only a questionably significant activity was found in the β -carbon of serine (while glycine was incorporated to a great extent), these experiments do not allow us to decide whether labile methyl groups may be transformed into the β -carbon by another route than via formate.

The oxidation of formate in homogenates and the simultaneous absence of formate incorporation into serine indicate that such an incorporation cannot be a necessary step in the oxidation of formate in the rat liver.

From the results obtained in the closer study of formate conversion to carbon dioxide in homogenate preparations (Tables 3–5) it seems likely that formate decomposition is caused by a non-particle-bound dehydrogenase, requiring Co I as a coenzyme and further dependent on the presence of a cyanide-sensitive electron mediator. Formate oxidation was also demonstrated in homogenates of rabbit liver and kidney cortex.

SUMMARY

1. In rat-liver slices formate carbon and the carbon of the labile methyl groups of choline are incorporated in the β -carbon of free serine.

2. Such incorporation could not be demonstrated in rat-liver homogenates even though the simultaneous incorporation of glycine indicated that new formation of serine took place.

3. Formate is oxidized to carbon dioxide in rat-liver homogenate; the enzyme system responsible for this process has been studied.

4. Based on these findings the conclusions are drawn that (a) formate is not the only (or is not the) immediate precursor of the β -carbon of serine, (b) the oxidation of formate in rat liver does not involve an incorporation into serine.

The author wishes to express his thanks for the help and encouragement received from Dr H. G. Wood and Dr W. Sakami.

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The Vitamin B₁ Sparing Action of Fat and Protein

4. THE EFFECT OF CARBOHYDRATE IN DIETS DEFICIENT IN VITAMIN B₁ UPON THE SURVIVAL AND VITAMIN B₁ CONTENT OF THE RAT

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(Received 22 September 1950)

Previous papers in this series (Banerji, 1940, 1941) have shown that rats, given no vitamin B₁, can thrive provided that the diet is free from sucrose. Subsequently it was demonstrated that on diets devoid of the vitamin, irrespective of the presence or absence of sucrose and so irrespective of the development or otherwise of signs of deficiency, kidney slices of rats show a specific metabolic defect, defective oxidation of pyruvate *in vitro*, which is restored when the vitamin is added to the respiring slices (Banerji & Yudkin, 1942). From these results, the conclusion was drawn that signs of deficiency are not due to the inability *per se* to carry out an essential metabolic function—the proper metabolism of carbohydrate—

but to an attempt to metabolize dietary carbohydrate in the absence of vitamin B₁. In other words, the animal can grow and thrive in the absence of vitamin B₁ provided it does not have to metabolize dietary carbohydrate. The signs of deficiency appearing when carbohydrate is fed would, on this hypothesis, be due to toxic products of incomplete or perverted carbohydrate metabolism.

Some of the consequences of this hypothesis have been examined in the work to be described. First, it was of interest to see whether and to what extent variation in the amount of dietary carbohydrate affected the rate of development of signs of deficiency. Especially it was interesting to see whether animals

were able to deal with small quantities of dietary carbohydrate in the absence of vitamin B₁. Secondly, an assessment was made of the amount of the vitamin in the tissues of animals with or without carbohydrate in the diet and receiving no vitamin B₁. Although the results of the experiments on respiration of tissue slices made it unlikely that there was any appreciable store of vitamin B₁ in the tissues, it is possible to explain the absence of signs in deficient animals receiving no carbohydrate by assuming a higher store of the vitamin in their tissues than in those of animals receiving carbohydrate. This might have arisen either by a decreased utilization of the vitamin when there was no dietary carbohydrate or by a synthesis of the vitamin in the alimentary canal on a carbohydrate-free diet.

EXPERIMENTAL

Animals. The rats used in all experiments were male piebald animals of the Hartwell strain, and experimental groups were constructed by adequate distribution of litter mates. The animals were kept in individual cages throughout the whole experiment which began when they were about 5 weeks old and weighed about 80 g.

