NUTRITION ON HIGH PROTEIN DIETARIES.

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It is generally agreed that protein can serve as the sole source of energy for the animal and that it can form carbohydrate and fat in the organism. Lusk(1) quotes Pflüger's experiment in which a dog was kept in active condition on meat alone. It is therefore reasonable to think that a diet consisting largely of protein supplemented with inorganic salts and those dietary factors (vitamins), the importance of which was unknown to the earlier workers, would suffice for the nutrition of the normal organism during the whole of its life cycle. It is of course apparent that such a diet is adequate for many of the carnivora.

Nevertheless, opinions have been repeatedly expressed that a high protein dietary is deleterious to omnivora or herbivora. We need scarcely deal in detail with the well-known views of Chittenden and his school but would refer briefly to the less known work of Chalmers Watson and his collaborators(2). From a prolonged experimental investigation mainly on rats Watson concluded that a high protein diet is harmful because the growth of the young animal is impaired and in the mature animal certain organs and tissues develop abnormally or become damaged, *e.g.*, hypertrophy of the thyroid, degeneration of the renal tubules. In reading through his papers, however, we were impressed by the similarity of the abnormalities he observed to those characteristic of certain vitamin deficiencies. We therefore decided to determine the effect of diets rich in proteins but containing in addition adequate amounts of salts and of the known accessory food factors.

Our experiments were carried out mainly on young growing rats as omnivorous animals and later on kittens as carnivorous animals. The rats and kittens were selected from normal litters so that some animals of each litter were on the high protein diet while the others were placed on normal diet to act as controls.

In the case of the rats the diets employed were as follows:

	Normal diet	High protein diet
Caseinogen	20 parts	83 parts
Starch	50	—
Yeast extract	5	5
Lemon juice	5	5
Butter[1]	15	—
Shark liver oil	—	2
Salt mixture	5	5
	100	100

Water *ad lib.*

In the high protein diet the quantities of yeast extract supplying the vitamin B, shark liver oil supplying the vitamin A, and lemon juice supplying the vitamin C had been proved by former experiments to be adequate for growing rats fed on a mixed artificial diet otherwise devoid of vitamins. The rats were given these dietaries when they had attained about 40 gms. weight (*i.e.* 20–30 days old) and in both cases consumed the diets readily, the average consumption being about 15 grm. dry weight per day. Fig. 1 shows the growth curves of the two groups of rats from the same litters when fed on these diets.

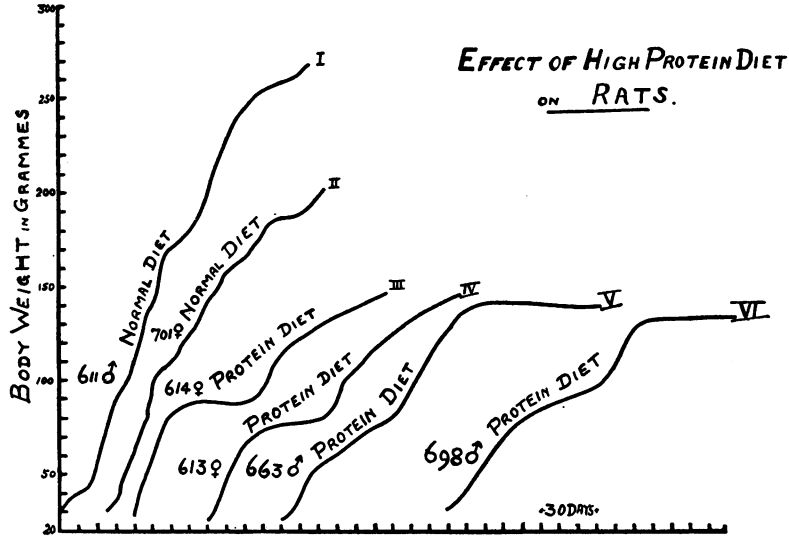

Fig. 1.

The rats on the balanced diet showed the normal growth that was expected, and at first the parallel group on the high protein dietary

[1] A later series of controls on a similar dietary in which the 15 p.c. of butter was replaced by 2 p.c. shark liver oil showed equally normal growth.

showed the same behaviour. They nearly trebled their weight before any abnormality was detected, which tends to confirm the preliminary results recorded by Osborne and Mendel(3). After about 30 days, however, they showed a retardation of growth. There is usually a slight temporary retardation of growth about this point in the normal growth curve, which probably represents the intermediate stage between two of the growth cycles studied by T. B. Robertson(4), but it appears to be much intensified in the case of our rats on the high protein dietary. After this period the latter group resumed growth but at a subnormal rate, although to outward appearances their condition and health were excellent.

The experiments were continued sufficiently long to allow for reproduction to take place and the group on the balanced diet produced and reared normal litters. Those on the high protein diet did not, however, show any disposition to breed. After four months feeding rats representative of both groups were killed for examination. The following table gives the weights of the control and experimental rats when 30 days old and when 130 days old, *i.e.* after feeding on normal and high protein diets for 100 days.

TABLE I.

