THE INFLUENCE OF CARBOHYDRATES AND FATS ON PROTEIN METABOLISM.

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THERE has always been a difficulty in drawing conclusions as to the effects of certain factors on endogenous metabolism since, unless in fasting, the excretory products may have come from either an exogenous or endogenous source, or from both of these.

Folin[1] in his paper on protein metabolism has stated that there are certain excretory substances characteristic of endogenous protein metabolism and others of exogenous. The substances which he classed in the first group were creatinine and neutral sulphur, and in the second urea and inorganic sulphates.

Experience has shown that it is very difficult to alter the daily output of creatinine to any considerable extent and yet if it were possible this would be the ideal product through which to investigate endogenous metabolism, as the method of quantitative estimation is so simple.

In 1907 I[2] found, as did also Benedict[3] about the same time, that creatine was a constant excretory product during inanition. I further noted that as soon as food was supplied the creatine practically disappeared. Here then was a product of pure endogenous origin the study of which might give some insight into the course of protein metabolism in the animal body. From the nature of the food given after the fast this diminution in the output of the creatine must have been due either to the influence of the carbohydrate and fat combined or to one of these alone. From some preliminary experiments carried out at the end of 1907 and from analogy with certain other feeding experiments which will be referred to later I came to the conclusion that the "active" part of the food was the carbohydrate.

In order to test thoroughly this supposition a series of experiments were designed, where, after a period of fasting in order to bring about the production of creatine as an excretory product, the food given would consist solely of one class of food stuffs—carbohydrates or fats.

I.

Each experiment from I to IV began with a complete fast of 40 hours duration which always brought about an output of some 150 mgrms of creatine. A fast of 60 hours was tried but without any corresponding gain in the output. This fast was followed by the ingestion of (1) carbohydrate food which consisted in two experiments of tapioca boiled with water, or made into small cakes with water, and eaten with a small quantity of honey. In another experiment the carbohydrate was in the form of banana meal made into small cakes with water and eaten with honey. (2) Fats. An attempt was made to live on pure butter and nothing else, but after two meals this food was abandoned on account of the nausea produced. Centrifugalised double cream (fat content 50—55 °/₀) was substituted. This contained very little nitrogen and was practically free from carbohydrate.

I myself was the subject of experiment in experiments I, II, III and V.

The methods of analysis employed were total nitrogen—Kjeldahl, urea—Folin or Morner-Folin, ammonia—Folin-Schaffer, uric acid—Hopkins-Folin, creatinine and creatine—Folin. The amount of carbohydrate in the fæces by Liebermann's method and the amount of fat by Soxhlet's method. The urine of the first 16 hours of fasting was always discarded; therefore the urine of the period marked fast in the tables was a 24 hours sample.

TABLE I.

Day of Exp.	Nitrogen in grms. Total	Urea	Ammonia	Uric Acid	Creatinine	Creatine	Per cent. of total nitrogen Urea	Ammonia	Uric Acid	Creatinine	Creatine	Diet
1	5·95	4·46	·396	·075	·445	·067	75·0	6·6	1·2	7·5	1·1	Fast ($-H_2O$).
2	7·85	6·22	·666	·146	·525	·021	79·2	8·4	1·8	6·7	0·2	Carbohydrate.
3	4·91	3·50	·493	·088	·509	·001	71·3	10·04	1·7	10·3	0·00	,,
4	9·15	—	—	—	·666	·000	—	—	—	7·2	0·00	Mixed.

Carbohydrate diet.

Tapioca	=454 grms.	Fæces =	Carbohydrate.
Sugar	=114 ,,		19·0 grms.
Honey	=227 ,,		4·5 ,,
Cornflour	= 85 ,,		

Calorie intake = 40 Cal. per kilo.

It will be noted here that instead of the usual fall in the output of nitrogen which is observed after the ingestion of carbohydrate there is a very distinct rise in all the products excreted with the exception of the creatine. This rise I believe is due to the washing out of the tissues as the fast was absolute, *i.e.* without either food or water. Naturally under these conditions the flushing out of the tissues during the fasting period was not thorough and it was to be expected that when the carbohydrate was begun the following day with a fair amount of fluid (about 500 c.c. of water was taken each day) there would be some washing out. In spite of this there is a very distinct fall in the output of creatine nitrogen from 67 mg. to 21 mg. The second day of the carbohydrate diet shows the usual effect, a fall in the total nitrogen below the starving total nitrogen with a similar result in the case of the urea. The ammonia has fallen below the amount excreted on the first day but is still above the fasting level. The uric acid is almost back to fasting level and the total creatinine output, *i.e.* preformed creatinine plus creatine, is also almost identical with that excreted during the fasting period (see Table VI). The preformed creatinine is considerably higher than on the day of the fast but the output of creatine has to all intents and purposes ceased. On the resumption of the ordinary mixed diet the mere trace of creatine present completely disappeared.

