CLVII. RELATION BETWEEN VITAMIN B AND PROTEIN IN THE DIET OF GROWING RATS.

PHYSIOLOGICAL RÔLE OF VITAMIN B. PART II.

BY VERA READER AND JACK CECIL DRUMMOND.

From the Department of Physiology and Biochemistry, University College, London.

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IN 1922 it was reported by Drummond, Crowden and Hill [1922] that cats and rats failed to grow at a normal rate when fed on dietaries containing 80–90 % of protein (caseinogen) but, to the best of our knowledge at that time, adequate as regards vitamins and salts. Furthermore, it was observed that in spite of the inhibition of growth the organs and tissues presented a normal appearance after several months on the high protein rations, and that the kidneys in particular exhibited no lesions of the type then rather generally believed to be caused by excessive nitrogen intake. Last year confirmation of these results was reported by the writers [1925].

Of possible explanations of the inability of the young animals to grow on the high protein dietaries, those based on the idea that a quantitative relation between certain dietary constituents may be necessary for normal nutrition seemed worthy of attention, particularly in view of the interesting observations of Hartwell on the relation between vitamin B and protein in the diet of pregnant and lactating rats [1924 and earlier papers].

It is uncertain what relationship exists between the symptoms described by Hartwell [1921] as being shown by young rats suckled by mothers fed on dietaries rich in proteins and the condition resembling beri-beri that can be produced by feeding these animals on a diet deficient in vitamin B. As we have remarked before [Drummond, Crowden and Hill, 1922], somewhat similar symptoms to those Hartwell described have been observed by us in very young rats being reared by mothers fed on a ration deficient in vitamin B. Later, Hartwell [1924, 1925] concluded from a continuation of her researches that for normal nutrition of the young it is necessary to maintain a *quantitative* relation between the level of protein and of vitamin B in the diet of the lactating mother. Assuming this relation to exist and also that it represents a relation between the vitamin and the metabolism of protein or its constituent molecules—for there is no evidence that in the absence of adequate amounts of vitamin B the digestion and absorption of protein is retarded or incomplete—it is of interest to enquire into the rôle of the former substance.

Drummond, Crowden and Hill [1922] suggested, on the grounds that it had been claimed that a quantitative relation exists between the vitamin B requirements of the organism and the total carbohydrate metabolised, that it might reasonably be argued that the vitamin would also be concerned in the metabolism of the glucogenic fragments of the protein molecules. Hartwell [1924] has pointed out, however, that this explanation does not serve to cover the results of a number of her experiments, and prefers to believe that "the rôle of vitamin B in the metabolism of protein is concerned with the nitrogenous part of the protein molecule or with protein *quâ* protein, and not with any fatty acid or carbohydrate residue." The following experiments were planned to throw further light on this question, in particular to determine whether the relationship traced by Hartwell serves to explain the failure of growth observed by us on high protein diets, and also whether any additional information could be obtained regarding other claims, namely those of Braddon and Cooper [1914], Funk [1914, 1919], Randoin and Simonnet [1923, 1924], that vitamin B is quantitatively related to the carbohydrate metabolised; of Tscherkes [1926] that the relation is between vitamin B and both protein and carbohydrate; and of Plimmer [1926] that the ratio between vitamin B and total food metabolised is normally a constant.

EXPERIMENTAL.

Two series of experiments were planned. In the first diets were employed of which the compositions were adjusted so as to give similar ratios between the amount of protein and the material supplying vitamin B, but to be of different calorific value. In the second series the proportion of protein was fixed whilst that of vitamin B was varied.

Series I. *Composition of Diets (parts).*

	A	B	C	D	E	F
Caseinogen	20	20	70	70	70	70
Starch	70	50	20	5	20	5
Hardened fat	0	18	0	18	0	18
Lemon juice	5	5	5	5	5	5
Salt mixture (McCollum)	5	5	5	5	5	5
Cod-liver oil	2	2	2	2	2	2
Yeast extract	4	4	16	16	4	4
Calories per 100 g. dry weight	360	480	360	480	360	480

Groups of young rats of approximately 50 g. weight were fed on each of these rations; close observation being made of their weight, food intake and general health whilst on the diet.

The growth curves of typical animals from each group are given in Fig. 1, from which it will be seen that the results of our previous work are generally confirmed, in that there is a failure to grow at a normal rate when the diet containing 70 % of protein is supplemented with only the same proportion (4 %) of yeast extract that suffices to produce good growth with 20 % of protein.

Fig. 1.

Observations of the amount of food consumed daily over periods of 14 days led to the following results being obtained (Table I).

Table I.

