FURTHER OBSERVATIONS ON NUTRITION WITH DIETS RICH IN PROTEIN.

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In spite of considerable study of the influence of diets rich in protein on growth and nutrition it remains uncertain whether they are adversely affected or not. Obviously in species which are carnivorous normal nutrition can be maintained when the food contains a large proportion of protein, but in omnivorous and herbivorous animals abnormal conditions have from time to time been ascribed to excessive nitrogen intake. Drummond, Crowden and Hill(1) found that rats fed on diets containing from 80–90 p.c. of the dry weight as protein (caseinogen) exhibited a subnormal rate of growth and failed to reach adult size. Post-mortem examination of the animals revealed no marked lesions other than a slight beading of the rib junctions; nor did the histological study of the tissues show abnormal changes. In particular, the kidneys, in spite of a relatively enormous daily excretion of nitrogen, appeared normal as compared with the controls and showed none of the degenerative changes observed in rabbits by Newburgh(2).

In 1923 Polvogt, McCollum and Simmonds(3) recorded experiments on rats fed on diet containing from 30–40 p.c. of protein. Their animals grew at a normal rate and reproduced satisfactorily for several generations, but usually showed evidence of kidney lesions in spite of the fact that their diet cannot be regarded as having been very abnormally rich in protein. More recently Osborne and Mendel(4) have claimed that normal growth to adult size may be obtained in rats fed on diets in which all carbohydrate and fat is furnished endogenously. They record hypertrophy of the kidneys but could detect no structural damage.

In view of these discrepancies a further series of experiments have been made.

Young rats weighing about 50 gms. were fed on diets compounded as follows:

	Group I	Group II	Group III
Caseinogen	20 parts	45 parts	90 parts
Starch	70 „	45 „	0 „
Cod liver oil	2 „	2 „	2 „
Yeast extract	5 „	5 „	5 „
Lemon juice	5 „	5 „	5 „
Salt mixture	5 „	5 „	5 „

Occasionally there was an initial drop in weight, especially in Group III, if the rats were less than 50 gms. in weight when first placed on the diet and a few rats died. The majority recovered and grew.

Figs. 1 and 2 show typical growth curves of the three groups of rats when fed on these diets.

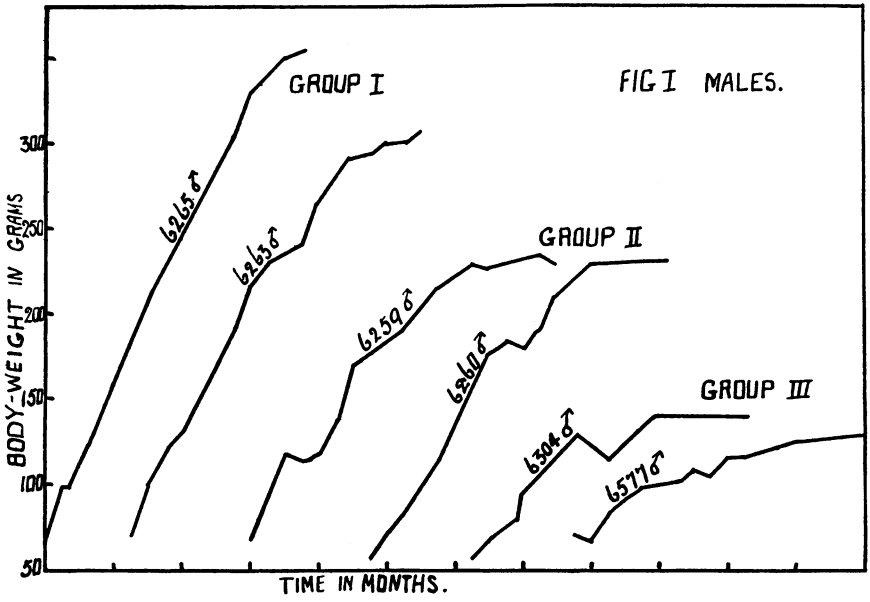

Fig. 1. Males.

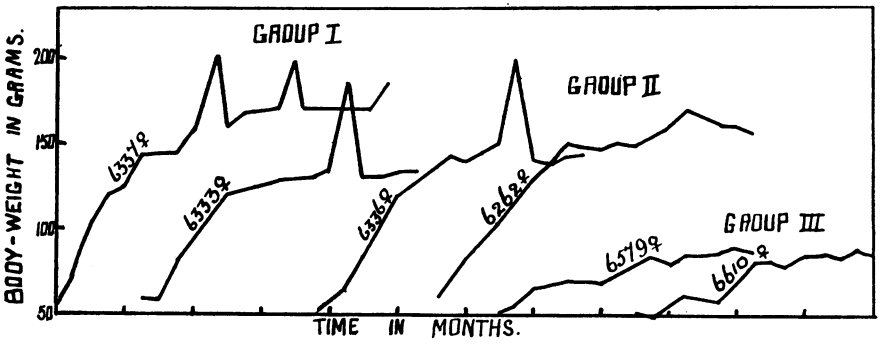

Fig. 2. Females[1].