In order to test whether the increase of nitrogen excreted after the commencement of the carbohydrate food was due to simple flushing out another experiment in every way comparable with the first was carried out except that during the fast water to the extent of 500 c.c. was taken.

TABLE II.

Day of Exp.	Nitrogen in grms.						Per cent. of total nitrogen					Diet
	Total	Urea	Ammonia	Uric Acid	Creatinine	Creatine	Urea	Ammonia	Uric Acid	Creatinine	Creatine	
1	6·80	5·33	·423	·076	·433	·057	78·3	6·2	1·1	6·3	0·9	Fast (+ H_2O).
2	6·85	5·61	·521	·089	·438	·007	81·9	7·6	1·3	6·4	0·1	Carbohydrate.
3	7·14	—	—	—	·535	·005	—	—	—	7·4	0·1	Mixed.

Here it will be noted that the taking of the food does not cause any appreciable rise in the output of nitrogen—a slight fall had indeed been looked for. The outputs of ammonia and of urea have risen somewhat but along with this there is a remarkably low excretion of uric acid. The total creatinine excreted is lower than on the first day of the fast although the preformed creatinine is somewhat higher. Here again under the influence of the carbohydrate feeding the output of creatine nitrogen has fallen from 57 mg. to 7 mg. With the resumption of the mixed diet there is a marked rise in the output of preformed creatinine and a very small output of creatine.

As the results of carbohydrate feeding were so definite it was next determined to try the effect of feeding with fat.

TABLE III.

Day of Exp.	Nitrogen in grms. Total	Urea	Ammonia	Uric Acid	Creatinine	Creatine	Per cent. of total nitrogen Urea	Ammonia	Uric Acid	Creatinine	Creatine	Diet
1	7·62	6·33	·396	·082	·424	·051	83·2	5·2	1·1	5·5	0·7	Fast (+H_2O).
2	11·67	8·54	·564	·043	·370	·141	73·1	4·8	0·36	3·1	1·2	Fat.
3	9·09	7·43	·945	·038	·353	·122	81·7	10·3	0·42	3·8	1·4	,,
4	15·44	12·41	1·60	·128	·372	·118	80·3	10·3	0·82	2·4	0·7	Fat-Protein (sugar free).
5	17·04	13·04	1·52	·193	·420	·130	76·5	8·8	1·1	2·4	0·8	,, ,,
6	14·28	—	—	—	·497	·085	—	—	—	3·4	0·7	Mixed, little carbohydrate.
8	—	—	—	—	·534	·000	—	—	—	—	0·0	Mixed, ordinary.

Fat diet. 570 grms. Cream (55 °/₀ fat) = 312 grms. fat. 40 Cal. per kilo.

Fæces = 18 grms. fat. [The fat on the whole was well utilised. Only very slight diarrhœa caused. The same is true of Exp. IV done on Dr Graham Brown.]

Fat-Protein diet.

Casein bread (Carbohydrate free)	= 170	grms.
Cheese	= 43	,,
Butter	= 128	,,
Eggs	= 12	,,

39 Cal. per kilo.

Here again as in previous fasts there was an output of creatine. On the first day of the fat feeding, although the usual 500 c.c. of water had been taken during the fasting period, there was a very definite rise in the output of total nitrogen. Along with this rise however there is a fall in the percentage output of urea and of ammonia but a rise in the absolute amount excreted. In the case of the uric acid and the creatinine there is both a percentage and an absolute fall. Creatine in contradistinction to the experiments with the carbohydrate shows a very marked rise both in the absolute and in the percentage amount excreted. This high output of creatine is continued on the second day of the fat feeding. The output of total nitrogen on the other hand has fallen on this day but there is a percentage rise in the output of urea. The ammonia, as was to be expected remembering the marked acidosis which follows pure fat feeding when all carbohydrate is absent, has risen very considerably, forming over 10 % of the nitrogen excreted. The preformed creatinine output has fallen still further, whereas the total creatinine output (see Table VI) which rose on the first day of the fat diet has on the second returned exactly to the fasting level.

