A TREATISE

On

THE EXCRETION OF CREATIN AND CREATININ.

Presented for the Ellis Prize

By

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C O N T E N T S.

CREATIN AND CREATININ.

Since the discovery of creatin in muscle a large amount of work has been done with the object of establishing its relation to the creatinin in urine. Many theories have been set forth. Of these one of the most important is that of Otto Folin.

In 1905 this observer determined approximately the analyses of 30 normal urines, and from the results so obtained he set up a new theory showing that the proteid metabolism consists of two parts, viz :- an endogenous or true tissue metabolism, and an exogenous catabolism, consisting of a series of hydrolytic splittings, resulting in a rapid elimination of proteid nitrogen as urea, a certain amount of oxidation also being present.

Creatinin is an excretory product resulting from the endogenous catabolism. This substance was shown by Folin to be constant as regards its elimination in the urine, and was, therefore, considered an ideal substance in the investigation of /

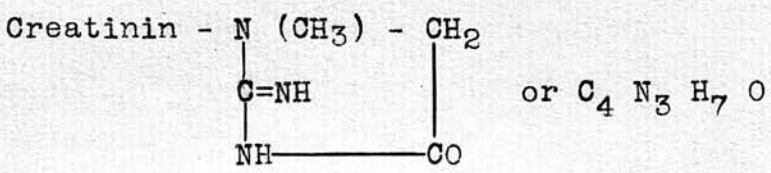
of the different influences affecting the endogenous metabolism. Attention has been directed to creatin, which, owing to its chemical relation to creatinin, and its constant presence in the muscles of vertebrates, was considered a substance useful in the study of proteid metabolism, as well as in its relationship to creatinin in the economy of the body, and how it is affected by physiological and pathological conditions.

The present writer, having investigated these substances variously, herewith presents an historical account of the literature on the subject as it stands, together with some results of his own research.

A Bibliography is appended.

CHEMISTRY/

CHEMISTRY OF CREATININ AND CREATIN.



First found by Liebig (2) in the crystalline deposit which Heintz and Pettenhoper (3) got from a concentrated urine treated with zinc chloride solution.

Creatinin, as such, separates out from a hot saturated solution in colourless, shining, anhydrous prisms (monoclinic), and has a pungent taste. From a cold saturated solution it separates out in large plates or prisms with two molecules of water of crystallisation, which it easily loses. It is easily soluble in hot water, 11.5 parts in cold, 102 parts in absolute alcohol (easier in hot) and very little in ether. Slightly alkaline reaction.

Creatinin has a reducing action, reducing mercuric oxide to metallic mercury, and at the same time one gets oxalic acid and methyguanidin formed. It has a disturbing influence on Trommer's test for sugar, partly as it reduces the copper hydrate in alkaline solution, and partly because it keeps the resulting cuprous oxide in solution. According to

Bang/

Bang seven parts of creatinin have the same reducing properties as 4.8 parts of glucose. On the other hand an alkaline bismuth solution is not reduced, and so Nylander's reaction for sugar is not interfered with by the presence of creatinin in the urine.

In alkaline solution it is supposed to be converted into creatin even in the cold.

When heated with bariumhydrat, creatinin splits up into ammonia and methylhydantoin

Salts of Creatinin

(1) Creatinin - zinc - chloride $(C_6H_2N_3O)_2$ Zn Cl_2 Got on adding a neutral concentrated alcoholic solution of zinc chloride to a concentrated watery or alcoholic solution of creatinin. After some time the compound so formed separates out as a crystalline powder. In a few days the precipitate is taken and washed with rectified spirit. The compound is recrystallised by dissolving in hot water. It is sparingly soluble in cold water. To set free the creatinin, dissolve the compound in hot water and boil for half-an-hour with lead carbonate. Filter hot, decolorise with animal charcoal/

coal and concentrate. Creatinin zinc chloride dissolves easily in hydrochloric acid, from which solution it is precipitated by sodium acetate.

(2) Creatinin Chloride - C_6H_7NOHCl

Easily soluble in water. Contains 1 mol, of water of crystallisation when slowly crystallised from water. More difficult to dissolve in alcohol.

(3) Creatinin Platinate - $(C_4H_7N_3O)_2$, $2HCl$, $PtCl_4$

Orange red prisms or needles, easily soluble in water, with difficulty in alcohol. Crystallises from water with two molecules water of crystallisation.

(4) Creatinin Gold Chloride - $C_4H_7N_3O \cdot HCl$, $AuCl_3$

Fairly easily soluble in water and in alcohol. Melting point 170 - 174°C.

(5) Creatinin - Picrate - $C_4H_7N_3O, C_6H_3N_3O_7$

Dissolves with difficulty, has no water of crystallisation, and can be used for separating out large quantities of creatinin. Melting point 212 - 213°C.

In 1886 Jaffe (1) whilst testing the action of picric acid on human urine found that the precipitate consisted of a part insoluble in hot water (uric acid), and a part which was soluble, consisting mainly of creatinin as a double salt, which he determined consisted of creatinin picrate and potassium picrate.

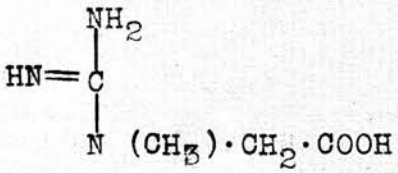
$(C_4H_7N_3O)\ C_6H_2\ (\overset{OH}{NO_2})_3$ & $C_6H_2(NO_2)_3OK$.

This/

This double salt crystallises in lemon yellow needles or thin prisms, is soluble in hot water or hot dilute alcohol, but sparingly in cold. Only slightly soluble in absolute alcohol, especially cold; hardly soluble in ether. It contains no water of crystallisation. With dog's urine he got a variable precipitate which consists mainly of creatinin and only now and then a trace of uric acid present. He also discovered that when one adds some picric acid to a sample of urine and then caustic soda in excess, there results a ruby red colour which attains its maximum intensity in about two minutes, and which according to Jaffe remains unaltered for hours. All these are added cold, for if hot, other substances, e.g. sugar, also give a colour reaction. In the cold acetone gives a similar reaction, but not so intense. Another and older test is that of Weyl. To the urine or creatinin solution add a few drops of freshly prepared sodium nitroprusside, then a few drops of caustic soda, the solution becomes ruby red, but soon changes to yellow. On the addition of acetic acid (making solution definitely acid) get a green colour which, on standing, deposits a blue precipitate.

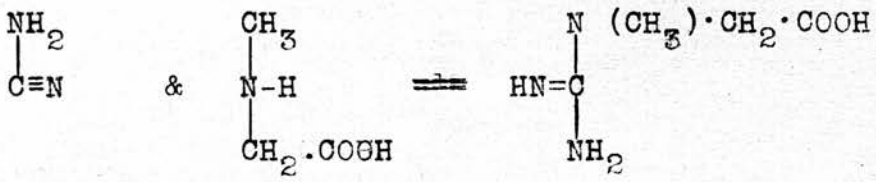
Creatin/

Creatin (Methylguanidinacetic Acid) $C_4H_9N_3O_2$ or



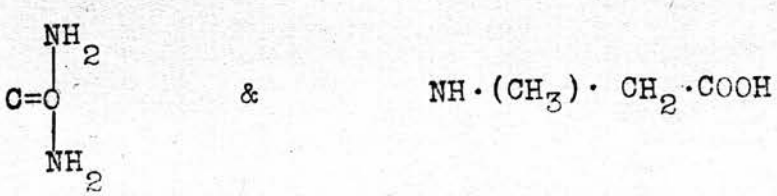
Discovered by Chevreul (4) in 1834, and more thoroughly investigated by Liebig (2) in 1847.

Synthetically prepared by J. Volhard (5) in 1869 by heating Sarcosin (Methylglicine) and Cyanamide in a closed tube for several hours at 100°C.



Cynamide Sarcosin Creatin

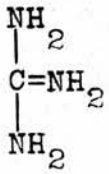
When a saturated watery solution of sarcosin is allowed to stand in the cold with the same quantity of cyanamide and a few drops of ammonia, creatin is formed. On heating with Baryta water, the creatin splits up into urea, sarcosin, etc., some methylhydantoin - $C_3H_3(CH_3)N_2O_2$ - being also formed with the evolution of ammonia.



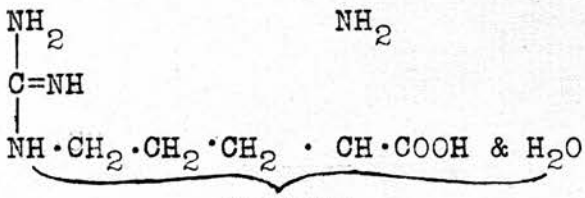
Urea Sarcosin

Because/

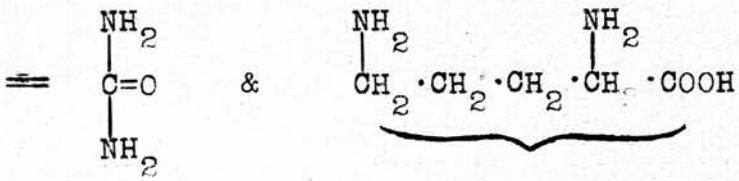
Because of this reaction some observers have looked upon creatin as a precursor of urea. It can be looked upon as a substituted granidin, whose formula is



Kossel also discovered a ferment capable of splitting off the preformed urea group from arginin, a homologue of creatin.



Arginin



Urea

Ornithin (Diaminovalerianic Acid)

On heating with acids creatin is converted into creatinin. If a creatin solution is heated with mercuric oxide, the latter is reduced to metallic mercury, especially in the presence of alkali; besides there are formed oxalic acid and the bad smelling methylguanidin. Creatin crystallises from a watery solution in transparent, hard crystals, with/

with one molecule of water of crystallisation, which is lost on warming to 100°, and there are left opaque crystals.

It dissolves in 74 parts of cold water, easier in hot; not easily soluble in alcohol, and not soluble in ether. The solutions are neutral, have a bitter taste, and are not precipitated by lead acetate.

No special test except crystals under microscope, or converting the creatin into creatinin by heating with acids.

METHODS FOR THE ESTIMATION OF CREATININ AND CREATIN.

The old methods in principle are similar to that which Pettenkofer (3) used when he discovered the substance in the urine, to which Liebig (2) later gave the name of creatinin. It was worked out for quantitive work by Neubaur (6). It consisted in adding calcium hydrate to the urine, evaporating the filtrate and making the residue up to an 80% alcoholic solution, then adding alcoholic zinc chloride, and weighing the precipitated zinc chloride compound.

In 1886 Salkowski (7) modified the above by certain/

certain improvements, amongst others he recommended, instead of using 95% alcohol, that absolute alcohol be added, after concentrating the urine. He also tried to substitute ammonia and calcium chloride for the $Ca(OH)_2$, but found no advantage. He, however, admits that "even with all care and without knowledge of any error," the above method may fail at times to give accurate results. Definite weak points were, the $Ca(OH)_2$ which was added to remove the phosphates, converts some of the creatinin to creatin, because of the alkaline reaction. Probably some of the creatinin is also precipitated with the phosphates. The alcoholic solution was found, by experiment, to contain a considerable amount of the creatinin in solution.

Hoogenhuyze and Verploegh (9) tried the solubility of creatinin in alcohol of different strengths, and found,

in 100 c.c.	Alcohol	99% -	a trace of	Creatinin
" 100 c.c.	"	93% -	5.6 mg.	Creatinin
" 100 c.c.	"	72% -	32.1 mg.	"
" 100 c.c.	"	50% -	104.5 mg.	"

With the Neubaur method the urine is concentrated, but still contains some water, and this of a variable quantity, and, therefore, on the addition of alcoholic zinc chloride all the creatinin is not precipitated. A variable quantity remains in solution.

If/

If on the other hand one evaporates the solution to dryness, on the addition of alcohol it becomes hard, and not easily extracted. On using absolute alcohol, in which creatinin is almost insoluble, and adding a few drops of hydrochloric acid to this, creatinin hydrochloride is formed, which is very soluble in alcohol. The difficulty, however, is to precipitate the creatinin with zinc chloride from this acid solution, for the crystallisation of this compound is hindered unless the liquid be neutral. The neutralising agent must not have an insoluble chloride. Lithium carbonate was tried, and though the creatinin was then precipitated by the zinc chloride, yet the creatinin zinc chloride was more soluble in alcohol owing to the presence of lithium chloride. Sodium acetate gave similar results. Equally unsatisfactory results were got with a double salt formation (with mercuric chloride.)

In 1901 Folin (8) introduced his colorimetric method, wherein he made use of Jaffe's colour reaction for creatinin (see page 6).

