Thiamine requirement of rats given a high-protein, carbohydrate-free diet

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Although much light has been shed on the biochemical aspects of thiamine deficiency, the relationship between the requirement for this vitamin and the level of dietary carbohydrate remains obscure. Banerji (1941) and Banerji & Yudkin (1942) found that characteristic signs of thiamine deficiency such as convulsions did not appear in rats fed on a carbohydrate-free, high-protein diet, though other signs, such as increased excretion of bisulphite-binding substances and the 'catatorulin effect' (Gavrilescu & Peters, 1931*b*; Peters, 1936), persisted.

On the basis of their results Banerji (1941) and Banerji & Yudkin (1942) suggested that, though thiamine-deficient tissues showed defective carbohydrate metabolism, convulsions (as a sign of deficiency) only appeared when carbohydrate was included in the diet, and were due to accumulation of some unknown toxic product of perverted carbohydrate metabolism. It was only when these workers (Banerji, 1940; Banerji & Yudkin, 1942) used high-fat diets that their animals thrived well, owing to the well-known thiamine-sparing action of fat. Recently Morgan & Yudkin (1957) have put forward the view that carbohydrate might be considered as a toxic substance, whose antidote is thiamine.

In view of these interesting suggestions of Yudkin and co-workers, the experiment of feeding rats on a high-protein diet in the absence of thiamine was repeated, particular attention being paid to the appearance of nervous signs of deficiency.

The effects of a high-protein, carbohydrate-free diet are reported here and the pharmacological responses of thiamine-deficient tissues are reported elsewhere (Boullin, 1960).

EXPERIMENTAL

Animals and diet

The synthetic diet, similar to that used by Banerji (1941) and Banerji & Yudkin (1942), consisted of purified casein (Genatosan Ltd) 80, deodorized groundnut oil (British Edible Oils Ltd) 15, and Dunn Laboratories salt mixture (British Drug Houses Ltd) 5 %. The percentage composition of the salt mixture was: $MnSO_4.4H_2O$ 0·022, NaF 0·004, KI 0·108, iron citrate 3·799, NaCl 5·427, $MgSO_4.7H_2O$ 8·684, $NaH_2PO_4.2H_2O$ 11·398, KCl 27·138, $Ca_3(PO_4)_2$ 43·420. In addition to this dry diet,

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the rats received orally in aqueous solution daily vitamin supplements as follows: choline 5 mg, *i*-inositol 1·1 mg, *p*-aminobenzoic acid 3·0 mg (all from British Drug Houses Ltd), nicotinic acid 0·5 mg, calcium D-pantothenate 0·5 mg, riboflavin 150 μg, pyridoxine 40 μg, biotin 1 μg (all from Roche Products Ltd) and folic acid 2 μg (Lederle Laboratories Inc.).

Control rats received 25 μg thiamine (Aneurin HCl, British Drug Houses Ltd) daily; the experimental animals did not. Other vitamins were administered to all rats once a week by stomach tube, each on a different day, as follows: cyanocobalamin 1 μg, vitamin A (as Adexolin) 120 i.u., ergocalciferol (as Adexolin) 20 i.u. (all from Glaxo Laboratories Ltd), vitamin K (menaphthone) 800 μg and vitamin E (DL-α-tocopheryl acetate) 15 mg (both from Roche Products Ltd).

The high-carbohydrate diet used by Boullin (1960) (see Fig. 1) was identical with the synthetic diet described above, except that 60 % of the purified casein was replaced by sucrose.

Expt 1. Albino rats of the Wistar Institute strain were placed on the experimental diet usually at 1 month (range 3–12 weeks). Litter-mate male animals were segregated into fifteen pairs of approximately equal weight ($\pm$ 10 %). One animal was given the thiamine-deficient diet and its litter-mate served as a pair-fed (eight rats), or *ad lib.* fed (seven rats), control.

Expt 2. The procedure was the same as for Expt 1 except that 200 mg phthalylsulphathiazole/day were added to the diet, twelve pairs of litter-mates being used. Five control rats were pair-fed and the remainder were fed *ad lib.*

Recognition of signs of thiamine deficiency

The signs of thiamine deficiency have been described by many authors, for example Kon & Drummond (1927), Drury, Harris & Maudsley (1930) and Sandels (1929–30). They include bradycardia, convulsions, and such 'biochemical lesions' (Gavrilescu & Peters, 1931*a*) as raised blood levels of pyruvate and lactate (Kinnersley & Peters, 1929; Peters, 1936). Apart from these, any other signs might be attributed to loss of appetite and consequent inanition (Reader & Drummond, 1926). The recognition by Drury *et al.* (1930) of the bradycardia as a distinctive feature of thiamine deficiency was used as a means of assay of the vitamin by Birch & Harris (1934).

