40 grams	Carbohydrates.	189 grams.
Dried skimmed Milk	30 grams.	Fat.	27 grams.
Margarine.	20 grams.		
NaCl.	2 grams.		

Water ad lib.

The animal was fasted on the 5th day after the commencement of the diet and injected on the 8th.

SYMPTOMS PRODUCED: Twenty minutes after the injection the dog vomited mucous and saliva as in Experiments I and II, but the vomiting was less profuse. After two hours there was no further vomiting. It was little upset and appeared quite active and lively. No food was offered on the day of the injection. Next day the animal was quite normal but only ate a small portion of its food. On the following day the full diet was taken.

URINARY ANALYSIS:

TABLE XVI. Showing excretion of Creatine on privation of Carbohydrates produced by Sodium Selenite.

Day.	Urine. c.c.	Specific Gravity. grms.	Total Nitrogen. grms.	Creatinine. grms.	Creatine.	Remarks.
1.	546	1015	3.33	.359	.029	full diet.
2.	650	1012	2.63	.321	.029	"
3.	930	1009	2.73	.311	.027	"
4.	985	1008.5	2.76	.310	.027	"
5.	210	1020	2.09	.288	.041	fast.
6.	840	1008	2.45	.303	.015	full diet
7.	690	1009	2.08	.319	.011	"
8.	760	1008	3.50	.339	.052	injection, no food
9.	420	1014	4.65	.329	.121	part of food
10.	1000	1009	3.67	.305	.011	full diet
11.	1000		2.86	.289	.005	"

EXPERIMENT

EXPERIMENT IV. March, 1913.

DIET:

Oatmeal.	30 grams.		
Tapioca.	100 grams.	Protein.	23.00 grams.
Cane Sugar.	80 grams.	Carbohydrates.	204.3 grams.
Dried skimmed Milk	50 grams.	Fat.	12 grams.
Margarine.	10 grams.		
NaCl.	2 grams.		
Water ad lib.			

SYMPTOMS PRODUCED: Salivation was profuse, but there was no vomiting. The dog appeared less lively than usual, but was otherwise quite unaffected by the injection. No food was offered on the day of the injection. Next day it appeared quite normal except that it only ate about one-third of the prescribed diet. On the following day it ate nearly the full amount, and thereafter full diet was taken.

URINARY ANALYSIS:

TABLE XVII. Showing excretion of Creatine on privation of Carbohydrates by Sodium Selenite.

Day.	Urine, c.c.	Specific Gravity.	Total Nitrogen. grams.	Creatinine. grams.	Creatine. grams.	Remarks.
1.	698	1009	2.84	.343	.026	full diet.
2.	690	1009	2.71	.341	.004	"
3.	695	1009	2.87	.359	.016	"
4.	640	1010	2.81	.354	.012	"
5.	780	1009	3.04	.352	.011	"
6.	320	1021	2.44	.395	.055	injection
7.	210	1027	4.47	.364	.035	One third food.
8.	350	1017	3.50	.373	.032	full diet.
9.	630	1012	3.22	.357	-	"

EXPERIMENT

EXPERIMENT V. May, 1913.

DIET:

Oatmeal.	30 grams.		
Dried skimmed milk.	30 grams.	Proteins	25.6 grams.
Casein.	9 grams.	Carbohydrates.	35.6 grams.
Margarine.	100 grams.	Fat	85 grams.
NaCl.	2 grams.		
Water ad lib.			

SYMPTOMS PRODUCED: Within 5 minutes profuse salivation was noticed. Vomiting then set in and consisted of saliva, mucous, and intestinal contents. The dog continued sick looking all day and inclined to lie or sit in a corner of its cage with head hanging limp. The sight and smell of its food seemed to produce nausea. Next day it appeared limp and listless and refused food. On the succeeding day it had much improved and was induced to eat all its food.

URINARY ANALYSIS:

TABLE XVIII. Showing excretion of Creatine on privation of Carbohydrates produced by Sodium Selenite.

Day.	Urine. c.c.	Specific Gravity.	Total Nitrogen. grams.	Creatinine. grams.	Creatine. grams.	Remarks.
1.	470	1017	3.34	.384	-	diet
2.	375	1018	3.23	.379	-	"
3.	410	1019	3.03	.370	-	"
4.	255	1027	2.94	.343	-	" no food
5.		urine lost.				injection
6.	275	1017	4.42	.342	.298	no food.
7.	220	1026	5.24	.351	.189	diet.
8.	510	1014	4.68	.343	.053	"
9.	680	1012	3.59	.331	.003	"
10.	340	1020	3.31	.322	-	"

EXPERIMENT VI. October, 1913.

In the previous experiments there is seen a decrease in the amount of Creatinine excreted following the injection. It was thought that the recorded decrease might be fictitious, being caused by alteration in the colorimetric readings due to the presence of Acetone bodies. A further experiment was therefore done to determine the accuracy of the above supposition. In this series of analyses, before readings were taken, these bodies were driven off by the method described in Section I. so that approximately the real amounts of Creatinine present were determined.

DIET:

Oatmeal.	70 grams.		
Margarine.	60 grams.	Proteins.	25 grams.
Dried skimmed milk.	30 grams.	Carbohydrates	100 grams.
Cane Sugar.	40 grams.	Fat.	52 grams.
NaCl.	2 grams.		
Water ad lib.			

URINARY ANALYSIS:

TABLE XIX. Showing excretion of Creatine on privation of Carbohydrates produced by Sodium Selenite.

Day.	Dog's Weight.	Urine. c.c.	Specific Gravity.	Reaction.	Total Nit.	NH_3 Nit.	Acetone plus Diacetic A.	Creatinine	Creatine.	Remarks.
					grm.	grm.	m.gr.	grm.	grm.	
1.	12.27	302	1024	acid	3.26	.144	.0115	.370	-	diet
2.		520	1016	neut.	3.13	.180	.0046	.380	.005	" "
3.		650	1014	"	3.76	.208	.0044	.388	.051	"
4.		480	1014	"	3.86	.245	.0041	.377	.060	"
5.		385	1020	"	3.86	.199	.0047	.377	.060	"
6.		305	1017	alk.	6.0	.347	.0054	.411	.227	inject;
7.		300	1020	"	6.76	.390	.0081	.397	.240	third food
8.		265	1021	"	5.21	.245	.0056	.385	.075	two-thirds food.

SYMPTOMS PRODUCED: Salivation began within 3 minutes after the injection. Over half a pint of saliva and mucus was vomited.

RESULTS OF SELENIUM EXPERIMENTS.

CREATININE: When the correct amount of Creatinine is registered as in Experiment VI. the previously recorded decrease is seen to be quite fictitious and due to the presence of the Acetone bodies. Any variation is in the direction of a slight increase. No parallelism can be traced between the excretion of Creatinine and that of Total Nitrogen or of Creatine. The excretion of Creatinine appears to be dependent upon some special metabolic process which is little disturbed by the catabolism of the protein molecule. The question is discussed in the appendix, which deals with the connection between Creatine and Creatinine.

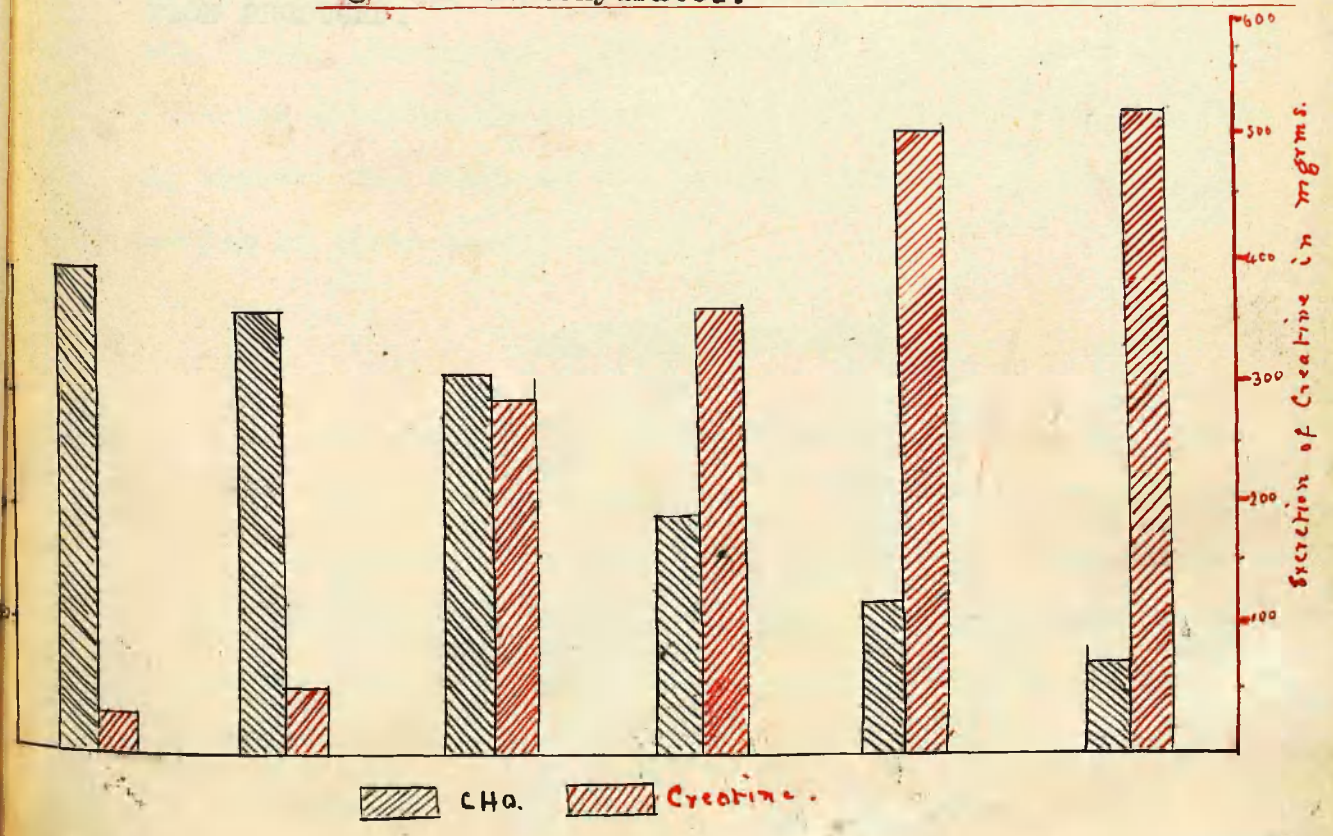
CREATINE: There follows upon each injection an output of Creatine which is in inverse proportion to the previous intake of carbohydrates as Table XX. and the accompanying diagram clearly show.

TABLE XX.

TABLE XX. Showing relation of Creatine excreted to previous ingestion of carbohydrates.

No. of Experiment.	4	3	1	6	2	5
Total output of Creatine on three days after injection, in grams.	.067	.137	.346	.540	.676	.545
Total output of Creatine in three days before the injection., in grams.	.039	.083	.044	.171	.151	-
Excess. grams.	.028	.054	.302	.369	.525	.545
Carbohydrates in food, in grams per diem.	204	189	161	100	64	34

DIAGRAM showing Relation of Creatinine excreted to previously ingested Carbohydrates.



The preliminary experiments confirmed the observations made by Jones (l.c.) that Sodium Selenite injected subcutaneously is carried by the blood stream to the tissues and there within the cells is reduced to Selenium by glucose which is thereby lost to the processes of metabolism. The injection of the Selenite therefore produces a condition of carbohydrate starvation in the tissues.

It is consequently concluded that - PRIVATION OF CARBOHYDRATES IN THE TISSUES LEADS TO AN EXCRETION OF CREATINE WHICH IS IN PROPORTION TO THE EXTENT OF THE PRIVATION PRODUCED.

SECTION I.

PRIVATION OF CARBOHYDRATES BY PHLORIDZIN POISONING.

In Diabetes Mellitus where the power of utilisation of carbohydrates by the tissues is defective, an excretion of Creatine has been recorded by Taylor and Krause. As in diabetes, Acetone bodies occur in the urine, the excretion of Creatinine recorded might be due, in part at least, to the error occasioned by these bodies.

By means of Phloridzin a condition of carbohydrate starvation may be established, as that drug exerts a specific action on the kidneys causing them to drain off sugar from the blood. Taylor and Cathcart, Wolf, Krause and Cramer observed Creatine in the urine in this form of poisoning, but no account was taken of the possible effects of Acetone bodies on their results.

