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CONTENTS

SOME PROBLEMS in ANIMAL METABOLISM

The really essential materials for the maintenance of life are the proteins. For its supply of these materials the animal kingdom is dependent on the vegetable kingdom, where, from inorganic precursors, these complex nitrogenous substances take origin. Without these substances neither animal nor vegetable life can exist.

How the animal tissues actually utilise the protein taken in in the diet, in what form or condition the digested protein reaches these tissues and what changes it undergoes after being taken up, whether it becomes, first of all, an integral part of the tissue protein already existing there before it can be utilised as is believed by Pflüger, or whether, as stated by Voit, no such conversion into tissue protein is necessary before it can yield up its energy, are questions which, principally owing to the numerous technical difficulties standing in the way of the experimental investigation, are still awaiting solution.

During the past decade information as to the real nature and constitution of the protein molecule has gradually been accumulating, thanks mainly to the brilliant work of E. Fischer and his co-workers. These workers have shewn us

how numerous are the nuclei which go to make up the large and complex protein molecule and at the same time they have clearly demonstrated the heterogeneity in composition of proteins of diverse origin. Further they have shewn how two proteins of practically the same origin may be very different in constitution, for example, on the one hand, gelatine which is obtained from the cartilaginous tissue of the animal body contains over 16% of the amino acid glycocoll, about 2% of leucin and neither cystin nor tyrosin and, on the other, globin, the protein obtained from the haemoglobin of the animal's blood, contains no glycocoll but has over 29% of leucin and quite definite amounts of both cystin and tyrosin.

The ordinary food then is made up of practically endless varieties of combinations of the different constituent parts, - of monamino acids, of di-amino acids, and of numerous aromatic and other substances. From this heterogeneous mixture the body has to form its tissue protein. Does it then simply take up the protein molecule intact and convey it to the tissue cells as Liebig believed, or does it break up the material before use? (For the question as to the form in which protein is absorbed see following section). Whatever the form in which the tissues take their protein food supply they exert a selective action. As illustrating this selective power the investigation of Abderhalden and Samuely (1) offers a very pretty

although perhaps an exaggerated example. These workers attempted to alter the constitution of one of the tissue proteins in a very clever fashion. They bled a horse almost to exhaustion and then fed the animal with a protein - gliadin - obtained from wheat, of very different composition to that of the blood protein. After the horse had been kept on the gliadin diet until the supply of blood had practically returned to normal, - that is feeding took place whilst there was an active reformation of the serum protein going on, - a sample of blood was taken and on analysis it was found to have the same percentage composition as the normal blood. In other words the tissues had demonstrated their selective power as regards the utilisation of food materials within the body.

Recent work on the so-called precipitin reaction tends to show that still more subtle differences exist in the various protein articles of diet. Indeed, in all probability, the proteins of one species of animal differ from those of another, as it has been said there is "a chemistry of species". Thus the necessity for the tissues to have the full power of selecting the substances most suited for their individual existence.

Formerly it was believed that the full requirements of the body were satisfied if there were an abundant supply of the three main foodstuffs, Proteins, Carbohydrates and Fats,

that they were present in proper proportion and in sufficient amount to supply the calorific needs of the body. Evidence is gradually accumulating to show that no such simple generalisation is longer possible. The result of many metabolism experiments has shewn that, in the higher animals at least, substances must be present in the diet, which although they yield no energy to the body, yet, nevertheless, are of absolute importance for the maintenance of life. Many years ago Lunin (2) shewed that mice fed on a mixture of fat, casein from milk and cane sugar but ash free lived for only a short time. If sodium carbonate were added to the diet, in order to neutralise the acids formed from the break down of the protein substances, the mice lived about a third of the time longer. If to this artificial food inorganic salts were added, in exact proportion as they exist in milk, life was not prolonged longer than in the case where the sodium carbonate was added. Mice fed on whole natural milk on the other hand lived and thrived. In a natural food then like milk there exists some "minimal" substance which is absolutely necessary for the animal. As Bayliss and Starling (3) say we are no longer justified in judging a diet simply on its calorific value and its content in protein carbohydrate and fat. Again Hopkins (4) has been forced to a like conclusion from certain feeding experiments on mice carried out with a protein - zein - obtained from maize. This protein contains

