CXLIX. THE PHYSIOLOGICAL FUNCTION OF VITAMIN B_1.

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More striking among the theories which have been proposed to explain the mechanism of action of "vitamin B" (using the old terminology) are those that connect the vitamin with biological oxidation processes. Some investigators have considered the vitamin to be concerned in cellular respiration in general, while others have claimed the existence of a definite relationship between the vitamin and certain aspects of metabolism. Some workers, however, have opposed the evidence on which such claims are based. *Post mortem* examination of avitaminous animals, while affording much valuable information, has not led to any definite knowledge as to the rôle that the vitamin plays in body economy. In some recent experiments with rats we observed that if the vitamin B_1-deficient animals were killed before they passed into the moribund condition, no specific lesions could be found in the internal organs, although the deprivation of vitamin B_1 was quite severe. These observations suggest that the gross abnormalities observed in the extreme stage of vitamin B_1 deficiency are most probably the secondary results of a primary disorder [*cf.* Drummond and Marrian, 1926].

The present investigation represents an attempt to throw some light on this problem.

Method.

All work was carried out with rats, most of which were of the black and white strain. They were kept in separate cages and 2 drops of a potent cod-liver oil were fed daily to each of them apart from the basal diet. All other supplements were also separately administered unless otherwise mentioned.

A. Vitamin B_1 and the protein/carbohydrate ratio of the diet.

The amylum-toxin theory originally proposed by Eijkman [1897, 1, 2], which was opposed by Grijns [1901, 1907] and later discarded, has more than once been revived in modified forms [Funk, 1914; Randoin and Simonnet, 1924]. Randoin and Simonnet, working with pigeons, considered that there was a definite relationship between the "antineuritic" vitamin and the carbohydrate content of the diet, while Hartwell [1925] and Reader and Drummond [1926] found that rats on high-protein diets required larger allowances of

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"vitamin B." It was, however, subsequently shown by Hassan and Drummond [1927] that the corrective agent for high-protein diets present in marmite was a heat-stable substance and, therefore, presumably not vitamin B_1.

In the present experiments the effect of the same optimum and sub-optimum doses of a relatively concentrated preparation of vitamin B_1 on the growth of groups of young rats subsisting on diets of varying carbohydrate/protein ratios was studied. It was considered that if vitamin B_1 plays a specific part in carbohydrate metabolism, sub-optimum doses of vitamin B_1 might have a greater influence on the growth of young rats receiving less carbohydrate than of those receiving more. The deleterious effect of excess protein in the diet, if any, was sought to be corrected by Hassan and Drummond's technique of increasing the quantity of alkaline-autoclaved marmite in the daily ration. In order to keep the conditions comparable, however, the same quantity of alkaline-autoclaved marmite was incorporated in high-protein as well as in low-protein diets. The animals were allowed to eat *ad libitum*.

Two litters containing six piebald rats each were broken up as evenly as possible as regards weight and sex into groups of two.

The following diets were used:

Diet 1	%	Diet 2	%	Diet 3	%
Sucrose	25	Sucrose	50	Sucrose	75
Caseinogen	71	Caseinogen	46	Caseinogen	21
Salt mixture (McCollum)	4	Salt mixture (McCollum)	4	Salt mixture (McCollum)	4

The caseinogen used was the "light, white casein" of the British Drug Houses. 20 cc. of a 50 % solution of alkaline-autoclaved marmite (prepared by autoclaving a concentrated aqueous solution of marmite with baryta at p_H 9 for $1\frac{1}{2}$ hours at 18 lbs. pressure and subsequently removing the baryta by sulphuric acid) were incorporated per 100 g. of the basal diet. The animals received cod-liver oil and the vitamin B_1 preparation separately.

Table I shows the diet and the dose of the vitamin B_1 preparation received by each group of animals.

Table I.

Group	Basal diet	Dose of vitamin B_1 preparation* (cc.)
A	1	0·075
B	2	0·075
C	3	0·075
D	1	0·15
E	2	0·15
F	3	0·15

* Obtained by extracting brewer's top yeast with 50 % aqueous alcohol, concentrating the extract *in vacuo*, precipitating with neutral lead acetate and removing the lead from the filtrate by hydrogen sulphide. The optimum dose of the concentrated solution was 0·15 cc., containing about 30 mg. organic material [see Guha, 1931, 1].

The growth curves, shown in Figs. 1–3, indicate that the need for vitamin B_1 is not diminished with a reduction in the carbohydrate content of the diet.

It will be observed that animals on the high-protein diet exhibit somewhat less vigorous growth than the others, though no definite signs of ill-health

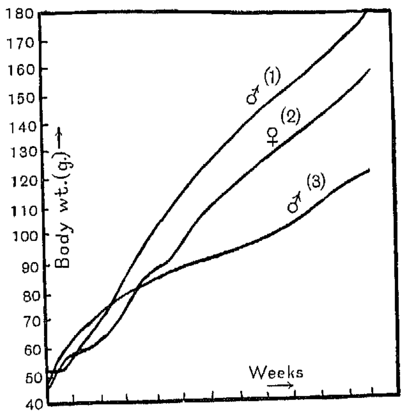

Fig. 1.