AUTHOR'S EXPERIMENTS.

The general condition of experiment and modes of analysis were as before described. The dog was put upon a fixed Creatine free diet for several days, and then one gram of Phloridzin was injected. For two days before and two days after the injection, the Acetone bodies in the urine were estimated. The results are tabulated in Table XXI.

The

The increase of Acetone bodies is negligible. The Diacetic Acid was driven off before estimation of the Creatinine was made. In any case, were the amounts of Creatine found fictitious and due to the error caused by the Acetone bodies, the sum total of Creatine plus Creatinine would not be altered, whereas there is a distinct increase in the total of more than 10%.

TABLE XXI. Showing excretion of Creatine in Phloridzin Diabetes.

ount of ine.	Specific Gravity.	Total Nitrogen. grams.	Creatinine plus Creatine. grms.	Creatinine grams.	Creatine grams.	Acetone bodies in grams.	Remarks
520	1014	2.49	.348	.348	-	?	
490	1013	2.17	.360	.360	-	5.4	
460	1012	2.21	.348	.348	-	5.3	
615	1028	2.32	.405	.365	.04	10.0	Inject: of Phloridzin.
550	1009	2.35	.375	.358	.017	5.1	
370	1016	2.15	.360	.345	.015	?	
370	1016	2.15	.360	.345	.015	?	
480	1013	2.31	.364	.364	-	?	

No marked alteration in the output of Nitrogen is observed, and the animal continued to take its full diet, so that the output of Creatine cannot be attributed to either an excess or deficiency of protein. No disturbance of health could be detected. The only abnormal condition was the draining off of the blood sugar in the urine.

It may be concluded that - THE PRIVATION OF CARBOHYDRATES INDUCED BY PHLORIDZIN POISONING CAUSES AN EXCRETION OF CREATINE.

SECTION I.

C. PRIVATION OF CARBOHYDRATES BY INANITION.

In starvation, an output of Creatine was observed by Cathcart. The observation was confirmed by Benedict and by Hawk, Hawe and Mattill. In inanition, however, Acetone bodies are excreted, and no account was taken by any of these workers of the possible effect of these on their results. All obtained a decrease in Creatinine which, together with an apparent presence of an amount of Creatine that would more or less compensate for the decrease, might be due entirely to the Diacetic acid.

AUTHOR'S EXPERIMENTS.

Urine was obtained from a subject, who in connection with another experiment was undergoing a three days' starvation. The Creatine and Creatinine were estimated in the ordinary way, so that a record of the apparent excretion of these bodies might be obtained. Then further analyses were performed in which the Acetone bodies were driven off so that the real amounts might be had. The urine was being collected in 12 hour periods. Unfortunately, the sample obtained in the period of 48 to 60 hours was lost and a further sample was unattainable. The presence of Creatine was tested for by the

the Diacetyl Reaction described by Walpole (l.c.)

TABLE XXII. Showing APPARENT excretion of Creatine in Starvation.

Period of Starvation.	Amount of Urine. c.c.	Creatinine grams.	Creatine. grams.	Walpole's Reaction.
24 to 36 hours.	190	.55	.077	Negative
36 to 48 hours.	257?	.382	.039	Positive
60 to 72 hours.	725	.587	.076	Positive.

TABLE XXIII. Showing REAL excretion of Creatine in Starvation.

Period of Starvation.	Amount of Urine. c.c.	Creatinine. grams.	Creatine. grams.	Walpole's Reaction.
24 to 36 hours.	190	.627	-	Negative.
36 to 48 hours.	257?	.413	.008	Positive.
60 to 72 hours.	725	.634	.029	Positive.

In Table XXII. the proportion of creatine to Creatinine corresponds rather closely to that recorded by Cathcart (l.c.). The presence of the Acetone bodies undoubtedly causes the apparent output of Creatine to be exaggerated.

In Table XXIII. which contains the true record, no Creatine appears until after 36 hours. It may be presumed that during that period the reserve store of glycogen is being drawn upon and that no deficiency of carbohydrates is experienced by the tissues. Beyond that period Creatine appears in increasing amounts.

It

It is concluded that - IN STARVATION CREATINE APPEARS IN THE URINE IN MEASURABLE QUANTITIES AFTER 36 HOURS.

SECTION II.

THE EFFECTS OF AN EXCESSIVE SUPPLY OF CARBOHYDRATES ON THE EXCRETION OF CREATINE.

In the preceding section evidence has been accumulat-ed to prove that in man and in the dog, in whose urine the presence of Creatine on a Creatine-free diet, may be regarded as abnormal, an excretion of Creatine can be produced by privation of Carbohydrates.

It would be of interest to ascertain whether an excessive supply of carbohydrates would diminish the Creatine excreted in animals, in whose urine it appears as a normal constituent.

In a paper published from this laboratory by Miss Lindsay, dealing with the protein metabolism of the foetus, there appears in the records of some analyses of urine indications that Creatine is present in the urine of herbivora. The figures are, however, too scanty and variable to found upon, and before undertaking any investigation as to the effects of carbohydrates, definite evidence based on experimental work was required that Creatine appears as a normal constituent in the urine of the animal experimented upon.

Through

Through the kindness of Dr.Leonard Findlay, a non-pregnant lactating Goat was rendered available for experiment. Examination of the urine showed the constant presence of Creatine which, however, might be attributed to the process of lactation or to the presence of an unsuspected pregnancy. As in the human, Creatine has been found both in lactation and in pregnancy (Mellanby, Krause). To avoid any obscurity therefore, the preliminary experiments to prove the constant presence of Creatine in Goat's urine were made upon a he-goat.

AUTHOR'S EXPERIMENTS.

A he-goat of age 6 months was put up in a metabolic cage of such construction that the urine could be collected separately from the faeces.

The urine was found to be invariably alkaline in reaction. Lest it might be objected that the Creatine found was due to a conversion of Creatinine to Creatine taking place in the alkaline urine in the bladder, an attempt was made to render the urine neutral or acid, by giving acid sodium phosphate, which was easily accomplished by pouring the solution of that substance on to the back of the tongue through a wide catheter. No influence on the reaction of the urine was produced. The diet was then changed from hay, turnips

turnips and bran, to maize and bread "ad lib.", upon which the urine became neutral in reaction as tested by litmus paper. It was then seen that the reaction of the fluid had no effect upon the Creatine content. This was proved quite conclusively later when working with the she-goat whose urine on a number of days was acid without in any way affecting the Creatine output.

Analyses of the urine were continued over a space of 25 days.

The condition of experiment and method of analysis are in these experiments similar to those described before, except that in this case no catheterisation was practised. Table XXIV. gives the results.

TABLE XXIV.

TABLE XXIV. Showing constant presence of Creatine in Goat's URINE.

Date.	Amount of Urine c.c.	Specific gravity.	Reaction	Total Nitrogen. g.	Creatine plus Creatinine. g.	Creatine g.	Creatinine. g.	Diet.
28	215	1031	alk.	3.12	.40	.03	.365	
29	180	1031	"	3.40	.45	.126	.324	
30	dil.to 600	?	"	1.65	.27	.146	.114	
1	450	1008	"	1.32	.30	.160	.140	
2	Urine lost.							Grams.
3.	187	1022	"	1.51	.244	.088	.158	bran 150
4	295	1015	"	1.49	.343	.160	.183	hay 200
5	125	1045	"	1.85	.430	.263	.168	turnips 600
6	56	?	"	.72	.250	.136	.114	
					25 grm.acid sod.phosp.			
7	Urine spoiled by being mixed with faeces due to							
8	diarrhoea caused by salt.							
9	75	?	alk.	1.34	.348	.219	.119	
10	59	?	"	1.55	.322	.159	.163	
11	65	?	"	1.25	.285	.153	.133	
12	No collection of urine. Maize and bread ad lib.fed							
13	to render the urine acid or neutral, from							
14	12th. to 15th.							
15	53		Slight alk.	2.19	.386	.143	.243	
16	500	1012	Neut.	5.65	.710	.394	.316	
17	190	1029	Slight alk.	2.91	.605	.316	.289	bran 150
18	157	1049	Strong alk.	2.99	.578	.285	.293	hay 200 turnips 600
19	85	1051	"	2.19	.426	.173	.253	
20	56	?	Slight alk.	1.34	.587	.306	.281	
21	52	?	"	.64	.532	.273	.260	
22	85	1050	"	1.64	.455	.235	.220	

The amount of Creatinine excreted in grams per day is .218 and of creatine .191

The

The foregoing is taken as affording ample evidence that Creatine is a constant and normal constituent of Goat's urine.

The lactating goat was put up in the metabolic cage. It was milked twice daily and the total nitrogen content of the milk was estimated so that the combined nitrogen excretion of milk and urine might afford some indication of the flesh catabolised when the low protein and high carbohydrate diet was adopted.

High protein feeding was practised for 5 days. The diet consisted of "Plasmon" 250 grams, Bean meal 150 grams, and Turnips 200 grams. About one-sixth of the diet was left each day uneaten. Following upon this a high carbohydrate diet of potatoes was given. The amount was increased from 1400 to 2200 grams per day. At the beginning and at the close of the experiment a natural diet of hay, turnips and bran was given.[x]. The results are tabulated below.

TABLE XXV.

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[x]The protein and carbohydrate content of the food are calculated from Crowther's Tables.

TABLE XXV. Showing effects on Creatine excretion of high and low Carbohydrate feeding.

Date.	Amount of URINE c.c.	Specific Gravity.	Reaction.	Total Nitrogen grams.	Creatinine. grams.	Creatine. grams.	Amount of MILK. c.c.	Total Nitrogen in MILK. grams.	Weight of Goat. kilos.	Diet.
6/7	187	1047	alk.	4.06	.65	.39	234	1.65		hay, bran, turnips.
7/8	115	?	"	1.09	.12	.12	240	1.65		fast.
8/9	1325	1027	"	24.04	1.30	1.98	234	1.57	33.1	prot.200
9/10	1052	1023	"	19.64	.74	1.31	192	1.34		CHO. 66
10/11	900	1030	"	23.88	.82	.86	158	1.12		fat. 1.6
11/12	742	1035	"	20.88	.64	.89	132	.99	33.6	
12/13	1145	1021	"	19.32	.81	.74	141	1.04	32.6	
13/14	1555	1009	"	5.81	1.11	.30	197	1.27	32.4	
14/15	860	1010	"	2.61	.91	.37	223	1.37	31.9	prot. 1.4
15/16	1162	1008	"	2.99	.87	.25	208	1.37	31.5	CHO. 266
16/17	1130	1009	"	3.89	1.16	.51	170	1.15	30.4	fat. 1.4
17/18	800	1013	"	4.65	.97	.42	160	1.09		
18/19	850	1013	"	2.72	.88	.25	166	1.13	30.2	
19/20	950	1613	"	4.00	.99	.40	177	1.20		prot. 1.7
20/21	1377	1010	"	3.03	.96	.16	180	1.22	29.5	CHO. 323
21/22	1033	1011	"	2.49	.82	.26	180	1.18	29.4	fat. 1.7
22/23	1237	1009	"	2.42	.98	.12	175	1.10	29.5	prot. 2 CHO. 380 fat 2
23/24	925	1010	"	2.46	.87	.01	136	.88	29.9	
24/25	1428	1012	"	1.92	1.01	.02	154	1.07	29.7	prot. 2.2
25/26	1450	1011	"	2.54	.95	-	43	.41	30.1	CHO. 418
26/27	1010	1005	"	2.29	.55	-	42	.52	30.2	fat 2.2
27/28	400	1020	"	1.19	1.30	-	30	?	30.2	attempt to feed on lin-seed meal.
28/30			goat at grass.							
30/31	1370	1009	acid	4.57	1.11	.97	97	.71		hay ad lib.
31/1	1400	?	"	5.87	.96	.60	120	.84		turnip "
1/2	1485	1007	"	5.37	.90	.27	117	.78	32.7	oats "
2/3	1695	1012	alk.	5.81	1.21	.32	127	.83	32.3	
3/4	1515	1013	"	4.91	.89	.40	140	.94		
4/11			goat at grass.							

TABLE XXV. continued.

Showing effects on Creatine excretion of high and low carbohydrate feeding.