no tryptophane (an amino acid not found among the decomposition products of zein) and when fed to mice fails to maintain life. When tryptophane was added to the dietary the survival period of the animal was greatly prolonged. In discussing this action of tryptophane Hopkins says. "But this result might well be expected, even if the tryptophane administered undergoes utilisation without directly contributing to tissue formation or structural maintenance. If it serves as a basis for the elaboration of a substance absolutely necessary for life - something, for instance, of an importance equal to that of adrenaline - ..... the utilisation of the constituent would seem to be of some direct and specific nature". These essential substances are looked on as perhaps precursors of secretions essential to life as in the work of Hopkins just referred to. (Tryptophane, it may be remarked, has been definitely stated by certain workers to be the precursor of adrenaline).

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II.

ABSORPTION of PROTEINS from the INTESTINAL CANAL

After ingestion the food protein is, under normal conditions, first subjected to peptic digestion followed by the digestive processes of the small intestine principally pancreatic in origin. The main question to be answered is, - In what form does the food protein leave the lumen of the alimentary canal? Unfortunately owing to the numerous technical difficulties attending experimentation on this part no absolutely definite although many suggestive results have been obtained.

The older workers believed (I will not here deal with the question of the absorption of the native or unaltered proteins; reference to this matter will be found in a reprint of a paper from Science Progress bound at the end of this Thesis) that the proteins left the intestinal canal in the form of proteoses and peptones, i.e., as the first comparatively simple products of gastro-intestinal digestion, and that after absorption into the cells of the intestinal mucous membrane immediate synthesis into tissue protein - serum protein - took place. Within the past decade it has been shewn that, in all probability, very little of the ingested protein is absorbed in this form. Kutscher and Seemann (1) Abderhalden (2) and others have shewn that under apparently normal conditions amino acids

can be obtained from the cavity of the small intestine. This method of investigating the intestinal contents is not however either very convenient or accurate as the protein food does not immediately decompose, it is not suddenly shattered into its nuclei by the action of the gastro-intestinal ferments, but is slowly disintegrated. Along with this slow setting free of the amino acids there is a constant absorption going on. Thus one cannot expect, even under the most favourable conditions, to find at any one time a collection of easily diffusible crystalline products - the amino acids - in the intestine. Another method which will attempt to obtain the products as they leave the intestine must be tried.

As this promised, on account of the diversity of opinion, to be a rather fruitful field for work in conjunction with Dr J.B. Leathes of the Lister Institute, London, I attempted to get some further information on this important subject. (Full paper at end of Thesis).

In the first place we attempted to repeat the work of Salvioli (3) carried out in Ludwig's laboratory in 1880 on whose authority the theory, that protein is absorbed as peptones and that after absorption these are converted by the cells of the intestinal mucous membrane into coaguable proteins, mainly rests. In the original experiments only a small piece of intestine was excised and perfused with a mixture of calf's

blood and normal saline solution. Into this loop of intestine a solution of Witte's peptone was put, and the perfusion carried on for several hours. At the end of the experiment the intestinal contents were examined but no peptone could be found either there or in the blood used for the perfusion.

Our first experiments only differed from those of Salvioli in that we used a much greater length of intestine. We too found that peptone disappeared from the intestine as such, yet no demonstrable absorption of nitrogenous substances took place. The perfusion of the intestine, even when undiluted blood of an animal of the same species as that from which the intestine used came, fails to keep the mucous membrane alive. We found that the mucous membrane in many cases had almost completely desquamated. No absorption, as has repeatedly been shewn, can take place when the intestinal mucous membrane is in any way injured. We believe that the conclusions drawn from Salvioli's work are quite unwarranted. The peptone could not be detected in the loop of intestine not because it was absorbed but because it was still further decomposed into simpler bodies - the amino acids etc - under the influence of the ferment erepsin. Cohnheim the discoverer of the ferment in question has shewn that it has the power to convert proteoses and peptones rapidly into simpler products. As this ferment is present in largest amount within the cells of the mucous

membrane the normal desquamation which took place actually aided its attack.