When the diet was changed by the addition of a large amount of protein but still kept strictly carbohydrate free there is no great alteration in the percentage amount of the products excreted. Naturally the total nitrogen has risen very considerably as the result of the increased nitrogen intake. The percentage output of urea is hardly altered and the ammonia on the first day of the new diet remains at its old high figure of some 10 % of the total nitrogen, but on the second day there is a slight fall. As regards the uric acid there is a steady rise during the two days (the diet is purine free) both in absolute and percentage amount well above the starvation figure. This is quite in agreement with the observation of Maurel[(4)] that alterations of the nitrogen content of a diet bring about alterations in the uric acid output especially during the condition of nitrogen hunger. Folin on the other hand does not believe that variations in the protein intake affect the uric acid output. In the case of the preformed creatinine there is quite a distinct rise in absolute amount excreted and this rise is continued on the second day of the fat protein diet, reaching then the fasting level. Creatine on the other hand is still present in large amount: a very slight drop occurs on the first day but this is followed by a well-defined rise on the second day. As regards the total creatinine (see Table VI) it will be noted that the output of the second day exceeds easily the output of any other day of the experiment. This is hardly in agreement with the statement of Folin that the output of creatinine is quite uninfluenced by alterations in the diet: this disagreement holds equally true if the amount of preformed creatinine excreted be considered alone.

Finally a mixed diet was given in which there was only a small amount of carbohydrate with the result that there was a still further rise in the output of preformed creatinine and a distinct although not a very great fall in the output of creatine. Two days later on an ordinary mixed diet an examination of the urine showed that there was an absolute increase in the amount of preformed creatinine excreted and an absence of any creatine. The total amount excreted on this day is less than the total amount excreted on the first day of the modified mixed diet following the fat protein diet.

As I desired confirmation on another subject of this effect of a fat diet Dr T. Graham Brown very kindly put himself at my disposal.

The diet in this experiment as in the last consisted of cream and was taken to the extent, after making allowance for the loss by the fæces, of 48 Cal. per kilo.

TABLE IV.

Day of Exp.	Nitrogen in grms. Total	Creatinine	Creatine	Per cent. of total nitrogen Creatinine	Creatine	Diet
1	7·16	·377	·042	5·2	0·6	Fast (+H_2O).
2	10·74	·324	·102	3·0	0·9	Fat.
3	17·05	·526	·072	3·1	0·4	Mixed.

Here again it will be noted that there is a very definite rise in the output of the creatine on the fat diet which diminishes when a mixed diet rather poor in carbohydrate but rich in protein is taken. There is a definite fall in the amount of preformed creatinine excreted but a rise in the amount of total creatinine. The amount of preformed creatinine and of total creatinine excreted is very markedly increased with the resumption of the mixed diet. This experiment also demonstrates the rise in the output of total nitrogen which follows the ingestion of a nitrogen poor fat diet.

Another experiment was carried out where the feeding period was not preceded by a fasting period. In this case the carbohydrate diet was continued for five days followed by a fat period of two days duration.

I. Carbohydrate diet.

Banana meal[1]	... ...	=454 grms.
Honey	... ...	=230 ,,

II. Fat diet.

Butter	... ...	= 65 ,,
Cream (55 %)	... ...	=340 ,,

Calorie intake = in I 32 Cal. per kilo; in II 35 Cal. per kilo. (In both allowance for the loss by the fæces is made.)

[1] Banana meal dried at 100° C. Nitrogen = 0·64 %, Sugar = 83·3 %, Fat = trace. Honey Nitrogen = 0·23 %. ,, Sugar = 75 %.

TABLE V.

Day of Exp.	Nitrogen in grms. Total	Urea	Ammonia	Uric Acid	Creatinine	Creatine	Per cent. of total nitrogen Urea	Ammonia	Uric Acid	Creatinine	Creatine	Diet
1	6·79	4·61	·375	·123	·478	·004	67·8	5·5	1·8	7·04	·06	Carbohydrate.
2	6·40	4·65	·134	·173	·460	·015	72·7	2·1	2·7	7·18	·24	,,
3	4·77	3·21	·132	·146	·413	·007	67·2	2·7	3·1	8·65	·15	,,
4	4·79	3·17	·121	·152	·450	·004	66·2	2·5	3·1	9·37	·08	,,
5	4·39	3·31	·104	·125	·436	·000	75·3	2·3	2·8	9·93	·00	,,
6	4·83	3·76	·238	·157	·400	·019	77·8	4·9	3·2	8·28	·39	Fat.
7	8·13	6·64	·527	·088	·347	·091	81·6	6·4	1·1	4·26	1·12	,,
8	16·21	13·45	1·13	·551	·477	·001	83·0	6·9	3·4	2·94	0·0	Mixed.