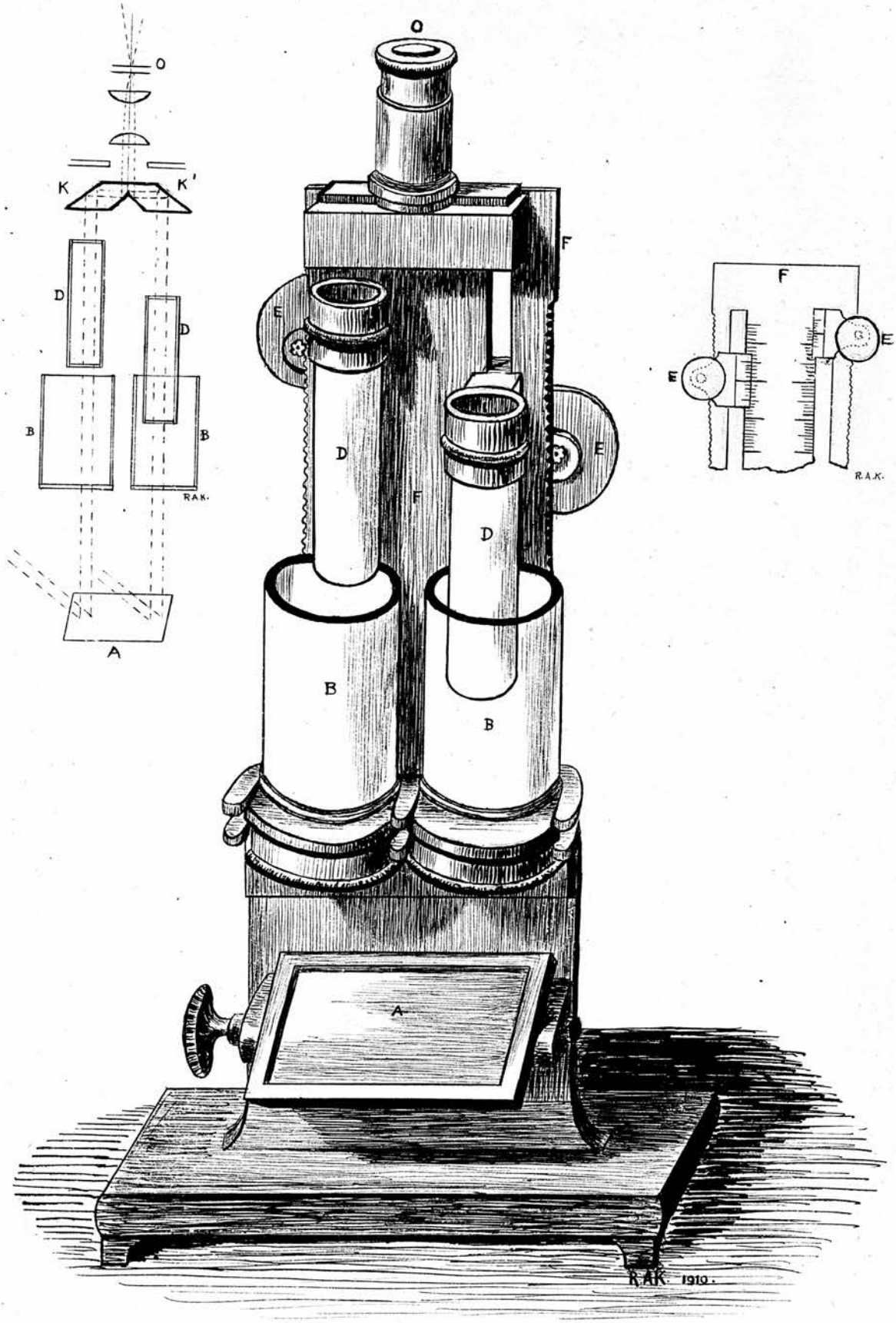
The method in practical use is the following :- 10 ccm. of the urine are pipetted into a 500 c.c. flask, then 15 c.c. of a saturated picric acid solution, and finally 5 c.c. of a 10% caustic soda solution are added. The mixture is then shaken and allowed/

allowed to stand for five minutes. Distilled water is added up to the 500 c.c. mark and the whole mixed. This solution is compared to a semi-normal potassium bichrom solution, in the Duboscq Colorimeter.

Different workers use different instruments, but in principle they are all the same, with only some structural variations.

The instrument used by the writer is a modified Duboscq Colorimeter. It consists (see illustration) of a vertical frame (F) fixed to a solid pedestal. To this vertical part are attached two glass cups (B), which are fixed but can be removed for cleaning, or to permit the addition of fresh solutions. Above these cups are two movable glass columns (D), one for each cup. These columns can be moved up and down by means of a screw (E) which is manipulated from behind, and the height at which they stand is measured on the graduated scale, also behind, the reading being rendered more precise by means of the vernier on the back of the metal bar supporting the glass columns. The graduated scale is in millimetres. Above is the eyepiece (O), by means of which one sees the two fields of colour, much the same as the half-shadow polarimeter. Below is a plane mirror (A), which reflects the rays of light up through the glass cups and glass columns. By means of a set

of/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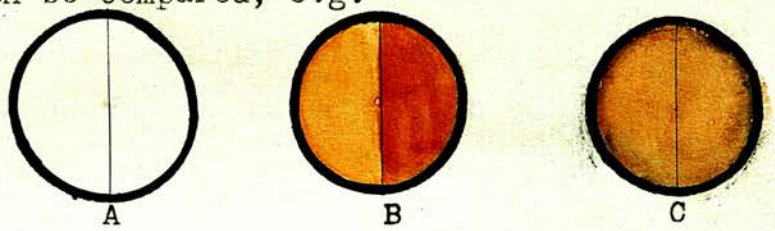
of prisms, the rays (K, K^1), having passed through the solution, are brought into juxta-position and so can be compared, e.g.-



A.- Represents the field without any solution in the glass cups.
B.- Shows the solutions present in each cup before the two fields have been equalised.
C.- Shows the two fields after equalisation of the solutions.

If into one of the cups be placed some of the $\frac{N}{2}$ pot.-bichrom solution, and the glass column be turned down until it stands at a height of 8 m.m., the same intensity of colour will be seen as with a creatinin solution, containing 10 mg. of creatinin treated with 15 c.c picric acid and 5 c.c. caustic soda solution (10%), the whole being made up to 500 c.c. with distilled water, and the glass column standing at 8.1 m.m.

The quantity of creatinin can be easily determined from the colorimetric readings. If, for example, the colorimetric readings be 9.6, 9.7, 9.5 and 9.6, the average being 9.6 m.m., and the creatinin content for 10 c.c. of urine will be $\frac{8.1}{9.6} \times 10$ or 8.43 mg.

Various factors influence the above estimation, and they have received attention by most observers.

One of the most important is the temperature

of/

of the reacting fluids. This, according to Folin (8) Hoogenhuyze and Verploegh (9) as well as Mellanby (10), must be the same for all the reagents. A difference of two or three degrees causes an appreciable error. This applies very markedly to the temperature of the distilled water. If fresh quantities of water are required whilst estimations are being made, there are likely to be differences in the results, and these in comparative work are to be avoided. Hoogenhuyze and Verploegh used to keep the water at a constant temperature of 15°C.

As regards time, Mellanby (10) states that although five minutes is the time which the reacting fluids should be allowed to stand before diluting, yet a certain latitude is not unsafe, and he adds that the readings are quite good any time from three minutes to fifteen minutes. Mellanby also states that the quantities of picric acid and caustic soda need not strictly be adhered to.

A point which has troubled the various workers is whether there is an alteration in the tone of the colour when working with varying depths or thickness of solution. The depths of fluid which should be used, according to Folin, are between 5 m.m. and 13 m.m., or in other words the amount of creatinin present in the 500 c.c. of solution should be between 7-15 mgm. He, however, does not say anything concerning/

concerning the difference he got in the various readings.

Hoogenhuyze and Verploegh as well as Gottlieb and Stangassinger mention that the average of their readings was about .1 m.m., and the maximum about .2 m.m. Klercker (16) as well as Baur and Barshall (18) give differences of .3 m.m. Dorner (15) states that his readings seldom varied more than .3-.5 m.m. Weber (12) says that where there is a lot of creatinin, for example as in the solution of a depth of 4.0 m.m, the difference is .2 m.m. When 8 m.m. the difference is .3 m.m., and with solutions with a layer greater than 11 m.m. the variation is .7 m.m. Weber considers the average unavoidable difference about .3 m.m., representing an error of 4%. Weber also indicates that the eyes soon become fatigued, and where a number of observations are to be made, errors may arise from fatigue alone.

Personally, I have found that the best depths to work with are those between 4 m.m. and 12 m.m. But this is by no means absolute, for certain urines give very good creatinin readings, the differences of the readings not exceeding .2 m.m,, with the column of liquid between 15 m.m. and 20 m.m.; which means a creatinin content for 10 c.c. urine of only 5.4 to 4.2 mg.

Again,/

Again, though on an average the difference in my readings have not exceeded .2 m.m., yet with some urines a difference of .4 m.m. has been recorded, even with the depth of the fluid about 7 m.m.

When a solution stands at about 2 m.m., a difference of .2 m.m. in reading means a variation of 3.7 mgm. of creatinin per 10 c.c. urine; whilst if standing at 8 m.m., a similar difference only means .3 mgm of an error. With a greater depth of solution, for example 15 m.m., the difference in the readings gives only .08 mg. of an error. On the other hand with greater depth of fluid or in other words smaller quantities of creatinin, the differences in shade are not so easily appreciated and there the readings may vary as much as .5 m.m. and even more, according to the height of column.

In those cases where the solution contains too small a quantity of creatinin one may have either to use double the quantity of urine and of reagents, or only dilute it to 250 c.c. with water, or sometimes both these have to be done. The calculation, of course, has to be altered accordingly. Hoogenhuyze and Verploegh state, that where double the amount of urine (20 c.c.) has to be used, because of the small quantity of creatinin present, only/

only the ordinary quantities of the reagents need be added, both for creatinin and creatin estimations. I have not been able to confirm this, as the following case illustrates.-

20 c.c of the urine were taken and the ordinary quantities of reagents added, namely 15 c.c picric acid and 5 c.c. caustic soda, and the creatinin estimated. The average of four readings was 14.5 m.m. To another 20 c.c of urine double the quantity of each of these reagents was added, the average of four readings was 12.55 m.m.

According to the first reading the amount of creatinin per 20 c.c. of urine was 1.39 mg., and with the second 1.6 mg, or a difference of about .2 mg., which in the large quantity of urine excreted by that patient meant a difference per day of .6 gm. preformed creatinin. Similarly with the creatin estimation. 20 c.c. of Urine treated with 5 c.c. of hydrochloric acid and neutralised by the same quantity of caustic soda, and the ordinary quantities of reagents added, gave as an average a reading of 10 m.m, or 2.02 mg. of total creatinin; with .20 c.c. of urine and all these reagents doubled in quantity, the average reading was 7.8 m.m. or 2.59 mg. of total creatinin. Here the difference per day would have been .77 gm. for the total creatinin.

Some workers have even concentrated the urine, e.g., Funaro (73) when examining the urine of infants found that to estimate the creatinin which was present in very slight quantities he had to evaporate some of the water and so increase the amount of creatinin per c.c. of urine.

As regards light, hardly sufficient attention has been directed towards how it affects the colorimetric readings. The best kind of light is the diffused light of a bright day. Under such conditions one/

one can readily get readings, the difference of which does not exceed .1 m.m.

Direct sunshine even with the use of the opaque glass reflector is too glaring, and readily fatigues the eyes. The light of a cloudy day, or twilight areequally unsatisfactory for distinguishing the different shades of colour. With the varying weather in this country one is often forced to use artificial light. I have not found an artificial illuminant by which I can take colorimetric readings easily and accurately. Further, when comparing a set of readings taken by artificial light with a set got in daylight an appreciable difference is got. For example, 10 c.c. of a urine were examined by artificial light (Nernst lamp), and as a result of seven readings, an average of 7.2 m.m. was got, the greatest difference being .2 m.m. This meant 11.25 mg. of creatinin per 10 c.c. or .862 gm. for the twenty-four hours specimen. The same specimen was also examined by good daylight, and the average reading was 7.9 m.m., the maximum difference here was .15 m.m. This meant 10.253 mg. of creatinin per 10 c.c urine, or .786 gm. for the total specimen. From this one sees that when doing comparative work in the two kinds of light, an error of 1 mg. per 10 c.c is seen, or 76 mg. for the total specimen. Of the/

the artificial lights, I have only found the Nernst lamp of any practical use. For comparative work on the same urine, daylight and artificial light should not be used alternately.