As the results here were so unsatisfactory we abandoned the perfusion method. The method which we finally adopted and which gave us very satisfactory results, was to leave the intestine in situ with circulation complete. The intestine we converted into a huge loop (the whole small intestine was employed as a rule) by ligaturing it off at the upper and lower ends and connecting tubes with the two ends of the intervening part. Through the upper tube we administered the peptone solution or other fluid as required, the lower tube served as a vent for the escape of gas and to permit of peristaltic movement. To investigate the question of absorption the blood was examined, a sample being drawn off before the experiment, and another at the end. This blood was examined as regards the total nitrogen present and for non-precipitable (by Tannic acid) nitrogen. In the majority of the experiments we tied cannulae into the ureters and the urine thus obtained served for the purposes of analysis. The result of our series of experiments was to show that, quite independent of the nature of the nitrogenous solution put into the intestine, the nitrogenous substances in the blood not precipitated by tannic acid were definitely increased in amount.

Our next attempt was to identify the nitrogenous

substances in the blood which were increased during absorption. As from the work of Nencki, Lang, Jacoby and others it was to be expected that ammonia would contribute largely to this increase. Such however was not the case as we were able to account for only about 4% of the non-precipitable nitrogen by means of the amount of ammonia we found.

We next turned to urea as a possible source of the increase noted. Our results shewed that urea accounted for almost one half of the increase of nitrogen not precipitated by tannic acid.

We then calculated the ratio between the increase of the non-precipitable nitrogen in the blood and the total amount of nitrogen which we knew to have been absorbed from the intestine and found on the average that about one seventh of the nitrogen absorbed could be accounted for in this way. As to the fate of the rest of the absorbed nitrogen we could obtain very little information. We thought that it might to a certain extent be stored in the liver and accordingly carried out some analysis on this organ with, however, but poor result. We found, it is true, a small gain after absorption, but at the very best this did not amount to more than 15% of the absorbed nitrogen, i.e., about an equal amount to that found in the blood.

As I already stated in some instances an examination of the urine during the course of absorption was made. The

figures we obtained shewed that, when the absorption was actively going on, there was a great increase in the urinary nitrogen. Taking the figures thus obtained into account we were able in one case at least to trace over 70% of the absorbed nitrogen. That we were justified in taking the urinary nitrogen into account is shewn by the work of Nencki & Zaleski (4) and others who have demonstrated that during absorption there is a great rise in the ammonia content of the portal blood. This ammonia as we know is conveyed to the liver and there converted into urea and immediately excreted by the kidneys.

Of course our failure to recover the whole of the absorbed nitrogen might be held to be due, as some workers do hold, to a resynthesis of the absorbed product in the cells of the intestinal mucous membrane, with the result that the absorbed products would reach the blood in a coagulable form. We, however, were never able to obtain the slightest proof of such a synthesis, as estimated by an increase of the total nitrogen, having taken place. Further confirmation of this belief was obtained by the haemaglobinometric examinations before and after absorption.

We incline to the belief that only a minimal amount, such an amount of nitrogen as is required to supply the actual nitrogen requirements of the tissues, reaches the systemic blood. The rest of the protein loses its nitrogen and reaches

the tissues in non-nitrogenous combination, probably largely as members of the fatty acid series, and not in the form of a resynthesised coagulable protein. We attempted to trace this non-nitrogenous material, but the results, owing to the poverty of reliable analytical methods in this particular field, were too irregular to enable us to draw any definite conclusions.

A point in favour of our contention that the protein is reduced to comparatively simple bodies before absorption is found in the work of Loewi (5) who discovered that the products of pancreatic digestion in vitro could serve, when given with a sufficiency of carbohydrates and fats, as sources of protein food supply. Another point in our favour lies in the work of Abderhalden and Samuely and the selective action of the tissue cells already referred to in Part I of this Thesis.

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III.