Group	Food consumption per 100 g. per day g.	Calorific intake per 100 g. g.	Approx. protein intake per day g.	Approx. intake of yeast extract g.	Average ratio Calories Yeast	Average ratio Protein Yeast	Growth
A Males	5.4	19.4	1.1	0.22	88	4.9	Good
A Females	5.7	20.6	1.1	0.23			
B Males	4.8	23.1	1.0	0.19	122	5.2	Good
B Females	6.0	28.8	1.2	0.24			
C Males	7.0	25.4	4.9	1.12	22.6	4.3	Good
C Females	8.9	32.2	6.2	1.42			
D Males	7.3	35.1	5.1	1.17	30.0	4.3	Good
D Females	7.8	37.5	5.5	1.25			
E Males	8.0	28.8	5.6	0.32	88	18	Poor
E Females	10.4	36.4	7.3	0.41			
F Males	10.5	50.5	7.3	0.42	120	17	Poor
F Females	11.0	52.9	7.7	0.44			

This analysis of the results of the estimations of food intake seems clearly to indicate a relation between the proportion of protein and the proportion of vitamin B in the diet as far as the effect on growth is concerned. On the other hand, there would seem to be no correlation between the total calories consumed and the vitamin B requirements, nor has any relationship been traced between the vitamin and the carbohydrate content of the diet, even when allowances are made in respect of the glucogenic value of the caseinogen.

The second series of experiments was made on young rats fed on rations containing the fixed proportion of 70 % of caseinogen, in which proportions

varying from 4 to 20 % of yeast extract were incorporated. Half the animals on each type of diet were given 15 % of fat (butter-fat) in their ration whilst the remainder were given no fat apart from a very small supplement of cod-liver oil to serve as a source of fat-soluble vitamins.

The growth curves of typical animals from each group and subgroup are reproduced in Fig. 2, from which it will be seen that a normal rate of growth is only attained when the 70 % of protein is supplemented with at least 16 parts of yeast extract, the addition of fat making no obvious difference. This is clearly illustrated by the curves in Fig. 3 in which are plotted the average body-weight of the rats in each group after a period of 10 weeks' feeding on the various diets against the percentage of yeast extract.

Fig. 2.

The ratios between the dietary constituents in these experiments are given in Table II.

Table II.

Diet	% protein	% yeast extract	Protein Yeast ex.	Calories Yeast ex.	Growth
1	70	4	17.5	90	Poor
2	70	8	8.6	45	Fair
3	70	12	5.8	30	Fairly good
4	70	16	4.4	22	Good, nearly normal
5	70	20	3.5	18	Normal
6	20	4	5.0	90	Normal

These results confirm those in Table I, in that there is no relation between total calories and vitamin B as far as correlation with growth is concerned, and that a normal rate of growth is not attained until the ratio between protein and yeast extract yields a value of about 5 or under.

Fig. 3. Improved growth with increased yeast extract.

Effect on thyroid gland.

The possibility of a hypertrophy of thyroid tissue being induced on the high protein diets, particularly when the percentage of yeast extract is below that adequate for a normal rate of growth, was suggested and at the end of the experiments the glands were dissected out and weighed. In all groups, however, the ratio between the weight of thyroid and total body-weight was within the normal limits given by Donaldson [1924].

Table III.

		Ratio $\frac{\text{Weight of thyroid}}{\text{Weight of body}} \times 1000$ on diets A—F.				
Groups	A	B	C	D	E	F
	2.3	2.3	2.7	1.9	2.4	1.9
	1.6	2.0	1.8	1.6	2.3	3.0
	1.4	1.9	1.9	1.7	2.4	2.4
	2.3	2.1	2.3	1.6	2.0	2.5
	2.8	2.4	2.3	1.8	—	2.4
	1.9	2.0	—	1.8	—	—
	2.1	1.7	2.3	2.2	—	—
	2.2	2.1	2.5	1.5	—	—
Av.	2.1	2.1	2.2	1.8	2.4	2.4

Effect on kidneys.

As in the previous studies from this Laboratory the weights of the kidneys were determined after the animals had been 4 months on their respective diets. The results are given in Table IV.

The hypertrophy of the kidneys in groups E and F, fed on the diets rich in protein but relatively low in vitamin B, confirmed our previous observations. It was of the same order as that formerly detected by us and also by Jackson and Riggs [1926].

We wish again to emphasise the normal condition and histological picture of the kidneys of these rats and to reassert our belief that the excretion of relatively very large amounts of the nitrogenous end-products of metabolism is not in itself harmful to the renal tissues.

Scarcely any hypertrophy was apparent in the kidneys of the rats fed on the diets rich both in protein and vitamin B, a fact in agreement with the observed normal rate of growth of these animals.

Table IV.

