XCII. THE VITAMIN B COMPLEX AND HIGH PROTEIN DIETS.

By FRANCIS THOMAS GARNET PRUNTY
AND MARGARET HONORA ROSCOE¹.

From the Division of Nutrition, Lister Institute, London.

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DRUMMOND, CROWDEN AND HILL [1922] showed that rats and kittens were retarded in growth when their diets contained large proportions of protein. This effect was again observed by Reader and Drummond [1925], who used three levels of protein intake and found that young rats grew progressively less as the protein in the diet was increased. The same authors showed [1926] that by increasing the vitamin B content the effect of the high protein diet could be counteracted and considered that this fact confirmed the theory put forward by Hartwell [1924; 1925] that the vitamin B requirements are dependent on the protein intake.

Hartwell's experiments were carried out with young suckling rats, in which spasms developed when the protein of the mothers' diet was increased, unless additional yeast extract was given. They are not therefore comparable with the above experiments or with those reported in this paper, which deal with the growth of young weaned rats, and it is not proposed to discuss them here.

Hassan and Drummond [1927] carried the matter further by showing that additional amounts of an autoclaved yeast extract were as effective in restoring growth as an unheated yeast extract. The differentiation between vitamins B₁ and B₂ had not at that time been completed, but the result of this experiment has since been taken as evidence that the more heat-stable vitamin B₂ is the factor in the vitamin B complex which counteracts the unfavourable effect of a high proportion of protein in the diet.

In addition to the effect of high protein diets on growth, their effect on the kidney has been studied. Polyvogt, McCollum and Simmonds [1923] found degenerative changes in the kidneys of rats receiving diets containing 31 to 41 % of protein. This has not been confirmed by later workers. Reader and Drummond [1925; 1926] and Hassan and Drummond [1927] observed hypertrophy of the kidneys but no degenerative changes, although their animals in some cases received as much as 90 % protein. Reader and Drummond [1926] claimed to have shown that this hypertrophy did not occur in rats which received additional amounts of vitamin B and grew normally, but only in those in which growth was retarded. It thus appeared that a balance must be maintained between the protein intake and the vitamin B₂ intake if normal growth and kidney development are to take place.

Since this work was carried out before the differentiation of vitamins B₁ and B₂ had been made, it was thought advisable to reinvestigate the question. The following experiments were therefore carried out.

¹ Beit Memorial Fellow.

EXPERIMENTAL.

Exp. 1. The effect on rats of a high protein diet at various levels of vitamin B₂ intake, vitamins B₁ and B₂ being given as separate daily doses.

Method. Two levels of protein intake were employed, 20 and 70 %. The diets had equal calorie values and were constituted as follows:

	%	%
Caseinogen ("Casumen"*)	20	70
Rice starch	60	10
Salt mixture†	5	5
Hardened cottonseed oil	15	15

* A commercial caseinogen not specially purified.

† Salt mixture No. 185 [McCollum, Simmonds and Pitz, 1917].

The diets were mixed with an equal amount of water and cooked in a steamer for 3 to 5 hours. Cod-liver oil was given as a separate daily dose (0.05 cc.).

Vitamin B₁ was given as an antineuritic concentrate from yeast [Peters, 1924; Kinnersley and Peters, 1925], vitamin B₂ as an autoclaved acetic acid extract of yeast (1 cc. $\equiv$ 1 g. dry yeast) [Roscoe, 1930].

Young 40 g. rats previously deprived of the B vitamins for 1 week were used, and the effects of the two diets observed at different levels of vitamin B₂ intake with respect to growth, kidney weight and incidence of dermatitis characteristic of vitamin B₂ deficiency. The food intake was also noted.

Vitamins B₁ and B₂ were given as separate daily doses, all rats receiving adequate vitamin B₁. The two diets with different percentages of protein were administered to two parallel series of rats of which group 1 received no vitamin B₂ and groups 2, 3 and 4 increasing amounts; the dose received by group 4 was more than adequate for normal growth. The rats were observed for 5 weeks, with the exception of those receiving no vitamin B₂, which were kept for 15 weeks or until dermatitis developed.

Results.

(i) *Growth* (Table I). At a given intake of vitamin B₂ growth was the same whether the percentage of protein in the diet was 20 or 70. A slight difference occurred in group 4, but this was probably not significant.

(ii) *Dermatitis*. Six of the eight rats receiving no vitamin B₂ (group 1) exhibited dermatitis. Three of these had received the diet with 20 % protein and showed symptoms in 7½, 8 and 9½ weeks, and three had received the diet with 70 % protein, showing symptoms in 7½, 7½ and 13 weeks. The amount of protein in the diet did not therefore appear to influence in any way the incidence of dermatitis.