PURIN METABOLISM

The subject of Purin Metabolism has always been one of great interest, but this interest has greatly increased in recent years since it has been shewn by Burian and Schur (1) that there are two quite independent sources of supply of the urinary uric acid.

The output due to one of the sources, the food supply, varies greatly with the nature of the food and the amount of the precursors of uric acid which it contains, whereas the output from the other source, the tissues themselves, is stated to be practically constant. That from the food is said to be exogenous in origin and that from the tissues endogenous. It is, of course, only the endogenous purin metabolism which is of real interest. From what tissues and in what form does uric acid arise? This question has caused much debate. The materials from which uric acid can be derived outside the body and which, when fed, give rise to an increased output in the urine are the nucleo-proteins. Does then uric acid normally arise in the tissues directly from the nucleo-proteins? Burian and Schur, who have carried out many laborious experiments, are inclined to think not.

Whilst carrying out my experiments on starvation

I had an unique opportunity of investigating this very question, as here any uric acid which might be excreted must be solely endogenous in origin (See reprint "Über die Zusammensetzung des Hungerharns"at end of thesis)

Burian and Schur in their papers question whether the results obtained during starvation can be admitted as evidence on this question. They however drew all their conclusions on this point from work done previous to their paper, both by old and modern methods of analysis. They did not attempt to carry out any investigations of their own along this line. They in one of their papers, admit that the results obtained from the investigations of the amount of purin material excreted during starvation, show that at one time the excretion may exceed the endogenous purin output, when the subject of the experiment is kept on a purin free diet under normal conditions, and at another the amount excreted may fall short of the normal endogenous amount. They make no attempt to reconcile in any way these markedly discrepant observations. Folin (2) in discussing the output of purins in his paper on the laws which govern the composition of the urine, during feeding with a nitrogen rich and a nitrogen free purin free diet, comes to the conclusion that it must be held to be extremely questionable whether all the purin eliminated, even under normal conditions, originates in the break down of living tissue protoplasm.

Although he admits that part of the purin excreted most certainly arises from the break down of protoplasm he does not offer any very definite opinion as to the site of origin of the rest of the purin excreted. He merely asserts that the metabolic processes which determine the purin output may be regarded as being in a relatively unstable equilibrium. His experiments show that the uric acid elimination is reduced when the total nitrogen elimination is very much diminished.

As to the explanation of the results which I obtained in my investigation. In a paper published several years ago Nemser (3) states that the portion of the cell which resists longest disintegration during starvation is the nuclein part. We may thus, if Nemser's results be accurate, explain how as the fast progresses there is a rise in the output of purin substances such as I observed in my subject. It would appear then, and this is borne out by my figures obtained during the pre-fast period when the man was on a nitrogen rich but purin free diet, and also by the results of Hirschfeld (4) Burian and Schur and others, that although the endogenous urinary purin output, is more or less constant for a given individual, this statement only holds good when the subject of the experiment is kept under ordinary dietary conditions. Here it would appear, according to Burian and others, that the break down of urinary purin giving substance is very steady but this does not seem to

hold during the course of starvation. In starvation the body is dependent on its own tissues to supply the essential materials to carry on the various processes necessary for life. It would seem that the tissues to be utilised first are those of least value, those of greater importance being preserved as long as possible. During the early days of fasting the tissues which suffer most are those which contain little or none of the materials which give rise to purin, the general protoplasm of the cells, probably mostly of muscle tissue, is attacked leaving the valuable nuclein part, i.e. the part concerned in reproductive activities intact. As the period of starvation lengthens out this material can no longer be so freely obtained, as is shewn in the diminution in the total nitrogen output. Accompanying this fall in total nitrogen there is a rise in the amount of purin excreted. I have interpreted this phenomenon as meaning that the body has now commenced to draw upon its more valuable preserves, the nucleins, which contain in largest amount the precursors of the purin bodies. Burian and Schur hold that the Hypoxanthin - a mon-oxypurin - of the muscle tissue is the most important source of urinary purin. It may well be then that this is the sole or chief source of supply during the early days of the fast but as the period of starvation lengthens other sources, more complex in nature, must be drawn upon.

