157. EFFECT OF A HIGH-FAT DIET ON THE EXCRETION OF BISULPHITE-BINDING SUBSTANCES IN THE URINE OF RATS DEFICIENT IN VITAMIN B₁

By G. G. BANERJI

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

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THE first indication that rats can be maintained on a low intake of vitamin B₁ provided that their ration contains a high proportion of fat was given by Evans & Lepkovsky [1928]. Since then a number of experiments have been done on the effect of fat in delaying the onset of beri-beri or polyneuritis. It has been found that even synthetic fats have this characteristic action.

In earlier work in this laboratory [Banerji & Harris, 1939], it was observed that rats on a diet deficient in the vitamin excreted large amounts of bisulphite-binding substances in the urine, and that the extent of the abnormality was proportional to the degree of the severity of the deficiency. It was therefore thought worth while to investigate the effect of high-fat diets on this feature of the avitaminosis. Another abnormality which has been intensively studied in this Institute is the bradycardia seen in the deficient rat [e.g. Drury & Harris, 1930; Birch & Harris, 1934] and the opportunity was therefore also taken to study the effect of the high-fat diets on the heart rate.

EXPERIMENTAL

The bisulphite-binding substances in the urine were measured according to the method of Clift & Cook [1932]. In one of the experiments, however, 5 ml. of 15% Na₂HPO₄ solution were used, in place of 5 ml. of saturated NaHCO₃ solution, as the alkali for breaking down the bound bisulphite. This small modification in the method, which has been adopted for later work, was found to give a more sharply defined end-point without causing any alteration in the titration value. The rest of the procedure was exactly as described in the paper cited above.

Heart rates were taken with the electrocardiograph in the way described by Drury *et al.* [1930] and Birch & Harris [1934].

Table 1

	High-fat diet	Standard basal diet
Sucrose	—	60
Lard	75	—
Arachis oil	—	15
Casein (light white)	20	20
Salt mixture	5	5
Autoclaved marmite*	12	12
Cod-liver oil	2 drops daily	2 drops daily

Rats receiving the high-fat diet in Fig. 1 had 10% autoclaved marmite instead of 12%.

* 1½ atmospheres at pH 8-9.

In all the experiments the rats used were young males weighing from 60 to 90 g. Six rats were taken for each experiment, one group of three receiving a high-fat diet, and the remaining three receiving a standard basal diet [Birch & Harris, 1934] and serving as controls (Table 1).

The animals were housed in separate cages throughout to reduce complications from coprophagy or refection.

Fig. 1. Effect of high-fat diet on growth, excretion of bisulphite-binding substances and heart rate in rats deficient in vitamin B₁. Each curve shows the average for three animals. Composition of diets as in Table 1. — Rats receiving high-fat diet. --- Rats receiving standard basal diet.

Fig. 2.

Fig. 2. Effect of high-fat diet, during partial deficiency of vitamin B₁ (5 µg. per rat per day), on growth, excretion of bisulphite-binding substances, and heart rate. Each curve is the average for three rats. Rats receiving high-fat diet + 5 µg. aneurin daily. -.-.- Rats receiving standard basal diet + 5 µg. aneurin daily.

Fig. 3.

Fig. 3. Effect of high intake of fat with nearly optimum allowances of vitamin B₁ (10 µg. of aneurin daily) as compared with normal intake of fat. —○— Rats receiving high-fat diet + 10 µg. aneurin daily. —×— Rats receiving standard basal diet + 10 µg. aneurin daily.

RESULTS

1. *Effect of high-fat diet in the absence of vitamin B₁*

It will be seen from Fig. 1 that the gradual increase in the excretion of bisulphite-binding substances in the urine, from 2 mg. to 20 mg., which occurred in the animals on the standard basal diet without vitamin B₁, did not occur in the animals taking a high proportion of fat. The control rats showed an initial gain in weight and then a fall, ending with the death of the animals after about 30–35 days, whilst those on a high-fat diet gained weight slowly but continuously throughout the experimental period. Lastly, whereas the control animals developed the bradycardia typical of aneurin deficiency, those on the high-fat intake showed no significant changes in their heart rate. Three similar experiments gave substantially the same results.

2. *Effect of addition of supplements of aneurin*

The effect of the provision of graded supplements of aneurin was then studied. Each rat received daily additions of aneurin, amounting to 5 μ g. (Fig. 2) or 10 μ g. (Fig. 3). The aneurin was injected intraperitoneally.

The addition of 5 μ g. aneurin resulted in a very slow rise in the bisulphite-binding substances in the control rats, the values reached being far less than those attained in the absence of aneurin. In the high-fat group the excretion showed no rise. Both groups showed a continuous gain in weight, but this was less in the animals on the high-fat diet. The heart rates remained normal in both groups. This experiment was repeated a second time with the same result.

Daily supplements of 10 μ g. aneurin almost-completely suppressed the rise of bisulphite-binding substances in the control groups, which, as with the lower supplement, showed a more rapid gain in weight than the group taking the high-fat diet.