The total nitrogen in this experiment during the carbohydrate period shows a very definite and steady fall in the amount excreted to be followed by just as well a defined rise in output on the fat diet. This is in absolute agreement with the observations of Landergren who was the first and as far as I am aware the only one to draw attention to the curious curve of the nitrogen excretion as the result of a carbohydrate rich followed by a fat rich but in both cases nitrogen poor diet. The percentage amount of urea excreted falls as low as 66·2 °/₀ on the carbohydrate diet but is about 80 °/₀ on the fat. The absolute amount of ammonia excreted falls steadily during the carbohydrate period although the percentage amount remains practically constant. With the fat diet there is a rise in the output of ammonia both in absolute and percentage amount as was to be expected from the acidosis which follows the ingestion of fat. On each of the two days of the fat diet the acetone and diacetic acid reactions were extremely well marked. On the carbohydrate diet the output of uric acid is to be regarded as very regular whereas on the second day of the fat diet quite a marked fall in the output takes place. This was followed by a very decided rise on the resumption of the mixed diet due to the ingestion of exogenous purine. The preformed creatinine on the carbohydrate diet in absolute amount excreted remained remarkably constant, with the result that in percentage amount of the total nitrogen there was a steady rise. With the fat diet there was at once a fall in the amount excreted followed by a rise when the mixed diet was resumed. As regards the creatine output it will be noted that throughout the carbohydrate period there is a trace present except on the last day of the period. The maximum output is 15 mg. on the second day of the experiment. This small output may have been due to undernutrition as the banana meal was not on the whole very well utilised. This is supported by the fact that during this experiment there was some loss of weight. During the two days of fat feeding there is a well-marked rise in the output of creatine: 19 mgs. on the first day rising to 91 mgs. on the second. With the resumption of the mixed diet the creatine present practically disappears.

Brief reference may now be made to the variation in amount of the different nitrogenous excretory products estimated, viewed in the light of all the experiments done.

Urea. As Folin (*loc. cit.*) showed, when a subject is put on a low nitrogen diet there is at once a fall in the percentage output of urea. This fall is very well marked in the case of the feeding with the carbohydrate as in Table V where the urea output has fallen to 66·2 % of the total nitrogen on the fourth day of the experiment. This fall is not so marked in those experiments where the carbohydrate was preceded by a fast. Of course it must be taken into account that in these fasting experiments the duration of carbohydrate feeding was short. In the case of the fat diet although again nitrogen free no such definite fall in the percentage output of urea is noted. On the second day of fat feeding in both III and V the urea forms a substantial proportion of the total nitrogen excreted (81·6 %).

Folin from his experiments with the nitrogen low and nitrogen high dietaries founded his theory of the dual protein metabolisms, the so-called exogenous and endogenous metabolisms. As principal excretory product of exogenous metabolism he puts down urea, basing his conclusions on the variations observed in its output during high and low nitrogen feeding. Folin however it must be remembered never states that no urea comes from endogenous sources. The results of my experiments would however seem to throw some objections in the way of the complete acceptance of the two forms of metabolism being absolutely distinct one from another. Here from a practically purely endogenous source we have a percentage excretion of urea not far short of that yielded by a protein rich mixed diet (cf. Noël Paton[5]). The present series of experiments was not however designed for the elucidation of this problem, and as the experiments done are too few in number to settle this particular point the question will be left for the present.

Ammonia. There is nothing exceptional in the excretion of this body. As was to be expected there was a marked rise in the output of ammonia during the fat periods due to the increased acidosis. The amount of acetone and diacetic acid excreted during the fat period was, judging solely from qualitative tests, very large. On the carbohydrate dietary the output during the period of feeding without previous fasting (Table V) was comparatively low and steady. In the experiment where fasting preceded the carbohydrate dietary I and II the output of ammonia rises markedly, in experiment I equalling indeed the output of the fat period. It is to be noted here that in this experiment although the acidity of the urine was not estimated it was probably high as there was a slight degree of alimentary glycosuria, therefore also probably some partially combusted acid carbohydrate compounds were excreted.

Uric Acid. The output on the carbohydrate diet without previous fasting (V) was remarkably steady and high if it be compared with the other experiments. On the fat diet it would seem that the output is lower. One curious point to which attention has already been drawn is that on the protein fat diet—a purine free diet—there is a rise in the output of the uric acid.

Creatinine and Creatine. The most interesting observation in the above experiments is that the excretion of creatine which is induced by starvation tends to disappear on a carbohydrate but to increase in amount excreted on the fat diet.

TABLE VI.

Exp.	Preformed creatinine in grms.	Total creatinine in grms.	Creatine in grms.	Diet
I	1·200	1·382	0·182	Fast.
	1·416	1·474	0·058	Carbohydrate.
	1·373	1·375	0·002	,,
II	1·168	1·321	0·153	Fast.
	1·182	1·200	0·018	Carbohydrate.
III	1·144	1·282	0·138	Fast.
	0·998	1·378	0·380	Fat.
	0·952	1·280	0·328	,,
	1·004	1·321	0·317	Fat-Protein.
	1·134	1·483	0·349	,,
IV	1·018	1·131	0·113	Fast.
	0·874	1·150	0·276	Fat.
V	1·240	1·280	0·040	Carbohydrate.
	1·112	1·136	0·024	,,
	1·212	1·224	0·012	,,
	1·169	1·159	0·000	,,
	1·080	1·128	0·048	Fat.
	0·936	1·182	0·246	,,

