120. THE RELATIONSHIP OF CARBOHYDRATE METABOLISM TO PROTEIN METABOLISM

2. NOTE ON THE EFFECT OF SEPARATION IN TIME OF THE PROTEIN AND CARBOHYDRATE MOIETIES OF THE DIET OF THE ADULT AND GROWING RAT

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PREVIOUS observations on the adult human subject have shown that when the protein and carbohydrate moieties of an adequate diet are separately ingested over short periods of time, fat being associated with both moieties, a negative N and S balance develops [Cuthbertson & Munro, 1939]. The following experiments were designed to determine if this phenomenon also took place in the case of the adult rat and, further, if the growth of young rats would thereby be affected. This is an extension of a previous brief note [Cuthbertson *et al.* 1939].

EXPERIMENTAL

The animals were housed in zinc cages and the arrangement for urine collection was that of Hopkins. All urines were made up to constant volume before their total N content was determined by the macro-Kjeldahl method. To prevent loss of NH_3 the filter funnels beneath the cages were washed down with dilute H_2SO_4 every morning before feeding and there was always some acid present in the collecting vessels.

Adult rats

Exp. 1. 4 adult male rats of some 300 g. body weight were placed on a mixed diet of the following composition for 1 week:

Dried egg yolk	12 %
Dried egg albumin	6 %
Potato starch (uncooked)	17 %
Rice starch (uncooked)	40 %
Cane sugar	10 %
Butter	10 %
De Loureiro's salt mixture [1931]	5 %
Brewer's yeast extract (untreated 0.01 % acetic acid extract of Chick & Roscoe [1930])	2 ml. daily supplied in separate dish
Cod liver oil	2 drops daily

The mean daily intake per rat during the week was 17.5 g.

During the next 12 days the diet was separated into two fractions:

A		B	
Potato starch	17 parts	Dried egg yolk	12 parts
Rice starch	40 „	Dried egg albumin	6 „
Cane sugar	10 „	Butter	5 „
Butter	5 „	Salt mixture	2.5 „
Salt mixture	2.5 „		
Cod liver oil	2 drops daily		
Yeast extract	2 ml. daily		

The A fraction (carbohydrate + fat) was fed *ad lib.* at 8.30 a.m. and the uneaten residue removed and weighed at 12 noon. 4.0 g. of the B fraction (protein + fat) were fed at 4.30 p.m. to each animal and were always consumed within a few hours. After 5 days on this diet the animals were weighed (fasting) and then there followed a 7-day period of urine collection on the same dietary regime.

At the end of a week the animals were reweighed (fasting) when it was found that all had lost weight, the mean loss per rat during this period (period 1) being 7 g. The rats were then given the A and B fractions in a mixed form (period 2), the amount consumed daily being the mean value of the daily intakes during period 1 for the animal concerned. During period 2 there was a gain in weight by all the animals, the mean gain per rat being 5 g.

The mean daily output of urinary N was greater in all 4 animals during the period of separation of their dietary protein and carbohydrate (0.165 g. N) than during the period of mixed feeding (0.145 g. N). The individual differences in the levels of N excretion were 0.0182, 0.0151, 0.0200 and 0.0266 g. per day (mean 0.02 g.).

Exp. 2. The foregoing experiment was repeated with a batch of 8 mature male rats of some 300 g. weight: margarine was substituted for butter. On an average they consumed 18 g. of the mixed diet during the pre-separation period. Thereafter they were subjected to a period of separation (period 1), 4.5 g. of the B moiety being fed per day. On the 5th day of separation urine collection was commenced and continued for a fortnight, when the A and B moieties were fed mixed for a further week (period 2), in quantities corresponding to the mean of the two preceding weeks.

During the 1st week of urine collection the average loss of weight was 6 g. per rat and by the end of the 2nd week they had lost a further 3 g. The mean daily output of urinary N for the 1st week of urinary collection during separation was 0.185 g. per rat and 0.200 g. during the 2nd week. This difference was probably due to the smaller intake of the carbohydrate + fat moiety (A fraction) during the 2nd week, 5.91 g. per rat as compared with 6.58 g. during the 1st week. This loss of appetite for A fraction of the diet is a constant feature in such separation experiments, but the B fraction was in all cases consumed ravenously.

During the week of mixed feeding the average gain in body weight was 5 g. per rat. Two of the animals, however, showed a further fall, one slight the other of 10 g. The mean urinary N output during this period was 0.180 g. per day, 0.0125 g. below the mean of the two preceding weeks of separation. This difference is undoubtedly less marked than that observed in Exp. 1, namely 0.02 g. This may have been due either to the more prolonged period of separation, with its progressive loss of appetite inducing a state from which recovery is slow, or to a gradual readjustment by the animal to the changed dietary conditions. 7 of the 8 animals showed N retention during period 2, the mean daily values being

0-0501, 0-0036, 0-0154, 0-0054, 0-0050, 0-0132 and 0-0140 g. The animal which had exhibited a further and considerable reduction in weight during this period also showed a diminished N retention.