Heart rates. The essential criterion of thiamine deficiency in the work now described was the presence of a bradycardia, and the method of recording the heart rate has been described by Boullin (1960). Heart rates were recorded at intervals in both the experimental and control animals. Animals with a heart rate of 360 beats/min or less were considered arbitrarily to be suffering from acute deficiency, and those with heart rates above this value and up to 408 beats/min were considered to show subacute or chronic deficiency.

Nervous signs. For a few animals of which the heart rate was not determined, the presence of convulsions was considered essential to indicate the deficiency state.

A method was devised whereby some indication of the degree of deficiency could be obtained by submitting the rats to an approximately standard stimulus that induced convulsions only in the deficient animals. The procedure (the 'spinning test') con-

sisted of picking the rat up by the tail, holding it about 1 ft above a flat horizontal surface, and spinning it rapidly by revolving the tail between the flattened palms of the hands. This manoeuvre produced an 'induced fit' in animals not sufficiently deficient to show a 'spontaneous fit'. A positive response to the 'spinning test' was taken as indicating advancing deficiency, and that gross deficiency might be expected to occur within a few days.

Three arbitrary stages were recognized and are described below in order of increasing deficiency.

Stage 1: the rat was able to stand in a normal posture, but walked with a raised gait; activity was normal with rapid movement and investigation; there was no response to the 'spinning test'.

Stage 2: the rat was unable to stand in a normal posture and tended to overbalance; the legs were splayed outwards and the gait raised; activity was low but the animal moved around and investigated its environment; the 'spinning test' elicited an 'induced fit'.

Stage 3: the animal swayed but did not fall owing to a tenacious grip on the cage mesh; the gait was raised; there was no tendency to move around and investigate unless disturbed, when a 'spontaneous fit' occurred; the rat responded to the 'spinning test' with an 'induced fit'.

The degree of deficiency was determined by the presence or absence of nervous signs according to these stages.

RESULTS

Experiment 1

The results are summarized in Table 1.

Seven control animals (nos. 1–7 in Table 1) received the diet *ad lib.* with the addition of thiamine. They survived for periods up to 62 weeks, but, in spite of prolonged survival, did not grow at a rate similar to that of rats fed *ad lib.* on a synthetic diet containing a high proportion of carbohydrate (60% sucrose) (see Fig. 1 and Boullin, 1960).

Though maximum body-weights of the protein-fed animals showed considerable individual variation, none showed any signs that could be attributed to thiamine deficiency at any stage.

Somewhat different results were obtained with the rats given the experimental diet. Though some survived for very long periods, they failed to grow like their litter-mate controls. The rates of growth were less, and the maximum body-weights attained were lower (Fig. 2). In comparison with control rats that were pair-fed these discrepancies were smaller, and the body-weight curves were of similar shape (Fig. 3). In addition all rats fed on the thiamine-free diet eventually showed signs of deficiency. Of the fifteen animals initially placed on the deficient diet, eight were found to be suffering from acute deficiency, assessed by the presence of a bradycardia of less than 360 beats/min (range 144–348 beats/min), and in two other rats chronic deficiency was evident from bradycardias of 384 and 408 beats/min. With one exception (no. 9, Table 1) all these deficient animals showed spontaneous fits (i.e. stage 3 deficiency) of great severity, lasting up to 30 sec.

Two control rats (nos. 6 and 10, Table 1) were transferred to the deficient diet after the death from deficiency of their litter-mates. The first was given phthalylsulphathiazole with the protein diet, the result being reported on p. 583. The second (no. 10, Table 1) was placed on the deficient diet immediately after its litter-mate had died of acute deficiency (after 48 days), and it subsequently showed signs of acute deficiency after 37 days. At this stage, 1 mg thiamine pyrophosphate was given, which abolished the convulsions and bradycardia and allowed survival for another 30 days before the animal finally died from acute deficiency.