Diets. The protein was light white casein (sodium caseinate, Glaxo Laboratories Ltd.); the fat was arachis oil when less than 40% was used and lard when a higher proportion was used. The carbohydrate was sucrose in all experiments, but in one of them glucose was also used. The protein, fat and carbohydrate made up 95 parts of the diet, to which were added 5 parts of salt mixture. Vitamins of the B group were supplied in the form of autoclaved marmite (12 parts to 100 parts of diet) and vitamins A and D in cod liver oil (2 drops daily to each rat).

Effect of amount of dietary carbohydrate on survival in the absence of vitamin B₁

Experiment 1. Twenty-four animals, in six groups of four, were placed on diets containing varying amounts of sucrose (Table 1). Diets 1, 2, 3 and 6

Table 1. *Effect of variation in dietary sucrose on survival time*

(All diets contained 4.5% salt mixture and 10.5% autoclaved marmite. Cod liver oil, 2 drops, was given daily to each rat.)

Diet	Composition (%)			No. of animals	Mean survival $\pm$ S.E. (days)
	Sucrose	Fat	Protein		
1	0	13	72	4	>140
2	0	67	18	4	>140
3	0	49	36	4	>140
4	18	31	36	4	46 $\pm$ 3.0
5	36	22	27	4	33 $\pm$ 0.5
6	54	13	18	4	31 $\pm$ 0.7

were of the same composition as those previously referred to as 'high-protein', 'high-fat', 'protein-fat', and 'standard basal' (Banerji & Yudkin, 1942).

All the animals on the first three (carbohydrate-free) diets survived in apparent health for 5 months, after which they were killed. Those receiving 18% sugar survived appreciably longer than those receiving 36 or 54% sugar.

Experiment 2. Five groups, each of four animals, were placed either on the high-protein diet, or on diets containing varying amounts of sucrose; in this experiment, the proportion of fat in the diet was constant. One of the animals on the high-protein diet died after 95 days; the others were apparently healthy when killed after 112 days. The animals on the other diets survived for the longest time with a diet containing about 9% sugar and for shorter times with increasing amounts of sucrose (Table 2).

Table 2. *Effect of variation in dietary sucrose on survival time*

(Proportion of dietary fat constant. For further details, see Table 1.)

Diet	Composition (%)			No. of animals	Mean survival $\pm$ S.E. (days)
	Sucrose	Fat	Protein		
1	0	13	72	4	>108
7	9	13	63	4	78 $\pm$ 4.2
8	18	13	54	4	55 $\pm$ 3.2
9	36	13	36	4	33 $\pm$ 2.6
6	54	13	18	4	43 $\pm$ 1.8

Experiment 3. In this experiment, the proportion of dietary protein was constant and the proportion of sucrose and fat varied. Again, there were four animals in each group. One animal on the high-fat (sucrose-free) diet died after 87 days; the other three survived until they were killed after 122 days. The survival of the rats on the diets containing sucrose decreased, as in Exp. 2, as the amount of sucrose increased (Table 3).

Table 3. *Effect of variation in dietary sucrose on survival time*

(Proportion of dietary protein constant. For further details, see Table 1.)

Diet	Composition (%)			No. of animals	Mean survival $\pm$ S.E. (days)
	Sucrose	Fat	Protein		
2	0	67	18	4	>114
10	9	58	18	4	63 $\pm$ 2.9
11	18	49	18	4	50 $\pm$ 2.1
12	36	31	18	4	35 $\pm$ 2.2
13	45	22	18	4	35 $\pm$ 1.9
6	54	13	18	4	32 $\pm$ 2.3

Experiment 4. It is hoped to study in detail the comparative effects of different carbohydrates on the development of signs of deficiency of vitamin B₁. One such experiment has been carried out, in which glucose was used instead of sucrose. The results were similar in that increasing amounts of either sugar