Creatinine (*preformed*). On the carbohydrate diet the output in the experiment without fasting (V) and in II remains fairly constant, but in II there is a tendency for the output to increase. Viewed in the light of total creatinine the output on the carbohydrate diet may be regarded as fairly constant throughout the different experiments. On the fat diet there is in each case a steady decrease in the amount of preformed creatinine excreted whereas when viewed in terms of total creatinine no such great diminution is to be noted. The average output of total creatinine (calculated from Table VI) on the carbohydrate diet is 1·27 grm. whereas on the fat diet it is 1·24 grm. It would seem then that there is after all some close connection between the creatine and the creatinine, almost amounting to the definite proof of the origin of creatinine from creatine. At any rate with the appearance of creatine in any amount as in the fat experiments above detailed there is a corresponding fall in the output of creatinine.

II.

As Bayliss and Starling[6] say, it is now practically certain that no longer is one justified in valuing a diet simply on its calorific value and its content of fat, carbohydrate and protein. Hopkins and Willcock[7] have also drawn attention to the same point as the result of their feeding experiments with zein and have suggested that certain of the substances taken in the food might be utilised without directly contributing to tissue formation or structural maintenance. Further, Folin[8] in his work has suggested the probability of a special tissue metabolism which would probably entail in turn the necessity of special food products. Again too in the question of the amount of protein—nitrogen—which must be consumed our ideas are undergoing a change. All workers now are at one on the point that the body can exist on a much lower intake of nitrogen-containing food per diem than was formerly believed to be possible. Chittenden[9], Siven[10], Landergren[11], Folin (*l.c.*) and others have all given demonstrations of this. The low intake of nitrogen is not the point. The nitrogen-containing food consumed must contain a sufficient amount of the particular nitrogenous substances required for repair. Thus if the body demands x amount of some particular substance for its reconstruction the food consumed must contain that amount although in getting it x^{10} of this and x^{20} of that other nitrogen rich substance, not required for the immediate process of repair, are consumed at the same time.

Take for example such feeding experiments as those of Kaufmann[12] and of Murlin[13] with gelatine where, in spite of a very large intake of nitrogen, equilibrium could not be maintained and at the same time note the improvement in the conditions when small amounts of certain aromatic bodies, normally absent from gelatine, are added. Or again consider the feeding experiments of Hopkins and Willcock (*l.c.*) with zein where improvement followed the addition of quite small amounts of tryptophane to the diet. The recent work of Michaud[14], as yet unconfirmed, is one of the most important as a demonstration of how on feeding with a food perfectly suited to a given animal's needs—in his case feeding a dog with dog flesh—the intake of nitrogenous bodies can be reduced to a minimum.

Formerly it was generally held that protein tissue alone contributed to the repair of waste in the organism and that the carbohydrates and fats were concerned in the supply of energy. Now however it is admitted that the carbohydrates do something more than supply the energy, but the nature of this something is not very definitely understood. It has been frequently observed that when carbohydrates are given in large amount the intake of nitrogen can be reduced and at the same time the excretion of nitrogen lowered, in other words the carbohydrate acts as a protein sparer as it is generally called. The same power has been ascribed to fats by some but denied by others. Carl Voit[15] admitted that the carbohydrates were more active in this respect than the fats although in his opinion neither equalled protein or gelatine. Others again like Frentzel and Reach[16] hold that fats and carbohydrates act in an equally economical fashion. Zuntz[17] found, by means of respiration experiments, that fats and carbohydrates can be utilised in equal degree during work for the saving of protein. He states further that a starving animal made to work obtains practically all its energy from fat sources. Lusk[18] found that carbohydrates have a greater power of conserving protein than fat either given in the diet or obtained from the body's supply. Chauveau[19] maintained that carbohydrate was the important body and that fat could only act after it had been converted in the liver into carbohydrate. He supported his own experiments by reference to the conversion of fat into carbohydrate in hibernating animals. The low respiratory quotient which at certain times has been observed in these animals by many workers, Pembrey[20], Weinland[21], and others, has been explained as indicating the combustion of the fat with the formation of sugar and the storing of the same in the muscles and liver as glycogen. The interesting point about this in the present connection is that the formation of carbohydrate would seem to take place just about the time the animal was going to awake and indulge in more active muscular exercise, *i.e.* increase the daily breakdown of tissue protein. The formation of carbohydrates from fatty bodies in seeds, or at any rate the observation that with the rise in the carbohydrate content there is a fall in the fat content of germinating seeds, must not be forgotten (Leclerc du Gablon[22], Mazé[23], and others). Further Pflüger[24] produces evidence which strongly suggests the possible conversion of fat to sugar.