Table 1. *Effect on rats of a carbohydrate-free, high-protein diet with and without thiamine*

Pair no.	Experimental life-time (days)		Time to induce deficiency (days)		Heart rate (beats/min) (bradycardia test)		Cause of death		Comments	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1*	404	404	404	—	144	430	K	K	AD, F, SC	—
2*	67	67	46	—	350	515	K	K	AD, F	—
3*	31	31	31	—	408	570	K	K	CD, F	—
4*	362	4	284	—	400	—	CD?	VS	SC, F	—
5*	438	438	?	—	384	480	CD	—	F, T	ET
6*	75	569	75	16	348	325	AD	AD	F	DD, SC, ADP
7*	77	201	44	—	—	—	AD?		F, T	—
8†	31	31	31	—	—	—	AD?	S	F	—
9†	10	4	—	—	—	—	VS	S	—	—
10†	48	117	48	37	—	240	AD	AD	F	DD
11†	78	78	50	—	336	455	K	K	F, T	—
12†	124	126	70	—	348	450	K	K	AD, F, T	—
13†	105	104	60	—	300	480	AD	—	SC, F	ET
14†	40	35	40	—	216	—	AD	S	F	—
15†	64	42	43	—	156	—	AD	S	F, T	—

a = experimental rat.
b = litter-mate control.
* = control fed *ad lib.*
† = control pair-fed.
AD = acute deficiency.
CD = chronic deficiency.
SC = 'spontaneous cure'.
F = fits (stage 3 deficiency, see p. 579).
S = starvation as a result of pair-feeding.
K = killed for pharmacological experiments.
DD = control given thiamine-deficient diet after death of litter-mate from deficiency (nos. 8 and 9).
ADP = acute deficiency induced by giving phthalylsulphathiazole (see p. 583).
ET = experiment terminated.
VS = voluntary starvation.
T = therapeutic test (administration of thiamine) successful.

In five rats administration of thiamine by stomach tube abolished bradycardia overnight, and caused a marked gain in weight due to increased food intake during the next few days (cf. Fig. 4).

Though some deficient animals were able to survive for prolonged periods (up to 50 weeks) without showing any signs of fits, and even longer before severe convulsions were evident (e.g. experimental rat no. 1, Table 1), other animals showed very severe fits and died comparatively rapidly (31–77 days). It was noted that many of the long-surviving deficient animals were coprophagous, but in no instance was the habit seen in control rats. Thus coprophagy may account for the variation in life-span.

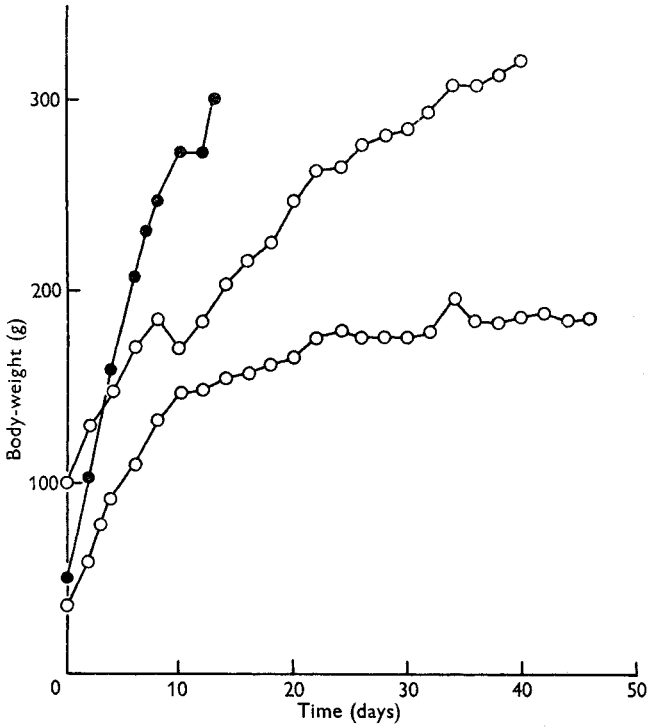

Fig. 1. Effect on the weight gain of rats of a carbohydrate-free, high-protein diet compared with that of a high-carbohydrate diet. ○, rats (control nos. 1 and 5) given the high-protein diet; ●, rat given the high-carbohydrate diet.