Exp.		Weight at 30 days grms.	Weight at 130 days Normal diet grms.	Weight at 130 days High protein diet grms.
A. 1	♂	42	257	—
2	♂	42	221	—
3	♀	36	—	139
4	♀	38	—	144
B. 1	♂	41	250	—
2	♀	42	182	—
3	♀	45	—	139
4	♀	42	—	121
C. 1	♂	36	—	146
2	♀	35	—	116
3	♀	40	148	—
D. 1	♀	42	197	—
2	♂	41	—	138

The letters A, B, C, D signify that the animals under each letter were of the same litter.

From the above table the average weights at the age of 130 days are as follows:

Male rats normally fed, 242 grams. High protein diet, 142 grams.
Female rats „ 175 „ „ „ „ 135 „

Although the groups are rather too small to use for deducing averages it is interesting to note that the male rats on high protein diet had only attained 58·6 p.c. of the weight of the normally fed male rats, while the female rats on high protein diet reached 75 p.c. of the weight of the

control female animals. Table II shows the relative increase in body weight of the male and female rats of both groups:

TABLE II.

		Relative body weights			
		Start	1 month	2 months	3 months
Normal.	Male	1	2	4	5·6
	Female	1	2·5	3·5	4·0
High protein.	Male	1	1·9	2·4	3·2
	Female	1	1·8	2·3	3·1

These figures show that in both groups male and female rats grew almost normally until the end of the first month, but later show the retardation which Chalmers Watson observed.

At the conclusion of the experiments the animals fed on high protein diet looked well, their coats were in good condition and their appetites were excellent; in fact they resembled normal animals younger than the controls. Detailed post mortem examinations were made of a number of the rats. In the high protein group plenty of body fat was found but the ribs showed marked beading as compared with the normal. The organs from rats of both groups were removed, weighed and their weights compared with the figures given by Donaldson(5) for the normal weights of organs for a given body-weight. The weights of the supra-renals, thyroid, ovaries, testes, spleen, liver and kidney in both sets were within normal limits of variation and microscopical examination showed no marked uniform difference between the experimental and control animals. The result confirms our suspicion that the pathological conditions in Chalmers Watson's rats were due to causes other than the high intake of protein. With regard to the beading of the ribs we hesitate to ascribe it to the high protein dietary, nor do we care to commit ourselves to an opinion as to the cause. It is well known that certain types of dietary deficiency tend to produce abnormal conditions at the costo-chondral junctions.

It was noticed that in some cases the contents of the abdominal cavity in the protein-fed rats had a markedly objectionable odour although they were freshly killed. It is possible that this was due to the exaggerated action of protein-putrefying organisms flourishing on the unusually large amount of protein products in the contents of the intestine.

In view of the normal condition of practically all the tissues of the high protein group it was difficult to explain their failure to grow. We first studied whether their daily consumption of the diet was sufficient to allow both for tissue building and for energy. Calculations showed that the average daily ration supplied approximately 60 cals. per 100 gm.

rat whilst the controls were consuming about 65 cals. per day. Previous experiments in this laboratory have taught us that rats of this weight can be maintained on 25 cals. per day and show normal development on 45 cals. It was therefore unlikely that the high protein group were showing retarded growth from an insufficient calorific intake.

The normal growth and condition of the rats fed on the mixed diet showed that the intake of the so-called vitamins was ample, and we therefore assumed that the same proportions of the ingredients of the food mixture which supplied these substances would be adequate for the high protein diet. To determine whether the failure to grow was a consequence of an omnivorous species being given a diet unnaturally high in protein we decided to study the question on carnivora which naturally subsist on such a food supply. The number of these experiments was small but the results were in general agreement.

Four kittens of the same litter were selected at the age of about six weeks. A male and female were used as controls and two females were fed on a high protein ration. The daily rations were made up as given below:

Normal diet	High protein diet
Raw beef (fat and lean) 110–170 grm.	Raw *lean* beef 110–170 grm.
Bread and milk *ad lib.*	Yeast extract 2–4 grm.
	Shark liver oil 4 c.c.
	Salt mixture 2 grm.[1]

The high protein ration was compounded by trimming the fatty tissue from lean beef, mincing and mixing the addenda to supply the salts and vitamins. Both lots of cats consumed their diets well and those on the normal ration grew and showed absolutely normal behaviour. The cats fed on the high protein diet, however, showed a retardation of growth at an early stage of the experiment and continued to grow at a somewhat sub-normal rate. At several periods during the experiment these two animals showed symptoms of joint trouble and lameness. These symptoms seemed to disappear when the supplement of shark liver oil was increased but no increase of the growth rate followed. The condition of these cats, apart from this lameness, was good although there was a tendency to excitability. Fig. 2 shows the growth curves of the cats. The experiments were continued for four months when one animal from the high protein group was killed for examination. The

[1] By an error the salt mixture was not incorporated in this diet for the first half of the experimental period. Little or no improvement occurred when the mistake was rectified and this fact taken together with the results of the rat experiments which were satisfactory from the outset leads us to conclude that the failure to grow was not due to deficiency of salts. The salts of the yeast extract probably made up any deficiencies of the meat.

second animal in this group was then given the normal mixed diet and immediately responded by a rapid increase in growth rate so that after another month it had regained the normal weight for its age. The tissues of the cat killed from the high protein group showed no abnormality beyond very marked beading of the ribs and the normal condition of the organs was confirmed by microscopical examination.