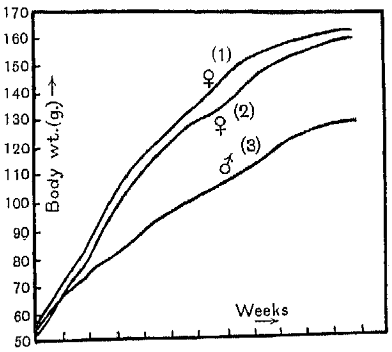

Fig. 2.

Fig. 1. Diet 1. Curves (1) and (2); dose = 0·15 cc. lead filtrate.
Curve (3); dose = 0·075 cc. lead filtrate.

Fig. 2. Diet 2. Curves (1) and (2); dose = 0·15 cc. lead filtrate.
Curve (3); dose = 0·075 cc. lead filtrate.

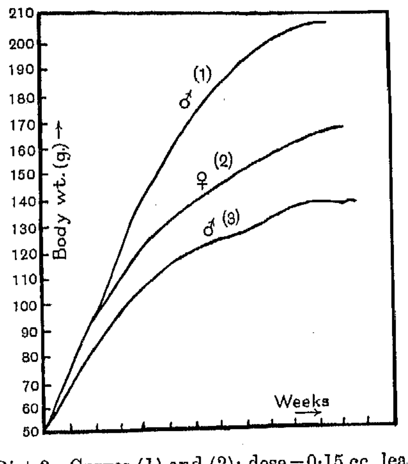

Fig. 3. Diet 3. Curves (1) and (2); dose = 0·15 cc. lead filtrate.
Curve (3); dose = 0·075 cc. lead filtrate.

were noticed. The high marmite content of these diets tended to give the rats slight diarrhoea during the first few days of experiment, which, however, rapidly cleared up.

B. The relation between vitamin B_1 and the nature of the dietary carbohydrate.

Considering the question of a possible relationship between vitamin B_1 and carbohydrate metabolism, it was of interest to investigate the effect of a sub-optimum dose of vitamin B_1 on the growth of young rats receiving diets containing different carbohydrates. It has been observed [Campbell and Soskin, 1928; Campbell and Maltby, 1928; Rose, Giragossintz and Kirstein, 1929–30] that the administration of dihydroxyacetone and fructose by way of the small intestine is followed by a rise in blood-lactic acid, whereas aldoses like dextrose and galactose have no such effect. As Kinnersley and Peters [1929] have stated that avitaminosis B_1 in the pigeon results in the production of excess lactic acid, it was clearly desirable to know whether the requirement of vitamin B_1 would vary according as dextrose or fructose formed the carbohydrate in the diet, although the observations of Campbell and of Rose constitute no evidence that lactic acid is produced in the normal metabolism of ingested fructose.

In these experiments the following basal diets were used.

Table II.

Diet No.	Constituents of diet		
4	75 % dextrose;	21 % caseinogen*;	4 % salt mixture†
5	,, fructose;	,,	,,
6	,, sucrose;	,,	,,
7	,, galactose;	,,	,,
8	,, lactose;	,,	,,
9	,, maltose;	,,	,,

* B.D.H. "light, white casein." † McCollum's.

A litter of 8 young rats was split up into 3 groups, (*a*), (*b*) and (*c*), and fed as shown in Table II. Their growth curves are illustrated in Figs. 4–6, and their average weekly gains in weight are given in Table III.

Table III.

Rat No.	Sex	Group	No. of basal diet	Daily dose of vitamin B_1 (lead filtrate) cc.	Av. weekly gain in weight over 18 weeks g.
1	♂	(*a*)	4	0·075	3·8
2	♀	(*a*)	4	0·075	3·8
3	♀	(*a*)	4	0·075	3·7
4	♂	(*b*)	5	0·075	3·3
5	♀	(*b*)	5	0·075	4·2
6	♀	(*b*)	5	0·075	3·4
7	♀	(*c*)	6	0·075	3·8
8	♀	(*c*)	6	0·075	3·8

The vitamin B_1 administered (0·075 cc.) represents a sub-optimum dose. Each animal received the vitamin B_1 supplement, 1·5 cc. of 50 % alkalised marmite and 2 drops of cod-liver oil daily in a dish separately from the basal diet.

The figures of the growth rates given in Table III show that there is no special relation between the requirement of vitamin B_1 and the presence of dextrose, fructose or sucrose in the diet. When some of these animals were later (after about 25 weeks) deprived of vitamin B_1, those that were receiving fructose were found to decline at a somewhat slower rate than those receiving dextrose or sucrose. This, however, could not be repeated when Kahlbaum's "crystallised laevulose" was used instead of the B.D.H. product. It is possible

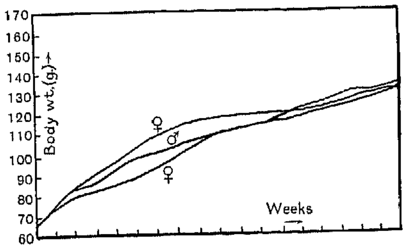

Fig. 4. Diet 4; dose = 0·075 cc. of lead filtrate.