Another point of some importance, especially when using artificial light, is that the light must be equal for both glasses, otherwise one gets a slight shade on the one field, which may prove very disturbing. Only two authors mention the source of light with which they worked. Klercker (16) used diffused light during the brightest part of the day. He was not able to get a convenient artificial light. Weber (12) used incandescent light and diffused daylight. He states that they both gave identical readings.

An expedient which was found very useful and also helpful towards accuracy was the insertion of a blue glass between the eyepiece and the glass columns. Thus the reddish tint was converted to a yellow-green tint, which is not so glaring, and whose shades of colour can be defined with greater precision.

There are substances in the urine which may interfere with the creatinin reaction or give reactions similar to it. These have been investigated by Mellanby, Weber, Hoogenhuyze and Verploegh, and/

and others have also tested the effects which different substances, normally present in urine, such as urea, destrose, sod. chloride, cal. chloride, phosphates (acid and normal) have on this reaction, and they conclude that they do not affect the readings when present in reasonable quantities, and certainly not in ordinary urines.

Urines and solutions to which glucose had been added in different quantities, viz :- .1%, 1% and 10% have been examined, but in none of these has any difference been noticed until about twenty-four hours after examination. When warmed at once, however, a red brown colour is got.

Acetone gives a similar reaction, as was pointed out by Jaffe in his original communication (1), the colour being a faint red-yellow tint. Hoogenhuyze and Verploegh (13) added .1% of acetone to 5 c.c. of urine, and they state that the same red colour reaction was got, but it appeared quicker and also disappeared fairly rapidly. With a reading of .01 m.m. lower at the commencement the reading soon comes to the proper creatinin level and remains stationary. This I have also been able to confirm. So it can be said as regards acetone, that it does not cause any error in colorimetric work, provided several readings are taken.

Klercker/

Klercker (16) states that acetone has the property of causing a rapid paling of the red creatinin picrate solution, so that it was absolutely impossible to take any readings. By heating the urine and so driving away the acetone and then bringing the urine to its original volume, the estimation can be performed with ease. The quantity present in the diabetic urines to be mentioned later, was only a trace in a few cases and certainly did not interfere with the readings. Besides Acetone, Folin (8) also noticed that diacetic acid, acetic ether and hydrogen sulphide also disturb the reaction, but does not mention in what manner.

Gottlieb and Stahgassinger (19) corroborate Jaffe's (20) statement that glycocyamin in the urine gives a similar reaction. According to the former the reaction is less intensive, reaches its maximum intensity very slowly, and according to Dorner (15) only after twelve hours, the resulting red being of a deeper tint. Half-an-hour after the addition of picric acid and caustic soda, 20 mgm, of glycocyamin equals in intensity of colour 10 mgs. of creatinin.

To estimate the creatin, Folin recommended 10 c.c. of the urine to be heated with 5 c.c. normal hydrochloric acid for three hours to convert the creatin into creatinin and then having neutralised the/

the urine with normal caustic soda (5 c.c.) the method employed is as indicated with creatinin.

The urine as well as the hydrochloric acid was pipetted into a CO_2 flask, a small broad-necked flask, with a capacity of about 40-50 c.c. This flask is then either fixed to a reflux condenser, or a cork is inserted (preferably rubber,) perforated for a long narrow glass rod, to prevent diminution in bulk of the solution. The heating is done on a water bath, the temperature, as recommended by Folin, being 90°C, and the heating is continued for three hours. By using a circular copper sheet, perforated with three or six holes suitable for the above mentioned flasks, one is able to work on a similar number of urines.

Benedict and Myers (21), from experimental work which they did, recommend heating in an autoclave at 117°C. for 15 minutes. They found that the conversion of creatin to creatinin was complete within 15 minutes, and no further change occurred even after heating for three hours. This also applies to where large quantities of creatin have to be converted into creatinin. Hoogenhuyze and Verploegh also got good results with the autoclave.

As regards the time, Mellanby in his paper states that the urine can be heated indefinitely without/

without any further change taking place, provided there is no alteration in bulk. Hoogenhuyze and Verploegh, as well as Dorner, held that three hours was the proper time, and that if heated longer the HCl acted upon the creatinin and destroyed part of it.

I have tried several experiments with ordinary urines without any added creatin or creatinin, and have found that where creatin was present the maximum amount was converted into creatinin within five to six hours.

Case 1. Amount of Creatinin present per 10 c.c. of urine being 5.06 mgs.

This urine was then heated for varying periods, controls also being taken.

Time Urine heated	Total Creatinin per 10 c.c.urine*
2 hours	5.625 mg.
5 "	5.328 "
18 "	6.04 "
20 "	6.04 "
24 "	6.06 "

Case 2. Preformed creatinin = 5.024 mg. per 10 c.c.

Time Urine heated	Total Creatinin per 10 c.c.urine*
2½ hours	5.56 mg.
4 "	5.74 "
6 "	5.86 "
8 "	5.76 "
10 "	5.76 "

From/

* Total Creatinin or Creatin (as creatinin) and preformed Creatinin.

From these two examples it will be seen that the conversion of creatin into creatinin is practically complete within six hours, and no further change occurs even on heating for twenty-four hours.

As regards the readings, if the preformed creatinin gives a reading of 8 m.m., and on heating with HCl the reading is 7 m.m., there is 10.12 mg. preformed creatinin and 11.57 mg. of total creatinin. Therefore it may be assumed that there is about 1.45 mg. of creatin expressed as creatinin. Here there is justification for stating that creatin is present, but if the difference in the reading be only about .1 m.m. or .2 m.m., then there is not certainty on the point.

PRESENCE OF CREATIN AND CREATININ IN THE ANIMAL KINGDOM.

Creatin seems to be confined entirely to the vertebrate kingdom. Valenciennes and Fremy in 1855 stated that they had been able to find these substances in the muscle of Crustaceans. Krugenberg (22) in 1886 was not able to verify this, though he used large quantities of lobster muscle. He thinks these authors were mistaken, as in lobster muscle, just as in the liver, there is a substance (not/

(not acetone) which also gives the nitroprusside reaction. Valenciennes and Fremy were not able to find creatin or creatinin in the muscles of Oysters or of the Cehpalopodia. Nor did Krugenberg in Molluscs (Ostria, Helix, Pectunculus, Dariopsis, Toligo), or worms (Lumbricus, Spirographis, Sipunculus). Ackermann and Kutscher (23) found creatinin in crab muscle extract.

Lettlier got crystals from the organ of Bojanus of the Mytilus (a Mollusc) which he thought were either creatin or creatinin. Mellanby (10) examined Limulus but found no creatin in the muscle; he found it present in Ammocoetes (nearly allied to vertebrates). He found nearly as much in adult Lamprey as in Skate. The amounts of creatin he got were :=

Lamprey	- .25 p.c.	Hedgehog (winter)	- .2 p.c.
Skate	- .24 "	" (summer)	- .2 "
Cod	- .3 "	Rats (2 ms. old)	- .3 "
Frogs	- .26 "	Bullock	- .3 "
Fowl	- .31 "	Pig	- .33 "
Guinea Pig	.32 "	Rabbits	- .44 "

Having found creatin absent in cross striated muscle of lobster, but present in that of ammocoetes, he concludes that this is some evidence that creatin does not depend solely on muscle metabolism. From his incubation experiments he states that for a considerable time the body weight, liver and creatin develope synchronously, yet towards and after hatching, the rapid development of the liver, due to the closing of/

of the ductus venosus, is accompanied by a corresponding increase in creatin formation, while at the same time muscular growth almost stops. He, therefore, concludes "That the gland of the mid gut of invertebrates has no morphological or physiological connection with the liver of vertebrates, and, therefore, the newly introduced liver might account for the development of creatin in vertebrates."

Creatinin has also been found in the urine of man, the horse, the calf, the cow, the dog, the pig and the rabbit. In 1868 Meissner (24) came to the conclusion that creatinin is not normally present in the urine of birds, while creatin is present. This was justified by Noel Paton (25) who concludes that creatin is an end product of the catabolism of avian muscle. Consequently, the amount excreted will give an approximate idea of the amount of muscle catabolised. Bubnow (26) states that creatinin is present in thyriod, whilst J. Forschbach (39) denies this.

Creatin is found in the blood, transudates, amniotic fluid, and sometimes in urines. In 1885 Baumstark (28) in a paper on the chemical examination of the brain, states that in the watery extract of that organ he was able to find all the substances present in meat extract except creatin. This I decided to investigate in view of the new methods in vogue/

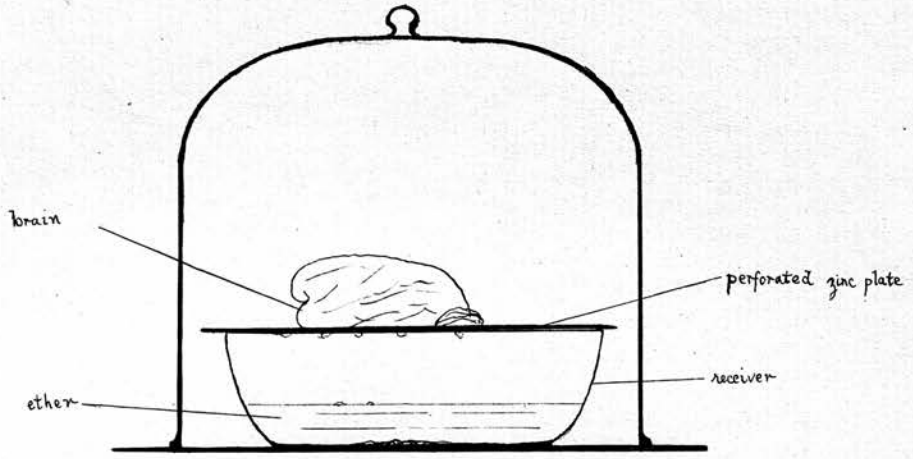
vogue as regards the estimation of creatin by the colorimetric method.

In extracting the brain for creatin one thing to be avoided, so far as possible, is the application of heat for if creatin is present in small quantities it may quite easily be converted into creatinin and so missed. I, therefore, adopted the method of displacing the water in the brain by means of ether, and as creatin is soluble in water but not in ether, the water so displaced will carry with it any creatin present in the brain tissues.

The method was as follows:- The brain of a newly killed ox was taken, one which had not been lacerated in killing, as it was essential to have as intact an organ as possible. It was carefully freed from all blood clots as well as the pia mater, care being taken to avoid injuring it. It was then cut several times longitudinally and placed on a perforated zinc plate, which was placed over a glass receiver, so that any fluid exuding from the brain might be collected. In this receiver was poured a fair quantity of ether and then the whole was covered by a bell jar. The ether on evaporating was brought into contact with the brain, into which it penetrated, displacing the water present in the cerebral tissues, Even within twenty-four hours an appreciable/

appreciable quantity of water had collected.

Where necessary ether had to be added.



This was overlooked in the first experiment with the result that slight putrefaction set in, before the fluid was examined for creatin and creatinin.

Expt. 1 : 20 c.c. of the fluid taken and examined for creatinin - no trace. Other 20 c.c. examined for creatin and found to contain 2.1 mg., and as the total amount of fluid collected was about 57 c.c. the total amount of creatin was 5.7 mg. In this experiment the slight putrefaction which had taken place did not convert creatin into creatinin.

Expt. 2 : Wt. of brain (inclusive of cerebellum) = 408 gms.
80 c.c. of fluid were collected;
10 c.c. found to contain 1.34 mg. of creatin;
80 c.c. therefore, contained 10.72 mg. of creatin per kilo of cerebral tissue.

On continuing to collect more fluid from the same brain I was able to get other 60 c.c., 10 c.c. of which yielded .77 mg. of creatin or 4.42 mg. per 60 c.c. The brain was then cut up, water added and extracted by means of heat, the result being a total of/

of about 7 mg. of creatin. This means approximately a creatin content of the whole brain being about 22 mgs. or .055 gm. of creatin per kilo of cerebral tissue.

Expt. 3 : Part of a spinal cord from an ox was taken, weighing about 75 gm. and subjected to a similar treatment. 30 c.c. of fluid were collected and found to contain 6.4 mg. of creatin, which would equal 85.3 mg. of creatin per kilo of spinal cord.

Expt. 4 : This experiment was made to find out whether there was any creatin present in the white tissue of the brain, and for this purpose the corpus callosum was used. The amount of fluid collected was 25 c.c., and it was found to contain about 4.3 mg. of creatin.

Expt. 5 : Human cerebro-spinal fluid was examined for creatin and creatinin, but none of these substances were detected. This was repeated with another specimen with the same result.

Expt. 6 : A brain was extracted by heating with water. This process was repeated several times and the total amount of fluid added together and concentrated to a definite bulk. 48.4 of watery extract resulted and was examined for creatin and creatinin.