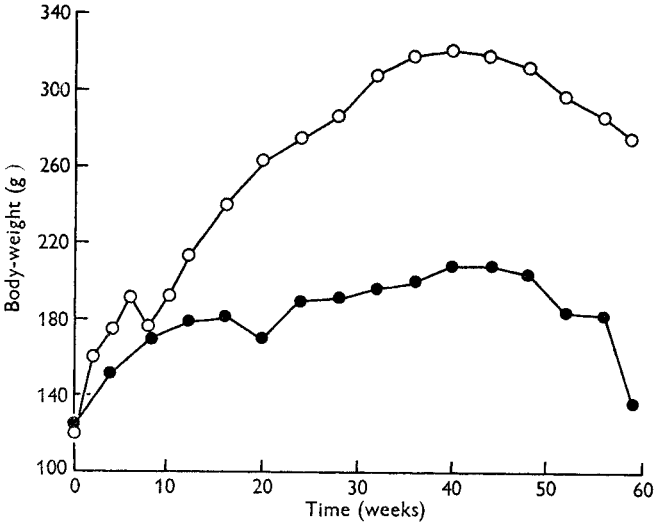

Fig. 2. Effect of coprophagy on weight gain and life-span of a rat given a carbohydrate-free, high-protein diet. ●, coprophagous experimental rat; ○, non-coprophagous control rat fed *ad lib.* (pair no. 1).

In four animals rendered thiamine-deficient at some stage fits appeared spontaneously several times during the experimental periods, but disappeared without the vitamin having been administered. This phenomenon, termed a 'spontaneous cure', was invariably associated with persistent coprophagy, and supports the view that the

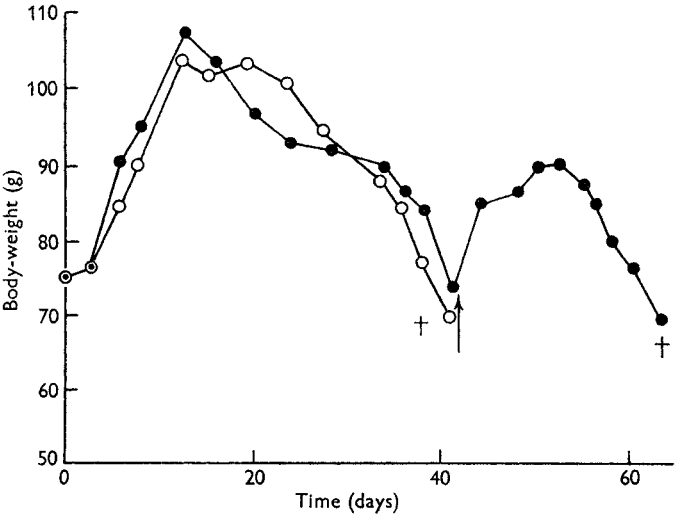

Fig. 3. Effect of a carbohydrate-free, high-protein diet with and without thiamine on body-weight of two pair-fed litter-mate rats, and the effect of a thiamine supplement given orally to the thiamine-deficient rat after death of its litter-mate at 42 days. ●, thiamine-deficient rat; ○, control rat (pair no. 15); †, died; ↑, thiamine given.

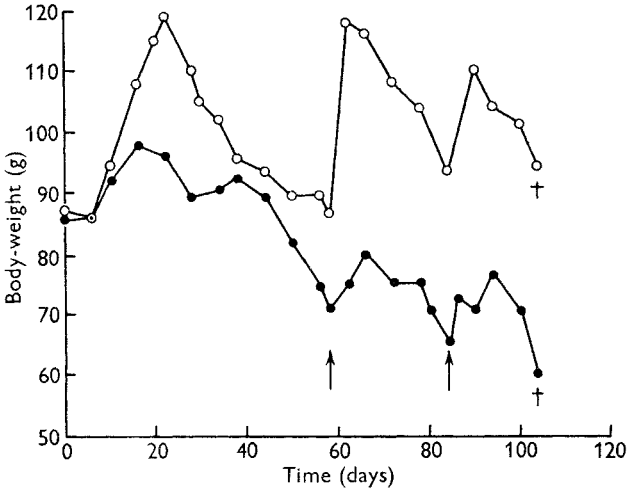

Fig. 4. Effect of oral administration of thiamine on weight gain of a pair of litter-mate rats given a carbohydrate-free, high-protein diet with and without thiamine. ●, thiamine-deficient rat; ○, pair-fed control (pair no. 13). ↑ thiamine given; †, died.

thiamine-deficient rats fed on the carbohydrate-free, high-protein diet were able to survive for long periods by virtue of their coprophagous habits, which enabled them to obtain sufficient quantities of thiamine from their faeces. As variations in the degree

of coprophagy might be expected to influence greatly the life-span, owing to variation in the amount of vitamin ingested, some ancillary experiments were performed in which phthalylsulphathiazole was added to the diets to reduce the intestinal flora and thus synthesis of thiamine.