The present experiments and those of Landergren (*l.c.*) however show that under our conditions—nitrogen hunger—the power of carbohydrate and fat to spare the breakdown of tissue protein is by no means equal. I may quote further the experiments of Kayser[25] who found that, even with a diet rich in protein, when carbohydrate was removed and the calorie value of the food made up with fat there was an immediate increase in the breakdown of the protein of the tissues. Landergren found that if he gave a diet made up of pure carbohydrate, practically nitrogen free, the output of total nitrogen steadily fell, but when the carbohydrate was replaced by a diet consisting solely of fat, again nitrogen free, the nitrogen output steadily rose in amount.

Landergren however does not utilise his figures for the discussion of a probable synthesis but develops a hypothesis as to the varying nature of the nitrogen present in the protein molecule. Table V in the present paper also demonstrates this rise in the output of total nitrogen after changing from a carbohydrate diet to a fat diet both containing very small amounts of nitrogen. Further in my experiments the behaviour of the creatinine-creatine output shows another difference between the effect of feeding with fat and with carbohydrate, probably connected however with the factor which influences the total nitrogen output.

Landergren drew attention to two other points in his paper which may be mentioned here. He showed that there is no relation between the so-called isodynamic ratios of fat and carbohydrate and this power of sparing tissue protein. He makes out that in this connection the carbohydrate is twice as active as an isodynamic amount of fat. Again he showed that the increased output of total nitrogen on a fat diet does not go on to an unlimited extent but that, as a rule, the maximun output is reached on the third day of fat feeding.

The effect of fat and carbohydrate diets has been most extensively studied in connection with muscle at work and not at rest as in the present instance. As however resting muscle may be regarded as undergoing the same changes only to a less degree as muscle doing voluntary work the conditions may be looked on as identical. Work probably merely increases the metabolism in the true sense of the word—the exchange of material.

Practically every investigator who has worked at this problem of the utilisation of material during work has found that the work done leads to no or only a very slight increase in the output of nitrogen, provided always the supply of food particularly carbohydrate and of oxygen be sufficient (Hirschfeld[26], Zuntz[27], Paton[28], Voit (*l.c.*) and others).

It is a fundamental mechanical law that every piece of machinery wears with work, the parts where there is the greatest friction and use more rapidly than the rest. Have we then to look on muscular tissue, the part most involved both at rest and during work, as being beyond wear and tear, a piece of mechanism on a level with the practically unwearable jewelled bearing? Far from it, because it is very readily demonstrable that if the work be carried on under unfavourable conditions we get immediate evidence of the utilisation of protein tissue (wear and tear of muscle?) in the form of an increased nitrogen output. What then is the course of events provided the conditions be favourable? Why is there no appearance or only a very small one of nitrogen as the result of work?

The small increased output of nitrogen from work may be explained in two ways at least:

(i) That the actual wastage of protein tissue is small in amount; that the protein tissue as it is broken down divides into two parts, a nitrogen free part which is utilised for the energy needs, and a nitrogen containing part which is resynthesised.

(ii) That the work increases the breakdown of protein tissue, but instead of this nitrogen appearing in the urine as an increased excretion there is a similar amount taken from the food nitrogen to replace the nitrogen set free by the work done. The result would be that no practical difference in the nitrogen excretion was to be observed although there had been an alteration in its source of origin, more coming from the endogenous source and less from the exogenous.

The first hypothesis presupposes that the actual nitrogen requirement of the body is low and that it could exist on a food of sufficient calorie value made up mainly of nitrogen free substances. Siven (*l.c.*) and others have shown that such is the case. The body cannot exist indefinitely in this condition, unless the source of the nitrogen supply be a perfect source, as there is a constant, although perhaps small, loss which has to be made good from some outside source. As I have already noted the low intake of nitrogen is not the point: it is not the quantity but the quality of the nitrogenous food which is of importance.

The second hypothesis presupposes that the slight excess of nitrogen which appears as the result of work must come from the breakdown of protein tissue and therefore if the hypothesis be right then all the nitrogen excreted must have come from broken down protein tissue, the result of the work. There would be too little nitrogen supplied by the food to make up the nitrogen loss. This hypothesis also would require the acceptance of the statement of Pflüger[29] that the need of food is satisfied first by protein, otherwise in those experiments where the intake of protein was high there should have been no appearance of excess nitrogen output. The work of Krummacher[30] shows that even with a very large intake of protein the increased output of nitrogen after work still takes place. The same worker also shows that the possible energy calculated from the nitrogen excreted does not equal the energy expended in the work. Frentzel[31] likewise demonstrated in his experiments that if not only the excess of nitrogen excreted be held as coming from protein utilised during work but that the total amount of nitrogen excreted on the day of the work be regarded as from this source not even then was there sufficient material combusted to furnish the energy expended. The energy calculated from the amount of nitrogen excreted in one experiment equalled only about two-thirds of the work done.