Date.	Amount of URINE c.c.	Specific Gravity.	Reaction.	Total Nitrogen grams.	Creatinine. grams.	Creatine. grams.	Amount of MILK. c.c.	Total Nitrogen in MILK. grams.	Diet.
11/12	980	1013	acid	7.46	.68	.20	105	.83	
12/13	478	1013	"	5.16	.82	.34	95	.74	prot. 20
13/14	270	1017	"	1.56	.31	.12	85	.71	CHO. 174
14/15	765	1021	alk.	4.25	1.14	.37	64	.69	fat 5.5
15/16	980	1011	"	2.86	.87	.27	56	.54	
16/17	610	1016	"	3.20	.84	.25	44	.48	
17/18	325	1017	"	3.39	.75	.17	25	.42	
18/19	no urine passed.						15		
19/20	940	1018	acid	7.90	1.56	.76	10		
	goat at grass.								

On the natural diet in which the carbohydrates averaged 174 grams per diem., the average daily excretion of Creatine was .38 grams. On the low carbohydrate diet of 66 grams per diem, it rose to 1.16 grams. On the first day of the high carbohydrate feeding period it fell to .30 and finally reached zero. On the days at which no Creatine was excreted the urine was alkaline, showing that the alkalinity of the urine in the bladder has no effect in converting Creatinine to Creatine.

The

The following Table summarises the results.

TABLE XXVI. Summary of Results of TABLE XXV.

Amount of carbohydrate fed. grams.	Creatinine average per day. grams.	Excretion of Creatine. average per day grams.
66	.86	1.16
174	.91	..38
418	.96	nil.

The daily excretion of Creatinine in relatively constant amounts is remarkable in view of the wide difference shown in the output of Creatine.

In this experiment the results are somewhat complicated as there are two concurrent factors in producing an excess of carbohydrate in the tissues. As will be seen from the Table, during the period of high carbohydrate feeding the milk flow falls from 197 to 30 c.c. per day. It was determined to separate these two factors and estimate their independent influence on the results.

Three weeks were allowed to elapse during which the milk supply decreased. When it appeared that lactation would soon cease, the goat was again put up in the cage, and its

its urine collected for analysis. The diet was of turnips, oats and hay. The results are tabulated below.

TABLE XXVII. Showing decrease in excretion of Creatine on cessation of Lactation.

Date	Urine Amount c.c.	Specific gravity.	Reaction.	Total Nitrogen grams.	Creatinine. grams.	Creatine. grams.	Milk c.c.	Diet.
3/4	343	1033	acid	6.49	.85	.33	12	Hay, oats and turnips.
4/5	605	1022	"	9.43	1.31	.26	8	
5/6	315	1026	"	2.55	.88	.14	7	
6/7	330	1034	"	3.66	.83	.14	-	
7/8	330	1034	alk.	3.66	.83	.14	-	
8/9	408	1026	"	3.31	.76	.16	9	
9/10	275	1036	"	4.67	.82	.24	-	
10/11	800	1016	"	3.52	1.01	.39	4	
11/12	645	1019	"	3.76	.97	.62	-	
12/13	535	?	"	2.6	.79	.42	3	
13/14	535	?	neut.	2.6	.79	.42	-	
14/15	635	1021	alk.	2.52	.93	.05	-	
15/16	402	1029	"	3.05	.94	.04	3	
16/17	588	1016	"	1.90	.86	-	no milk	

The foregoing results can not be regarded as unequivocal. The decrease in the Creatine output does not proceed

proceed "pari passu" with the decrease in the secretion of milk. Further work would require to be done before the effects of lactation on the metabolism of Creatine could be stated with precision.

It appears however, reasonable to conclude that CESSATION OF LACTATION WHICH LEAVES AVAILABLE FOR TISSUE METABOLISM SUGAR FORMERLY EXCRETED IN THE MILK TENDS TO CAUSE A DECREASE IN THE OUTPUT OF CREATINE.

The influence of increased feeding with carbohydrates, uncomplicated by variation in the milk flow, was then determined. The goat now non-lactating, was put upon a diet of hay, oats, and turnips. After six days the diet was changed to potatoes, which were increased in amount, and finally given "ad lib."

TABLE XXVIII.

TABLE XXVIII. Showing effect on excretion of Creatine of increased amounts of carbohydrates in the diet.

Date. March 1913.	Amount of Urine. c.c.	Specific Gravity.	Total Nitrogen. grams.	Creat-inine. grams.	Creat-ine. grams.	Weight of goat. kilos.	Diet.
18/19	615	1017	2.55	.88	.20	-	
19/20	640	1012	2.25	.87	.16	35.3	
20/21	405	1021	2.32	.84	.12	35.2	turnips.
21/22	732	1014	2.12	.84	.12	35.7	hay
22/23	565	1018	2.18	.77	.11	35.6	oats.
23/24	506	1021	2.39	.89	.16	35.4	
24/25	815	1015	3.29	.90	.15	-	potatoes
25/26	780	1012	3.11	.92	.07	34.1	beginning
26/27	720	1012	3.78	.89	.39	33.8	with 840
27/28	915	1013	3.89	.91	.15	34.4	grams per
28/29	915	1013	3.89	.91	.15	-	day and
29/30	1526	1010	2.89	.90	.08	33.7	increased.
30/31	1133	1010	2.35	.92	-	33.5	
31/1	1316	1009	2.42	.97	.01	33.3	Potatoes
1/2	1240	1010	2.27	.86	-	33.4	ad lib.

The average output of Creatine on the natural diet for six days is .145 grams per day compared with .38 grams per day in the preceding corresponding experiment. The low excretion in this case may be due to the contimued influence of the cessation of lactation which had only taken place a few days prior to the beginning of this experiment.

On increasing the amount of carbohydrates in the food, the Creatine output diminishes and twice touches zero.

Taken in conjunction with the previous finding with which

which this is in entire agreement, the results are regarded as affording evidence that - IN THE GOAT IN WHOSE URINE CREATINE APPEARS AS A NORMAL CONSTITUENT, THE EXCRETION IS DIMINISHED BY INCREASING THE AMOUNT OF CARBOHYDRATE IN THE FOOD.

Attention is directed here to two points of interest arising out of the work done in this Section, which have an important bearing on the discussion to be taken up later as to the function of Creatine.

During the 14 days upon which the animal is on the high carbohydrate diet (Table XXV.) there is an output of 49.97 grams of Nitrogen (Milk 14.96 plus Urine 35.01) which represents the catabolism of (49.97 × 6.25) 312.3 grams of protein. The intake amounts to 24.3 grams, leaving a negative balance of 278 grams derived from the tissues. These figures represent dried material, amd as the tissues contain approximately 75 per cent of water, the amount of protein tissue consumed would be 278 × 4, i.e. 1112 grams. During the period the weight of the animal falls from 32.4 to 30.2 kilos, a loss of 2.2 kilos. The potato diet is excessively low in fat, (.1%) and the utilisation of body fat may account for the remainder of the weight lost. The same loss of body weight

weight and heavy negative nitrogen balances indicating an extensive "flesh catabolism" occurs in the experiment recorded in Table XXXVIII.

Assuming that the protein is derived from muscle flesh, there should be during the 14 days, 3 to 4 grams of Creatine set free in addition to the average daily output. It is under these conditions that the excessive supply of carbohydrates produces a reduction in the Creatine output.

The Creatine is not excreted as Creatinine, the output of which suffers but little variation. Thus, during the carbohydrate period the average daily output is .96 grams, and on the normal diet, when the .38 gram of creatine is being excreted daily, the average is .91 grams. On the high protein diet when 1.16 grams of Creatine is being excreted daily, the daily output is .86 grams.

Evidence will be adduced later which affords grounds for the belief that the variation in the Creatinine output depends upon factors quite other than those which govern the Creatinine output. That the Creatinine excretion is independent of the Creatine output is well seen in comparing the periods during which the animal was upon the same diet - hay, turnips and oats, - and in the same condition, except that lactation proceeded in one case and not in the other. During

During the lactating period the daily output was Creatinine .860 grams, and Creatine .38 grams, and during the non-lactating period the figures are .865 and .125 grams respectively

The subject will be referred to later when the experimental results are considered in the light of each other.

SECTION III.

THE EXCRETION OF CREATINE IN PATHOLOGICAL CONDITIONS AFFECTING METABOLISM OF CARBOHYDRATES.

In Sections I. and II. a close inter-relationship has been shown to exist between the excretion of Creatine and the supply of carbohydrate available for metabolic processes.

Certain pathological conditions exist in which the tissues are unable to use the carbohydrate, however freely it be supplied. Of these the most outstanding is Diabetes Mellitus where Creatine has been found to be excreted more or less in proportion to the severity of the condition. Taylor (l.c.), Krause (l.c.).

Many authors have called attention to the tendency to glycosuria that exists in diseases affecting either the thyroid gland or the pituitary body. Van Noorden, after a discussion of the literature on the subject, concludes that "an increase of thyroid activity in the body makes the normal use of carbohydrates more difficult" and in treating of the connection between acromegaly and diabetes says "an interdependence between the two conditions cannot be doubted".

While

While the various hypotheses as to the nature of the connection between the disturbance of the function of the thyroid or pituitary glands and the occurrence of glycosuria may be regarded as still "sub judice", there appears general agreement in both clinical and experimental evidence that a quantitative or qualitative alteration in the secretions of these organs tends to have the effect of rendering the utilisation of carbohydrates by the tissues more difficult.

Greenwald observed an excretion of creatine in parathyroidectomised dogs, an observation which was repeated by Gino Frontali (l.c.) who showed that the amount excreted exceeded what could be accounted for by the inanition which followed the operation.

In 1907, Forschbach examined the urine of patients suffering from exophthalmic goitre where there occurs an excessive or a perverted secretion, but his subject was upon a meat diet which contains Creatine, and he used the Neubauer Salkowski Method. The figures he states, e.g. the excretion of .01 gram of Creatinine per day in a subject weighing 72 likos. appear impossible. His results are undoubtedly vitiated by the method used. The matter was investigated by Schaffer who found Creatine present in the urine in amounts

amounts varying from .03 to .18 gram per day.

Several cases have been reported which show that in the treatment of obesity and of myxoedema the exhibition of thyroid extract has led to a transient glycosuria or a true diabetes. (Van Noorden).

No reference was found in the literature to the effects on the excretion of Creatine of giving thyroid extract to a normal, healthy individual, or tc the presence or absence of Creatine in the urine of patients suffering from acromegaly. It was considered that it would be of interest to ascertain whether the connection between the metabolism of carbohydrate and the excretion of Creatine would be so close that in these conditions Creatine would appear in the urine.

AUTHOR'S EXPERIMENTS.

A. EXCRETION OF CREATINE IN THYROIDISM.

A healthy adult male of weight 65 kilos. was put upon a fixed diet, and 10 grams of dried thyroid extract (Burrough & Wellcome's Preparation) was given daily for seven days. Examination of the urine was continued for other four days. Account was taken of the possibility of the production of an acidosis. The Acetone bodies were estimated and the possibility of error due to these was safe-guarded against. The

The urine was collected in 24 hour samples and the methods of analyses were as before described.

DIET: Protein. 118 grams. (approx.)
Fat. 76 grams. "
Carbohydrates. 417 grams. "

TABLE XXIX. Showing excretion of Creatine in Thyroidism.

Date Febry. 1914.	Urine Amount c.c.	Total Nitrogen grams.	Ammonia grams.	Creatinine grams.	Creatine grams.	Body weight kilos.	Remarks.
9	1120	14.8	.21	1.478	-	65.1	10 grams dried thyroid extract.
10	1150	16.0	.31	1.479	-		
11	1250	18.1	.32	1.468	-		
12	1060	17.7	.36	1.491	.004		
13	1105	18.2	.27	1.495	-		"
14	1095	18.0	.29	1.451	.051	64.1	"
15	1060	18.2	?	1.409	.134		"
16	1090	18.2	.36	1.440	.115	64.1	no thyroid
17	1005	18.2	.30	1.457	.065	64.3	"
18							"
19	1025	16.6	.31	1.484	-	64.4	"

Since the above work was completed a reference was found to some work done on the effects of thyroid feeding by Krause and Cramer. They gave the results of two short three-day experiments. In one, in which the diet was almost pure carbohydrate with some fat, no Creatine appears on any of the three*

x To save space details of diet are omitted in these and subsequent experiments.

three days. In the other, which was poorer in carbohydrate a trace appears on the second day, and .131 on the third.