Table 2. *Effect on rats of a carbohydrate-free, high-protein diet and phthalylsulphathiazole with and without thiamine*

Pair no.	Experimental lifetime (days)		Time to induce deficiency (days)		Heart rate (beats/min) (bradycardia test)		Cause of death		Comments	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
16*	3	98	—	24	—	300	VS	AD	—	DD
17*	37	68	37	—	—	—	AD?	?	—	—
18*	35	52	35	17	360	348	AD	AD	—	DD
19*	37	5	37	—	—	—	AD?	TT	—	—
20*	5	5	—	—	—	—	TT	TT	—	—
21*	25	103	25	—	192	516	AD	—	—	ET
22*	51	103	22	—	290	480	AD	—	T	—
23†	26	108	24	33	—	—	AD?	AD?	—	DD
24†	24	81	24	—	—	—	AD?	—	—	ET
25†	37	81	37	—	264	480	AD	—	T	ET
26†	36	22	36	—	330	—	AD	S	—	—
27†	30	75	30	—	360	—	AD	—	—	ET

a = experimental rat.
b = control rat.
* = control fed *ad lib.*
† = control pair-fed.
DD = control given thiamine-deficient diet after death of litter-mate from deficiency.
ET = experiment terminated.

AD = acute deficiency.
VS = voluntary starvation.
S = starvation.
T = therapeutic test (administration of thiamine) successful.
TT = death with severe diarrhoea (? toxicity of phthalylsulphathiazole).

Experiment 2

The results are given in Table 2.

Three rats in which the phthalylsulphathiazole appeared to have some toxic effect (pairs nos. 19 and 20, Table 2) were very young and of low initial body-weight. With older rats no toxic effects were seen. Animals given the complete diet with phthalylsulphathiazole usually grew at a rate similar to that of rats given the protein diet alone and survived for periods of up to 14 weeks (compare control rats nos. 2, 3, 5, 7, Table 1 with nos. 17, 21 and 22, Table 2).

When thiamine was omitted from the diet, the growth of these deficient rats was depressed in comparison with that of litter-mate controls either pair-fed or fed *ad lib.* (Fig. 5). The effect of the introduction of phthalylsulphathiazole was to induce deficiency in a short time (range 17–37 days, Table 2). No fits were observed in any deficient rats, but in each a severe bradycardia was present. In two animals the bradycardia was abolished overnight by the administration by mouth of 12–25 μg thiamine. The rapidity of the appearance of classical signs of thiamine deficiency was most probably due to a reduction in the intestinal flora by phthalylsulphathiazole, resulting in a reduction in intestinal synthesis of the vitamin, with a consequent fall in the effective-

ness of coprophagy. In this respect one control rat (no. 6, Table 1) was noteworthy; it was given the protein diet *ad lib.* with thiamine for 365 days, its litter-mate having died after 75 days on the deficient diet with signs of acute stage 3 deficiency. After 1 year the control rat was given the deficient diet. It gradually lost weight (from 175 to 129 g) and then regained weight until after 188 days on the deficient diet it weighed

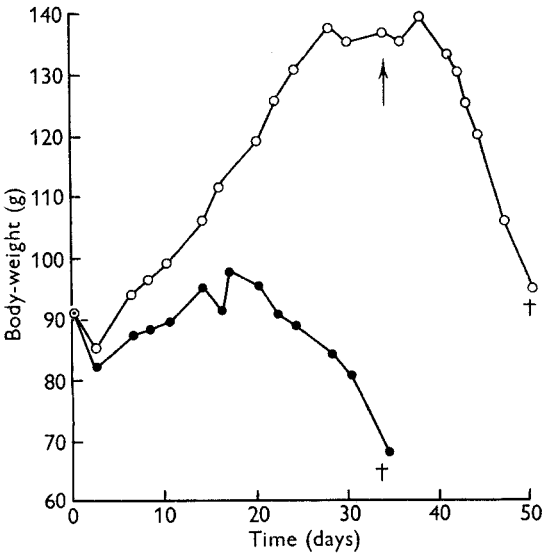

Fig. 5. Effect on the weight gain of a pair of litter-mate rats of a carbohydrate-free, high-protein diet containing phthalylsulphathiazole (200 mg/day) with and without thiamine, and of withholding the thiamine supplement from the surviving rat after the death of its litter-mate at 35 days. ●, thiamine-deficient rat; ○, control rat fed *ad lib.* (pair no. 18) and given thiamine; † died; ↑ thiamine supplement withheld.