In the following table I have attempted to calculate

the amount of endogenous purin which might have been expected from the amount of protein broken down, as evidenced by the output of total nitrogen. The purin content was calculated from data obtained by Burian and Schur. As in addition to muscle, glandular material rich in nucleins, also breaks down during starvation, I have given an increased theoretical yield in a second column.

Day of Starvation	Muscle Tissue Total N × 6.25 × 5	Purin Nitrogen 0,03% (Muscle)	Purin Nitrogen 0,06% (Muscle + Gland)	Actual Excretion Uric Acid	Actual Excretion Total Purin
I	328,5	0,098	0,197	0,12	0,15
II	449.4	0,134	0,269	0,06	0,11
III	428.8	0,128	0,256	0,06	0,09
IV	428.8	0,128	0,256	0,08	0,14
V	353,1	0,106	0,212	—	—
VI	336,5	0,100	0,200	0,10	0,14
VII	302.2	0,090	0,180	0,12	0,15
VIII	297.5	0,089	0,178	0,12	0,15
IX	293.4	0,088	0,176	—	—
X	261,8	0,078	0,156	0,16	0,20
XI	265,3	0,079	0,158	0,16	0,20
XII	274.0	0,082	0,164	0,17	0,19
XIII	280,3	0,084	0,168	—	—
XIV	243,1	0,073	0,146	0,17	(0,19?)

From these figures it will be seen that there is a steady decrease in the possible supply of purin nitrogen calculated from the hypoxanthin source whereas actually there is found to be a steady rise in the amount excreted. I therefore infer that tissues or parts of tissues - probably the nuclein

parts - rich in the mother bodies of the endogenous urinary purins are being utilised.

In conjunction with Dr Leathes I investigated another phase of the metabolism of the purins (uric acid) and that was on the relation between the output of uric acid and the rate of heat production in the body. (See reprint at end of thesis) In this investigation the experiments were designed to show how the reaction of the body to loss of heat on the one hand, and the heat produced by ordinary muscular exertions on the other, might affect the amount of uric acid in the urine. In the course of the experiment one of us was put on a purin free diet and then an experiment in which the subject was exposed in such a condition (stripped at a swimming bath) that there would be great heat loss was carried out. This experiment a few days later was again repeated but in addition active muscular exertions were introduced. Another experiment was carried out in which the subject performed again severe muscular exertions but this time heavily clothed and finally still another experiment was carried out under the same conditions as the first.

The results of our experiments point to the inference that at least one of the factors largely concerned with the production of uric acid in the body and its appearance in the urine is the reaction of the body to the loss of heat, and

further that this reaction involves some form of activity other than voluntary movements of the muscles.

A further series of experiments which I have carried out in conjunction with Dr Steel (Glasgow) and which are not yet published would seem to point to the fact that variations in the output of uric acid after severe voluntary muscular exertions depends to some extent on the conditions under which the muscular exercise is taken. In our experiments hard exercise was at one time taken in the open Fives Court and at another in the hot room (37° C) of the Physiological Laboratory with both the doors and the ventilator tightly closed, with the result that under the conditions of the first experiment the rise in uric acid output was the less marked.

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IV.

CREATINE and CREATININE METABOLISM

The physiological history of these bodies is as yet unknown. Creatinine is a constant constituent of the urine and after urea and ammonia it forms the most abundant nitrogenous body there present. Creatine however under normal conditions is never found in the urine but in certain pathological states, especially where there is apparently a large break down of muscle tissue as in cancer (particularly in hepatic carcinoma according to E. Mellanby) it is constantly present in fair amount. Creatine is a constant and probably the most important nitrogenous extractive body present in muscle and the inference, a perfectly fair and warranted one, has been drawn that the creatinine of the urine is derived from this creatine. Chemically the two bodies are very closely allied, the only difference being that Creatine contains a molecule of water more than Creatinine.