B. EXCRETION OF CREATINE IN ACROMEGALY.

Through the kindness of Professor T.K.Monro, opportunity was afforded for the examination of the urine in a case diagnosed by him as one of Acromegaly.

The subject was a woman 25 years of age. She had not menstruated for two years previously so that the results are not complicated by the incidence of any gynaecological phenomena which have been shown to be sometimes accompanied by the output of Creatine[x]. The temperature was (maintained) normal during the whole period of investigation, and no sign or symptoms other than those due to acromegaly were detected. The urine was free from sugar.

The patient was kept confined to bed for some days prior to, and during the whole course of the experiment. A fixed diet was given. The collection of the urine, which was entrusted to the nurses, was evidently imperfect as appears from the comparison of the protein intake and the excretion of total nitrogen, so that except perhaps on the first

x Reference: Murlin, Krause, Krause and Cramer, Van Hoogenhuyze and A ten Doeschate.

first and last days, only a portion of the 24 hours' secretion was obtained.

DIET: Protein 55 grams. (approx.)
Fat 93 grams. "
Carbohydrates 360 grams. "

Caloric value = 2565.

URINARY ANALYSES:

TABLE XXX. Showing excretion of Creatine in Acromegaly.

Date. Decr. 1913.	Amount of Urine c.c.	Specific gravity.	Reaction	Total Nitrogen grams.	Ammonia grams.	Creatine grams.	Creatinine grams.	Body Weight.
10/11	1206	1016	acid	7.94	.83	.390	.886	9st.6 lb.
11/12	1661	1008	"	5.18	.64	.189	.682	
12/13	662	1016	"	4.90	.73	.121	.684	
13/14	1355	1008	neut.	3.92	.68	.033	.471	
14/15	1090	1012	acid	5.77	.68	.090	.852	9st.7 lb.

There is seen to be an irregular excretion of Creatine. That the Caloric Value of the food was ample is evidenced by the fact that the subject increased one pound in weight during the five days, and no indication can be gathered from the comparison of the protein intake with the total nitrogen output of the occurrence of catabolism of tissue flesh which might account for the presence of the Creatine in the urine.

Further

Further research on the metabolism in acromegaly is required before definite deductions can be made, but in the light of the results recorded previously, it is suggested that the constant presence of Creatine in the urine is due to some interference with the normal carbohydrate metabolism, a tendency to which is admittedly present in acromegaly.

The experimental results obtained in this Section follow upon the conclusions arrived at in Sections I. and II. are taken as being sufficient to warrant the suggestion that - IN PATHOLOGICAL CONDITIONS WHICH ADVERSELY INFLUENCE THE NORMAL CARBOHYDRATE METABOLISM CREATINE TENDS TO APPEAR IN THE URINE.

SECTION IV.

THE INFLUENCE OF WORK ON THE PRODUCTION AND EXCRETION OF CREATINE.

All the conclusions of the work recorded in the preceding Sections lead to the suggestion that the metabolism of Creatine is intimately connected with the utilisation of carbohydrate by the tissues. A large proportion of the carbohydrate of the food may be regarded as devoted to the liberation of energy expended on work. The effect of work upon the metabolism of Creatine was therefore investigated.

The literature on the subject prior to the introduction of the colorimetric method of estimation is somewhat voluminous, but as the results are vitiated by the faulty methods it will only be briefly referred to.

A. INFLUENCE OF WORK ON THE CREATINE CONTENT OF MUSCLE.

Liebig (l.c.) found in the tissues of the hunted fox ten times as much Creatine as in the tissues of the same animal kept in confinement. Sarokow observed an increase of Creatine in tetanised frog's muscle, and Sczelkow in tetanised fowl's muscle. Monari also found an increase after work and

and a tendency for the conversion of Creatine to Creatinine, which latter result, however, was almost certainly due to faulty methods of analyses, which involved the presence of conditions capable of converting Creatine to Creatinine, as was shown by Mellanby (l.c.).

In opposition to these, Nawrocki could find no greater difference after stimulation than could be attributed to errors of estimation. Voit, after somewhat exhaustive investigation, concludes "Jedenfalls steht aber fast, dass durch die Arbeit die Summe von Kreatin und Kreatinin nicht grösser wird," a conclusion which is at variance with his own results, which on analysis show an increase of Creatine after work.

Using the newer method, Mellanby (l.c.) stimulated isolated frog's muscle with the circulation intact. He concludes that work has no effect on the metabolism of Creatine. His figures show that, in the controls of the isolated frog's muscle, the percentage of Creatine found is .255, while in the stimulated muscle the percentage on only two occasions is as low as in the controls and in all other experiments is higher, being twice .260. In intact rabbit muscle the percentage in unstimulated is .440, and in stimulated .430. His results therefore show a slight increase of creatine on stimulation of isolated muscle and a decrease with circulation intact.

Brown

Brown and Cathcart investigated the question. Their methods of experiment are clearly defined and their data are ample, and shows uniform results. They conclude that in isolated nerve muscle preparations stimulation increases the creatine content of the muscle, while with the muscle "in situ" and circulation intact, stimulation brings about a constant though small decrease, which conclusion is in accordance with Mellanby's results but at variance with his deductions.

The balance of evidence in the literature is clearly in favour of the view that the metabolism of Creatine is associated with muscular activity.

AUTHOR'S EXPERIMENTS.

N.B. In this and in the heart perfusion experiments the author had the active assistance of Dr.Cathcart.

A rabbit was taken. The abdomen was opened and a canula inserted into the descending aorta. An isotonic salt solution was run in until the fluid returned through the common iliac veins was seen to be clear. In this way an "in situ" muscle preparation was obtained in which not only was there no circulation of blood, but from which the blood and at least some of the lymph had been abstracted. A sartorius

sartorius muscle was then exposed and stimulated for about 30 minutes with rapid alternating faradic shocks, short intervals of rest being allowed. Stimulation was direct, the electrodes being brought into contact with the muscle at the point of entry of the nerve.

The two sartorius muscles, both of which had been subjected to the washing out process, but only one of which had been stimulated, were then removed and the Creatine content estimated by the method described by Myers and Fine. In the stimulated muscle .348% of creatine was found, and in the non-stimulated muscle .312%.

Taken in conjunction with the findings of Brown and Cathcart the above result is regarded as evidence that Muscular activity "per se" produces Creatine in muscle.

B. THE INFLUENCE OF BODILY EXERCISE ON THE EXCRETION OF CREATINE and CREATININE.

Meissner, working with dogs, which he kept running for periods of five hours, found an increased excretion of Creatinine. (In most of the papers prior to 1904 Creatinine may

N.B. In both cases the figures obtained by our use of this method are rather low, but as care was taken to have the treatment exactly similar in all details in both cases, the results are believed to be accurate for comparison, but not for absolute values.

may be taken as including both Creatine and Creatinine. It is so in this case.) On the day of the exercise Voit found a decrease in Creatinine and an increase on the following day. Grocco found an increase on work days. Moitessier who maintained a uniform diet, and Gregor who adopted a Creatine-free diet, both found an increase on work. Oddi and Tarulli found an increase if the work were excessive. Dunlop, Paton, Stockman and Macadam, obtained a like result. On the other hand Hoffman concluded that there were variations in the excretion, but that these were not connected with the work. Gregor (l.c.), however, on analysis of Hoffmann's results finds evidence of an increase following the work. Van Hoogenhuyze and Verploegh, using the Folin Method, concluded that with an ample diet, work left the excretion of Creatinine unaffected, but on an insufficient diet as in fasting, the excretion was distinctly increased. They give no separate figures for Creatine and Creatinine. Pekelharing found an increased excretion of creatinine on increased muscle tonus, the metabolism of which he regards as differing from that of contraction. The balance of evidence is in favour of the view that there occurs an increased production and excretion of "total Creatine", i.e. Creatine plus Creatinine, following work.

Information

Information was desired as to whether the increase affected the Creatine or the Creatinine, and the following experiments were undertaken.

AUTHOR'S EXPERIMENTS.

I. On the days prior to the work-day ordinary laboratory work was done, the evenings being spent reading. On the work-day the same amount of work approximately was done, at the close of which, six hours varied and strenuous exercise were undertaken, of such a nature as to exercise as far as possible all the muscles of the body, and to such a degree as to cause dyspnoea and excessive perspiration. At the end of the work period and for two days after, a feeling of lassitude and a soreness of the muscles of arms and legs was experienced. The urine for two days afterwards was loaded with urates.

DIET: Creatine free.

Protein	56 grams.)	
Fat	284 grams.)	Caloric value 2166
Carbohydrates	284 grams.)	

URINARY ANALYSES:

URINARY ANALYSES:

TABLE XXXI. Showing excretion of Creatin on work.

	Total Nitrogen. grams.	Creatinine. grams.	Creatine. grams.
Average of days before work.	11.08	1.61	-
Work day.	11.68	1.76	.06
ay after work.	9.52	1.61	.02

II In this experiment the work consisted of a 40 miles' cycle ride on a hilly road where the hills were of such a gradation as could be ascended without dismounting, but which required the exertion of all the physical force which the subject could command. Care was taken as far as possible not to push the exercise to such a degree of excess as to produce dyspnoea, so that the results might be free from the effects of deficient oxidation of the tissues or the production of lactic acid. The extra exercise was superimposed upon the same amount of work as that performed on the previous days (ordinary laboratory work) by arranging that part of the laboratory work should be completed at the close of the exercise period. Unfortunately a strong head wind arose on the afternoon which delayed the return journey and increased the amount of work done beyond the originay intention

intention. The ordinary laboratory work was completed, but the work had been so excessive that frontal headache, giddiness, and nausea were produced to such a degree that it was found impossible to finish the diet. On the work day therefore the evening meal - about one third of the total - was not taken. It was added to the next day's food.

DIET: Creatine free.

Protein	30.2	grams)	
Fat	56.7	grams)	Caloric value 2206
Carbohydrates.	381	grams)	

URINARY ANALYSIS:

TABLE XXXII. Showing excretion of Creatin on work.

	Total Nitrogen. grams.	Creatinine. grams.	Creatine. grams.
	9.53	1.365	.02
	8.09	1.355	-
Work day →	8.06	1.400	.05
	8.96	1.336	.05
	7.52	1.390	-

III. A Third experiment was performed under the same conditions as those recorded in Experiment II., except that potatoes were substituted for tapioca to which had been ascribed the incidence of intestinal colic which probably accounts for the presence of the .02 grams of Creatine on the first

first day, of which record is given in Table XXXII. The diet contained protein 30, fat 70, carbohydrates 387: caloric value 2360.

Only the results of two days' analyses of urine are given as owing to circumstances not connected with the research the experiment had to be abandoned after the completion of the work.

TABLE XXXIII. Showing the excretion of Creatine on Work.

	Total Nitrogen. grams.	Creatinine. grams.	Creatine. grams.	
Day before work.	6.74	1.28	-	
Work day.	6.92	1.25	.08	

Even an approximate estimation of the amount of work done in these experiments is impossible, but it may be taken that the amount of energy expended was in each case far in excess of what could be derived from the food taken on the work day. The food reserves of the body would be drawn upon. There is no evidence however from the total nitrogen output, that any flesh catabolism occurred to account for the Creatine excreted.

A rise in the excretion of Creatinine is observed. Whether this depends directly upon the work or upon concomitant

concomitant conditions produced by the work is discussed later.

It is concluded from these experiments that - EXCESSIVE WORK CAUSES THE EXCRETION OF CREATINE IN THE URINE.

C. HEART PERFUSION EXPERIMENTS.

Weber demonstrated that on perfusing an isolated heart by Langendorff's Method the Ringer Solution used contained Creatin.

It was thought that valuable information could be obtained by repeating Weber's experiment with certain variations.

AUTHOR'S EXPERIMENTS.

A modification of J.A.Gunn's Apparatus was prepared, and Tyrode's solution was used as the perfusing fluid. A guinea pig was killed and the heart extracted while still beating. It was plunged into cold Ringer's solution to stop the action, so that the minute dissection necessary could be more easily accomplished. It was then fixed by the aorta to the apparatus and Tyrode's solution without any sugar, was allowed to flow through the apparatus, which by the circulation

circulation of water at 38 degrees C. through the water jacket, was kept at that constant temperature in which it collected, and was transferred by hand back into the receiver of the apparatus again.