It is evident from these two experiments that the separation of the A and B fractions of the diet, essentially a separation of the time of ingestion of the protein and carbohydrate moieties, leads to a loss in weight and tissue N. In this respect these observations on the adult rat agree with those on adult man [Cuthbertson & Munro, 1939].

Young rats

Two paired feeding experiments were carried through. In the first the diet was similar to that already described (Exp. 1) protein being associated with some of the fat + salt mixture. In the second the diet was slightly different and the protein component was fed alone.

Exp. 3. 12 pairs of recently weaned male litter-mates were fed the mixed diet *ad lib.* for a period of 12 days. One member of each of the first 10 pairs was then selected for the separated diet and was fed 5 g. of the B moiety at 8.30 a.m., the uneaten residue being taken away at 12 noon. The A moiety (fed *ad lib.*) was given to these rats ("X" rats) at 4.30 p.m. and taken away at 10 p.m. The second member of each pair (control or "Y" rats) then received the exact amount of food eaten the previous day by its opposite, but in a mixed form. There was thus always a lag of 1 day in the quota of food supplied to the controls. The two remaining pairs ("Z" rats) were fed the mixed diet *ad lib.* throughout the whole growth period.

In the earlier stages of the experiment, when the controls had not finished their food by 12 noon the dish was taken away and replaced at 4.30 p.m. In all instances the controls ate all the food provided. With increasing weight the intake of the B fraction which averaged 2.5 g. initially, rose to 5 g./day. It was kept stationary at that level for a further 10 days and was then reduced to 2.5 g. daily as the higher protein intake appeared to be accelerating the growth of these animals to a greater extent than was occurring in the Z group fed the mixed diet *ad lib.* By reducing the B fraction to 2.5 g. daily it was possible to bring the protein intake more into line with this 3rd group. The mean intake per rat per day at the end of the experiment was 2.5 g. B + 7.27 g. A.

The animals in this and the experiment to follow were weighed in the fasting state. In the 2nd experiment the precaution was taken of reserving until 4.30 p.m. a quota of the total food due to the controls. This was carried out on the days preceding the weighing of the rats, i.e. every 4th day.

Growth data. The total period of separation of the A and B components of the diet was 42 days. During this period the mean increase in body weight per rat was 106 g. (mean initial weight 66 g.), that of the controls fed the same quota of food but mixed, 114 g. (mean initial weight 69 g.). Those animals fed the mixed diet to full satisfaction showed an increase of 130 g. (mean initial weight 69 g.). The growth curves have not been included as no real difference existed between the "separated animals" and the "controls" fed the same amount of food, but mixed.

Exp. 4. The two moieties, C and D, were constituted as shown on the next page.

The mixed diet was fed to 12 pairs of recently weaned male litter-mates. 2 pairs continued on the mixed diet and were fed *ad lib.*, the remaining 10 pairs after being on the mixed diet for 10 days were separated and a paired feeding

experiment was carried through which was similar to the preceding one except that moiety D, was fed in the late afternoon and C in the morning.

C		D	
(Fed in excess at 9 a.m. Residue removed at 12 noon)		(Fed at 4.30 p.m. Residue, if any, removed at 10 p.m.)	
Potato starch (uncooked)	17 parts	Ashless casein (Glaxo A/E)	12 parts
Rice starch (uncooked)	40 "	Dried egg albumin	6 "
Cane sugar	10 "		
Lard	10 "		
Salt mixture	5 "		
Cod liver oil	3 drops daily		
	per rat		
Yeast extract	2 ml. daily		
	per rat		

The urinary N excretion of 4 pairs of these rats was determined. The collection of urine was from the 2nd to the 10th day of the period of separation, and for the controls fed the same quota of food but in a mixed form, from the 3rd to the 11th day.

The D moiety never exceeded 1.5 g. throughout the whole growth experiment. In order to minimize any possible differences in weight which might have been attributable to a difference in the time of feeding, 1.5 g. of the total quota of food due to the controls were reserved until 4.30 p.m. on the day preceding the actual weighing.

N balance data. The mean daily urinary N excretion per rat fed the separated diet (mean body weight 87 g.) was 0.0928 g., that of the controls receiving the same quota of food but in a mixed form was 0.0930 g. (mean body weight 87 g.).

In contrast to the adult rat the young rat exhibits no significant differences in N excretion when placed on a diet in which the protein element is separated from the carbohydrate.

Growth data. The period of separation of the C and D fractions of the diet lasted 32 days. During this period the mean increase in body weight of the rats receiving the separated diet was 32 g. per rat, that of the controls fed the same quota of food but mixed, 32.5 g. Those animals fed the mixed diet to full satisfaction exhibited a mean increase in weight of 67 g.

The differences in the rates of growth observable in these two growth experiments is probably primarily due to the qualitative and quantitative differences in protein intake. The second group had a more restricted protein intake and qualitatively the proteins may have been slightly inferior.