Creatinin = 3.5 mg.
Creatin (as Creatinin) - 13.0 mg.
Total Creatinin for the whole brain was 16.5 mg.
or expressed as Creatin, 19.2 mg.

Here some of the creatin was converted into creatinin by the application of heat. This showed that creatin as such is present in the brain.

BACTERIAL ACTION ON CREATIN.

Mellanby (10) could find no trace of creatin in a sample of Liebig's extract which had stood for several/

several months. He indicates that the methyl guanidin which Kutscher discovered in meat extract was probably due to bacterial action on the creatin, for when he distilled the above mentioned extract with HCl he smelt ammonia.

N. Antonoff (27) states that most bacteria form creatinin. There is a connection between acid formation and creatinin formation in some cases, though not generally. In most cases some other factor has to do with this creatinin formation. Bac. Typhosus forms no creatin, whereas the Bac. Coli does.

Hoogenhuyze and Verploegh (13) agree with Gottlieb and Stangassinger that there are substances, probably enzymes, which have the power of converting creatin into creatinin.

METHODS FOR ESTIMATING CREATIN IN MUSCLE.

There are quite a number of methods for extracting creatin from muscle, of which Liebig's is the oldest. The following three have been chosen as being the latest, the easiest and the most useful for quantitative estimation of creatin in muscles, and also applicable to the colorimetric method.

(1)/

(1) Mellanby (10). The muscle is ground up with 95% of alcohol; the alcohol is poured off and the mass is then extracted by adding four portions of water, the last portion being filtered through muslin. The alcoholic and watery extracts are then put together and evaporated to dryness (to get rid of the proteids), and then extracted with 75% alcohol, which dissolves the creatin and creatinin. This alcoholic extract is then made up to a certain volume (generally 150 c.c.) and filtered, about 100 c.c. being taken and evaporated on a water-bath and made up to 50 c.c. with water.

(2) E. P. Cathcart and J. Brown (29), took the excised mass of muscle, removed rapidly fatty and fibrous tissue, as well as blood, then weighed and put into a small mortar, minced and rubbed up by means of powdered glass, water being gradually added until a fine suspension was got. This was carefully put into an Erlenmeyer flask and filled up to 150 c.c. with distilled water, some chloroform and thymol solution being added (to prevent putrefaction). The solution was thoroughly shaken and placed in a hot water oven at 50°C where it was left for 8-10 hours, being occasionally shaken. Then the flask, after faintly acidifying its contents with acetic acid, was put on the water-bath (boiling) for thirty minutes/

minutes, and filtered. The solid residue was extracted with boiling water 5-7 times and again filtered. The united filtrates were then concentrated to 40 c.c. and the creatin and creatinin content estimated.

(3) Pekelharing and Hoogenhuyze (30). The muscle being freed from fat and connective tissue was cut up and put into a flask with 1% HCl, and weighed. It was then heated on a water-bath for 4-5 hours till all the muscle was broken up, diminution in bulk being avoided. The turbid fluid was first neutralised and heated to precipitate the proteids, then cooled and made to a definite bulk and finally filtered. A part of the filtrate was then taken and concentrated, leaving a solution with approximately a creatin content of .1%.

PRESENCE OF CREATIN IN MUSCLE AND FACTORS AFFECTING IT.

Since creatin was discovered in muscle many experiments have been carried out, and different views formed as to how it is affected by physiological activity, and whether creatinin is present in muscle.

Heintz/

Heintz (31) regarded creatinin which was supposed to be present in muscle as produced from creatin in the manipulation of the muscle by the action of the acids. With this Liebig (2) did not agree. He stated that a mineral acid of equal concentration to the organic acids present in meat could not convert creatin to creatinin even when heated. In his classical experiment in 1847 on a hunted fox, he found an increase of creatin in muscle after work.

Borszezow and Johnstone (36) in 1861 held that creatinin was present in muscle, but some was changed to creatin in the process of manipulation. Sarokin (Sarokow - 32) in 1863 found in frogs that muscle contained double as much creatin as creatinin, and also that work converted creatin to creatinin. Similarly, Ranke in a treatise on Tetanus (1865) stated that creatin was converted into creatinin as a result of work. Scgelkow (33) in 1866 found the same in hens; whilst with rest he got a decrease of creatin. He also stated that different groups of muscles contained different quantities of creatin. On the other hand, Nawrocki (34) in 1866 found no increase of creatin in hens or mammals. He found that different muscles of dogs contained the same quantity of creatin.

Voit (35) in 1868 investigated frog's and calf's heart/

heart muscle, and found creatinin present, it being .066%, .019% and .038% respectively. He stated also that there was a decrease of creatin with work, the total quantity of creatin and creatinin, however, was not increased. Monari (37) in 1889 tetanised the muscles of dogs, and got an increase in the total creatin to creatinin. The amount of creatinin which he found in muscle was .066%. These workers all used the old methods for estimating creatin.

Grindley and Woods (41) found that creatinin is not present in fresh meat, and also that if any is present, it is got after heating in the presence of the meat acids.

Weber (38) in 1907, experimenting with mammalian heart, using Langendorff's method, found that when working well, definite amounts of creatin or creatinin are passed into the perfused Ringer's fluid, whilst with a resting heart the perfused fluid contained no creatinin. In a dog in which he had divided the sciatic nerve, and examined the muscles several weeks later, he found a marked decrease of creatinin. In a dog (fasting) with pronounced spasm, he observed a distinct increase of creatinin in urine, but a marked diminution when compared to the total nitrogen. He also found a diminution of creatinin by forcible work/

work in a dog. He did not estimate the creatin as he considered the method of converting creatin to creatinin with hydrochloric acid did not give sufficiently reliable results. He, however, used Folin's method for creatinin estimation.

Mellanby (10) in 1908 having worked with the muscles of rabbits and frogs states that creatinin is never present in muscle in quantities capable of detection, and that creatin is not changed to creatinin, either by ordinary or prolonged work. F. Urano (42) made diffusion experiments with fresh muscle and partly with muscle juice. He supports the view that creatin is not simply an excretory product dissolved in muscle, but that it is as an organic combination an integral part of the muscle protoblasm.

T. G. Brown and E. P. Cathcart (40) in 1909 published their results of work on muscle. They found that with the circulation intact and stimulation of the muscles, that there is a constant although small decrease in the quantity of total creatinin got from stimulated muscle. The same authors found an increase in total creatinin when ordinary nerve-muscle preparations are stimulated; Whereas, with intact circulation there is always a slight decrease in the amount of total creatinin.

Recently/

Recently Pekelharing and Hoogenhuyze (30) have published the results of experiments on cats, rabbits and frogs in connection with the effect of Tonus and Rigidity on the creatin of the muscle. They have shown that different groups of muscles in rabbits have different creatin contents. Then they have examined normal and paralysed muscles and have found a decrease of the creatin in the paralysed muscle. In order to eliminate various disturbing factors, e.g. altered circulation after section of the sciatic nerve, a cat was narcotised, the cervical cord was then exposed and the posterior nerve roots of the first and second dorsal to the fourth and fifth cervical extradurally divided. The animal was then decerebrated and the chloroform discontinued, when the decerebrate rigidity generally set in within half-an-hour. Special attention was paid to the Triceps muscle, which was rigid on the right side and flaccid on the left. The vasoconstrictor nerves not being severed caused no circulatory disturbance. The result obtained was that the muscles with Tonus present have larger amounts of creatin than the flaccid muscles. These investigators also tried the effects of chemical agents which have the property of increasing Tonus, e.g. Veratrin,/

Veratrin, Nicotin, Calcium Chloride, Sod. Sulphocyanide and Caffein. They found the creatin increased when the muscle was stimulated whilst in the fluid containing the reagents; but by simply putting the muscle into the solution without stimulation no alteration was caused as regards the creatin. Muscle in Rigor Mortis always had a higher creatin content than normal muscle.

They conclude that in the muscles of vertebrates in heat rigor, as well as rigor mortis, and in Tonus, a chemical change takes place which causes creatin to be formed. They also indicate that their experiments support the view that the creatin formation as well as the proteid metabolism in the body are to a great extent influenced by muscle tonus, just as Pfluger in his Archives (Bd.18) indicated how important the muscle tonus was for the production of body heat.

The behaviour of Creatin during the Autolysis of muscle has within the last few years aroused the interest of a number of observers, one of the first being Schmidt-Nielsen (43) who in 1903 found that in the autolysis of fish muscle there is an increase of amido-acids and xanthin bases. Seemann (44) in 1907 indicated that in autolysis there is an increase of the creatinin in the muscle, and this is still further increased if gelatine be added to the autolysing/

autolysing muscle.

The most extensive work on this subject has, however, been carried out by Gottlieb and Stangassinger (19) and they conclude that -

(1) in autolysis of muscles and other organs, e.g. liver, kidneys, etc., creatin is first formed.

(2) The creatin present in autolysing muscle (as well as any added creatin) is changed by ferment action partially into creatinin.

(3) In progressing autolysis, the creatin and creatinin are destroyed by ferments (creatase and creatinase.)

(4) This ferment action is also present in the urine.

In 1908 R. Stangassinger (45) gives some further results on autolysis, and states -

(1) That creatase and creatinase develope their best action in slightly acid media. Also that if left standing quietly they show better action. Toluol as an antiseptic has the least injurious effect on their action. Urea or salt in great concentration as well as all metabolic poisons retard their action.

(2) In the autolysis of dog's liver and blood, creatin is first formed.

(3)/

(3) Liver extracts destroy added creatinin, and at the same time show creatin in definite quantities.

Gottlieb and Stangassinger (46) state further that creatin is destroyed both in the liver and kidneys and by the blood perfusion experiments demonstrate that creatin can be formed in the liver.

Voit says, concerning the metabolism of creatin, "One cannot understand in which way creatin in the alkaline blood is changed to creatinin, for the latter is converted into creatin in alkaline solution," and so he holds, that the change takes place in the parenchyma of the kidney. Gottlieb and Stangassinger suggest that a study of the ferment action may explain the dependence of the creatinin excretion upon the reaction of the urine. They hold that creatinin is an intermediate product of metabolism. Also that from the quantitative estimation in the urine it cannot be determined how much creatin and creatinin is formed in the tissues. A defect in their experiments is that they evaporate the urine to dryness on a water-bath, and thereby expose their results to the possibility of great error as regards preformed creatinin.

In 1908 Mellanby (10) in pointing out the above defect in Gottlieb and Stangassinger's experiments, also/

also carried out autolysis experiments, and concluded that wherever the muscle became thoroughly septic all the creatin in the muscle entirely disappeared; and that autolytic action (antiseptic or aseptic) leaves the creatin in the muscle untouched. The results of experiments on autolysing tissues of rabbits, cats, guinea-pigs and hedgehogs do not confirm the presence of ferments.

A. Rothmann (47) 1908, repeated the autolytic experiments both by Gottlieb and Stangassinger's and Mellanby's methods, he confirms the results of the former observers.

On reviewing the work which has been done on creatin in muscle there is at first a liability to confusion because of the varying and conflicting results presented, but it is best to put out of count the older results because their differences are really all due to the defective technique of the Neubaur method of estimating creatinin. Since Folin's method has come into vogue the results, though not yet all similar, are more in harmony. Differences are no doubt due to defects in method. Weber points out in his paper (38) regarding the creatin estimation that conversion of creatin into creatinin by means of HCl is governed by inestimable differences/

differences and this applies more especially where muscle is concerned. He, therefore, only estimates the total creatinin and does not try to estimate the creatin. According to Neubaur and Newrocki simply boiling creatin in water can change creatin to creatinin, and if this be so the various methods of extracting the creatin and creatinin by heating are bound to cause a certain conversion of creatin into creatinin. On the other hand Folin equally emphatically asserts that the conversion of creatin into creatinin or vice versa, are by no means so easy as commonly held. Still the present writer thinks that recent research has proved that creatin alone is present in muscle in chemical combination.

As regards the effects of work on the creatin content of muscle, I do not think there is any justification for taking a decided view, for although the preponderance of results indicate that work has a definite effect on the creatin, still I rather agree with Mellanby that the results are too low and the differences observed well within the limits of technical error.

FATE OF INGESTED CREATIN OR CREATININ.

(injected or ingested)

What/

What becomes of creatin or creatinin taken per as or injected subcutaneously, and what is their relation to metabolism, have repeatedly been subjects of experiment. In 1868 Meissner (24) published results of experiments. He found that creatinin as well as creatin when ingested or subcutaneously injected, were all or nearly all recovered in the urine, and in the form of creatinin. In the same year C. Voit (35) gave a dog 8.6 gm. of creatin with food and recovered 4.2 gm. of creatinin and 3.2 gm. of creatin in urine. Mallet (48) in 1900 found that the human body possessed a practically unlimited capacity for manufacturing and eliminating creatinin from creatin absorbed from the digestive tract. More recently results have differed from the foregoing.