The heart was allowed to contract for 90 minutes at the end of which the fluid was examined for Creatinine and Creatine. No trace of Creatinine could be detected. Creatine was present as shown both by Folin's indirect method, and by Walpole's direct diacetyl reaction[x].

The above was repeated in exactly the same manner except that the Tyrode solution contained sugar. The perfusing fluid in this instance showed traces of neither Creatinine nor Creatine. The results of four experiments are summarised below.

TABLE XXXIV.

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[x]The Creatine was present in such quantity that an attempt was made to estimate the amount, but the reading on the colorimeter scale was beyond the limits of accuracy. In view of the quantity got from such a small heart as the guineapig's it appeared evident that with a large heart measurable amounts could be obtained and the rate of production of creatine in muscular activity estimated.

TABLE XXXIV. Showing the effect of heart perfusion.

No. of Exper.	Fluid used.	Time of Perfus-ion.	Jaffe Reaction for Creat-inine.	Folin Method for Creatine.	Diacetyl Reaction for Creatine.
1.	Tyrode sol. NO sugar.	90 min.	neg.	pos.	pos.
2.	Tyrode sol. WITH sugar.	90 min.	neg.	neg.	neg.
3.	Tyrode sol. NO sugar.	90 min.	neg.	pos.	pos.
4.	Tyrode sol. WITH sugar.	90 min.	neg.	neg.	neg.

It is concluded that - CONTRACTION OF THE HEART MUSCLES PRODUCES NO CREATININE: CREATINE IS PRODUCED AND CARRIED OFF BY THE PERFUSING FLUID IF NO CARBOHYDRATE BE AVAILABLE. IF CARBOHYDRATE BE SUPPLIED NO EXCRETION OF CREATINE TAKES PLACE.

SECTION V.

ON THE CONNECTION BETWEEN CREATINE AND CREATININE.

In recent years a considerable amount of work has been done to ascertain whether either a conversion of Creatine to Creatinine or the obverse takes place within the body. It is almost unanimously agreed that Creatinine given by the mouth or injected hypodermically is excreted as such, up to between 80 and 90 per cent. of the amount given, and that there are no indications of its conversion to Creatine (Myers and Fine, Van Hoogenhuyze and Verploegh).

The question of the conversion of Creatine to Creatinine however, must still be considered unsettled. Czernecki, Folin (l.c.), Klercker and Lefmann, found no evidence of a conversion. On the other hand Pekelharing and Van Hoogenhuyze, Plimmer, Dick and Lieb, Myers and Fine (l.c.) Voegtlin and Van Hoogenhuyze and Verploegh (l.c.) found a conversion which, however, was small in amount, in most cases under 5%, the rest of the Creatine being retained or excreted unchanged.

Some of the workers used in their experiments, flesh which contains about .3 per cent of Creatine. Others used preparations

preparations of Creatine isolated from flesh or urine.

The matter was investigated afresh.

AUTHOR'S EXPERIMENTS.

A. USING FLESH AS A SOURCE OF THE CREATINE.

The subject of the experiments, apparently healthy, normal males in their third or fourth decade of life, were put upon a Creatine-free diet for several days. Then, on a given day, a weighed quantity of flesh meat was added to the diet, after which the creatine free diet was resumed. The excretion of total nitrogen, creatine, and creatinine were estimated by the methods already described. The results are recorded below.

TABLE XXXV. Showing the effects of Meat ingestion on the excretion of Creatine and Creatinine.

EXPT. I. D.B.

Amount of Urine. c.c.	Specific Gravity.	Total Nitrogen grams.	Creat-inine. grams.	Creat-ine. grams.	Diet.
Average of 2 days preceding.		11.68	1.405	-	Creatine free.
1897	1017	15.13	1.500	.06	½lb.meat added
1300	1019	11.88	1.430	.01	Creatine free
1500	1018	11.55	1.410	-	"

EXPT: II.

EXPT: II. D.B.

Amount of Urine c.c.	Specific Gravity.	Total Nitrogen grams.	Creat-inine. grams.	Creat-ine. grams.	Diet.
Average of 4 days preceding.		12.12	1.610	.004	Creatine-free
1150	1021	13.17	1.650	.10	¼lb.raw meat added.
1495	1021	13.19	1.640	.02	Creatine free
2280	1017	11.91	1.610	.02	"
EXPT: III.M.C.					
Average of 3 days preceding.		11.1	1.29	.026	Creatine free
700	1027	12.4	1.45	.13	½lb.meat added
900	1026	12.2	1.33	.10	Creatine free
700	1028	11.5	1.23	-	"
EXPT:IV. N.M.					
Average of 6 days preceding.		11.4	1.57	-	Creatine free
1100	1023	12.9	1.86	.001	½lb.meat added
1100	1023	12.9	1.64	.002	Creatine free
900	1032	11.7	1.46	-	"

EXPT: V.J.B.O.

In the foregoing experiments while the diet was Creatine free except on the days meat was given, (it) the Creatine varied in amount and in composition. In the two following experiments a fixed diet of known composition was maintained. It consisted of oatmeal, peasemeal, white bread, dried milk, sugar, butter and cocoa, containing protein 63 grams, fat 80 grams,

grams, carbohydrates 230 grams. It was continued for 10 days. Upon this diet was superimposed on the 8th day 125 grams of flesh, which was eaten raw. An Analysis of 125 grams of the same flesh was made by the method described by Brown and Cathcart (l.c.). There was found .38 grams of Creatine and no Creatinine.

URINARY ANALYSES:

TABLE XXXVI. Showing the effect of Meat ingestion.

Amount of Urine. c.c.	Specific Gravity.	Total Nitrogen grams.	Creatinine. grams.	Creatine. grams.	Body Weight kilos.	Diet.
Average of 7 preceding days.		10.30	1.45	-	68.5	prescribed.
960	1031	12.52	1.62	.05	68.5	plus 125 grams meat, containing .38grams. Creatine.
1184	1024	13.60	1.47	-	68.5	prescribed.
757	1027	10.96	1.32	-	69.1	"

EXPT: VI.J.B.O.

In this experiment the conditions are as before described except that bread is substituted in the diet for peasemeal which had become unpalatable - the protein being thus reduced. Only 107 grams. of meat were taken.

TABLE XXXVII.

URINARY ANALYSES:

TABLE XXXVII. Showing the effect of Meat ingestion.

Amount of Urine c.c.	Specific Gravity.	Total Nitrogen. grams.	Creat-inine. grams.	Creat-ine. grams.	Body Weight. kilos.	Diet.
Average of 2 preceding days.		9.31	1.74	-	68.7	prescribed
680	1023	9.99	1.85	-	-	plus 107grms. meat containing .3 gram Creatine.
665	1033	9.61	1.60	-	68.7	prescribed.

B. USING A PREPARATION OF CREATINE.

The Creatine used was a preparation of Merck's. It was examined and found to contain a trace of Creatinine. The contained Creatine was estimated and found to be about 80%. .5 gram was taken, i.e., .4 gram of Creatine.

URINARY ANALYSES:

TABLE XXXVIII. Showing the influence of Creatine ingestion on the excretion of Creatine and Creatinine.

EXPER:VII.J.B.O.

Amount Urine c.c.	Specific Gravity.	Total Nitrogen grams.	Creat-inine. grams.	Creatine grams.	Remarks.
670	1030	8.71	1.80	-	Creatine free diet
640	1032	7.96	1.80	-	do.
890	1025	8.68	1.77	.03	.4 gram Creatine
760	1030	8.71	1.80	-	Creatine free diet
730	1029	8.71	1.808	-	.4 gram creatine
660	1033	8.46	1.80	-	Creatine free diet
790	1027	10.64	1.80	.2	.4 gram Creatine

It is seen that in every case the eating of flesh causes a pronounced rise in the Creatinine excretion. Thus, in Experiment V. the flesh which contained .38 grams of Creatine causes an increase of Creatinine of .17 grams and in Experiment VI. an increase of .11 grams is caused by flesh containing .3 gram of Creatine. In Experiment VII. where .4 gram of Creatine - not in flesh - is taken, no change in the excretion of Creatinine is brought about.

I have analysed the data of Van Hoogenhuyze and Verploegh (l.c.) who took 2 grams of Creatine by mouth, and concluded that there resulted therefrom a slight increase in the Creatinine excretion. Of six experiments on Verploegh, one may be discarded, as grave doubts are cast on its accuracy by the authors themselves, the other five show no increase of excreted Creatinine. Of six experiments on Van Hoogenhuyze, one shows a doubtful result, four show no increase, and of the other two, one shows an increase of .05 grams, and one of .06 grams. Considering the amount of Creatine taken - 2 grams - and the daily fluctuations in the Creatinine excretion, the results are not such as would lead to the conclusion, that the body affords facilities for the conversion of Creatine to Creatinine.

No deductions are drawn from the results of the experiments of this Section other than that necessary for the

the main argument of this thesis, viz: that WHILE THE EATING OF FLESH CAUSES AN INCREASE IN THE CREATININE EXCRETION, THERE IS NO INDICATION THAT CREATINE TAKEN BY THE MOUTH IS CONVERTED BY THE BODY TO CREATININE.

SECTION VI.

THE EFFECTS OF EXCESSIVE WATER INGESTION ON THE EXCRETION OF CREATINE AND CREATININE.

Frequent observations have been made upon the effects on metabolism of the ingestion of large quantities of water. All observers are agreed that there results an increased excretion of the nitrogenous constituents of the urine. As to the cause of the increase opinion is divided, some workers holding that it results from a mere mechanical washing out of waste products from the tissues, and others, that it is dependent upon a stimulated metabolism.

An account of the literature is given by Hawke and Fowler, who did some work on the subject and extended the field of observation to include Creatine and Creatinine. From somewhat limited data these workers concluded that excessive water drinking produces in a Creatine-free diet, a marked excretion of Creatine and a decrease in the excretion of Creatinine.

These results could not be accounted for by the theory of the function of Creatine propounded in Section VII. and it was determined to undertake the following research to confirm

confirm or confute these findings.

AUTHOR'S EXPERIMENTS.

EXPERIMENT I.

The subject, a male, was put upon a fixed Creatine-free diet for seven days, the amount of water (being) taken with the food being maintained as nearly as possible a constant. On the 8th, 9th and 10th day in addition to the prescribed diet, there was taken at intervals throughout the day 3 litres of water. On the 11th, 12th, and 13th day the diet alone was taken as before.

The diet consisted of oatmeal, white bread, butter, sugar, cocoa, salt and milk, containing protein 58 grams, fat 98 grams, and carbohydrates 348 grams.

The results of the urinary analyses are given in Table XXXIX.

URINARY ANALYSES

URINARY ANALYSES:

TABLE XXXIX. Showing effect of Water ingestion on the excretion of Creatine and Creatinine.

Day	Body Wt. kilos.	Vol. Urine. c.c.	Sp.Grav.	Total Nitrogen grams.	Creat-inine. grams.	Creat-ine. grams.	Remarks.
1.				Diet commenced.			
2.	57.8	570	1029	10.95	1.43	-	Prescribed diet
3.	57.7	730	1027	10.42	1.43	-	"
4.	57.7	790	1027	10.14	1.42	-	"
5.	57.7	600	1028	10.89	1.40	-	"
6.	57.7	600	1028	9.07	1.35	-	"
7.	57.7	670	1028	8.79	1.42	-	"
8.	58.4	2980	1008	8.83	1.36	-	3 litres water
9.	58.2	3105	1008	8.96	1.36	-	"
10.	58.2	3400	1006.5	9.24	1.38	-	"
11.	57.7	790	1025.5	8.88	1.42	-	prescribed diet
12.	57.7	880	1025	9.38	1.34	-	"
13.	57.7	870	1022	9.46	1.35	-	"

EXPERIMENT; II.

In this experiment the protein of the diet was doubled approximately by substituting "plasmon" and cheese for oatmeal. It contained 115 gram of protein, otherwise the conditions of experiment were as before.

The results of the urinary analyses are given in Table XL.

URINARY ANALYSES

URINARY ANALYSES:

TABLE XL. Showing effect of Water ingestion on the excretion of Creatine and Creatinine.