(iii) *Kidney weight*. The kidneys of the rats in groups 2, 3 and 4, receiving different amounts of vitamin B₂, were weighed at the end of the experimental period of 5 weeks (Table II). Those of the rats receiving 70 % protein were in all cases larger than those of the rats receiving 20 % protein. This hypertrophy did not decrease with increasing amounts of vitamin B₂. It was, in fact, found, as may be seen by reference to Table II, to be roughly proportional to the increase in protein consumption, whatever the dose of vitamin B₂.

(iv) *Food intake*. In Table II are also shown the average amounts of food consumed by each group of rats during the 5 weeks of the experiment. The rats

Table I. *The growth of rats receiving diets containing 20 or 70 % protein at different levels of vitamin B₂ intake.*

	Vitamin B ₂ daily dose cc.	Increase in weight in 5 weeks g.					
		20 % protein diet			70 % protein diet		
		Rat	Average		Rat	Average	
1	0.0	676 ♂	18	22	680 ♂	29	21
		692 ♂	21		696 ♂	18	
		677 ♀	15		681 ♀	22	
		693 ♀	36		697 ♀	15	
2	0.15	729 ♂	47	42	733 ♂	59	43
		730 ♂	38		734 ♂	39	
		731 ♀	44		735 ♀	45	
		732 ♀	40		736 ♀	40	
3	0.5	684 ♂	83	76	698 ♂	87	78
		685 ♀	72		699 ♂	87	
		695 ♀	73		688 ♀	73	
					689 ♀	66	
4	1.75	678 ♂	133	111	682 ♂	100	92
		686 ♂	129		690 ♂	100	
		679 ♀	89		683 ♀	97	
		687 ♀	95		691 ♀	71	

Table II. *Kidney weights and food intakes of rats fed for 5 weeks on diets containing 20 or 70 % protein and on different amounts of vitamin B₂.*

Dose of vitamin B ₂	Averages of 4 rats.							
	(1) 0.0 cc.		(2) 0.15 cc.		(3) 0.5 cc.		(4) 1.75 cc.	
	g.	% increase	g.	% increase	g.	% increase	g.	% increase
Kidney weight g.:								
20 % protein	—	—	1.068	33	1.535	17	1.724	27
70 % protein	—	—	1.420	—	1.790	—	2.176	—
<u>Kidney weight</u>								
<u>100 g. body weight</u>								
20 % protein	—	—	1.209	—	1.220	—	1.110	—
70 % protein	—	—	1.540	—	1.396	—	1.476	—
Food intake g. dry weight in 5 weeks								
20 % protein	202	—	201	—	399	—	431	—
70 % protein	147	-27	173	-14	263	-34	316	-27
Protein intake g. in 5 weeks								
20 % protein	40	—	40	—	80	—	86	—
70 % protein	103	157	121	202	184	130	220	156
<u>% increase in kidney weight</u>								
<u>% increase in protein intake</u> × 100	—	—	16	—	13	—	17	—

fed on the 70 % protein in every case ate considerably less than those on 20 %, but notwithstanding they grew as well.

This, in conjunction with the fact that in the experiments of Drummond *et al.* [1922], Reader and Drummond [1925; 1926] and Hassan and Drummond [1927] the vitamin B complex was given as a percentage of the diet, suggested a possible reason for the fact that the present experiments fail to confirm the observations of those workers. A second experiment was therefore carried out in which vitamins B₁ and B₂ were given as a percentage of the diet incorporated in it.

Exp. 2. The effect on rats of a high protein diet, when vitamins B₁ and B₂ are incorporated as a percentage of the diet.

Method. The diets were the same as those in Exp. 1 with the exception that a yeast extract containing both vitamins B₁ and B₂ was added, in the proportion of 10 cc. per 100 g. dry weight of diet. (This yeast extract was prepared in the same manner as that used as a source of vitamin B₂ but was not autoclaved.)

Two groups of four rats of one litter were fed on each diet for 5 weeks.

Results. In Table III are shown the total weight increases of these rats, the amounts of food which they ate during the 5 weeks of the experiment, and, calculated from this, the average amounts of yeast extract consumed *per diem*.

Table III. *The growth of rats receiving diets containing 20 or 70 % protein and 10 % yeast extract to supply vitamins B₁ and B₂.*

	Rat	20 % protein diet				Av.	70 % protein diet				Av.
		824 ♂	825 ♀	826 ♀	827 ♀		828 ♂	829 ♂	830 ♀	831 ♀	
Increase in weight in 5 weeks g.		100	86	88	92	91	52	58	34	33	44
Food eaten in 5 weeks g.		285	298	297	325	301	175	159	145	162	160
Yeast extract average <i>per diem</i> cc.		0.81	0.85	0.85	0.93	0.85	0.50	0.45	0.41	0.46	0.45

The rats receiving the high protein diet showed a much smaller weight increase than those receiving the low protein diet, but they also ate much less and thus obtained smaller quantities of the yeast extract incorporated in the food. It is calculated that the two groups actually received 0.85 and 0.45 cc. respectively of this yeast extract daily, and the weight increase was of the order that would have been expected in such circumstances.