The rats in Group I showed normal growth, as was to be expected. Those in Group II at first showed a similar behaviour, but later a retarda-

[1] The sharp peaks in these curves indicate pregnancies.

tion of growth set in and they failed to reach full weight. The animals in Group III failed to show normal growth at any stage and attained a constant weight of approximately one-third the calculated normal weight. Throughout the experiment the animals in all three groups were quite healthy and presented a satisfactory appearance, apart from the cases of stunting to which reference has already been made. Records of food consumption over periods of ten days were made several times during the experiments, and revealed the fact that more food is eaten when the percentage of protein in the diet is raised. This may be seen from the figures given below:

Group	% protein in diet	Average weight of rat	Daily consumption of dry food in gms.	Consumption per 100 gms. body weight
I	20	100	10·5	10·5
II	45	100	12·7	12·7
III	90	75	17·9	23·9

In view of the earlier experiments in which it was suspected that there was interference with ossification in the animals receiving much protein, periodic X-ray examination was made of the rats in the three groups in this experiment. Careful study of the radiographs disclosed no abnormalities and it is concluded that calcification had proceeded normally even in the stunted animals on diet III. This was later confirmed by histological examination of the costochondral junctions in a number of cases.

The experiments were continued sufficiently long for reproduction to take place, but while Group I produced many litters, and Group II a few, there was no reproduction in Group III.

A number of animals were killed after they had been about four months on their respective diets, and were subjected to careful examination. The organs presented a normal appearance; there was plenty of body-fat and apart from the small size of the animals in Groups II and III they might all have been normal rats. In the earlier experiments made in this Laboratory it appeared that the kidneys of the animals on the protein-rich diet were enlarged, but insufficient were then examined to justify a statement. Osborne and Mendel(4) found the average weight of the kidneys on the protein-rich diet to be almost twice that of the kidneys of control animals, whilst their size was about one-third greater. We have now obtained ample confirmation of this hypertrophy.

The following figures give the ratio of kidney weight to body weight for a number of rats of the same age from each group:

I (20 % protein)	II (45 % protein)	III (90 % protein)
·0063	·0103	·0163
·0069	·0099	·0149
·0079	·0102	·0150
·0090	·0094	·0196
·0079	·0101	·0237
·0084	·0104	·0174
·0090	·0098	·0155

Donaldson(5) gives the normal range for this age as ·0070–·0090. Histological examination was made of the livers and kidneys, using Zenker's fixative and staining with hæmatoxylin and eosin, but no abnormalities were detected which could be ascribed to the diet.

Discussion.

We are unable at this stage of the enquiry to offer an explanation of the failure of the rats to grow normally on the diets containing 45 and 90 p.c. of protein. Both Polvogt, McCollum and Simmonds(3) and Osborne and Mendel(4) obtained better growth than we. In the former case their diets never contained more than 41 p.c. of protein and therefore may reasonably be compared with our Group II, but there are differences between the rations employed for we used only one protein, caseinogen, whilst they used mixtures of caseinogen with the proteins present in such natural foods as wheat, maize, navy beans and liver. On the other hand, Osborne and Mendel observed better growth on a diet containing 50–55 p.c. of caseinogen, together with some fat and carbohydrate, than we recorded on the ration containing 45 p.c. caseinogen. There appear to be no experiments strictly comparable with ours in which 90 p.c. of caseinogen was used. In view of the different types of diets employed by the various investigators it is almost impossible without further experimentation to suggest the cause of the discrepancies, but it is just possible that the recent work of Hartwell(6) may throw some light on the matter. She has traced what she believes to be a relation between the amount of vitamin B required in the food and the protein content of the diet. As yet the evidence appears insufficient to prove this relation, but if further work should establish that it does exist it may provide an explanation of the discordant results we have outlined.