Day.	Body Weight. kilos.	Vol. of Urine. c.c.	Sp.Grav.	Total Nitrogen. grams.	Creat-inine. grams.	Creat-ine. grams.	Remarks.
1.			Diet begun.				
2.	57.2	1220	1022	17.55	1.38	-	prescribed diet.
3.	57.3	1100	1024.5	16.63	1.35	-	"
4.	57.3	1040	1023.5	17.47	1.38	-	"
5.	58.8	3450	1007	19.04	1.33	-	3 litres water
6.	58.8	3895	1009	19.66	1.41	-	"
7.	58.9	2730	1011	18.72	1.34	-	"
8.	57.6	1350	1021	19.49	1.40	-	prescribed diet.
9.	59.4	1220	1022	20.12	1.34	-	

EXPERIMENT: III.

If Creatine be a mere waste product that can be washed out of the tissues, the more water drunk, the greater the chance of flushing it out in the urine. If it be a sort of protein food and liable to excretion where an abundance of protein is available - as Folin (l.c.) believes, - the greater the quantity of protein in the food, the greater is the chance of washing it out by excessive water ingestion.

These conditions were arranged in this experiment. The amount of water ingested per day on the water days was 9.6 litres which it was found impossible to exceed. The diet contained

contained 319 grams of protein instead of 58 grams as in Experiment I. The diet was continued for 15 days. On the 5th. and 9th. 10th and 11th. days the water which was in addition to that contained in the food was drunk at intervals throughout the day.

On the water days there was profuse perspiration and an uncomfortable feeling of abdominal distention.

The records are as follows:-

DIET:

Dried skimmed milk	600 grams.)		
Cheddar Cheese.	300 grams.)	Protein	319 grams.app.
Cane Sugar.	100 grams.)	Fat	153 grams "
White bread.	100 grams)	Carbohydrates	179 grams."
Water.	3 litres.		

URINARY ANALYSES:

TABLE XLI.

URINARY ANALYSES:

TABLE XLI. Showing influence of water ingestion on the excretion of Creatine and Creatinine.

Day.	Urine Amount. c.c.	Total Nitrogen. grams.	Creatinine. grams.	Creatine. grams.	Remarks.
1.	1930	39.82	1.660	-	prescribed diet.
2.	1980	40.24	1.480	-	"
3.	2180	42.19	1.563	-	"
4.	2960	43.88	1.554	-	"
5.	7400	40.19	1.533	-	9.6 litres water.
6.	2200	34.69	1.588	-	diet
7.	2110	38.19	1.650	-	"
8.	2040	40.22	1.604	-	"
9.	8370	40.79	1.586	-	9.6 litres water.
10.	8500	40.57	1.459	-	"
11.	8070	39.74	1.547	-	"
12.	2440	34.16	1.587	-	diet.
13.	2150	36.34	1.605	-	"
14.	2160	36.89	1.619	-	"
15.	2300	40.39	1.642	-	"

The results of the foregoing experiments are summarised below

TABLE XLII. Showing Summary of Water Ingestion Results.

Exp.	Average excretion of Creatinine on non water days.	Average excretion of Creatinine on water days.	Creatine.	Water Ingested.
1	1.406	1.366	-	3 litres.
2	1.370	1.360	-	3 litres.
3 A.	1.605	1.533	-	9.6 litres.
3 B.	1.605	1.531	-	9.6 litres.

CREATININE

CREATININE: There is a constant decrease in the amount of Creatinine excreted on the days of excessive water ingestion, a result which accords with that obtained with Hawke and Fowler (l.c.). On these days however, the bulk of the urine is enormously increased and the Creatinine is consequently in great dilution. To enable the quantity to be estimated by the Folin method, either the sample taken must be increased, or the dilution after development of the colour must be decreased. In Experiments I. and II. the latter expedient was adopted, dilution being carried to 250 c.c. instead of to 500 c.c. as on the non-water days. In Experiment III. as the quantity of urine on the water days was about three times that on the non-water days, 30 c.c. of urine was taken instead of 10 c.c. In Part I. of this thesis, it is shown that both of these deviations lead to error, causing an apparent reduction in the amount of creatinine found. It will be noticed that the reduction of Creatinine is in proportion to the dilution of the urine. Thus in Experiment III. where the urine excreted exceeded 8 litres, a reduction of .072 grams is found. In Experiment I. and Experiment II. where the amounts never reach 4 litres, the reduction is in Experiment I. .04 gram and in Experiment II. .01 gram.

It is believed that not only does the dilution of the urine account for the apparent reduction of Creatinine but

but in view of the amounts of reduction produced by this method as found in Part I. a positive increase of Creatinine would be registered, if experiments were conducted in which the dilution of the urine were maintained a constant throughout the whole series of analyses.

CREATINE: In addition to the routine estimation of Creatine by Folin's Method by which none was found, the urine was tested for Creatine by the direct Diacetyl Method of Walpole (l.c.) No trace could be discovered either on water or on non-water days.

It is concluded that - EXCESSIVE WATER INGESTION CAUSES NO EXCRETION OF CREATINE AND THE DECREASE IN CREATININE WHICH HAS BEEN NOTED IS PROBABLY FICTITIOUS.

SECTION VII.

DISCUSSION OF RESULTS OBTAINED IN SECTIONS I. to VI.

Connection between Creatine and Carbohydrate.

Cathcart (l.c.) in 1907 observed the presence of Creatine in starvation, an observation which was made about the same time by Benedict (l.c.) Benedict and Diefendorf later found Creatine in the urine of starving insane women. These results have been repeatedly confirmed in animals by various workers. Pushing the investigations further, Cathcart found that in inanition in man, the output of Creatine was unaffected by feeding with protein or fat, but that it ceased on taking carbohydrates. Mendel and Rose, whose experiments are virtually a repetition of these, found that in the rabbit the Creatinuria of starvation ceased on feeding dextrose or starch, but continued on feeding protein, fat or alcohol, or a combination of these. Excretion of Creatine has also been found in Diabetes Mellitus and Phloridzin Diabetes. Cathcart and Taylor (l.c.), Wolf and others.

Graham and Poulton (l.c.) made objection to these findings on the grounds that the presence of Creatine was fictitious, and due to the generation in those conditions of Acetone bodies, which cause an error of analysis. The effects of these bodies were investigated in Part I. and Greenwald's

Greenwald's observation as to the error confirmed.

In part II. Section I, the effects of inanition on the excretion of Creatine was investigated afresh, the error due to the acetone bodies being guarded against. The results show that the Creatine excreted is less in amount and later in appearance than was formerly thought, but that the original observation of Cathcart is substantially correct - Creatine is excreted in starvation. The accuracy of the findings as to the excretion of Creatine in Phloridzin Diabetes was also investigated, the error caused by acetone bodies being again guarded against, and the original observation as to the presence of Creatine in the urine confirmed. It is impossible that the Creatine found could be due to the acetone bodies, as the sum total of Creatine plus Creatinine is increased, whereas, in the error caused by acetone bodies, any increase in Creatine must be balanced by a decrease in the Creatinine.

In the Selenium experiments it was seen that privation of carbohydrates in the tissue is caused by the ingestion of Sodium Selenite and causes an excretion of Creatine in inverse proportion to the amount of carbohydrate previously fed. It is further shown that in Thyroidism and Acromegaly where there exists some influence adversely affecting the utilisation

utilisation of carbohydrates by the tissues, Creatine appears.

The accumulated evidence undoubtedly confirms the view that an inadequate supply of carbohydrate or inability of the tissues to use the carbohydrate leads to the excretion of creatine in the urine.

The results of Section II. show that in the goat, where Creatine appears as a normal urinary constituent, the cessation of lactation which leaves for utilisation by the tissues an excess of carbohydrate equivalent to the amount formerly excreted in the milk, produces a reduction in the Creatine output, and that in increasing the carbohydrate content of the food the output is diminished in proportion to the increase.

The impossibility of ascribing the excretion of Creatine, to the breaking down, or the retention of Creatine, to the building up of muscular tissue in the body, has been shown by analysis of the results in Sections I. and II. page 33 and 44. In the goat it was shown that on the higher protein diet where there would be least tendency to break down protein tissue, the creatine excretion per day is 1.16 grams., and that on the low protein and high carbohydrate diet where there is a loss of weight of 2 kilos. and as calculated from nitrogen excreted a loss of protein tissue of over 1 kilo, the

the Creatine output decreased to zero. It is evidently not the case that the excretion of creatine and carbohydrate privation can be ascribed solely to flesh catabolism causing a break down of muscle tissue and an excretion of the contained Creatine, or obversely, that carbohydrate prevents the excretion of Creatine where it normally occurs by causing a building up of Creatine containing tissue. While the view admittedly contains an element of truth which explains the excretion of Creatine observed in conditions such as wasting fevers, Leathes, muscle degeneration, Schaffer, involution of the uterus, Murlin, it obviously fails to cover all the facts as now presented.

The more reasonable assumption, which accounts for all the results here obtained, is that in normal conditions the metabolism of Creatine is intimately connected with that of carbohydrate, the utilisation of the two being concurrent and interdependent.

If it be assumed at this stage, that there occurs a continuous production of creatine in functioning muscle, (grounds for which are adduced later), the results would lead to the suggestion that, where the production is in excess of the amount of carbohydrate available for its utilisation, it is excreted, and in cases where there is a continuous excess and

and excretion, increase of the carbohydrate leads to its utilisation and disappearance from the urine

The Connection between Creatine and Muscle Activity.

In Section IV. it is shown that the weight of opinion in the literature on the subject is in favour of the view that work increases the Creatine content of muscle and increases the "total creatine" output, i.e. Creatine plus Creatinine, in the urine. In that Section also there is recorded data to show that thirty minutes' electrical stimulation of an "in situ" muscle whose blood vessels and lymph spaces had been washed out with an isotonic salt solution, increased the creatinine content from .312% to .348%: and heart perfusion experiments showed that the beating heart when fed only with salt solution excreted Creatine into the perfusing fluid. There are also recorded various experiments whose results give evidence that in bodily exercise, which in energy expended exceeds the energy equivalent of the food taken, there appears Creatine in the urine. Together with the findings of previous workers to which reference has been made, these are considered as affording sufficient data for the conclusion that Creatine is a product of muscle activity.

The results recorded in the literature afford ample evidence

evidence of the connection thus existing between Creatine and Work. An analysis of Beker's investigations as to the Creatine content of various organs shows that Creatine is situated throughout the body in those places where the liberation of energy occurs, and in amounts proportionate to the ability of the tissue to liberate energy rapidly. Thus voluntary muscle contains from .30% to .45%; heart muscle from .21% to .25%; involuntary muscle about .03%. In rabbits in the white (i.e. the rapidly contracting) muscle there is .45%, and in the red (the slowly contracting muscle) the amount is .33%.

Not only do the different organs vary in Creatine content, but the same organ may vary at different times in proportion as to the amount of work being done, as in the case of the uterus. The non-pregnant uterus is comparatively inert. As pregnancy proceeds, tonic contractions at the rate of one every few minutes occur, of such intensity that in the later months they can be felt by the palpating hand on the abdominal wall, and are regarded as diagnostic of a pregnancy as opposed to a tumour. During the course of the pregnancy in the human, the creatine content of the muscle rises from .045% to .077%. In the bicornuate uterus of the cow, the non-pregnant horn increases in size, but it may be presumed

presumed that the tonic contractions there are less than in the pregnant horn. The non-pregnant cow's uterus contains .037% Creatine: in pregnancy the non-pregnant horn contains .060% and the pregnant horn .084%.

In the foetus, and after birth, the appearance of Creatine in the muscle corresponds with the incidence of muscular activity in the animal. In the embryo pigeon of 225 m.m. Mendel and Leavenworth found .03% as against .45% in the adult. The chick immediately on hatching begins to run about and pick. Mellanby showed that the total Creatine of the chick increases from 13 to 23 m.grm. on the day of hatching, and on the third day it amounted to .2% of the muscle. By the 14th day it had reached .29%, i.e. practically adult proportion (.31%).[x]

The rabbit on the other hand, is comparatively inactive for several weeks. The percentage of Creatine in the rabbit's muscle at various ages are - 7 days .19%, 19 days .32%, adult .43% (Mellanby, l.c.) Myers and Fine give the following figures in the kitten where active movements develop slowly: 2 weeks .22%, 6 weeks .34%, 7 weeks .47%.

The

[x]Mellanby connected the sudden increase in Creatine content with rapid growth of the liver. Noël Paton in the fowl, and Foster and Fisher, and London and Boljarski in the dog, cut the liver out of the circulation by an Eck's fistula and found the metabolism of Creatine and Creatinine practically unaltered. The rapid growth of the liver is more likely to be connected with the sudden demand for the exercise of its glycogenic functions as the chick feeds as soon as hatched.