DISCUSSION.

These experiments show that although rats fed on a high protein diet grew less well than did those on a low protein diet, this only occurred if some growth-promoting factor was supplied as a percentage incorporated in the diet. Reduced intake was a constant feature with the high protein diets and thus produced a reduced intake of the B vitamins when these were incorporated in the diet. This is not in agreement with the data given by Reader and Drummond [1925]. Here the food intake of the rats on the high protein diets (45 and 90 % caseinogen) was recorded as being greater than that on a 20 % protein diet. In a later paper however [Hassan and Drummond, 1927], the food intake was recorded for rats on 70 and 20 % caseinogen diets and was definitely lower on the former (average of 39 against 58 g. in 4 days). The respective amounts of yeast extract consumed were thus 0.4 and 0.6 g. *per diem*. In this case, therefore, it appears probable that it was the reduced intake of yeast extract that was limiting growth.

In the same paper [Hassan and Drummond, 1927] it was also shown that addition of extra amounts of the heat-stable fraction of the vitamin B complex (B₂) restored growth. Vitamins B₁ and B₂ were supplied together in the yeast extract used (marmite) and this extract has since been shown to supply vitamin B₁ in doses smaller than suffice for vitamin B₂. The deficiency of vitamin B₂ thus became apparent before that of vitamin B₁. This was also the case in Exp. 2 of the present series where the amounts of the yeast extract consumed in the low and high protein groups, 0.85 and 0.45 cc. *per diem*, contained sufficient vitamin B₁ for normal growth while vitamin B₂ was present in limiting amounts.

It is however possible that the supply of any factor necessary for growth or maintenance, when incorporated as a percentage of the food, might become insufficient when a high protein diet, of which the intake is low, is being fed. In this connection it may be noted that Drummond, Crowden and Hill [1922], giving vitamins A and D as shark-liver oil, 2 to 3 % of the diet, noted beading of the ribs, lameness and symptoms of joint trouble in rats and kittens on high protein diets.

The fact that in Exp. 1 the food intake was higher on the 20 % protein diet than on the 70 % protein, suggested, at first, that 20 % protein (in the form of caseinogen) is below the optimum, so that the rats on this diet ate more in order to acquire sufficient protein. This does not seem probable as Osborne and Mendel [1915] have shown that when rats received a diet containing a sub-optimum percentage of protein they ate less, rather than more, of that diet. These authors also showed that good growth occurred when the diet contained 15 % caseinogen and that increasing the percentage to 18 did not result in more growth; 15 % caseinogen therefore was the optimum amount. Their rats receiving the 18 % caseinogen diet ate more of the diet than did those receiving 15 %, although growing to the same extent. It therefore appears that the food consumption of these rats was more than was necessary and this, it is suggested, was the explanation of the high food intake of the rats in the present experiments receiving the 20 % caseinogen diet. They ate more food than they needed, while the rats fed on the 70 % protein diet, possibly owing to the unpalatability of the diet, ate enough but no more.

The results obtained in Exp. 1 also prove that the intake of vitamin B₂ directly affected the rate of weight increase independently of any effect it may have had on appetite. Growth was constant for any given dose of vitamin B₂, whereas the calorie intake varied with the food consumption within wide limits, according to whether the diet had a high or low protein content. Also the food consumption of the rats fed on the 20 % protein diet receiving no vitamin B₂ (group 1) was the same as that of those receiving the smallest dose of vitamin B₂ (group 2), but the rats with vitamin B₂ grew twice as well as those with none.

That vitamin B₁ has a direct effect on growth has already been demonstrated by another method by Sure, Kik and Smith (1932).

SUMMARY.

1. No relation has been found between the protein intake and the vitamin B₂ requirements of rats.
2. Excess protein in the diet had an indirect depressing effect on growth, since it caused a reduced food intake. This resulted in a lowered vitamin intake when the vitamins were incorporated as a percentage of the diet.
3. The supply of vitamin B₂ had a direct effect on growth independent of any effect on appetite.
4. A high protein diet did not affect the incidence of the dermatitis characteristic of vitamin B₂ deficiency.
5. Hypertrophy of the kidneys of rats on a high protein diet was independent of the vitamin B₂ supplied.

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