Czernecki (49) fed rabbits with creatin, and about 1/3rd of the creatinin was recovered from the urine as the double salt zinc chloride. The creatin did not appreciably augment the creatinin elimination. The urine was not examined for unchanged creatin.

Mendel in a paper (56) stated that the amount of creatinin excreted bears a possible relationship to the quantity of proteid metabolised. He drew his comparisons from different cases, and so the variations which he got in his tables may be due to the differences/

differences in weight in the various individuals.

Achaelis (50) experimented with men and dogs giving large quantities of creatinin with the food, and he concludes that a large quantity of this was destroyed.

V. Klercker (17) failed to find any indication that creatin given with food is converted into creatinin before being eliminated. He took large quantities of meat and found no apparent increase in the creatinin excretion. He holds that the urinary creatinin is of endogenous origin, as no creatinin was present in the food, but a change from creatin to creatinin did not take place. A connection between the urine creatinin and muscle creatin is not probable; more likely it is formed in the ordinary proteid metabolism.

Folin (14) in 1906, as a result of various experiments, concludes that the animal organism does not convert the creatin of the food into creatinin. He also concludes that creatin in contradistinction to creatinin is a food and not a waste product. He adds that the results of feeding experiments with creatin depend largely upon the character of the food. With a rich carbohydrate and fat diet, poor in proteids, a larger amount of the ingested creatin was retained in the body than when a proteid rich diet was taken, and so the retention of the creatin/

creatin when fed together with a N-poor diet, clearly indicates that creatin is not a waste product.

In a former paper (51) Folin says that creatin may be one of the nitrogen substances which serve to maintain the N-equilibrium in the living body, and which do not easily take part in the urea forming processes, and that probably it belongs to the endogenous metabolism, just as Uric Acid which he found was likewise unaffected by proteid rich diet, which agrees with Burian and Schur (52) as well as Siven (53).

Klercker (17) in 1907 as a result of experiments carried out with meat extract and pure creatin confirms his earlier as well as Folin's views, that ingested creatin and creatinin are partially excreted by the kidneys as such, without any change. The only difference between meat extract and pure creatin is that none of the latter is recovered in the urine. This he was able to prove to be due to a proteid poor diet. Wolf and Shaffer (54) have confirmed these results by injecting creatin, and finding the excretion of creatinin wholly unaffected.

Mellanby (10) found that creatin and creatinin feeding has no effect upon the creatin content of muscle after the muscle has reached a certain saturation/

saturation point. In one lot of six chicks there was an increased quantity of creatin after creatin feeding. Weber (38) tried feeding experiments with meat extract. He found that the increase of creatinin in the urine exceeded the amount given in the food, and that some of the creatin had been converted to creatinin. Hoogenhuyze and Verploegh (13) recovered some of the ingested creatin as creatinin in the urine. Lefmann (55) also made experiments with meat extract. When he gave a dog small quantities the creatin and creatinin excretion was increased. He agrees with Weber that the quantities of creatin and creatinin in meat extract are very variable. He was not able to determine any conversion of creatin to creatinin, as the amount of creatin excreted corresponded with that ingested. He, therefore, agrees with Folin and Klercker in opposition to Meissner that ingested creatin is not excreted as creatinin. He also found that the excretion of creatinin was not increased with a nitrogen rich diet though the creatin might be through the large amount of ingested creatin; and he thus shows that dogs at any rate are not able to convert ingested creatin into creatinin. He also shows that the creatinin excretion is controlled by factors other than those of uric acid and urea.

Besides/

Besides the study of the effects of creatin and creatinin, ingested or injected, various observers have endeavoured to find out whether feeding with substances of a somewhat similar constitution to creatin might help to throw some light upon the formation of creatin or creatinin within the body.

Burian (61) holds that the purin bodies as well as creatin are derived from the same chemical basis and that in the formation of these substances the nucleins and nucleo-proteids play no part. Jaffe (62) holds a similar view. Forschbach (39) fed a patient with large quantities of pancreas (which is rich in guanin) but there was no effect on the creatinin excretion.

The following experiments were carried out before the above as well as the under-mentioned papers were known. On different days, liver, thymus and pancreas were taken, but no alteration in the creatinin excretion was observed (a creatin free diet was also taken during this period and on several days preceding it.)

Expt. 1.

The following diets were taken:-

(a) Creatin free diet on first three days.
(b) 1 lb. of pancreas and 1 lb. of thymus on the fourth and fifth day respectively.
(c) About $\frac{3}{4}$ lb. raw liver on the sixth day.
(d) Creatin free diet on the seventh day.

Day/

Day of Expt.	Urine	Creatinin.
1	1412 c.c.	1.284
2	1210	1.195
3	1385	1.179
4	1136	1.209
5	1542	1.249
6	1242	2.076
7	1700	1.327

No change in the creatinin excretion is seen after a diet with a large amount of pancreas or thymus present. A rise in the creatinin excretion is, however, seen after a meal of raw liver. No creatin was found in the above samples of urine.

Expt.

The following diets were taken:-

(a) Creatin free diet on the first day.
(b) On the second and third day, beef which had been boiled for two to three hours so as to extract as much of the creatin and extractives.
(c) The solution got from the foregoing on the fourth day.
(d) Creatin free diet on the fifth day.

Day of Expt.	Urine	Creatinin.
1	1356 c.c.	0.972 gm.
2	1138	2.362
3	1198	2.876
4	1022	1.097
5	944	1.158

In this experiment the beef or "suppenfleisch" as it is known in Germany, causes a definite increase in the creatinin excretion. Not, however, with the broth. Small quantities of creatin (about .033 gm.) found on the fourth or fifth day.

This/

This latter experiment was tried with a patient suffering from diabetes. On a creatin free diet about 1.12 gm. of creatinin, and .4 gm, of creatin.*

Day of Expt.	Urine	Creatinin	Creatin*
1	2386 c.c	1.098	.458 gm.
2	2841	1.307	.755
3	2841	1.343	.826

In this case there is only a definite rise in the creatinin on the second day of the diet. The same applies to the creatin. The broth does not diminish, but rather increases the creatin excreted. Compared to the total nitrogen excretion, the creatinin nitrogen remains fairly constant. The creatin nitrogen however, shows a rise in percentage.

Before Expt.	Total Nitrogen	Creatinin N.	Creatin N.
	19.03 gms.	2.09%	0.86%
1	22.119	1.8	0.66
2	23.864	1.99	1.10
3	24.091	2.03	1.09

Dorner (15) from experiments with glycocyamin, concludes that creatin can by methylated from gylcocyamin in the body of a rabbit. This is also probable in frogs, but more slowly. A dog was fed with thymus, but there was no increase in creatinin excretion.

Lefmann (55) fed a dog with liver, thymus, and spleen, but there was no increase in creatinin, although/

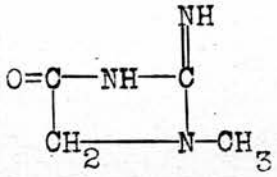
*The Creatin is here expressed in terms of creatinin, into which it was converted for estimating.

although the total nitrogen excretion was increased.

Czernecki (49) on feeding a rabbit with glycocyamin, increased creatinin excretion. Jaffe also fed with gylcocyamin, and got creatin in urine. Neubaur's method was employed and the urine decolorised with charcoal. He also found an increase of creatin in muscle.

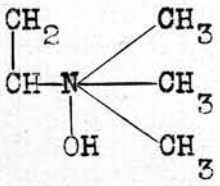
Mellanby (10) on trial feeding experiment with chicks, giving them glycocyamin, concluded that there was no effect on the creatin content of muscle. In one batch there was a large quantity of creatin, but this Mellanby thought insignificant.

Koch has published a report (57) of some investigations in which he pointed out that lecithin might possibly have something to do with the excretion of creatinin. He shows that creatinin is the only constituent in the urine in which a methyl group is attached to nitrogen.



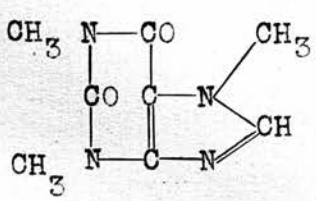
On the other hand lecithin and kephalin are the only substances in the diet, except the small quantities of caffeine in tea or coffee, which contain such/

such methyl groups attached to the nitrogen.

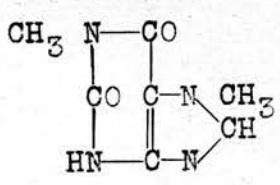
(Stearyloleyl lecithin) $C_{39}H_{73}NO_7O$ 

From the feeding experiments which Koch carried out he concludes that under ordinary conditions of diet, the methyl group of the lecithin and kephalin ingested, can all be accounted for by the methyl groups of the creatinin excreted. This is, however, not the case when an excess of these substances is ingested. Some increase of the creatinin is noted, and this increase must be due to the lecithin and kephalin of the eggs and not to some other substance. From this he conjectures that creatinin probably is a better indicator of the methyl metabolism than of the amount of lecithin and kephalin metabolised, although ordinarily they seem closely allied.

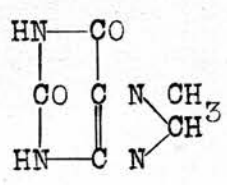
That a transference of methyl groups is possible has been shown by Albanese (58) with caffeine, which in man is changed to dimethyl xanthin, and to monomethylxanthin in the dog.



Caffeine



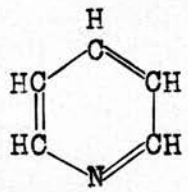
Dimethylxanthin
or
Paraxanthin



Monomethylxanthin
or
Heteroxanthin

His/

His (59) also found that pyridin in its passage through the body has a methyl group attached and is thus eliminated as methyl-pyridin or picoline $C_5H_4N(CH_3)$.



Whilst Hofmeister (60) was able to demonstrate that when selenic or telluric acid is ingested it is excreted combined with a methyl group.

Besides the foregoing a number of analyses have been made on the urine of vegetarians. In 1900 Long published (64) the analyses of urines of vegetarians. Using the Neubaur-Salkowski method he found that on an average for twenty-four hours about .8 gm of creatinin was excreted. No body weights were noted. Caspari and Glaessner (66) using the same method found no creatinin excreted by vegetarians. They, however, found creatin (by the Voit and Meissner method).

The average amounts being -

In 69 kilo, man - 0.111 gm. per diem
In 58 kilo, woman - 0.074 gm. per diem

Closson (65) using Folin's method for the creatinin estimation, found that the excretion of creatinin was still very considerable in the urines of several persons who had lived for a long time on a vegetarian diet. He also noted that the output of creatinin was/

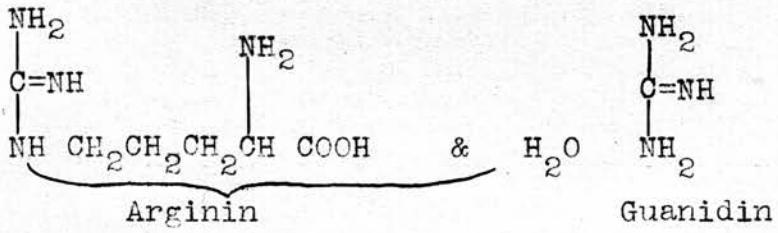
was very constant even though there were some very marked variations in the total nitrogen output.

The individual constants in his cases were -

	Body wt. kilos	Creatinin mg. per kilo.
I.	70	17.0
II.	61.5	18.0
III.	57.2	19.0

In 1906 Kutscher and Lohmann (75) found in dog's urine a base which was indentified to be dimethyguanidin. The dog had previously been fed on Liebig's meat extract.

Achaelis (50) examining the human urine for methylguanidin, found it present in three different cases. He also found it present in the urine of a horse. He concluded methylguanidin must be looked upon as a precusor of the creatin resulting from the proteid catabolism, and probably derived from the guanidin containing derivatives of the proteid molecule. He, however, was unable to find a definite increase of methylguanidin in the urines after large quantities of creatinin had been ingested or methylguanidin was injected. This Methylguanidin may possibly connect the creatin with the guanidin part in arginin.



PHYSIOLOGICAL/

PHYSIOLOGICAL CONDITIONS AFFECTING CREATININ IN URINE.

The Physiological conditions affecting the excretion of creatinin in the urine are of some interest, as some observers have by this means attempted to elucidate the life history of creatin and creatinin, with, however, variable results.

Voit and Zantl (63) found no increase of creatinin in urine of a dog which had run for eight hours. In another experiment Voit (35) got no increase of creatinin in the urine of man after work. Meissner (24) got a decrease of creatinin in urine on the day of exercise in a dog which had run for five hours, and an increase next day. Altogether he does not get an increase of the creatinin, and he concludes that the creatinin in mammals has not the same origin as urea. He indicates that to investigate the metabolism of creatin and creatinin, feeding with meat is not permissible.