Both these latter observers however apparently believed that the amount of nitrogen excreted represents exactly the amount of protein tissue metabolised in the body. Such a belief is not now so generally held. Take for example the case of inanition. Here it must be admitted that part at least of the nitrogen liberated by the breakdown of protein tissue within the body is resynthesised, as certain tissues and organs retain practically their original weight up to the last (Voit, Chossat).

The idea of a resynthesis taking place within the tissues particularly the muscles is not a new one. Hermann[32] 40 years ago put forward the hypothesis that the protein in all probability was broken down into a nitrogen containing part which was reutilised in some way and a nitrogen free part which was used. He believed further that an increase in the output of nitrogen took place only when the work done was very prolonged and severe, when an actual destruction of muscle fibres was brought about. Pflüger[33] has offered the hypothesis that all metabolism is a partial breakdown with a subsequent regeneration of the living protein molecule. Verworn[34] supports also such a view holding it conceivable that under certain circumstances regeneration of the nitrogenous residues can take place at the expense of the food stuffs and oxygen. As he says "such economy with the costly nitrogen would be wholly in accord with the methods of the organic household."

To elaborate this hypothesis further was the object of the present investigation. In order to get some insight into the metabolic changes it was considered necessary to attempt the study of one product of protein breakdown and preferably a product which was not one of the ordinary end products. Creatine was the substance chosen as it never appears in the urine as an excretory product under normal conditions (Folin, *l.c.*) but which can be readily caused to appear having as its chief source of origin the muscle tissue.

As has already been pointed out the ingestion of a diet consisting of pure carbohydrate practically nitrogen free leads to a very marked fall in the output of creatine present as the result of fasting and at the same time as Landergren and others have noted to a fall in the output of total nitrogen. On the other hand a diet again practically nitrogen free but consisting of fat does not bring about the fall in the output either of total nitrogen or of creatine during two days although there is reason to believe that after three or four days such a fall in the case of the total nitrogen at least begins. It is further to be noted in this connection in Exp. III the output of creatine is lower on the second day of the fat diet than on the first, which points to a reduction in the breakdown of protein.

It is believed that the above experiments may be regarded as giving ground for the acceptance of the hypothesis that a resynthesis takes place in the tissues (muscle ?) and that the greater part of the muscle protein tissue nitrogen which is set free as the result of work does not appear in the urine but is reutilised. Further that the food stuff which plays the most important *rôle* in this resynthesis is carbohydrate. Fats apparently must undergo some change which requires time to produce—the fall which follows the preliminary rise in the output of total nitrogen on a fat diet does not take place until the second or third day. This change which fats undergo may be the conversion into carbohydrates advanced by Chauveau and supported by the observations on hibernating animals, germinating seeds, etc.

The following diagrams which are based on one which appeared in a paper by Professor Noël Paton (*l.c.*) and which he has kindly permitted me to use will perhaps aid in making the position clear. It is presumed that N_{10} is the amount of nitrogen set free by protein breakdown in a muscle. In diagram I the supply of oxygen and carbohydrate has been sufficient so that N_9 is resynthesised and N_1 is passed on for excretion with the CO_2 the product of the combustion of nitrogen free material. In II the supply of carbohydrate has been deficient, with the result that there is a resultant loss in the nitrogen set free so that only say N_6 is resynthesised and N_4 is passed out as waste.

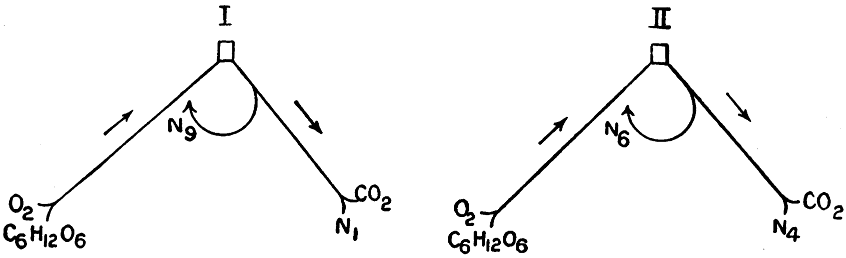

Fig. 1. I. Carbohydrate adequate. II. Carbohydrate inadequate.

That carbohydrates play an all important part in the utilisation of protein given in the diet has been repeatedly demonstrated. The best example of this is in the feeding of animals with biuret free digest products where unless there is an abundant supply of carbohydrates there is no retention of nitrogen. The experiments of Lesser[35] which followed the publication of Loewi's[36] epoch making work are a most excellent demonstration of this very point. Lesser was unable to confirm Loewi's results but an examination of his protocols shows that in his experiments he omitted carbohydrate altogether from his diets, using fats and protein digest products alone. Lüthje[37] in an elaborate paper has proved beyond any manner of doubt the absolute importance of the carbohydrate moiety in the utilisation of protein. He suggests that some form of amino sugar is first of all elaborated.