From the work of Folin (1) it may be taken that the Creatinine excreted represents an end product in the metabolism of living tissues as it is found that when an examination is made under constant conditions the output of Creatinine is quite independent of the quantity of protein consumed. If we take it that Creatine is the precursor of Creatinine another experiment of Folin's (2)

is rather antagonistic. He found that if Creatine was fed to a person on a constant diet, in not too large amounts, there was no increase in the urine either of Creatinine or Creatine itself, but if larger doses were given creatine appeared but without any increase in the amount of creatinine. On the other hand when he fed creatinine the creatinine can be again recovered from the urine. He therefore concludes that muscle creatine cannot be justifiably regarded as a precursor of creatinine.

During my study of the urine during starvation (See reprint Hungerharn) I discovered that during the course of the fast the output of creatinine steadily fell and that creatine, which was not present before the fast except in minute amount rose in amount excreted as the fast progressed. This observation has been again recently made both by Benedict and Folin (3) in the case of starving people and in starving rabbits by Dorner (4).

The amounts of creatine which Benedict found agree fairly well with my results, the output in his case varying from 0.03 to 0.16 grm whereas in mine it varied from 0.02 to 0.17 grm. In Benedict's case as in mine the output of preformed creatinine fell. On the resumption of feeding with a milk diet the creatinine increased in amount but again as in my case it did not reach the level it stood at before the fast began.

Creatine was still found although in small amount after the feeding started, it was also present in just such small amount in the starvation experiment during the prefast period. In both cases although the diets previous to the fast were in every way ample for the bodily requirements yet in neither were they the ordinary every day mixed diets.

In one of his papers Benedict says "As pointed out in a discussion of the creatine determinations on fasting men the creatine excretion during fasting appears to be the result of the disintegration of flesh, and the hypothesis was there advanced that as the flesh was katabolized or broken down the creatine which existed in the flesh as such was excreted by the body as such and not converted to creatinine. According to this hypothesis, therefore the creatine and the creatinine of the urine have two entirely distinct origins.".

In another paper Benedict (5) shows that the output of creatine in certain pathological states is more or less a constant feature. The experimental evidence up to the present is too small yet to make a general statement but as Benedict says, such information as has been obtained would rather point to creatine being excreted in wasting diseases, i.e. where flesh is breaking down.

A review of Benedict's cases on which the observations on creatine output were made, shows that a fair proportion are

noted as under nourished but with one exception no details of their diets are given except that it was both creatine and creatinine free. Benedict had of course the work of Folin to go on as a basis for his theory that the creatine arising from the break down of flesh is excreted as such and not converted into creatinine. Folin, as already mentioned, found that the administration of creatine by the mouth did not give rise to any increase of urinary creatinine, but it must be remembered that Folin was quite unable to recover any creatine unless he gave very large doses. Folin therefore imagined that creatine taken into the body in the food had some particular role to play in the tissues as a very highly specialised food stuff, which served to maintain the nitrogen equilibrium in the living tissues. Another reason for the non appearance of the creatine either as creatine or creatinine in the urine may exist and that is that the creatine administered, unless in very large dose, never leaves the lumen of the intestine. The mere fact that when given in large doses creatine appears in the urine shows that it can be absorbed. Mr Mellanby of Cambridge has informed me that he has satisfactory evidence that creatine is readily destroyed by bacterial action but that such is not the case with creatinine. Here then, in all probability, lies the explanation of Folin's results that the creatine given was lost trace of whereas if creatinine be given it appeared again

in the urine. This fact of course decidedly weakens the case against the ordinary assumption that from muscle creatine the urinary creatinine takes origin.

The nature of the food would appear to play some part in the utilisation of the creatine. Folin shewed, when working at the investigation dealt with above, that on a nitrogen poor diet - the starch and cream diet - there was a greater retention of fed creatine than on a nitrogen rich diet. I do not think that the stress should be laid on the relative content in nitrogen, but in the light of Mellanby's observation attention should be directed as to which diet would form the most suitable medium for bacterial growth. May it not be however in the various cases examined by Benedict and others, and certainly during starvation, that some essential substance is absent or present in insufficient amount in the diet to bring about the conversion of creatine to creatinine? Folin in his original paper has pointed out that normal persons fed on a normal diet eliminate creatinine but no creatine. Is it not possible that the normal conversion of creatine to creatinine may be somewhat analogous to the conditions under which the utilisation of the decomposition products of proteins by the body tissues takes place? Lüthje (6) has recently pointed out that before these decomposition products can be utilised by the body and so replace "whole" protein, carbohydrates must be present in suffic-

ient amount. Again reference may be once more made to the work of Bayliss and Starling and of Hopkins on the selective action of the tissues and the apparent necessity of certain very special materials in the diet (See Section I of this thesis)