K. B. Hofmann (67) used a mixed diet. He got no increase of creatinin with work but marked variations, which variations had no apparent connection with the work performed. Whilst P. Grocco (69) assumed there was an increase of creatinin from his experiments in which he got an increase of creatinin on the day of work over succeeding days.

Moitessier/

Moitessier (74) found a greater excretion of creatinin on the day of work than on the following days. Oddi and Tarulli (76) repeated the experiments of Hofmann and Grocco, and concluded that only over-exertion has any influence. Gregor (77) concluded that with muscular work an increase of creatinin was obtained.

Hoogenhuyze and Verploegh (9) tried several experiments on themselves, two of which consisted of a certain kind of work being performed, the person living on a mixed diet. A third experiment in which work was done, whilst living on a proteid poor diet. The fourth consisted in overworking to a certain extent with a slightly insufficient quantity of food. In none of these did they find any difference in the creatinin excretion. The same was true with a set of experiments in which with mixed diet was also included Casein and Gelatine (Casein because of the high arginin content) but still there was no difference. They conclude that with varying diets as long as they are sufficient, muscular work has no effect on the creatinin excretion in man. Only in conditions of starvation does one get an increased creatinin excretion. Similarly, Shaffer (78) found that muscular work with adequate food has <u>per se</u> no effect on the excretion of creatinin, which in his experiments/

experiments was constant, and also quite independent of both the amount of proteid and of the amount of muscular activity. And still further confirmed by Cathcart, Kennaway and Leathes (80) who were unable to detect a rise in the output of creatinin even after severe work under different conditions, when diet was ample.

Shaffer gives 8.1 as being the average creatinin co-efficient. This creatinin co-efficient is the ratio in milligrams of creatinin-nitrogen per kilo of body weight. He shows that there exists a greater regularity in the hourly excretion of creatinin than indicated by Hoogenhuyze and Verploegh as well as by Klercker. Hoogenhuyze and Verploegh have shown that creatinin during rest at night is diminished. According to Benedict and Myers (21) the creatinin excretion in women is, in general, much lower than in men. According to Folin (51) the chief factor determining the amount of creatinin eliminated appears to be the weight of the person. The amount of the fat must also be noted. The fatter the patient the less creatinin will he or she excrete per kilo of body weight. He concludes that the amount of creatinin excreted primarily depends upon the amount of active protoplasmic tissues. On the other hand, Shaffer emphasises the importance of the varying degrees/

degrees of muscular development, and more especially muscular tonus as affecting creatinin elimination. This is also indirectly in accordance with the view expressed by Pekelharing and Hoogenhuyze regarding the effect of muscle tonus on the creatin excretion.

Age is also a very important factor as regards the creatinin excretion in the urine. Elderly people eliminate less creatinin than younger people, when the body weight is the same. As regards infants a variety of results have been published. Hofmann (67) as well as Pouchet (68) found no creatinin. Grocco (70) found a few crystals, microscopically, of creatinin zinc chloride in some cases. On the other hand, Rietschel (71) failed to detect creatinin in the urine of suckling infants, either by Neubaur's method, or by Weyl's reaction. All his cases were in a more or less weak condition. Closson (65) was able to find creatinin in the urine of puppies and kittens. Amberg and Morrill (72) using Folin's method examined the urine of the newly born, and concluded that the creatin was present in too small quantities to permit a conclusion regarding its definite presence. They recommended concentrating the urine. Acting on this suggestion, Funaro (73) concentrated the urine and was able to definitely establish the presence of creatinin in the urine of infants. The individual differences/

differences were not great whether the case was normal or pathological. Neither was it affected by differences in the food. The quantities which he found present were only about 50-60 mg. of creatinin per day on the average.

APPEARANCE OF CREATIN IN URINE IN STARVATION.

The examination of the urine of a fasting person was considered of importance, as it was thought that it might help towards the determination of the true proteid catabolism. As there is no proteid ingested, the proteid present in the urine of a fasting person in all probability is derived from the catabolism of tissue proteid, or that proteid metabolism which is constant, but normally cannot be determined because of the ingested proteid. Most of the publications dealing with the condition of the urine during fasting do not state anything concerning the creatin and creatinin excretion. Voit's records (35) show that creatin might be expected during fasting.

Meissner (24) indicated, that in rapidly growing pigs with full feeding and a flesh free diet, the excretion/

excretion of creatin is at its minimum, whereas with an insufficient diet the amount excreted increases. He holds that this is due to the rate at which the muscle tissue is used up. He also records a case in which he got more creatin in the urine of a hen at the end of a thirty-six hours fast than at the beginning of it.

Baldi (80) and Grocco (70) both found that the creatinin excretion during fasting is low. The former found only traces of creatinin on the seventeenth day of the fast.

Dorner (15) experimented on rabbits and found that during a fast the creatin, where present, was increased. In one case the increase was very marked. It ranged from 4.6 mg. to 223.9 mg. On the other hand, the creatinin showed a diminution. The total creatinin, however, showed an increase. He concludes that in the breaking down of larger quantities of body proteids, creatin is got in increasing quantities in the urine of rabbits.

E. P. Cathcart (81) examined the urine of a man who fasted for fourteen days and who for the first three days following the fast lived on a purely carbohydrate and fat diet. The constancy in the creatinin excretion, as indicated by Folin, in normal/

normal urines, was not got in this case. There was a gradual diminution from .52 gm. to .24 gm. of creatinin-nitrogen, and a slight increase on the resumption of food. Creatin was found from the commencement of the fast. It was slightly irregular in its excretion, but did not show the same tendency as creatinin to diminish, as the period of fasting increased. The average was .1 gm. creatin nitrogen. With the carbohydrate and fat diet the creatin excretion diminished practically to nil. Cathcart, in his analysis of the results sets the question whether in the condition of hunger, there may be an absence of a substance usually present in the diet, in consequence of which the creatin is not converted into creatinin. He indicates that it may be, as Luthje (82) pointed out, that before the products of proteid catabolism can be utilised by the body, carbohydrates must be present.

In 1907 Folin in a private communication reported the appearance of creatin in the urine of a fasting man. Benedict and Diefendorf (83) also found creatin in the urine of a fasting patient, the amount increasing gradually with the progress of the fast (seven days). The creatinin on the other hand gradually diminished, while with a milk diet the creatinin increased. Benedict holds that the creatin/

creatin excretion in fasting persons is due to a going into solution of the muscles, the creatin being excreted as such, and not changed to creatinin first.

Cathcart (84) made a series of experiments on himself consisting of a fast of forty hours duration followed by a carbohydrate diet (tapioca, sugar, honey and cornflour) with and without water on the days of the fast. He also made a fast of similar duration followed by a fat diet (butter and cream); an experiment in which carbohydrates were taken for several days (no fast) was also made and followed by a fat diet. "On the fat diet there is in each case a steady decrease in the amount of preformed creatinin excreted, whereas when examined in terms of total creatinin no such great diminution is noticed. The average output of total creatinin on the carbohydrate diet is 1.27 gm. whereas on the fat diet it is 1.28 gm. It would seem then that there is after all some close relationship between the creatin and the creatinin, almost amounting to the definite proof of the origin of creatinin from creatin."

The conclusions he came to were that with a carbohydrate diet, practically nitrogen and fat free, there is a diminution in the output of nitrogen/

nitrogen in the urine; whilst with a fat diet, practically carbohydrate and nitrogen free, there is a decided increase in the urinary nitrogen. During the fast creatin appears in the urine, but disappears when a carbohydrate diet is taken. On the other hand the creatin is increased when a fat diet is taken, and this increased amount of creatin with a fat diet is not diminished to any great extent by adding proteids, when no carbohydrates are taken.

Cathcart goes on to quote C. Voit (35) Lusk (85) and Chauveau (86) who point out the relative importance of carbohydrate over fat as a protein sparer; whilst Frentzel and Reach (87) and also Zuntz (88) looked upon carbohydrates and fats as equal protein sparers. Cathcart points out that his experiments as well as those of Landergreen (89) indicate that under conditions of nitrogen hunger this power of carbohydrates to spare the protein is greater than that of fat. Kayser (90) got similar results.

Cathcart further indicates that various authors (Hirschfeld, Zuntz, Paton, Voit, etc.) have found that work leads to little or no increase in the nitrogen output provided a proper supply of food (especially carbohydrates and oxygen) are given. From this he suggests that there is a breaking down of/

of the proteid in the body and a resynthesis, and this might explain the presence of creatin under different conditions. According to Cathcart's view there is as a result of work, a setting free in the tissues (e.g. muscle) of the protein-tissue nitrogen which, however, does not appear in the urine, but is resynthetised. The food substance which is of the greatest importance in this connection is the carbohydrate. Fats may also have this effect but must first be converted into carbohydrates, and this requires some time. If no carbohydrate is given, this resynthesis cannot take place and an increase in the output of the nitrogen will be noticed. From the above Cathcart puts forward the hypothesis that the carbohydrates are absolutely essential for endo-cellular synthetic processes in connection with protein metabolism.

Noel Paton in a recent paper (91) states that creatin is an end product of the catabolism of avian muscle and the amount excreted thus gives a measure of the muscle catabolised. In fasting the creatin excretion is generally increased, both absolutely and also proportionately to the excretion of the total nitrogen. He states that in well nourished birds and during the first day of the fast, non-muscle "flesh" is catabolised. In poorly nourished birds/

birds and later in the fast, muscle flesh is chiefly catabolised, and some of the nitrogen is retained possibly to be resynthetised.

APPEARANCE OF CREATIN IN URINE IN PATHOLOGICAL CONDITIONS.

Since Folin ( ) noted that creatinin excretion in man remains constant without regard to the quantity and quality of the food, and that creatin is absent from normal urine, a number of investigators have confirmed his observations, and have also endeavoured to find out how the creatin and creatinin excretion would be altered, if at all, under various pathological conditions.

The older observers examined subjects with some affection of the muscular system, because of the supposed production of creatinin in muscle. Rosenthal (92) found a diminution in the excretion of creatinin in progressive muscular atrophies. Weiss (93) Langer (94) and Jacubowitsch (95) got similar results. Pinter in a case of Myositis ossificans progressive found also a diminution. J. Forschbach (96) gave a practically creatin free diet/

diet to a patient 19 years old suffering from juvenile progressive muscular atrophy and found the average creatinin excretion to be .88 gm., or 17.3 mg, pro kilo body weight. He also examined a case of Myelogenous Leukaemia in which the average creatinin. excretion was .662 gm., or pro kilo body weight as 12.3 mg., which is low. He also examined cases of Basedow's disease. Here the output per diem of creatinin was .552 gm., or 12.1 mg. creatinin per kilo body weight. With increase of body weight an increase of creatinin was also noticed. On feeding patients with pancreas and thymus no difference was found, nor was there any marked increase after the removal of the greater part of the thyroid.

Forschbach ascribes to the thyroid an important function as regards the metabolism of creatinin. In Basedow's disease he holds that it has the power of destroying the creatinin.

W. Scholz (97) concludes from his cretins that creatinin, as well as the other nitrogen constituents, is diminished in the urine. Stejskal and F. Erber (98) publish a case of lymphatic leukaemia in which pro kilo body weight the creatinin excretion is .137 mg. or .01 - .02 gm. per diem. They also give a case of Myelogenous leukaemia in which the daily average excretion of creatinin was .4 gm.

Hoogenhuyze/

Hoogenhuyze and Verploegh (13) examined urines of different kinds of patients, and got the following results. In Fevers, and in cases of pathological exaltation, the output of creatinin was increased. It was diminished where the vitality is lowered e.g. marasmus, whether due to disease or old age, and in such cases creatin may be found in the urine. They conclude that creatinin is formed from creatin in the body, especially in the liver.

Benedict and Myers (21) published results of examination of twenty-five urines of insane women, and found that the form of insanity had no marked influence on the creatinin elimination. In another paper of the same issue they publish work on urines, in various pathological conditions, in which creatin was also found in the urine.

If the hypothesis of Folin is correct, that the creatinin excretion is dependent on the metabolism, it may be expected that an alteration in the metabolism will cause a change in the excretion of the creatinin. An increase where the metabolism is increased and a diminution where there is lessened proteid catabolism. Various papers have been published concerning the creatinin elimination in fevers in which there is generally an increase in the metabolism./

bolism. The most important are those of Leathes (99), Hoogenhuyze and Verploegh (13), Schottin(100), Shaffer and Munk. Generally speaking a rise of body temperature is associated with an increase in the creatinin output. After prolonged fever when the organism has reached a state of inanition, a fall in the creatinin content of the urine was noticed sometimes. Also an appearance of creatin in the urine. Leathes from the figures of the febrile cases he examined, found that although in each case there was increased tissue metabolism, the proportion of the nitrogen leaving the body in the form of creatinin was low. His average $\frac{CN}{TN}$ % being about 2.0. He injected into himself anti-typhoid vaccine. With a rise of temperature, he got an increase of about 20% in the output of creatinin, but an increase in the total nitrogen of about 50%, so that relatively there was a diminution rather than an increase in the creatinin. Hoogenhuyze who has a rise of temperature for several days (Influenza), noted an increase in the creatinin of about 50%, without any corresponding rise in the total nitrogen.