Table 4. *Effect of variation in dietary sugar on survival time*

Diet	(Proportion of dietary fat constant.) Composition (%)				No. of animals	Mean survival $\pm$ s.e. (days)
	Sucrose	Glucose	Fat	Protein		
7	9	0	13	63	4	65 $\pm$ 2.4
7G	0	9	13	63	4	83 $\pm$ 1.0
8	18	0	13	54	4	46 $\pm$ 1.5
8G	0	18	13	54	4	63 $\pm$ 4.0
9	36	0	13	36	4	35 $\pm$ 1.7
9G	0	36	13	36	4	53 $\pm$ 2.7

Table 5. *Effect of sucrose on vitamin B₁ content of tissues*

Diet	Daily supplement vitamin B ₁ (μ g.)	No. of animals	Vitamin B ₁ (μ g./g.) in			
			Brain	Liver	Kidney	Muscle
Standard basal	0	6	0.74 $\pm$ 0.049	0.43 $\pm$ 0.038	0.51 $\pm$ 0.040	0.26 $\pm$ 0.024
High-protein	0	6	0.81 $\pm$ 0.050	0.50 $\pm$ 0.041	0.45 $\pm$ 0.037	0.22 $\pm$ 0.020
High-fat	0	6	0.73 $\pm$ 0.045	0.41 $\pm$ 0.032	0.48 $\pm$ 0.032	0.25 $\pm$ 0.021
Standard basal	10	6	2.3 $\pm$ 0.22	2.9 $\pm$ 0.31	2.3 $\pm$ 0.29	1.0 $\pm$ 0.09
High-protein	10	6	2.3 $\pm$ 0.25	2.7 $\pm$ 0.30	2.0 $\pm$ 0.20	1.0 $\pm$ 0.10
High-fat	10	6	2.1 $\pm$ 0.25	3.0 $\pm$ 0.29	2.1 $\pm$ 0.18	1.1 $\pm$ 0.09
Standard basal	4	4	—	0.46 $\pm$ 0.41	—	0.24 $\pm$ 0.022
Standard basal	8	6	—	2.9 $\pm$ 0.30	—	0.65 $\pm$ 0.044
Standard basal	27	4	—	4.8 $\pm$ 0.42	—	1.1 $\pm$ 0.11
Standard basal	40	5	—	6.2 $\pm$ 0.54	—	1.15 $\pm$ 0.12

lessened the times of survival, but survival was somewhat longer on glucose than on sucrose (Table 4).

Effect of sucrose upon the content of vitamin B₁ in the tissues

Experiment 5. Thirty-six rats were used in this experiment. They were given one of three diets; the high-protein diet (1), the high-fat diet (2), or the standard basal diet containing sucrose (6). Six animals on each diet were given no supplement and the other six were each given 10 μ g. vitamin B₁ daily. The animals on the standard basal diet with no supplement developed severe deficiency after about 4 weeks and each was killed a day or two before it would have died. The remainder grew well and were killed after 5 months.

Vitamin B₁ was estimated in the brain, liver, kidney and skeletal muscle by the thiochrome method of Wang & Harris (1941). The whole brain, both kidneys and samples of about 2 g. liver and thigh muscle were used.

In the results given in Table 5, figures are also added for analyses done on liver and muscle in the course of other work with rats receiving the standard basal diet and 4, 8, 27 or 40 μ g. vitamin B₁ daily (Yudkin, 1949). The animals receiving 4 μ g. daily were killed when moribund and the others after 4 or 5 months.

It will be seen that the presence of sucrose in the diet did not affect the amount of vitamin B₁ re-

maining in the tissues. More particularly, animals receiving no vitamin B₁ in the diet had similar stores of the vitamin, whether or not the diet contained sucrose and whether or not the animals developed signs of deficiency.

DISCUSSION

The results of these experiments support the view that animals are able to thrive with no vitamin B₁ provided their diets contain no carbohydrate. With smaller amounts of dietary carbohydrate they are able to survive longer than with larger amounts, but they succumb when the diets contain as little as 9% sucrose or glucose. The stores of vitamin in animals receiving no sucrose are as low as in those receiving sucrose and consequently dying of deficiency. The survival of the former, therefore, is due to an ability to carry out their essential metabolic functions in the absence of vitamin B₁, although this ability does not extend to the proper metabolism of dietary carbohydrate. When, therefore, carbohydrate is fed without the vitamin, an abnormal metabolism of carbohydrate occurs and this is responsible for the sign of deficiency.