The fact that the creatine output in my case of starvation fell almost to nil and the creatinine output rose when the diet, although it was only the starch and cream diet, was resumed - a diet which may have contained the "essential" substance in insufficient amount - lends an air of probability to the above view.

At present in conjunction with Dr T. Graham Brown I am attempting to obtain further facts bearing on the theory that some article of diet plays an important part in the conversion of creatine into creatinine. Our results, so far as they go, distinctly favour such a theory. We have been able to influence the creatine content of frog's muscles by exercising them but so far we have been unable to <u>definitely</u> determine that the body formed from the creatine is creatinine although, from certain observations we have made, we believe that the inference may safely be drawn. From purely theoretical considerations we have been led to the belief that here again it may be the carbohydrate moiety of the diet which is the important item. In certain of our experiments we found that when we lowered the amount of carbohydrate stored in the body and then

exercised the various muscles that the diminution in the amount of creatine present in the muscle tissue was less than when the same experiment was performed when much carbohydrate was present. In other words it would seem that the presence of the carbohydrate leads to the more ready conversion of the creatine into some body as yet unknown but which may turn out to be creatinine.

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V.

EFFECT of STARVATION on the NITROGENOUS and NON-NITROGENOUS METABOLISM as indicated by the composition of the URINE.

About a year ago I had an excellent opportunity of studying the effect of starvation on the output of the various urinary constituents in the case of a professional starving man - Beauté - (See reprint at end of thesis). Many such observations have been made in the past but the present investigation differs from all the preceding in that the subject of the experiment was fed on a purin free standard diet devised by Folin, the effect of which on the composition of the urine was known, for a week before the actual fast began. This period of feeding was followed by a fast, during which the subject was under strict observation night and day, of two weeks duration, and this in turn was followed by another week on standard diet. In this way one was able to judge how the metabolism of the subject before the fast compared with that of normal people and gave at the same time results with which those obtained during the fast could be compared. Finally the period of feeding at the end of the fast may be divided into two quite distinct parts (1) when the subject was on a so called nitrogen free diet immediately following the close of the fast and (2) the period of a nitro-

gen rich diet which succeeded. This feeding after the fast gave some very interesting information as to the effect of food on the starving tissues of the body.

As regards some of the information obtained from the investigation. It was found that during the course of the fortnight's fast that Beauté lost 7.83 kilos or about 17 pounds. The most rapid loss of weight took place during the first four or five days thereafter the daily amount lost, fell and remained fairly constant until the end of the fast. The amount lost equalled about 12% of the original weight. This loss was made up of about 9½ lbs of protein and about 7½ lbs of fat and carbohydrate.

As regards his body measurements there was no very great diminution, the greatest loss was round the abdomen at the level of the umbilicus. During the course of the fast the muscular power of the subject did not seem to suffer much as, on the last day of the fast, he carried two 25 kilo weights about 6 yards with perfect ease.

With reference to the urinary constitutents as the uric acid and the creatine and creatinine output have already been dealt with (Sections III and IV) attention will be directed to the other important substances present.

The total nitrogen output fell from 16.45 grams on the last day of feeding to 7.78 grams on the last day of the

fast. On the first day of the fast the output fell suddenly to 10.51 grams but on the second day it rose again to 14.38 grams. This result agrees very closely with Prausnitz's results and his theory that the nitrogen waste during the early hours of fasting is prevented to some extent by the body drawing upon its stores of carbohydrate material. It is of course very well known that the giving of carbohydrate material prevents the waste of protein tissue, it is indeed one of the chief functions of the carbohydrates to act as "protein sparers". This protein sparing power is particularly well exemplified during the first three days of feeding after the fast on a nitrogen free diet but one rich in carbohydrate material. Here the output of nitrogen fell from the 7.78 grams of the last day of the fast to 2.84 grams on the third day of feeding. When the nitrogen rich diet was resumed, the same which before the fast caused an excretion of about 16.5 grams of nitrogen daily, the urinary nitrogen rose in amount but still just to about half of the pre fast amount. In other words an enormous retention of nitrogen took place in order to satisfy the demands of the tissues for this material. On the last day of the experiment the nitrogen output was still low.