The same connection between activity and Creatine content of muscle is seen in different species of animals: thus in the lethargic hibernating hedgehog the percentages are in October .197%, in July .230%: in the pig .31% and in the rabbit .45% Mellanby (l.c.).

Various interpretations have been put upon these results by their authors, but viewed unitedly they all point to a genetic connection between Creatine and Muscular Activity.

The Metabolism of Creatine and Carbohydrate connected in Muscle Activity.

So far two main conclusions have been reached, viz. (1) that Creatine is constantly produced by active muscle, and (2) that Creatine so produced is utilised (and fails to appear in the urine) in proportion to the amount of carbohydrate consumed by the tissues.

The consumption of carbohydrate may lead to either liberation of energy or production of heat. In fevers, Creatine is excreted in considerable amounts, as shown by Leathes and others. Van Hoogenhuyze and Verploegh, doing work in another connection, made estimations of the Creatine and Creatinine excreted during oxygen inhalation. In Verploegh's case there appears in the urine on both occasions of his oxygen inhalation .014 gram. of Creatine. On no other day was creatine present

present. The oxidation of Glucose in the production of heat either in oxygen inhalation or in fevers, apparently fails to utilise the Creatine. It would appear, therefore, that the point in the metabolic field, where the utilisation of carbohydrate and of creatine coincide is the liberation of energy.

The heart perfusion experiments confirm this finding. In the beating heart perfused with sugar-free Tyrode's solution, there is an excretion of Creatine: when glucose is added to the solution no excretion occurs. Account has already been taken, p.55 and p.95. of the possibility of the view that the Creatine excreted in the absence of carbohydrate might be due to the catabolism of flesh used as a source of energy. An additional argument may be added here. In the isolated muscle experiment the Creatine content rose from .312% to .348% an increase of over 10%. These figures agree rather closely with those found by Cathcart and Brown whose increases varied from 7 to 15%. If it be taken that the Creatine produced in contraction is that liberated from tissue catabolised, one is forced to conclude that 30 minutes' interrupted work is sufficient to cause the destruction of one-tenth of the body of muscle performing the work. That muscle would continue to contract under those suicidal conditions is unbelievable. Even in excessive work where Creatine appears

appears in the urine the increase in output of total nitrogen is either very small or entirely absent.

The only possible deduction that seems permissible by the facts available, is that on muscle contraction there is a production of Creatine which is utilised in further contraction, provided the energy be supplied by carbohydrate.

The conclusion to which the foregoing argument leads is that - THE FUNCTION OF CREATINE IN THE ECONOMY IS INTIMATELY CONNECTED WITH THE LIBERATION OF ENERGY FROM CARBOHYDRATE IN MUSCLE CONTRACTION AND THAT MUSCULAR ACTIVITY MAKES CONTINUOUS PROVISION FOR FUTURE LIBERATION OF ENERGY BY THE FORMATION OF CREATINE.

Collateral Evidence in support of this Conclusion:

If the theory enunciated be correct, in deficiency of carbohydrate, Creatine should accumulate in the tissues up to a saturation point and then be excreted. Demant showed that in the pigeon in starvation the Creatine content of muscle is increased. Mendel and Rose found in the fowl, that the content on starvation rose from .411% to .495%, and in the rabbit from .498% to .571%. In Section II. it is shown that in privation of carbohydrate, when fat or protein must be drawn upon as sources of energy, the excretion

excretion of creatine is in direct proportion to the degree of privation engendered.

Again, if fat and protein be with-held in the diet so that all the energy must be derived from carbohydrate there should follow a utilisation of creatine in excess of the normal production, which would occasion a decrease in the Creatine content of muscle. Myers and Fine fed a rabbit upon a pure starch diet. The Creatine content fell from .52% to .48%. In the goat we found that on an excessively low fat and protein diet when the animal was forced to live exclusively on carbohydrate, an excess of Creatine excreted previously in the urine decreased to zero.[x].

Connection of the Liver with Creatine Metabolism:

In view of the number of authors, Mellanby (l.c.) Van Hoogenhuyze and Verploegh (l.c.) and others, who assume that the liver is the chief organ connected with the metabolism of Creatine, it is necessary in putting forward the above theory as to the origin and function of Creatine to discuss the

---

[x] The excretion of Creatine in the goat may be due to its confinement. Its natural habitat is the hills and rocks where it leads a very active life, for which doubtless its muscular metabolism is adapted and provision made for the production of creatine in proportion to the muscular exercise indulged in. In the rabbit kept in hutches the excretion of Creatine may be due to the same cause. Van Hoogenhuyze & Pekelharing (l.c.) ascribed it to a conversion of Creatinine to Creatine due to the alkalinity of the urine, That this is fallacious appears on page 49 where it is seen that the alkalinity or acidity of the goat's urine has no appreciable influence on the amount of Creatine excreted.

the influence of this organ.

Mellanby (l.c.) found that in the chick the growth of the liver coincided in time with the increase of Creatine in the muscle, and that pathological conditions affecting the liver function influenced the Creatine excretion. Upon the first of these findings a different interpretation has already been put and the second is discussed below.

The belief of the other workers rests upon two different sets of observations. The first is that made by Gottlieb and Strangassinger, who alleged they had found in the liver and to a lesser degree in other organs, a number of different enzymes which acted in various ways upon Creatine and Creatinine. This work was repeated by Rothman, who found in autolysis of liver, a conversion of Creatine to Creatinine. He admits, however, that some of his results were discarded owing to the evident presence of bacterial action. Rose claimed to finding a "Creatinase" in the thyroid gland.

Valid objections to the acceptance of the above results are offered by Mellanby (l.c.), who showed that the chemical conditions and the presence of bacteria accounted for the changes observed. Gottleib and Strangassinger claim that urine also contains the enzymes. The experience of the Author is that urine may be kept for 72 hours at 37 degrees

degrees C. or for at least three weeks at room temperature without any change taking place in the contained Creatine or Creatinine, provided they be neutral and kept antiseptic by the presence of a small quantity of thymol dissolved in chloroform. If however they be made acid, a slow conversion of Creatine to Creatinine takes place, and if decomposition sets in, as indicated by the ammoniacal smell, both bodies rapidly disappear.

Even if Creatine be added to an organ or an extract, and after several days' incubation at body heat Creatinine be found, it is a gratuitous assumption to claim that there has been a conversion of Creatine to Creatinine. In Section V. it was shown that the ingestion of meat caused a very distinct rise in the Creatinine output which was quite unaffected by the ingestion of Creatine. The possibility of the derivation of Creatinine from some other constituent of the tissue (probably a common precursor of both Creatine and Creatinine) has to be considered. It is interesting that an analysis of Rothman's results show that on one occasion he obtained almost twice as much Creatinine as could be accounted for by the Creatine originally present.

Mellanby (l.c.) was unable to confirm the results of Gottleib and Strangassinger, and Dakin could find in the extract

extract of liver, kidney and duodenal mucous membrane no ferment acting upon Creatine or Creatinine.[x]

Until the presence of "Creatinase" has been repeatedly proved under conditions that prohibit the possibility of bacterial action, with controls that undergo identical chemical treatment, and using Creatine which has been proved free from Creatinine (a neglected precaution), the real existence of a Creatine-splitting enzyme must be considered doubtful. In any case the utmost caution should be exercised in drawing conclusions from such experiments, as it has not been established that the chemical changes that occur in an isolated organ in process of dissolution are analogous to those that take place in the living organ, where the chemical changes act in connection with, and under the influence of, what may be regarded as a resultant of the sum total of the activities of every organ in the body. The influence which the liver exerts on the metabolism of Creatine by means of an alleged "Creatinase" may be disregarded until definite proofs of its existence are forthcoming.

The

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xSince the above was written there appeared a paper by Folin and Dennis in which they state that "The various hypothetical ferments of Gottleib and Strangassinger for the transformation of Creatine to Creatinine into one another as well as for their destruction - in so far as they have any existence at all-represent post mortem changes."

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The other series of experiments that connect the liver and Creatine consist of a vast amount of work that shows that in any pathological condition affecting the liver there occurs an excretion of creatine in the urine. With these conditions have been classed starvation, and it has been stated that the reason Creatine appears in starvation is, that the liver becomes inactive in all its functions because the glycogenic one is in abeyance (Mellanby, Van Hoogen-huyze). Noël Paton on the bird, Foster and Fisher and London and Borjarski on the dog, cut out the liver from the portal circulation by an Eck fistula and allowed the nutriment from the alimentary canal to pass direct to the tissues. The excretion of Creatine and Creatinine were little affected, showing that the liver is neither the sole, nor even the chief, organ connected with the metabolism of these bodies.

In so far as the liver is at once the gate-house and the store-house for carbohydrates, and it has been shown that the utilisation of Creatine is dependent quantitatively upon that constitution of the food, any circumstance whatever that depresses its glycogenic function is calculated to influence adversely the utilisation of carbohydrate by the tissues - the very condition in which it has been shown in Sections I. and III. Creatine appears in the urine. The amount

amount of work therefore, that proves the excretion of Creatine in cases where the glycogenic function of the liver is upset - e.g. in liver neoplasms Mellanby (l.c.) in chloroform poisoning Rowland and Richards, in cyanide poisoning, Richards and Wallace, in Hydrazine poisoning, Macadam, in Phloridzin poisoning, Graham Lusk; is simply an accumulation of collateral proof of the intimate connection that exists between the metabolism of Creatine and Carbohydrate.

CONCLUSION.

While it is admitted that the theory as to the function of Creatine enunciated in this thesis must undergo extensive modification and probably complete alteration as new facts arise, it is claimed that it co-relates and affords an explanation for all the varied accepted results obtained by the different workers and offers a hypothesis and a basis for future investigation of the origin, function, and fate of Creatine and Creatinine which is one of the most important and certainly one of the most fascinating of the problems of metabolism that await solution.

APPENDIX.

THE METABOLISM OF CREATININE.

From the results of experiments recorded in Sections I. to VI. some interesting deductions can be drawn relative to the Metabolism of Creatinine. As they do not affect the main argument of this thesis, they are simply stated here without discussion.

Throughout the experiments on the human the protein intake varies from 58 grams. to over 300 grams per day, without any relative variation in the Creatinine excreted. In the goat, where the protein intake decreases from 200 grams to 2 grams per day, the Creatinine excretion remains practically identical (Table XXV.) Any variation is in the direction of a slight increase on the low protein diet. In the dog, following on the injection of the Sodium Selenite there is a breaking down of protein tissue, as shown by the great increase in the excretion of total nitrogen. The Creatinine excreted shows no corresponding increase. This constancy in the Creatinine output under those very diverse circumstances shows that the production and excretion of Creatinine is dependent on some special metabolic process which is independent of the catabolism of the protein molecule whether derived

derived from food or tissues.

In the bodily exercise experiments Nos.1 and 2, a very slight increase of Creatinine is observed on the work days. On these days the amount of work must have been several times that performed on the other days, and out of all proportion to the increase of Creatinine, which is more probably dependent upon some concomitant factor, e.g. increased temperature or blood pressure, or the acceleration of some process caused by them. In the heart perfusion experiments no Creatinine could be detected in the perfusing fluid on any occasion. It is evident that the formation and excretion of Creatinine are not directly dependent upon muscular activity.

After the injection of Selenite in the dog there is liberated in the tissues and excreted in the urine a larger quantity of Creatine which leaves the Creatinine excretion practically unaffected. In the goat, Table XXV. when 1.16 grams of Creatine are being excreted daily, the excretion of Creatinine is .86 grams per day, and when the Creatine output decreased to zero the Creatinine output suffers a slight increase to .96 grams per day. Together with the observations made in Section V. that Creatine taken by the mouth has no influence in increasing the Creatinine excretion

excretion these tend to subvert the view that Creatinine is derived from Creatine or can be formed from Creatine in any way analagous to the formation of Urea from Ammonia. If Creatinine is derived from Creatine, between the two there lies some special and constant metabolic process which is neither accelerated nor retarded by variation in the amount of protein metabolism or of muscle contraction.