In 1908 Shaffer(101) published his paper dealing with creatinin and creatin excretion in health and disease./

disease. He states that a creatinin co-efficient below 7 is normal only in elderly, inactive, poorly developed or excessively fat subjects. He also emphasises that a low creatinin excretion is found in a large number of diseased conditions - in Chronic Nephritis, flat foot (very inactive), Diabetes, and Lymphatic Leukaemia. When the excretion is abnormally low, it is not peculiar to any one disease. Creatin he shows to be an abnormal product of endogenous metabolism, and not normally found in urine unless it has been taken in the food. It may be excreted by subjects of acute fevers, in the acute stage of exophthalmic goitre, and also in other conditions, where there is a rapid loss of muscle protein; also in women during the post-partum resolution of the uterus. He concludes that the source of endogenous creatin is probably the creatin of the muscle tissue, and its appearance in the urine most likely indicates an absoprtion of the muscle proteid.

As regards creatinin, this he does not regard as an index of the total endogenous proteid metabolism, for in cases of exophthalmic goitre in which this metabolism is probably greatly increased, very low creatinin excretion may be obtained. The creatinin which/

which is slightly increased in acute fevers, is not in these cases regulated by the muscular efficiency of the patient. The creatinin excretion appears to be the result of a normal metabolism, of which the greater part, if not all, takes place in the muscles. The muscular efficiency of the person seems to depend upon the intensity of this process. In another paper(107) which he published dealing with a patient with a permanent biliary fistula, he also noticed a low creatinin excretion.

C. N. Longridge in a paper (103) on the involution of the uterine, found that the creatinin (as nitrogen) excretion during the first week after delivery is about 3% as compared to the total nitrogen excretion. He used a creatinin free diet containing about 13.5 gms. of nitrogen per diem. He noticed a rise in the total nitrogen at the end of the first week.

The low figures which he gets are probably due to his not estimating the total creatinin, i.e. the preformed creatinin + creatin (as creatinin). For the amount of creatin excreted after delivery is fairly high. This may be illustrated from a case of a woman who was on a creatin free diet, and whose urine I examined on the 4th and 6th days after delivery.

1./

	Urine	Creatinin	Creatin (as Creatinin)
1.	1562 c.c.	2.178 gm.	.665 gm.
2.	680	.812	.270

or expressed as nitrogen.

	Total Creatinin N.	Creatinin N.	Creatin N.
1.	1.037 gm.	.795 gm.	.242 gm.
2.	.394	.296	.098

The total nitrogen was not estimated, yet these figures show a fairly large amount of nitrogen excreted as creatin.

From the foregoing it was of interest to see whether creatin was also excreted in pregnant women before delivery. The results which I have got up till now are shown below.

Case	Creatinin	Creatin (as Creatinin)
1	5.74 mgm.	.3 mg.
	8.57	.07
2	7.94 mg.	.75 mg.
	12.01	1.59
	12.4	1.56
3	5.54 mg.	.32 mg.
	6.13	.20
4	7.64 mg.	.62 mg.
5	6.43	.10

The urines of these cases were only examined to find out whether creatin was present. It was therefore decided to estimate the creatin per diem. This could not be done very satisfactorily owing to the difficulty in getting all the urines carefully collected. But the following figures give a fairly approximate idea.

Case/

Case	Urine	Creatinin	Creatin (as Creatinin)
1	1192 c.c.	1.0847 gm.	.066 gm.
	1420	0.999	.116
	1988	1.123	.04
2	1079	.919	.2
	992	.821	.13
	1363	.883	.06
3	965	.697	.07
	1363	.953	.13
4	965	.531	.043
5	1533	.472	.045
6	2044	.973	.061
	2050	.803	.108
7	1760	1.250	.120
	1910	1.212	.063

All these cases were expected to be delivered within one month, when the urine was examined. They were healthy young women, though one of them, (case 2), was troubled with varicose veins.

The foregoing table rather indicates that creatin is present in all these cases even though in some of them only in very small quantities. These latter, one must look upon as doubtful. Certainly before one can say definitely that creatin is present in the urine of pregnant women, it will be necessary to investigate a large number of cases.

All these cases were put upon a creatin free diet, namely, porridge, bread, butter, eggs, vegetables, coffee or tea; meat of any kind being carefully excluded.

Spriggs/

Spriggs(104) examined a number of conditions in which the muscular system was either directly or indirectly affected. He found that the creatinin excretion is lowered where the bulk of the muscle tissue is diminished, e.g. in the primary myopathies. The same is seen where the muscular activity and the muscular tone are depressed by an affection of the muscle or motor apparatus, e.g. Myasthenia gravis and amyotonia conjenita. But in cases where the muscular tone is lowered by an interference with the sensory path, e.g. locomotor ataxia, it is unaffected. In cases of abnormal muscular activity e.g. in tetanus and spasticity, he was unable to note more than a slight increase in its excretion. His conclusions are that "creatinin is probably connected with the nutritional metabolism of the muscle fibre and is not a substance formed in the act of contraction."

Mellanby performed some incubation experiments on chicks. He studied the growth, the development of the liver and the increase of creatin, in their relationship to one another and was able to show that these three developed synchronously up to near hatching, when an increase in the creatin formation was observed corresponding with the growth of the liver. The muscular growth on the other hand had

almost/

almost ceased. This Mellanby held suggests that the liver plays a very important part in the formation of creatinin, and consequently he examined a number of patients with disease of the liver. He found that the excretion of creatinin in disease of the liver is low. Patients suffering from cancer of the liver excrete a large amount of creatin, whereas in cirrhosis and engorged livers there is no, or practically no, creatin in the urine. Mellanby states that the diminished creatinin excretion is more likely to be due to depressed liver activity than to any circulatory disturbance. This small amount of creatinin in liver disease would give additional support to the suggestion that the liver is responsible for the formation of creatinin. As regards the presence of creatin in the urine, he concludes that in carcinoma of the liver with accompanying loss of body weight it is probable that the creatin set free by the breaking down of the muscle cells is excreted without being changed to creatinin.

Hoogenhuyze and Verploegh (13) noted a low excretion of creatinin in some cases of liver disease while in others the excretion was normal. In certain cases it was even excessive. They also found creatin in the urine in cases of carcinoma of liver where the disease had destroyed the greater part of the/

the organ. But in patients with liver disease, where the function of the organ was depressed, creatin was only present in the urine in small quantities, or not at all. No creatin was found in cases of carcinoma of any part of the body, when the liver was unaffected. These investigators add that the presence of creatin might be explained by the metabolic processes being reduced to a minimum in all the organs, and the liver consequently rendered unable to convert the creatin into creatinin. But they favour the idea according to which the muscle disintegration is increased and an increased amount of creatin set free, which owing to the liver being functionless as such (because of the cancer) passes on and is excreted with the urine. This would also explain the diminution of the creatinin, for in the above disintegration, the creatinin formation in the various tissues will be diminished if not stopped, and so the amount excreted from this source will be small. Underhill and Kleiner(105) as well as Richards and Wallace(106) got similar results with cases of liver disease which they examined.

Leffmann (55) induced organic disease of the liver in a dog by giving it amylalcohol as well as phosphorous, in order to study the proper relationship of creatin and creatinin to the liver function./

function. His conclusions are :-

(1) The creatin and creatinin excretion in a well nourished animal is fairly constant. When creatin or creatinin are given with the food in such an animal, they are again conpletely excreted.

(2) Creatin given per os or injected is never changed to creatinin. In hunger this creatin is, however, practically all retained.

(3) When the liver is damaged, and there is increased proteid breakdown, there is a larger amount of excreted creatinin, followed by a diminution. With the diminished creatinin excretion an increased output of creatin is got. From this he concludes that the liver is probably the seat of formation of creatinin. Leffmann forms the following hypothesis from this :- When the muscle requires a supply of creatin, a ferment comes into action, which converts the necessary amount of creatinin into creatin. This he holds is proven by the feeding experiments with meat extract, in which case because of the large amount of proteid present, the creatin is mostly excreted and not retained. Whereas when pure creatin is ingested, there being no abundance of proteid with it, such creatin is retained in the body.

He also poisoned the kidneys of dogs with potassium chromate. The output of creatinin was continually/

tinually lowered with the progress of the lesions, but, in proportion with the fall in the creatinin, the output of creatin rose so that ultimately the ratio of $\frac{\text{creatinin}}{\text{creatin}} = 1.2$. A still greater increase in creatin output was observed after intravascular injection of creatin or after a beef diet.

The author concluded that creatinin and creatin formation have to be regarded as two phases in the metabolism of one substance.

Cathcart (84) puts forward another theory according to which the liver is the organ most deeply concerned in one stage at least of the carbohydrate metabolism. If the gylcogen storing capacity of the liver were interfered with there would no longer be a proper supply of sugar available, with the result that faulty and incomplete synthesis would take place.

As regards blood diseases apart from Myelogenous and lymphatic leukaemia, Hofmann studied Chlorosis (67), and Stejskal and Erber, cases of Pernicious Anaemia (98). They found a low creatinin excretion in these conditions.

Levene and Kristeller(108) examined a number of pathological conditions, classifying them under three headings :-

(1) Those which are associated with a cellular activity/

activity of a very high intensity, e.g. convulsions, maniacal conditions, fever, etc.

(2) In which the cellular activity is depressed, e.g. paralysis, fasting, etc.

(3) Conditions in which a deficiency in the function of an individual organ is marked, e.g. liver and kidney diseases.

From their results they hold that there are various factors which regulate the output of creatinin, such as the formation of the substance, and its oxidation. Any disturbance of either of these two factors may lead to an abnormal creatinin output. The second factor may only be partially deficient, so that ingested creatin fails to be further oxidised.

Levene and Kristeller also hold that creatin and creatinin are different phases of one substance, for they observed that a diminution in the creatinin excretion was accompanied by a rise in creatin. They also found that a high proteid diet (creatin free) caused in some patients an increased excretion of both creatin and creatinin. They explain the normal creatin excretion during conditions of high muscular activity by assuming that the tissues have greater power in oxidising the creatin, even though it is produced in a larger amount than under normal conditions.

conditions.

On examining a large number of urines of patients suffering from various diseases I found that creatin was present in most of them, but in rather variable quantities.

In one condition, diabetes, I always found creatin excreted in large quantities. As creatin is present in the urine after a meat diet it is essential to eliminate the exogenous creatin by adjusting the diet. As it may also appear in the urine as the result of the complete withdrawal of carbo-hydrates from the diet, it was essential to give the patients a creatin free diet containing a moderate amount of carbo-hydrates.

The diet given to the following cases unless otherwise indicated consisted in the main of porridge, bread or toast with butter, milk soups and puddings, vegetables, eggs and milk, and tea.

----- The following four cases show the presence of creatin in the urine.

	Urine.	Creatinin.	Creatin.*
1.	3182 c.c.	.795 gm.	.238 gm.
	3921	1.117	.337
	3694	.443	.109
	3654	.706	.142
2.	2273	1.702	1.115
	2273	1.266	.454
	2387	.811	.561
3.	4404	.920	.745
4.	1790	1.145	1.029

* (Expressed as Creatinin) The creatinin coefficient or the creatinin nitrogen per kilo body weight.

------The first and second cases, which were of moderate severity, both show a definite creatin excretion. The creatinin coefficient+ of the first case was 5.4 mg., which is somewhat low. The third and fourth cases were both fatal. Extended observations were not possible, but the cases show a creatin excretion almost equalling that of creatinin.

----- The following case, a man, whose weight was 53.9 kilos., and who suffered from diabetes shows a creatinin coefficient of 8.8 for the average excretion, irrespective of diet, which would be considered normal, whereas on a creatin free diet his creatinin coefficient is 3.2, which is decidedly low. The creatin excretion in this case is also high. It nearly equals the creatinin in amount on the day in which a creatin free diet was given. The rise in creatin, as well as in creatinin on the days on which a half diabetic and a full diabetic diet were given, (namely on a meat rich diet) is due to the ingestion of exogenous creatin and creatinin.

5.