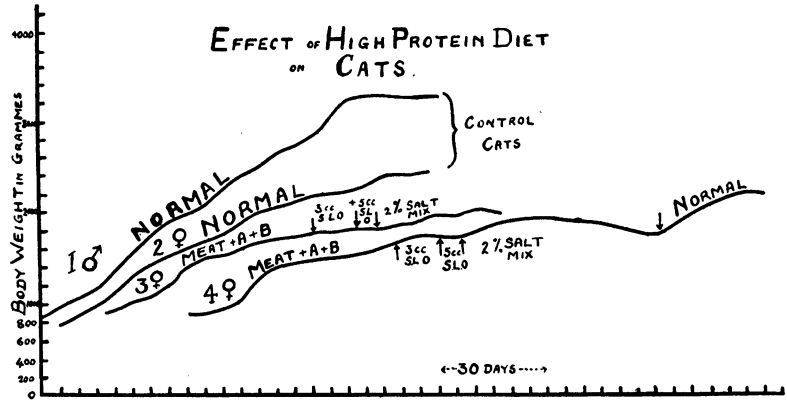

Fig. 2.

Table III shows the relative weights of the animals throughout the experiment.

Diet		Start	Relative weights 1 month	2 months	3 months
Normal.	Male	1	2	2·9	3·5
	Female	1	1·9	2·5	2·9
Protein.	Female	1	1·7	1·9	2·1
	Female	1	1·7	1·9	2·1

Remarks. The failure of a typical carnivorous species to show normal growth on a high protein dietary supplemented with what were believed to be adequate amounts of inorganic salts and vitamins was surprising but curiously confirmative of the results with the rats. The normal condition of the organs in our experimental animals leads us to infer that Chalmers Watson's results were largely attributable to the fact that his dietaries were deficient in the accessory factors.

In our experiments on rats with a high protein diet, microscopical examination showed no change in the tissues. The kidneys remained normal in appearance although they excreted 1·5 to 2 grms. of nitrogen daily for long periods. Thus the statement that excretion of large amounts of nitrogen end-products causes degenerative changes in the kidney is not confirmed.

In her studies of lactation on high protein diets Miss Hartwell has, in her last paper(6), to a certain extent considered the obvious criticisms of her earlier experiments and has taken some account of the possible influence of other dietary factors. She finds that the adult rat and the young growing rat can live normally on the diet containing an excess of protein (proportion 15 gm. bread to 5 gm. protein) but that the same ration is not adequate for normal milk production in the lactating female unless definite quantities of some other constituent or constituents are supplied. Before this paper appeared we commenced a series of experiments designed to throw light on her results and their bearing on our high protein feeding tests already described. These latter experiments are as yet incomplete but we have obtained definite evidence that a high protein ration (83 p.c. of dry weight of diet in the form of caseinogen) can be adequate as a diet for lactating female rats and enables them to rear healthy litters of normal weight provided that it is also adequate in components serving as sources of the vitamins. We have also found that the diet of the mother prior to pregnancy and lactation is a factor of fundamental importance and one to which Miss Hartwell may not as yet have given sufficient attention. Miss Hartwell's preliminary results with yeast extract, milk, butter, etc., tend to show that a factor present in yeast extract and milk is protective. There is a certain amount of scattered evidence that vitamin B which is contained in yeast is directly or indirectly concerned quantitatively with the metabolism of carbohydrate (subject reviewed by Funk(7)) and if this is correct it is justifiable to assume that it will be equally concerned in the metabolism of the non-nitrogenous fragments of the amino-acids which normally undergo breakdown along the paths of carbohydrate metabolism. In this connection it is interesting to note that one of us has frequently observed the typical symptoms described by Hartwell in litters of rats but only in the case of those being reared by mothers on diets deficient in vitamin B. Her negative results with butter confirm our one observation that raising the quantity of shark liver oil did not restore the normal growth of the cat on the high protein ration.

Summary.

1. Growth, but at a sub-normal rate, was shown by rats and cats fed on dietaries containing 80–90 p.c. of the dry weight in the form of protein (caseinogen) but apparently adequate as regards vitamins and salts. On changing to a normal diet the animals reach normal weight.

2. The rats maintained excellent health throughout but did not reproduce whilst on the special diet.

3. The organs and tissues presented a normal appearance at the end of the experiment and there is little doubt that the abnormal conditions found in similar experiments by other workers were due to causes other than the high protein intake, *e.g.* vitamin deficiency, etc.

4. The excretion of very large amounts of nitrogenous waste products over considerable periods of time does not appear to cause damage to the kidney in these species.

5. The failure to grow normally and to reproduce is in our opinion due not to the high protein intake itself but to a lack of balance between this constituent and some other component or components of a normal diet. It is tentatively suggested that the vitamin B, which is believed to be quantitatively concerned in the metabolism of carbohydrates, may likewise be concerned in the metabolism of those non-nitrogenous fragments of the amino-acids which follow a similar fate.

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