150. EFFECT OF DIETS RICH IN PROTEIN UPON RATS DEPRIVED OF VITAMIN B₁

By GOUTOM G. BANERJI

*From the Nutritional Laboratory, University of Cambridge
and Medical Research Council*

(Received 7 November 1941)

IN a previous communication [Banerji, 1940] it was noted that the ingestion of a diet rich in fat caused a diminution in the excretion of bisulphite-binding substances in the urine of rats deficient in vitamin B₁. It was hence thought worth while to investigate the effect of a high-protein diet on the excretion of these compounds by the deficient rats.

Experimental

The bisulphite-binding substances in the urine were measured by the modified method used in the last experiment of the previous communication [Banerji, 1940].

The electrocardiogram was used for taking heart-rates in the way described by Drury & Harris [1930] and Birch & Harris [1934].

The rats used in these experiments were young males weighing between 60 and 90 g.

The compositions of the diets used are given below (Table 1).

Table 1

	High protein diets		Standard basal diet
	(A)	(B)	
Sucrose	0	0	60
Lard	15	0	0
Arachis oil	0	15	15
Light white casein	80	80	20
Salt mixture	5	5	5
Marmite ('laboratory' brand), autoclaved at pH 8-9, 1½ atmospheres pressure for 1 hr.	12	12	12
Cod-liver oil	2 drops daily		2 drops daily

Each rat was kept isolated throughout the experimental period to lessen complications from coprophagy, refection etc.

Results

(1) *Effect of high-protein diet in the absence of vitamin B₁*

As is seen from Fig. 1, the rats on a diet deficient in vitamin B₁ but containing the high proportion of protein, although they excreted a large amount of bisulphite-binding substances (B.B.S.), neither died nor developed bradycardia. Similar results were obtained in a duplicate experiment.

(2) *Comparison of the excretion of B.B.S. with and without high protein, and with graded supplements of aneurin*

Diet B of Table 1 was used in this experiment with graded doses (0, 2 and 10 μ g. daily) of vitamin B₁. The vitamin was injected intraperitoneally. It had

Fig. 1. High-protein diet, fat as arachis oil (average of 3 rats).

Fig. 2. ... High protein diet + no vitamin B₁.
--- Ordinary basal diet + no vitamin B₁.

Fig. 3. ... High protein + 2 μ g. vitamin B₁.
--- Ordinary basal diet + 2 μ g. vitamin B₁.

Fig. 4. ... High protein diet + 10 μ g. vitamin B₁.
— Ordinary basal diet + 10 μ g. vitamin B₁.

the usual accelerating effect on growth, and the level of the excretion of B.B.S. varied inversely with the amount of the vitamin injected (Figs. 2-4). The rate

of growth was higher on the high protein diet for each level of intake of the vitamin.

The group of rats on the standard basal diet started losing weight after about a fortnight. The animals then developed bradycardia and died in 4 weeks from the beginning of the experiment. The group on the vitamin B_1 -free high-protein diet, on the other hand, went on gaining weight week by week (Fig. 2). The excretions of B.B.S. by the rats on the two diets when having no vitamin B_1 were found to be very much the same.

A comparison of the two groups of rats having $2\mu\text{g.}$ of vitamin B_1 per rat per day (Fig. 3) shows that the group on the standard basal diet maintained its growth after attaining a given weight, whereas there was a gradual increase of growth in the group having the high protein. There was, however, no significant difference in their excretion of B.B.S. (Fig. 3).

Daily supplements of $10\mu\text{g.}$ of vitamin B_1 per rat per day (Fig. 4) caused a continuous gain in weight in both the groups. The rate of growth, however, was greater in the group on the high-protein diet. The excretion of B.B.S. was very low and was maintained at approximately the same level in both groups.

Examination of casein for vitamin B_1 . In order to determine whether the action of the casein in preventing loss of weight and bradycardia was due to its possible contamination with vitamin B_1 , the casein was analysed for the vitamin according to the method of Wang & Harris [1939] and Harris & Wang [1941].

50 g. of the light-white casein were heated on a water-bath with about 500 ml. of distilled water in a beaker until dissolved. The reaction was then adjusted to pH 2 and the beaker was kept in an incubator at 40° overnight with the addition of 1 g. of pepsin. The reaction was next adjusted to pH 4 and the beaker was left in the incubator at 40° overnight with the addition of 0.5 g. takadiastase. The pH was then adjusted to 4.7 to precipitate the casein, and the mixture was centrifuged. The clear centrifugate was then shaken with 1 g. of fuller's earth at pH 4 for the adsorption of the vitamin. It was next filtered and dried. The weight of the dried fuller's earth was found to be 1.66 g. 0.05 g. of this fuller's earth was then examined for its vitamin B_1 content by the thiochrome method, according to the procedure of Wang & Harris [1939], and was found to contain $0.18\mu\text{g.}$ of vitamin B_1 .

Hence 1 g. of light-white casein contains $0.119\mu\text{g.}$ of vitamin B_1 .

Though the amount of vitamin B_1 present in the light-white casein is thus too small to explain the significant difference observed in the above experiments, the next experiment was nevertheless carried out with light-white casein which had been extracted by the acid-alcohol method of Guha & Drummond [1929]. This extracted casein was tested in the above way and found to be almost free from vitamin B_1 .

The vitamin B_1 -deficient high-protein diet containing this extracted casein permitted a gradual increase of growth and there was some increased excretion

Fig. 5. High-protein diet, with extracted casein (average of 3 rats).

of bisulphite-binding substances (Fig. 5). In this experiment, however, the B.B.S. excretion was not very high: this was possibly due to the fact that the rats only consumed small quantities of the diet.

Discussion

The principal conclusion which stands out from these experiments is that protein, in the form of light-white casein, spares vitamin B₁. Further proofs have been secured, based on (a) the vitamin content of the tissues and (b) the catatorulin effect of the rats' kidney on vitamin B₁-deficient high-protein, high-fat and standard-basal diets. This work has been done in collaboration with Dr J. Yudkin, and an account of it is in the press [Banerji & Yudkin, 1941].

Investigations are in progress to determine whether proteins other than the light-white casein spare vitamin B₁. It is also intended to extend these experiments by using individual amino-acids.

SUMMARY

1. The presence of a large amount of protein in the diet prevented the bradycardia and loss in weight usually associated with deficiency of vitamin B₁. It failed however to prevent the increased excretion of bisulphite-binding substances. In this last respect, protein differs from fat, which has previously been shown to relieve this as well as the other effects of deficiency.

2. The vitamin-sparing action of the protein was demonstrated in further experiments in which the growth rates on various graded levels of vitamin B₁ were found to be better in each case with excess of protein than without it. There was a similar effect on the urinary excretion of B.B.S.

The author wishes to take this opportunity to express his gratitude to Dr L. J. Harris for his continual interest and helpful criticisms.

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