One other observation is of interest in view of the work done by Spriggs, Shaffer, Pekelharing and others, which seems to indicate that Creatinine is in some way connected with the condition of the efficiency of muscle. In Tables XXXI. and XXXII. the average daily excretions of Creatine are about 1.60 and 1.36 grams. respectively. Prior to the date of Table XXXI. there occurred a period of about six months outdoor life involving an amount of muscular exercise. The data of Table XXXII. is fifteen months later. The intervening period was almost completely occupied in laboratory or sedentary work, reading &c., during which there would come about a deterioration in the muscular efficiency.

That the metabolism of Creatine and Creatinine are quite distinct appears abundantly evident. On the other hand their connection is manifested by the close resemblance of their

their chemical structure; by their connection in function with muscle; and by the notable fact that in birds Creatine replaces Creatinine in the urine. (Noël Paton, l.c.).

It is possible that both may originate from a common precursor, which in view of the results obtained by the group of workers represented by Achelis , Seeman, and Inouge, is most probably some body containing the guanidin nucleus, and it may be, that in the bird with a more primitive form of metabolism Creatine represents both the Creatinine and the Creatine found in the urine of mammals, in which by a further differentiation, part of the common precursor is diverted to some special constant metabolic process by which Creatinine is formed, the rest of the precursor forming Creatine whose metabolism has been herein discussed.

SUMMARY OF RESULTS.

PART I. ON THE USE OF FOLIN'S METHOD:

(1) Acetone in quantities exceeding 3% and Diacetic Acid in minute quantities cause a decrease in the apparent amount of Creatinine and a corresponding increase in the amount of Creatine in the urine.

(2) The optimum time for development of the colour lies indifferently between 6 and 10 minutes for room temperature.

(3) In a dilute urine, unless special precautions be taken, the amount of Creatine found tends to be too low.

PART II. THE METABOLISM OF CREATINE.

Section I. Privation of carbohydrate induced by (a) Injection of Sodium Selenite, (b) Phloridzin poisoning, or (c) Starvation, causes a true excretion of Creatine.

Section II. In the goat in confinement there occurs a constant output of Creatine which varies inversely with the amount of carbohydrate rendered available for metabolism.

Section III. Pathological conditions as thyroidism and acromegaly which affect adversely the tissue of carbohydrate metabolism produce an excretion of Creatine.

Section IV. Work in muscle increases the Creatine content. Excessive work leads to the appearance of Creatine in the urine

urine on the day of work and the following day.

In the perfusion of the heart if Tyrode's solution be used without sugar the perfusing fluid accumulates Creatine; if sugar be added no Creatine appears.

Section V. The ingestion of meat causes an increase in the Creatinine excretion which is not brought about by the ingestion of Creatine.

Section VI. Creatine is not flushed out of the body in excessive water drinking. The decrease of Creatinine which has been found is most probably fictitious.

Section VII. It is suggested that Creatine is a product of muscle contraction, and that the metabolism of Creatine and of Carbohydrate are interdependent in the liberation of energy for muscle contraction.

APPENDIX:

I. The excretion of Creatinine is not directly dependent upon the amount of protein catabolised or work performed.

II. Creatinine is not derived directly from Creatine.

III. It is suggested that while there exists a definite connection between these bodies which may be derived from a common precursor the function and metabolism of each is distinct.

REFERENCES.

1. Neubauer. Anal. des Harns.
2. Myers. Amer. Jour. of Med.Sc. Vol.CXXXVI. p.256, 1910.
3. Meisser. Zeitschr. f. Physiol. Chem.Vol.LXXXVI.p.415, 1913.
4. Liebig. Ann. d. Chem. u.Pharm. Vol.LVII. p.257, 1847.
5. Mallet. Bulletin No.66 U.S. Dept.of Agr. 1889.
6. Meissner. Zeit. f.Rat. Med. Vol.XXXI, 1866.
7. Folin. Amer.J.Phys. Vol XIII. p.44, 1905.
8. Folin. Amer Jour. Phys. Vol.XIII p.66, 1905.
9. Noël Paton. Jour. Phys. Vol.XXXIII. p.6 1905.
10. Folin. Zeit. f. Phys. Chem. Vol.XLI. p.223, 1904.
11. Jaffe. Zeit. f. Phys. Chem. Vol.X. p.391, 1886.
12. Thomson. Proc. Internat.Congress of Med. London, 1913.
13. Chapman. The Analyst, Vol.XXXIV. p.475, 1909.
14. Van Hoogenhuyze & Verploegh, Zeit.f.Phys.Chem.Vol.XLVI.p.415.'05
15. Mellanby. Jour.Physicl.Vol.XXXVI. p.447, 1908.
16. Taylor. Biochem.Jour. Vol.V.p.362. 1911.
17. Greenwald. Jour.Biol.Chem. Vol.XIV.p.87, 1913.
18. Thomson. Wallace & Clotworthy. Biochem.Jour.Vol.VII.p.445.1913
19. Krause. Quart.Jour.Exper.Physicl.Vol.III.p.3, 1910.
20. Graham & Poulton. Proc.Royal Soc.Vol.LXXXVII. p.205, 1914.
21. Klercker. Zeit. Biochem.Vol.VIII.p.59, 1906.
22. Van Hoogenhuyze & Verploegh. Zeit.Physicl.Chem.Vol.LVII.p.161'08
23. Rose. Jour.Biol,Chem. Vol.XII.p.73. 1912.
24. Wolf & Osterberg. Amer.Jour. Physiol. Vol.XXXVIII p.71.1911.
25. Dorner. Zeit.Physicl.Chem. Vol.LII.p.225, 1907.
26. Gino Frontali. Arch.Inter.Physicl.Vol.XIII.p.431, 1913.
27. Walpole. Jour.Physiol. Vol.XLII. p.301. 1911.
28. Folin. Hammarstens Festschrift, 1906.
29. Jones. Biochem. Jour, Vol.IV. p.405, 1909.
30. Taylor. Brit. Med.Jour. Vol.XIII. p.43, 1910.
31. Taylor. Biochem.Jour. Vol.V.p.362, 1910.
32. Krause. Quart. Jour.Exper.Physiol. Vol.III. p.3, 1910.
33. Taylor & Cathcart. Jour.Physiol. Vol.XLI. p.276, 1910.
34. Wold & Osterberg. Amer.Jour.Physicl.Vol.XXVIII.p.71,1911.
35. Krause & Cramer. Proc.Physicl.Soc. July 1910.Jour.Physicl.Vol.XL
36. Cathcart. Biochem.Zeit. Vol.VI. p.109, 1907.
37. Cathcart. Jour Physicl. Vol.XXXV. p.590, 1907.
38. Benedict. Carbegie Insti.Report. Washington ;1907.
39. Hawk, Howe & Wathill, Jour.Amer.Chem.Soc .Vol.XXXIII. p.568,'11
40. Lindsay. Biochem.Jour. Vol.V.p.407, 1911.
41. Mellanby. Proc. Royal Soc. Vol.LXXXIII. 1913.
42. Krause. Quart.Jour.Exper.Physiol. Vol.IV.p.3, 1911.
43. Von Noorden. Metabolism & Practical Medicine. 1907.
44. Greenwald. Amer.Soc.Physicl. Vol.XXVIII. p.103.
45. Krause & Cramer. Proc.Physicl Soc. May 1912.
46. Murlin. Amer.Soc.Physicl.Vol.XXVII. p.422.

REFERENCES contd.

47. Froschbach. Arch.f.exp.Path. u.Phys. Vol.LVIII. p.113, 1907
48. .Murlin. Amer Soc.Physiol. Vol.XXIII. 1908.
49. Krause & Cramer. Proc.Physiol.Scc. July 1910.
50. Van Hoogenhuyze. A ten.Doeschate. Annal.de Gynea.et d'Obstet.
51. Sczelkow. Centrall.f.d.Med.Wiss. p.481 1866 (cit in 14)
52. Sarakow. Arch.f.Path Anat. XXVIII.p.544 1863.
53. Monari. Arch. ital.de Biol. Vol.XIII.1890 (Cit.14)
54. Nawrocki. Centrall. f.d.Med.Wiss. p.416, 1896 (cit.in 14)
55. Voit. Zeit.f.Biol. Vol.IV. p.77, 1868.
56. Brown & Cathcart. Biochem.Jour.Vol.IV.p.420, 1909.
57. Graham,Brown & Cathcart. Proc.Physiol.Soc.March.1908.
Jour.Physiol. Vol.37.
58. Myers & Fine. Jour.Biol.Chem.Vol .XVII.p.65, 1914.
59. Grocco. Malay's Jahresbericht. Bd.VI.p.179,1889 (cit.14)
60. Moitessier. Thesis Montpelier. 1891 (Cit.in 14)
61. Gregor. Zeit.f.Phys.Chem. Vol.XXVI. p.78. 1900.
62. Oddi and Tarulli. Malay's Jahresbericht. Vol.XXIV. p.542.
1895, (cit.in.14)
63. Dunlop, Paton, Stockman and Macadam. Jour Physiol.Sept.1897.
64.Hoffmann. Anal f.Path. Vol.XLVIII p.358½ 1869 (cit.14)
65. Pekelharing. Zeit.f.Phys.Chem. Vol.LXXV. p.207, 1911
66. Weber. Arch.f.exp.Path.u.Phys. Vol.LVIII p.93. 1908.
67.Gunn. Jour.Physiol. Vol.46. p.506 1913.
68. Myers & Fine. Jour.Biochem. Vol.XVII.
69.Czernecki. Zeit.f.Physiol.Chem.Vol.XLIV.p.296. 1905.
70. Lefman. Zeit.f.Physiol.Chem. Vol.LVII. p.476. 1908.
71. Pekelharing & Van Hoogenhuyze. Zeit.Physiol.Chem LIX.p.101
1909.
72. Plimmer Dick. & Leib. Jour.of Physiol.Vol.XXXIX.p.101.1909
73. Voegstin & Fowler. Jour.Biochem.Soc.Vol.X.p.429, 1911.
74.Hawk & Fowler. Jour.Exper.Med. Vol.XII.p.385. 1910.
75. Benedict & Diefendorf. Amer Jour.Physiol. Vol.XVIII.p.263.1907
76. Cathcart. Jour.Physiol. Vol.XXXIX. p.311, 1909.
77. Mendel & Rose. Jour.of Biol.Chem. Vol. X.p.213, 1911.
78. Leathes. Jour.Physiol.XXXV. p.205, 1906.
79. Schaffer. Amer.Jour.Physiol.XXIII, 1905.
80. Schaffer. Jour.Biol.Chem. Vol.III. XIII, 1907.
81. Beker. Zeit.f.Physiol.Chem. Vol.LXXXVII p.27, 1913.
82. Mendel & Leavenworth. Amer.Soc.of Physiol. Vol.XXI.p.95 1908
83. Van Hoogenhuyze & Verploegh. Zeit.Physiol.Chem.Vol.LIX.
p.61. 1909.
84. Demant. Zeit. Physiol.Chem.Vol.XXXIII.p.81. 1879.
85. Meldel & Rose. Jour.Bio.Chem.Vol.X.p.213.1911.
86. Myers & Fine. Jour.of Biol.Chem. Vol.XVII.
87. Gottleib & Strangassinger. Zeit. f.Physiol.Chem.Vol.LII.1907
88. Rothman. Zeit. Physiol.Chem. Vol.LVII. p.131, 1908.
89. Rowe. Amer.Jour.Physiol. Vol.XXXI.p.169, 1912.
90. Dakin. Jour.Biochem. Vol.I.p.221, 1906.
91. Noel Paton. Jour.Physiol. Vol.XLV. p.115, 1912.
92

REFERENCES contd.

92. Foster & Fisher. Jour.Biochem. Vol.IX.p.359, 1911.
93. London & Borjarski. Zeit.Physiol.Chem.Vol.LII.p.465.
94. Rowland & Richards. Jour.Exper.Med. Vol. IX. p.344.1909.
95. Richards & Wallace. Jour.Biol.Chem.Vol.IV. p.129, 1908
96. Macadam. Jour.Path.and Bact. Vol.XVIII. p.281. 1913.
97. Graham. Amer.Soc.Physiol. Vol.XIX. p.461, 1907.
98. Spriggs. Biochem. Soc. Vol.II. p.206. 1907.
99. Pekelharing. Zeit. Physiol.Chem. Vol.LXXV. p.207. 1911.
100. Achilis. Zeit. Physiol.Chem. Vol.L. p.10 1906.
101. Seeman. Zeit. Physiol. Vol. XLIV. p.259, 1905.
102. Inouge. Zeit. Physiol. Vol. LXXXI. p.71, 1912.