In a recent publication, Gruber (1950) has attempted to distinguish between two meanings of the phrase 'vitamin sparing' as used to describe the effect of increased dietary fat in delaying the onset of signs of deficiency of vitamin B₁. He says, 'The verb "to spare" can have a double meaning here, namely,

“to dispense with” or “to use less of”.... According to the first meaning, an increased carbohydrate ingestion would provoke symptoms of aneurin deficiency at a level of this vitamin in the tissues at which these symptoms would not yet appear with lessened carbohydrate intake. According to the second meaning, the increased consumption would entail a bigger loss of aneurin from the tissues.’ He estimated vitamin B₁ in the tissues of pigeons fed for 13 days on three diets, containing about 90, 70 and 0 % of sucrose, and found increasing amounts of the vitamin with decreasing amounts of dietary sucrose. These results he interprets as showing that the sparing action of fat means that the fat decreases the use of the vitamin, rather than that the fat allows the animal to dispense with it.

The work reported in this and the previous paper, however, make it clear that fat (and protein) are able to spare vitamin B₁ in both senses; not only do animals use less vitamin B₁ with decrease in dietary carbohydrate, but in the complete absence of dietary carbohydrate they are, in fact, able to dispense with it altogether. The effect of sparing is, therefore, quantitative in the sense that the animals use the vitamin less rapidly, and so survive longer, when the proportion of dietary carbohydrate is decreased. But it later becomes qualitative, in the sense that the small amount of vitamin B₁ remaining in the tissues after a shorter or longer period of deprivation is adequate for survival in the absence of dietary carbohydrate, but inadequate in its presence.

However, even with no carbohydrate in the diet, the animals are still presumably metabolizing carbohydrate, produced from the metabolism of both fat and protein. It is possible that the animal has, in fact, two pathways for the metabolism of carbohydrate, one involving vitamin B₁ and the other not. The latter must then be considered to be limited, and sufficient only to deal with an amount of carbohydrate such as that produced from fat and protein. Alternatively, it is possible that the organism deals differently with carbohydrate derived from fat or

protein, and carbohydrate taken in the diet. Further, it may be that, with no dietary carbohydrate, the consumption of larger amounts of fat and protein increases, by a process of adaptation, the utilization of these substances without the intermediate formation of carbohydrate. The observations of Gilmore & Samuels (1949) are of interest in this connexion; they showed that the diaphragm of the rat after a high-fat diet utilizes *in vitro* far less carbohydrate than one from an animal fed carbohydrate. More recently, Wertheimer & Ben-Tov (1950) have demonstrated adaptive changes in the metabolism of carbohydrate in rats which have been given a restricted diet. There was an increased storage of glycogen in the liver and in the muscles and a deposition of glycogen in the adipose tissue; the latter is taken as indicating an increased conversion of carbohydrate to fat.

Further work is now in progress to study the metabolism of carbohydrate, fat and protein in animals on carbohydrate-free diets.

SUMMARY

1. Rats consuming as little as 9 % sucrose or glucose in their diets were not able to survive in the absence of vitamin B₁ as did those receiving no carbohydrate. They did, however, survive longer than those consuming diets with higher proportions of carbohydrate.

2. The amount of vitamin B₁ in the tissue of animals receiving no vitamin B₁ was unaffected by the presence or absence of carbohydrate in the diet, even though those consuming carbohydrate were moribund and the others were well.

3. The results support the hypothesis that signs of deficiency are due to the disturbed metabolism of carbohydrate in the absence of vitamin B₁.

I am grateful to Messrs Allen and Hanbury Ltd., British Drug Houses Ltd. and H. W. Carter and Co. for grants made to this Department.

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