As urea is by far the most abundant nitrogenous constituent of the urine it followed as is only natural, the line of the output of total nitrogen. As urea mostly comes from the

utilisation of protein tissue the result of the nitrogen free diet at the close of the fast is very striking in this connection. Urea formed 77% of the total nitrogen on the last day of the fast but on the third day of feeding it only amounted to 62% rising immediately the nitrogen rich diet was started to the prefast figure.

The output of ammonia at first fell during the period of starvation then steadily rose until the eighth day when it again began slowly to fall. An attempt was made to trace some connection between the variations in the output of ammonia and the output of the acetone and di-acetic acid which were shewn to be present throughout the fast. As a rule the variations in ammonia output can be traced to the formation of different acids in the body which combine with the ammonia and prevent its conversion into urea.

The undetermined nitrogen formed a fair percentage of the total nitrogen during starvation but not, on the whole, more than what is regarded as normal. As Brugsch has shewn the amino acids do not account for any very large part of this nitrogen. The maximum output of undetermined nitrogen for the whole experiment was on the second day of the nitrogen free diet at the close of the fast.

As regards the output of the inorganic constituents previous investigations were confirmed.

The excretion of Chlorine was absolutely characteristic of the fasting condition i.e. a steadily progressive fall in the daily output till the end of the fast. With the resumption of feeding the output rose slightly but not to the pre fast figure, in other words a marked retention took place amounting to between ten and eleven grams during the first four days of feeding. During the fast only about 66% of the Chlorine was excreted as Sodium Chloride whereas before the fast about 80% of the Chlorine was in this form.

As in the case of the Chlorine excretion the output of Phosphates fell slowly but steadily right to the end of the fasting period. Here again with the resumption of food a marked retention of the phosphates took place. Part of the phosphates excreted were demonstrated to have come from the break down of tissue other than protein, in all probability from bone. This agrees with the observations of Munk and others.

With the excretion of sulphur the same fall was observed as in the two previous inorganic constitutents. The sulphur output followed very closely the output of total nitrogen thus agreeing with the statement that the sulphur excreted during starvation comes from the break down of protein tissue. In my investigation the inorganic sulphates, the ethereal sulphates and the neutral sulphur present were all estimated. The most marked fall took place with the inorganic and ethereal

sulphates. Viewed as percentages of the total sulphur excretion it was found that the ethereal sulphates remained practically constant throughout the fasting period.

A limited number of estimations of the bases present in the urine during the experiment were carried out for me by Dr C.E. Fawsitt. A fall in output of all during the fast took place.

Finally an attempt was made on three of the fasting days to get some insight into the diurnal variation, if any, in the excretion of various nitrogenous substances. A control experiment was carried out on the sixth day after the fast when presumably conditions were again practically normal.

Leathes has shewn that the diurnal variation in the excretion of various nitrogenous bodies is very well marked and practically constant for each particular substance. He found that the maximum output of total nitrogen took place during the hours of sleep and the minimum during the early hours of the day, whereas in the case of uric acid, the maximum output takes place during the day up to about 4 p.m. and the minimum excretion during the night. In the present investigation the normal observation, i.e. on the sixth day after the fast, corresponds with Leathes's observation, but when the fasting urine figures were examined it was found that the maximum output of total nitrogen took place during waking hours. The ammonia output

followed the course of the total nitrogen fairly closely both in respect to the fasting and normal periods. In the case of the uric acid excretion both observations were much alike and further both conformed with the observations of Leathes.

For the Literature of this section see reprint "Über die Zusammensetzung des Hungerharns," bound with this thesis.