An admittedly inadequate attempt to test whether her theory is correct was made by administering extra amounts of yeast extract to a few rats the growth of which had been retarded by the protein-rich diets. Sometimes there was a slight response but the results were inconclusive and call for further work. In a few other cases rats from the

same group were given an extra supplement of cod liver oil in case a larger amount of the vitamins in that foodstuff was necessary, but no acceleration of growth took place. When rats from Groups II and III were given the ration of Group I there was usually a resumption of growth, sometimes at a nearly normal rate, but before long it ceased and the animals failed to reach a normal size. (See Fig. 3.) Here again we offer no explanation, preferring to wait for the results of further experiments.

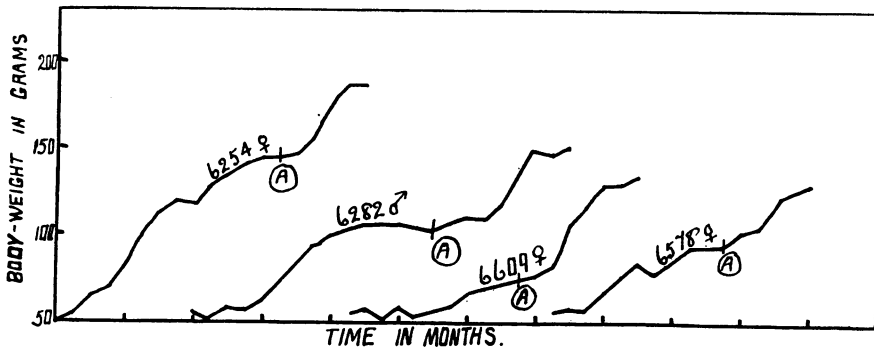

Fig. 3. Group III changed to Group I diet at (A).

The hypertrophy of the kidneys, which we observed, doubtless results from the increased work which the kidneys are called upon to perform when so large an amount of nitrogenous waste products must be excreted. It has been frequently stated, largely from clinical observation, that kidneys may become enlarged from this cause, and it has also been repeatedly denied. These results, confirming those of Osborne and Mendel, definitely prove that physiological hypertrophy may occur. It has also been widely held that the prolonged excretion of abnormally large amounts of nitrogenous waste products will induce degenerative changes in the kidneys and no small part of the literature on Bright's disease concerns this point. Here again, this view has been established largely on the results of clinical observation and has lacked controlled experimental support.

What at one time appeared to be adequate support for this widely held opinion was furnished by the paper of Newburgh(7), who stated that rabbits fed on diets rich in protein almost invariably show, after a few weeks, marked nephritis. Careful examination of his experimental records, however, tends to shake one's confidence in his conclusions. Many of his animals were fed on ill-balanced and inadequate rations, such as a diet consisting solely of egg-white, which might, and probably

would, produce pathological changes. Furthermore, many of the kidneys examined were removed from the body after the animals had been found dead from inanition or as a result of the diet. This is not justifiable. Certainly other experiments of his are less open to severe criticism and if the results in these cases are reliable it would indicate that the rabbit may show kidney damage as a consequence of feeding on diets rich in protein.

It is less easy to understand why degenerative changes in the kidneys of rats were observed by Polvogt, McCollum and Simmonds(3) when their animals were fed on well-balanced diets containing the relatively small proportion (30–40 p.c.) of protein. As a matter of fact they state that the characteristic picture contrasted with that observed in the kidneys of control rats was one of enlargement and congestion. In some cases there was degeneration of the tubular epithelium and hyaline casts were present. The enlargement we have already considered. Some congestion was noted in practically all the kidneys we examined, the differences between the three groups being insignificant. The degeneration may possibly be due to the longer period that their animals were kept on a protein-rich diet, but we do not ourselves think this is the explanation. Slight abnormalities of the type they describe are, we believe, fairly common in rats, and may possibly be associated with the many minor ailments to which they are subject, particularly from internal or external parasites, and about which little or nothing is known. In our rats one or two cases showed extremely slight degeneration of tubules with occasionally small patches of leucocytic infiltration. These abnormalities were observed, however, as frequently in the control group as in the groups receiving large amounts of protein, and we, therefore, cannot agree that the excretion of abnormally large amounts of nitrogenous end-products of metabolism causes damage to the kidney in the rat.

Summary.

1. Rats failed to grow to adult size when fed on diets containing a high proportion of protein (45–90 p.c.).

2. The animals appeared to be in good health and the consumption of food was satisfactory. Post-mortem examination revealed no abnormality other than hypertrophy of the kidneys, as measured by the ratio of kidney weight to body weight.

3. The slight degenerative changes which were observed in some of the kidneys of rats fed on the diets rich in protein could not be ascribed

to the action of the food as similar changes were seen in the controls. Excretion of relatively very large amounts of nitrogen (as much as 2·5 gm. daily) over periods of four months does not appear to damage the kidneys of rats.

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