152 g and showed no convulsions, though a moderate bradycardia (400 beats/min) was detected. At this stage 200 mg phthalylsulphathiazole/day were added to the diet. The rat lost weight very rapidly and died of acute deficiency only 16 days later, with severe bradycardia (325 beats/min, Table 1) and severe convulsions. The convulsions were of much shorter duration (1–2 sec) than those seen in any other experiment.

DISCUSSION

Experimental diets used for inducing thiamine deficiency usually contain a high proportion of carbohydrate. Under these conditions signs of deficiency such as bradycardia and convulsions generally appear in 3–4 weeks (Sandels, 1929–30; Boullin, 1960).

The production of classical signs of acute thiamine deficiency in rats given the carbohydrate-free, high-protein diet used in my work, together with the remedial action of thiamine, clearly show that a requirement exists for this vitamin in the absence of dietary carbohydrate.

It is difficult to reconcile the findings of my study with those of Banerji (1941) and

Banerji & Yudkin (1942) that their rats were able to thrive on a thiamine-free, high-protein diet, free of carbohydrate, when their results showed that a mild bradycardia was present, and that a 'catatorulin effect', that is an increased oxygen uptake induced in vitro in deficient tissues by thiamine in the presence of pyruvate, was also present. Perhaps their animals were suffering from incipient chronic thiamine deficiency which might have been accentuated by continuation of their experiments. Sarett & Morrison (1958) suggest that the finding of Morgan & Yudkin (1957), that the addition of sorbitol to a carbohydrate-free, high-protein diet deficient in thiamine and other vitamins of the B complex produced good growth and survival in rats, may be explained by access of the animals to their faecal material. Recently Morgan & Yudkin (1959) have stated that this effect of sorbitol is dependent on coprophagy.

The short time taken to induce deficiency when phthalylsulphathiazole was added to the diet is compatible with the idea that the intestinal synthesis of thiamine was greatly reduced, with the result that, even in the most coprophagous rat, insufficient thiamine was available to it to prevent the appearance of a severe bradycardia characteristic of acute deficiency. The absence of convulsions under these conditions may be explained by the fact that there was no time for the vitamin level in the brain to fall sufficiently low to cause convulsions, but that the level at some other unknown site was low enough to cause the death of the animal, as suggested previously by Gruber (1953).

The finding that rats fed on a carbohydrate-free diet containing a high proportion of protein require thiamine, is in agreement with the work of Wainio (1942) who noted that the thiamine requirement of rats was reduced by only 39 % when the proportion of dietary carbohydrate was reduced by 70 %. He suggested that thiamine participated in the metabolism of proteins to the extent of the glycogenic amino acids which they contained. The present biochemical view of the mode of action of thiamine gives no reason to suppose that it is necessarily associated exclusively with carbohydrate metabolism, for it is required for the decarboxylation of α-ketoglutarate and pyruvate, as well as having a function in the transketolase reaction (Fruton & Simmonds, 1958, pp. 475, 504, 529).

SUMMARY

1. The effect of a high-protein, carbohydrate-free diet without thiamine was observed on body-weight, heart rate and the appearance of nervous signs of thiamine deficiency in young male albino rats, and compared with the effect of the same diet with 25 μg thiamine/rat daily on litter-mate rats either fed *ad lib.* or pair-fed.

2. Rats fed on a high-protein, carbohydrate-free diet were found to have a requirement for thiamine.

3. In the absence of this vitamin, rats showed classical signs of deficiency such as bradycardia and fits. Prolonged survival on the deficient diet was probably due to coprophagous habits allowing thiamine to be obtained from faecal material.

4. Addition of phthalylsulphathiazole to the diet induced acute deficiency indicated by a severe bradycardia in 17–37 days, probably by reducing intestinal synthesis of thiamine available for ingestion by coprophagous rats.

5. These results do not support the concept that the fits characteristic of the thiamine-deficiency state are due to the toxic products of perverted carbohydrate metabolism, or the view that carbohydrate is a toxic product the antidote of which is thiamine.

This work was undertaken in partial fulfilment of the requirements for the degree of M.Sc. (Nutrition) in the University of London. I should like to thank Dr J. P. Quilliam for his advice and encouragement, and Professor John Yudkin for much helpful information in connexion with the feeding techniques.

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