		Ratio $\frac{\text{Kidney-weight}}{\text{Body-weight}} \times 1000$.				
Groups	A	B	C	D	E	F
	10.0	7.4	12.6	11.1	12.0	13.0
	10.4	7.8	13.5	9.1	14.5	14.0
	9.1	8.0	12.0	11.0	13.9	14.3
	8.6	9.6	13.3	8.0	15.0	14.8
	8.1	9.0	12.7	8.5	—	14.4
	10.2	14.0	11.3	10.0	—	—
	9.3	9.4	11.9	8.0	—	—
	10.1	14.0	—	10.0	—	—
Av.	9.4	9.6	12.4	10.9	13.9	14.1

DISCUSSION.

From these results, as well as from the researches of Hartwell, it would seem established that a definite relation must exist between the intake of protein and of some factor in yeast, presumably the vitamin B, before normal nutrition is possible. Moreover, both investigations cast some doubt on the existence of a quantitative relation between the vitamin and the carbohydrate intake; a relation which, as has been pointed out, a number of investigators have claimed to have demonstrated.

Actually, however, a careful examination of the literature on this matter reveals a rather unsatisfactory state of affairs, in that many of the experimental results and the conclusions drawn from them seem unconvincing.

Against the claims of Braddon and Cooper [1914], Funk [1914, 1919], Randoin and Simonnet [1923, 1924], all of whom trace a relation between vitamin B and carbohydrate metabolism, can be set the work of Eijkman and van Hoogenhuyze [1916], Vedder [1918] and of Anderson and Kulp [1922], which lends no support to this view.

Much of this work was concerned with determining the time of onset of the so-called "polyneuritic" symptoms in pigeons fed on different types of diets and seems on critical examination to be lacking in precision and adequate control.

By employing a similar method, Tscherkes [1926] believes he has traced a relation between the requirements of the organism for vitamin B and the intake of carbohydrates and protein, but that the vitamin is unrelated to the fat of the diet. Naturally, many of the points raised by this work are bound up with the question whether the growth-promoting factor in yeast (vitamin B) is identical or not with that protecting the organism against the so-called beri-beri.

In view of the fact that the evidence on this matter is still far from complete and that the assumption that only one factor is concerned is not at variance with any recognised facts, it seems wiser in the present state of our knowledge to accept the simpler hypothesis. Assuming, then, that only one substance is concerned, it is not possible with our results in mind to subscribe to the opinion that the vitamin B is quantitatively related, as far as growth in the rat is concerned, either to the carbohydrate intake or to the total calories consumed.

What part the vitamin plays in protein metabolism remains a matter for further investigation, but at first sight it would seem that Hartwell is right in believing that it is concerned with the fate of the proteins or their constituent units as a whole rather than with the metabolism of the nitrogen-free residue of the amino-acid molecules, as was at one time tentatively suggested.

SUMMARY.

1. Further confirmation has been obtained that diets consisting largely of protein (caseinogen) may be inadequate for the growth requirements of young rats.

2. This inadequacy may be made good by raising the proportion of yeast extract (vitamin B). Normal rates of growth were obtained on dietaries in which the ratio of protein to yeast extract has a value of 5 or under.

3. The results of these experiments seem to confirm the views of Hartwell and of Tscherkes that vitamin B is related quantitatively to the amount of protein in the diet.

They do not lend support to the opinions that the vitamin stands in such relation to the amount of carbohydrate ingested or metabolised or to the total calories consumed, at any rate as far as the effect on growth is concerned.

4. Additional evidence has been obtained of the hypertrophy of the kidneys in animals fed on diets consisting largely of proteins. This hypertrophy is only marked when the composition of the diet is such that the animal does not grow at a normal rate. As pointed out in previous publications the excretion of large quantities of nitrogenous end-products of metabolism does not cause damage to the kidneys.

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REFERENCES.

- Anderson and Kulp (1922). *J. Biol. Chem.* **52**, 69.
Braddon and Cooper (1914). *J. Hygiene*, **14**, 331.
Donaldson (1924). *The Rat* (2nd edition. Philadelphia).
Drummond, Crowden and Hill (1922). *J. Physiol.* **56**, 413.
Eijkman and van Hoogenhuyze (1916). *Proc. K. Akad. Wetensch. Amsterdam, Afd.* **24**, 1471.
Funk (1914). *Z. physiol. Chem.* **89**, 373.
— (1919). *J. Physiol.* **53**, 247.
Hartwell (1921). *Biochem. J.* **15**, 140.
— (1924). *Biochem. J.* **18**, 785.
— (1925). *Biochem. J.* **19**, 1075.
Jackson and Riggs (1926). *J. Biol. Chem.* **67**, 101.
Plimmer (1926). *Brit. Med. J.* Feb. 6th.
Randoin and Simonnet (1923). *Compt. Rend. Acad. Sci.* **177**, 903.
— (1924). *Bull. Soc. Chim. Biol.* **6**, 601.
Reader and Drummond (1925). *J. Physiol.* **59**, 472.
Tscherkes (1926). *Biochem. Z.* **167**, 203.
Vedder (1918). *J. Hygiene*, **17**, 1.