DISCUSSION

The above results show that the consumption of a diet rich in fat is able to counteract, in a quantitative manner, two of the abnormalities associated with vitamin-B₁ deficiency, or partial deficiency, in the rat—namely the increased excretion of bisulphite-binding substances and the slow heart rate. The high intake of fat not only alleviated these two symptoms of the deficiency, but apart from a slightly depressing effect on the growth did not appear to have any injurious action upon the rat. This latter point seems of interest because there can be no doubt that in the human subject so high an intake of fat would have given rise to a ketosis. In the rat, on the contrary, so far from producing ketosis, the fat counteracted the excretion of acetone-bodies resulting from the vitamin deficiency. The reason for the apparent difference between humans and rats is not clear and is being made the subject of further experiments.

Some authors have claimed that the delay in the onset of symptoms of deficiency brought about by the consumption of large amounts of fat is probably due to the aneurin-carrying capacity of the fat. As against this, it has been proved that the esters of some of the fatty acids exert a sparing action while others do not. Salmon & Goodman [1937] have claimed that the effectiveness of the esters of fatty acids regarding their sparing action is maximum with 8-carbon acid and decreases in both directions.

Whether lard contains any aneurin has been investigated recently by Melnick & Field, Jr. [1939], using their own chemical method of estimation.

They concluded that lard is devoid of aneurin. The lard used in the above experiments was examined several times for its aneurin content in this laboratory by the thiochrome method. No aneurin was detected on any occasion.

Whether there is any stored aneurin or cocarboxylase in the tissues of the rats receiving aneurin-free high-fat diets requires further study, and experiments are now in progress.

The results of these experiments have an important bearing on the whole question of the methodology used for the biological assay of vitamin B₁. As we have seen, fat spares the more specific symptoms of deficiency such as the excretion of bisulphite-binding substances, polyneuritis and bradycardia, but on growth the effect is initially one of retardation and only later does it prevent the loss of weight. It is therefore conceivable that the determination of the "antineuritic" value of a specimen rich in fat might give different results depending on whether the method used was a growth test or a more specific test such as the bradycardia method.

More important however is the bearing of these results on the biological as compared with the chemical estimation of vitamin B₁. The sparing action of fats on the symptoms of deficiency has, as already stated, led to the statement by many observers that lard for example contains the vitamin. Chemical tests on the other hand have failed to confirm this. It might be argued that, since one's concern with vitamins is in the end always biological, it is the "antineuritic" action of a substance which one desires to estimate rather than its actual chemical content of vitamin B₁.

It is clear, nevertheless, that the antineuritic action of a fatty substance in preventing the onset of polyneuritis or bradycardia may be influenced by the proportion of fat in it. In view of this and of possible species differences in the vitamin-sparing action of fats, the ideal method of expressing the vitamin B₁ activity of a diet would be to state the actual quantity of the vitamin as determined chemically, together with the composition of the diet in respect to fat, protein and carbohydrate, with possibly an indication also of the relative availability of the vitamin in the food in question. (There are indications for example that in fibrous and bulky foods which are not always readily digested, the vitamin is sometimes less readily absorbed.) The method of expressing antineuritic potency as not necessarily identical with vitamin B₁ content would resemble the customary procedure in indicating the antirachitic activity of a diet by stating the number of units of vitamin D together with such factors as the Ca-PO₄ ratio and the acid-base balance of the diet.

Finally, these results are of interest in connexion with the proposed use of a carbohydrate tolerance test (cocarboxylase function) for detecting hypovitaminosis-B₁ in the human subject. The experiments with rats emphasize once again the need, already indicated in an earlier paper [Banerji & Harris, 1939], for adequately controlling the basal diets of individuals submitted to such tests. Actual experience of tests on human beings has indicated for example that there may occur a rapid increase in B.B.S. in the urine almost at the very beginning of a period of restriction to a diet of polished rice, and this cannot be attributed solely to the low intake of vitamin B₁. As mentioned by Banerji & Harris [1939] the most suitable conditions for applying a cocarboxylase function test are not the same for the human as for the rat. With rats the administration of lactate and estimation of excretion of pyruvate or B.B.S. is found to be a sufficiently satisfactory procedure. With human beings on the other hand the direct administration of pyruvate seems preferable. This work on human beings is still

in progress and will be discussed in a later paper, but it seems advisable to allude here to the differences in technique, as between the human and the rat, to avoid the possibility of misunderstanding.

SUMMARY

The ingestion of a high-fat diet by rats deprived of vitamin B₁ diminished the excretion of bisulphite-binding substances in their urine and alleviated the severity of the bradycardia. The aneurin-sparing action of fat, therefore, is not limited to its effect in protecting such animals against loss of weight or the development of the better known symptoms of deficiency, but shows itself likewise in the absence of these two characteristic signs of the avitaminosis. The significance of these results for vitamin assays, and for the assessment of deficiency, is discussed.

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