Urine.	Creatinin.	Creatin*	
5115 c.c.	1.0127	.6443	Fish at dinner.
6194	2.156	.483	
4830	0.454	.391	
2855	1.048	.604	½ diabetic diet.
2955	1.573	.642	½ diabetic diet.
2728	1.096	.841	Full diabetic diet.

Case 6 /

* (Expressed as Creatinin)

+ Creatinin Coefficient or the Creatinin Nitrogen in Milligrams per Kilo Body Weight.

Case 6. The following is a case of a girl suffering from diabetes. Her weight was about eight stone and her average creatinin coefficient was about 5.7, which is not particularly low. The creatin excretion is not so high as in the preceding case.

6.

Urine.	Creatinin.	Creatin*
2955 c.c.	.564 gm.	1.06 gm.
2614	.732	.397
3012	.844	.107
3182	.792	.423
4546	.663	.181
4092	.630	.433

The preceding when expressed as nitrogen, and compared to the total nitrogen are as follows :-

Total Nitrogen.	Creatinin - N.	Creatin - N.
32.600 gm.	.206 gm. (.6%)	.387 (1.1%)
21.37	.267 (1.2%)	.116 (.5%)
18.13	.308 (1.6%)	.038 (.2%)

This table is of interest as with an increase in carbohydrates the creatin excretion gradually diminishes.

The creatinin, as such, does not show a similar rise per diem. When, however, the total percentages of creatin and creatinin - Nitrogen taken together, are compared to the total nitrogen per diem the results are constant.

----- Case 7 is that of a patient suffering from epileptic fits/

* Expressed as Creatinin.

fits as well as glycosuria. His creatinin coefficient on the last day of a creatin free diet was 4.1. In this case the creatin was more or less constant when considered from the standpoint of the daily excretion. Even on the day of a fast** there was no definite alteration. The excretion of creatinin increased definitely on the day of the fast.

Urine.	Creatinin.	Creatin.*
3410 c.c.	1.079 gm.	.218 gm.
2671	.940	.197
3410	.723	.229
** 2784	1.256	.207

-----Case 8 is that of a diabetic, a middle-aged man of much the same type as Case 7. His creatinin coefficient is about 3.3. In this case the creatin excretion is constant.

Urine.	Creatinin.	Creatin.*
3636 c.c.	.483 gm.	.306 gm.
4204	.744	.487
3124	.474	.481

-----Case 9, a young man of 22 years (diabetic) with creatinin coefficient of 3.9. This case also shows a diminution in creatin on a creatin free diet, as in Case 6.

Urine	Creatinin.	Creatin.*
4887 c.c.	.723 gm.	.504 gm.
4460	.551	.436
4602	1.194	z739 (Diabetic diet.)

Case/

* Expressed as Creatinin.

----- Case 10. A woman aged 40. Diabetic.

This case is of interest owing to the relative constancy in the excretion both of creatin and creatinin on a creatin free diet. The sugar in the urine of the patient gradually diminished from about 130 gm. per diem to about 60 gm. on a reduced diet, creatin free. Still the case does not show the constancy in creatinin excretion which Folin and Shaffer say was present in normal cases.

Urine.	Creatinin.	Creatin.*
2671 c.c.	1.374 gm.	.667
2273	1.416	.585
2216	1.136	.311
2230	1.374	.375
2728	1.674	.390
2955	1.300	.360

This case was again dealt with at a later date, and given a similar diet. On this occasion however the sugar remained about 140 gm.

Days	Urine.	Creatinin.	Creatin.*	
1.	2273 c.c.	1.736	.482	
2.	2427	0.895	.432	
3.	2386	1.123	.517	
4.	2386	1.098	0.458	(Extracted meat)
5.	2841	1.307	.755	" "
6.	2841	1.343	2.17	broth
7.	2784	1.149	.777	
8.	2557	1.02	.573	
9.	2727	1.162	.647	
10.	2614	2.64	.775	fast
11.	509	.808	.351	fat diet.
12.	624	.835	.201	" "
13.	738	.790	.174	" " with toast.

The/

* Expressed as Creatinin.

The patient was at first on a creatin free diet. Then she got meat which had been boiled for three hours and the broth strained off. The broth she got on the sixth day. A rise of creatinin and creatin noticed after feeding with meat, and a marked rise in the creatin excretion after ingestion of the broth. She later had a 36 hours' fast, followed by a diet of cream, and the second day after the fast by cream, butter, cheese and buttermilk. On the day of the fast a rise in creatinin and creatin observed. On the fat diet a marked decrease in both creatinin and creatin got.

Days	Total Nitrogen.	Creatinin - N.	Creatin - N.
3.	19.03 c.c.	.409 gm. (2.09%)	.165 (.86%)
4.	22.119	.40 (1.8 %)	.146 (.66%)
5.	23.864	.477 (1.99%)	.281 (1.1 %)
6.	24.091	.490 (2.03%)	.264 (1.09%)
7.	21.047	.419 (1.9 %)	.289 (1.3 %)
8.	18.819	.372 (1.9 %)	.209 (1.1 %)
9.	21.761	.424 (1.9 %)	.236 (1.08%)
10.	35.498	.963 (2.7 %)	.383 (.8 %)
11.	9.364	.294 (3.1 %)	.128 (1.3 %)
12.	12.966	.304 (2.3 %)	.073 (.5 %)
13.	11.881	.288 (2.4 %)	.063 (.5 %)

The above table shows the creatinin and creatin expressed as nitrogen, and their percentage to the total nitrogen is also indicated. This shows that the creatinin and creatin compared to the total nitrogen show no increase after ingestion of the meat/

The creatin on the first day of the meat diet shows a diminution. Also a rise on the day after the broth diet.

meat or the broth. On the day of the fast a rise in the creatinin excretion is noticed and this is further increased the next day when the patient only got cream, and remains high while the fat diet is continued. The creatin only shows an increase the day after the fast and then on the fat diet sinks down, to remain constant at about .5%. This may be due to the buttermilk which the patient drank ($\frac{1}{2}$ pint per diem) and which contains some carbohydrates.

It was of interest to see what effect the experimental production of diabetes would have on the creatin and creatinin excretion.

A dog was put on a creatin free diet. At first there was some creatin in the urine, but this soon disappeared. The dog then got two injections each of about 1 gm. phloridzin dissolved by adding some sodium bicarbonate to the solution.

Three injections of phloridzin were not given as the material ran short, and so the maximum effect could not be attained, Lusk (110) and Cremer (111).

	Urine.	Creatinin.	Creatin.*
1.	360 c.c.	.214 gm.	-
2.	265	.171	-
3.	300	.229	-
4.	*.*. 610	.240	.024 gm.
5.	** 550	.227	.051

* Expressed as Creatinin.

** Specimen after injection of 1 gm. Phloridzin.

In this table the creatinin excretion shown is fairly constant in amount. On the first three occasions no creatin or glucose was found in the urine. After the injections, however, both appeared. The sugar was very abundant on the second day of the injection.

	Total Nitrogen.	Creatinin - N.		Creatin - N.	
1.	3.646 gm.	.078 gm.	(2.1%)	-	
3.	2.430	.082	(3.3%)	-	
4.**	4.453	.087	(1.9%)	.008 gm.	(.17%)
5.**	4.158	.082	(1.9%)	.016	(.39%)

In this table the creatinin and creatin nitrogen are compared to the total nitrogen excreted. At first no creatin was present. After injection there was a rise in the total nitrogen, as well as in the appearance of creatin in the urine, and this was greater on the second day. The creatinin, however, showed very slight variations.

I tried to confirm the above experiment on myself by taking the phloridzin per os, but found no sugar in the urine. This was due to the phloridzin being badly absorbed from the intestines as was shown by Loewi (112) and I did not have sufficient phloridzin to get even the small amount of sugar which Mering(113) got./

** Specimen after injection of 1 gm. Phloridzin.

got.

---Case 11. An acute case of progressive muscular atrophy of a man of 38. The average creatinin coefficient was 4.6, which is low.

	Urine.	Creatinin.	Creatin.
1.	852 c.c.	1.232 gm.	1.120 gm.
2.	625	.713	.145
3.	852	1.069	.430
4.	426	.547	.013
5.	852	1.077	.133

Here the total amount of creatinin excreted per diem, shows no constancy. Creatin is also present and in variable amounts.

The creatinin nitrogen compared to the

	Total Nitrogen.	Creatinin - N.	Creatin. - N.
2.	8.880 gm.	.260 (2.9 %)	.053 gm. (.58%)
3.	13.919	.390 (2.8 %)	.156 (1.1 %)
4.	6.858	.199 (2.9 %)	.005 (.07%)

Total nitrogen, however, shows a constancy in the percentage. The creatin does not show this constancy in the percentage.

----- Case 12. A case of progressive muscular atrophy. (Man aged 45) Not so acute as the preceding.

Urine.	Creatinin.	Creatin.
1705 c.c.	.321 gm.	.145 gm.
1705	.451	.121
1278	1.025	.162

This case shows the presence of creatin, also variability/

variability in creatinin excretion.

---- Case 13. Case of Progressive Muscular Atrophy.

(Man aged 60 years)

Urine.	Creatinin.	Creatin.
1136 c.c.	.752 gm.	.008 gm.
1420	.568	.027

Here only a very small quantity of creatin was present. The creatinin coefficient was about 4.

---- Case 14. Case of Addison's Disease. (Man about 40 years of age) Creatinin coefficient on average about 4.0.

Urine.	Creatinin.	Creatin.
852 c.c.	.419 gm.	.011 gm.
1077	.773	.04
795	.536	.00

In this case the creatin excretion diminished on a creatin free diet, and eventually disappeared.

---- Case 15. A girl aged 15, suffering from inanition which terminated fatally.

	Urine.	Creatinin.
1.	-	.092 gm.
2.	660 c.c.	.860
3.	225	.08
4.	465	Inestimable.

The creatinin excretion per diem is here very low, the creatinin coefficient for the 1st and 2nd specimens being about 1.4.

The/

The fourth sample was got two days before the patient died, and it shows no creatinin in the urine.

From the preceding cases I conclude that endogenous creatin has been definitely proved to be present in the urine of diabetic subjects. The total excretions of creatin and creatinin per day are however variable, but when the nitrogen content of these substances is compared with the daily nitrogen excretion a constancy in creatinin excretion is sometimes noticeable. There is not however this constancy in creatin excretion.

Case No. 10 was much longer under observation, and of it, it is possible to speak with confidence as regards the food taken and the total quantity of urine passed. The patient excreted about 1 gm. of creatinin per diem, while on a creatin free diet. The excretion expressed as nitrogen was 1.9 % of the total nitrogen.

Folin in his analyses of thirty normal urines, gives the creatinin nitrogen percentage as from 3.2 - 3.8 of the total nitrogen.

The above observation shows that the creatinin excretion is diminished.

Another/

Another point which this case brings out is that the creatin and creatinin excretions are not necessarily interdependent.

With reference to Case No. 6, when the patient was put on a creatin free diet, an increase in the creatinin was noticed, but the creatin excretion was diminished. The combined nitrogen excretion with these substances remained constant, namely 1.8 %. This suggests that with a creatin free diet consisting of a fair amount of carbohydrates, the patient was apparently able to oxidise some of the carbohydrate which acted as a protein sparer and caused a greater conversion of creatin into creatinin. That this does not take place in Case No. 10 is probably but evidence of a deficiency in the oxidising power of the patient.

When the diabetic patient (Case No. 10) fasted there was an increase in the creatin excretion on the day of the fast, which nearly equalled the average amount of creatin excreted by a normal subject during fasting, (Cathcart (81)) . This goes to prove that the creatin present in diabetes is not wholly due to starvation.

Case No. 10 also shows that the creatin excreted is not an evidence of muscle catabolism, for the patient/

patient in this case showed no loss of weight. She rather gained weight.

From the phloridzin experiment we see that the increased excretion of carbohydrates, which prevents the organism from utilising the potential energy of them, plays an important part in the appearance of creatin in the urine, probably in great part owing to the defective oxidation which results. This experiment also shows an increase in the total nitrogen, ~~a condition~~ which is also found in the urine of diabetics (109) even when not on a strict diabetic (meat rich) diet, but simply owing to the fact that the carbohydrates in the above experiment as well as in diabetes are not acting as protein sparers.

SUMMARY OF RESULTS.

1. THE IMPORTANCE OF CONSIDERING THE DEFECTS OF EXPERIMENTAL METHODS.

2. THE PRESENCE OF CREATIN IN THE BRAIN.

3. THE PRESENCE OF SMALL QUANTITIES OF CREATIN IN URINES OF NORMAL PREGNANT WOMEN.

4. THE PRESENCE OF CREATIN IN DIABETIC SUBJECTS AND DIMINUTION OF CREATININ.

5. THE APPEARANCE OF CREATIN IN URINE OF A DOG AFTER INJECTION OF PHLORIDZIN.

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