Again we have the support of the botanical physiologists (Hansteen[38], Iwanoff[39] and others) who have time and again demonstrated that the presence of carbohydrate is absolutely essential before protein synthesis takes place.

Then we may cite the rise in the output of total nitrogen which is frequently observed in cases of diabetes when the sugar instead of being utilised in the body is excreted. Further during starvation the sugar content of the blood keeps high, a probable provision for the reutilisation of the products of protein disintegration formed during this condition.

Except for the suggestion of Pflüger (*l.c.*) that the nitrogen containing part of the broken down protein molecule combines with "alcohol radicles" to form new protein no experimental work that I am aware of on this synthesis hypothesis exists. It is of course well known that aldehydes will form compounds with many different substances containing nitrogen. Take for example the most simple of all these compounds, the union of aldehyde with ammonia to form aldehyde ammonia. Not only will a simple aldehyde like acetaldehyde unite with ammonia but Lobry de Bruyn [(40)] has formed compounds from sugars and ammonia. Again Morrell and Bellars [(41)] have combined guanidin with dextrose and lævulose and Wolff [(42)] found that amidoguanidin would form compounds either with a simple aldehyde or with sugar. Schoorl [(43)] has formed a compound of glucose and urea and Jaffe [(44)] found that creatine (and also creatinine) could unite to form a compound with formaldehyde. Tryptophane can also combine with aldehydes. The formation of methylimidazol which Knoop and Windaus [(45)] observed from glucose in the presence of ammonia and zinc oxide is also of interest in this connection as this imidazol ring occurs in histidine, creatinine and purine. Sorensen [(54)] has shown that the simple monamino acids can unite with formaldehyde. Spiegel [(46)] has suggested that certain peptide groups in the protein molecule are combined by means of carbon atoms and has attempted to get a synthesis of protein from the decomposition products of the protein molecule by means of formaldehyde. He states that he has obtained products which suggest a synthetic change.

In the body if some similar synthesis does take place it is much more likely to occur between the nitrogen containing rest of the decomposed protein molecule and one of the lower aldehydes resulting from carbohydrate decomposition than with the complete carbohydrate molecule. One product which can readily be obtained from the decomposition of glucose *in vitro* is lactic acid and this same acid is also obtained from isolated muscle as the result of certain conditions for example work. Further if the supply of oxygen is deficient as in the experiments of Araki [(47)] lactic acid appears in the urine; it also appears when excessive exercise is taken (Ryffel [(48)]). Now the steps of the formation of lactic acid from glucose as given for instance by Wohl [(49)]

show the production of two actively reacting aldehydes before the final lactic acid is reached, namely glyceric aldehyde and pyruvic aldehyde (methyl glyoxal), either of which might unite with the nitrogen rest. (*In vitro* experiments with both these substances are at present being carried out.) Or again it is quite possible that the necessary aldehyde may be formed from the lactic acid by further decomposition.

Fletcher and Hopkins[50] in their paper on the production of lactic acid in muscle state (p. 295) that "it is conceivable that the disappearance of lactic acid during recovery from fatigue does not involve its oxidative removal in the form of carbonic acid and water but may be due to the occurrence of reconstructive processes." Leathes[51] has also suggested that lactic acid may not be an end product but an intermediate one in some synthetic or transformative change.

The hypothesis here put forward could also explain the curious observations of Mellanby[52] and of Van Hoogenhuyze and Verploegh[53] that in cancer of the liver there is much creatine present in the urine, as the liver is the organ most deeply concerned in one stage at least of the carbohydrate metabolism. The glycogen storing capacity or the glycolytic power of the liver being interfered with there would no longer be a proper supply of sugar available, with the result that faulty and incomplete synthesis would take place. This same hypothesis might also explain to a large extent the appearance of such large quantities of nitrogen in the urine after phosphorous poisoning, a condition where the liver is again principally involved.

Conclusions.

With a carbohydrate diet, practically nitrogen and fat free, there is a fall in the output of urinary nitrogen.

With a fat diet, practically nitrogen and carbohydrate free, there is a marked rise in the output of urinary nitrogen.

During starvation creatine is constantly present in the urine.

The output of creatine induced by fasting at once falls when the diet consists of carbohydrate, whereas with the fat diet the amount excreted increases.

The amount of creatine excreted during the fat period is not markedly reduced by the addition of protein food (carbohydrate free) to the diet.

The hypothesis is put forward that the carbohydrates are absolutely essential for endo-cellular synthetic processes in connection with protein metabolism.

It is probable that food stuffs should be valued, as Chauveau suggested, more on account of their isoglycogenic than their isodynamic value.

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