DISCUSSION

The growth behaviour of these young rats indicates that the separation in the time of ingestion of the protein and carbohydrate moieties of the diet leads to a loss of appetite which is not subsequently overcome. This loss of appetite is related to the carbohydrate plus fat fraction and not to the protein fraction which is always eaten voraciously. In contradistinction to the adult rat and adult human subject the metabolism of protein in the young rat remains unaffected. In the adult organism there is a loss of body N which although of small degree and very close in amount to the minimum endogenous expenditure, is almost always present.

The experiments on the adult organism have admittedly been of relatively short duration as compared with the growth experiments and it might be considered that, given time, the organism would adapt itself to the abnormal conditions. We have, however, noted that over like periods of time during the early days of the "separation" period, this difference in behaviour does exist between the young and mature rat. In the second experiment on the adult rats it was thought that some evidence on the time factor might be obtained by observing the difference in N excretion during the first and second weeks of urine collection during "separation". Undoubtedly there was a greater loss of N during the second week but this was complicated by the fact that the animals, although eating the same amount of protein plus fat, had definitely reduced their intake of carbohydrate plus fat. It is difficult to see how this experimental difficulty can be overcome.

The resiliency of the growing organism must be such as to lead to a readjustment to the abnormal dietary conditions so as to maintain development as nearly as possible at the optimal level permitted by the gross intake. How does this come about and why cannot the adult organism similarly respond? Undoubtedly the endocrine system acts as a regulator of metabolic phenomena. Does the lack of dietary carbohydrate during a considerable part of the day lead to a defective secretion of insulin with consequent defective oxidation of carbohydrate and increased protein metabolism? Young [1940] considers that the growth-promoting action of the anterior lobe in the young animal may possibly rest on the ability of the pancreas to secrete sufficient insulin to induce N retention and promote carbohydrate oxidation. Mirsky & Swadesh [1938] believe that the anterior lobe of the pituitary exerts two types of action on protein metabolism in the normal animal: (1) direct stimulation of protein catabolism in the muscles; (2) anabolic, by way of its effect on the pancreas. During growth the influence of the pituitary would appear then to be contingent on its pancreatic activity. There is the evidence of Anselmino *et al.* [1933] and Richardson & Young [1937] that treatment of rats with anterior lobe extracts increases the islet tissue in the rat. More recently Marks & Young [1940] have shown that the insulin content of rat pancreas is greatly increased under the same conditions. In view of this evidence experiments have now been commenced to determine if injections of growth-promoting extracts of the anterior pituitary can induce retention of N during periods when the carbohydrate and protein elements in the diet are being separately ingested.

In view of our observations on the adult human organism it might be asked how the Eskimo metabolizes his protein. The observations of Rabinowitch [1936] and many others go to show that the Eskimo does eat carbohydrate. For 2 months in the year berries, lichens etc., are available and throughout the rest of the year he obtains his supplies from the glycogen present in the liver, muscle and skin of animals. He relishes the stomach contents of the caribou and musk ox and the clams found in the walrus stomach.

The evidence of Mirsky *et al.* [1937] indicates that in the eviscerated dog, which is essentially a muscle preparation, the spontaneous output of amino-acid N is removed under the stimulus of insulin and the amino-acids are presumably utilized for storage by the muscle or for synthesis into peptides or proteins. It may be that the restitution of the endogenous N expenditure is dependent on some such action by insulin and that in the absence of a stimulus this cannot be effected. In the adult the necessary stimulus is dietary carbohydrate ingested along with protein; in the young the anterior pituitary creates the necessary stimulus to insulin production even in the absence of the dietary stimulus.

From the observations on mature animals it would appear that the association of fat with protein does not affect the loss of N during the separation in time of the ingestion of protein and carbohydrate. This is of particular interest with reference to the problems of the interconversion of these three proximate principles. The stimulus of dietary carbohydrate is presumably the missing factor. The minute amounts of carbohydrate normally associated with proteins are not sufficient in themselves to effect this.

In the light of these and our earlier findings [Cuthbertson & Munro, 1937; 1939]¹ we are led to the conclusion that there is an intimate relationship between the metabolism of carbohydrate and protein and that the time factor and also the relative and absolute quantities of these two components in the diet as well as the frequency of feeding can effect the metabolism of protein. Fat undoubtedly also plays a part but this is more obscure and is chiefly related to the relative and absolute amounts associated with carbohydrate. Lovell & Rabinowitch [1939] have confirmed that time is a factor which influences the protein-sparing action of carbohydrates in the treatment of diabetics on low calorie diets.

SUMMARY

Separation in the time of ingestion of the protein and carbohydrate moieties of an otherwise adequate diet leads in the case of the young rat to a loss of appetite but to no apparent disability to metabolize protein. On the other hand in the mature rat such a procedure is coupled with a small loss of nitrogen and body weight. In respect of this behaviour the mature rat corresponds to the adult human subject.

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¹ In Table 5 of this paper [Cuthbertson & Munro, 1939], for calories derived from fat in period II read 748 for 493 and 776 for 521.

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