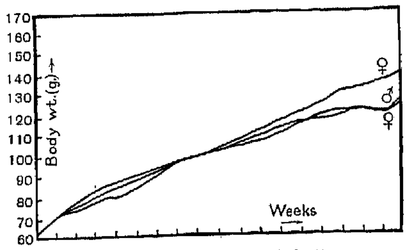

Fig. 5. Diet 5; dose = 0·075 cc. of lead filtrate.

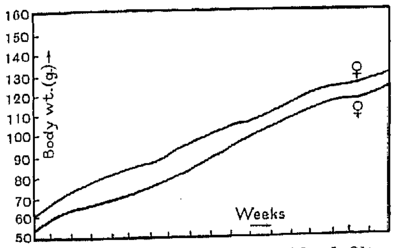

Fig. 6. Diet 6; dose = 0·075 cc. of lead filtrate.

that this latter carried traces of vitamin B_1, although it gave no perceptible precipitate with phosphotungstic acid.

When diets 7 and 8, containing galactose and lactose respectively as the sole sources of carbohydrate, were used, it was found that they caused a rapid decline of both young and adult rats. In the case of diet 7 final collapse could not be prevented even by incorporating whole dried yeast (5 %) in the diet. This phenomenon has been further investigated separately [Guha, 1931, 2].

Diet 9 containing maltose produced as good growth as the diet containing dextrose.

C. The relation between vitamin B_1 and dietary fat.

In the course of investigations on the effect of purified rations on the growth of rats, Evans and Lepkovsky [1929] made the striking observation that the presence of fat in the diet diminished the need of the rat for vitamin B_1. As the importance of this observation in relation to the suggested rôle of vitamin B_1 in carbohydrate metabolism is obvious, this question was investigated with reference to the following points.

(1) The effect of a sub-optimum dose of vitamin B_1 on the growth of young animals receiving diets containing (*a*) varying amounts of the same fat; (*b*) different fats in the same proportion; (*c*) varying proportions of fat but the same restricted amount of food every day.

(2) The effect of a complete deficiency of vitamin B_1 on rats subsisting on diets containing varying amounts of fat.

The following basal diets were used. The protein in each of the diets consisted of "light, white casein" (B.D.H.).

Table IV.

	Diet 12	Diet 13	Diet 14
	%	%	%
Sucrose	65	35	10
Palm kernel oil	10	40	65
Caseinogen	20	20	20
Salt mixture (McCollum)	5	5	5

	Diet 24	Diet 25	Diet 26
	%	%	%
Sucrose	65	35	10
Olive oil	10	40	65
Caseinogen	20	20	20
Salt mixture	5	5	5

	Diet 28	Diet 29	Diet 30
	%	%	%
Sucrose	65	35	10
Lard	10	40	65
Caseinogen	20	20	20
Salt mixture	5	5	5

	Diet 44 Parts		Diet 45 Parts		Diet 49 Parts
Lard	50	Sucrose	50	Sucrose	70
Caseinogen	36	Caseinogen	36	Caseinogen	20
Salt mixture	4	Salt mixture	4	Salt mixture	4

Of these, diets 44, 45 and 49 were incorporated with 20 cc. of 50 % alkalised marmite per 100 g. of the diet. The caseinogen content in diets 44 and 45 was raised in order to bring the nutritive ratio approximately to 1:3 and thus to make them more balanced than the other high fat diets [see Evans and Lepkovsky, 1929]. Diets 44 and 49 were made up so as to resemble Evans and Lepkovsky's diets 559 and 542 respectively. When the other basal diets were used, 1 cc. of alkalised marmite was fed separately every day. This dose was considered sufficient as 2 cc. gave no better growth. Two drops of cod-liver oil were, as usual, fed separately to each rat. Vitamin B_1, whenever fed, was administered separately in a sub-optimum dose (0·05 cc. of a lead filtrate preparation from brewer's yeast, different from that used for work described in the preceding sections).

Litters were evenly broken up as regards weight and sex and litter-mates used for the same batch of experiments. Some animals were kept under observation for periods exceeding 25 weeks. Figs. 7–9 illustrate some of the results obtained. Food intake records, which were kept for a period of 3 months, show that in general the gain in weight goes hand in hand with the food consumption. It will be observed that while olive oil and palm kernel oil do not give any definite indication of a sparing action on vitamin B_1 when the animals are receiving a sub-optimum dose of vitamin B_1, lard appears to have a definite effect. The decline in weight of rats completely deficient in vitamin B_1 is also slower when lard is present in the diet.

Four rats which were receiving diets 28 and 29, containing respectively 10 and 40 % lard, supplemented by a sub-optimum dose of vitamin B_1, were

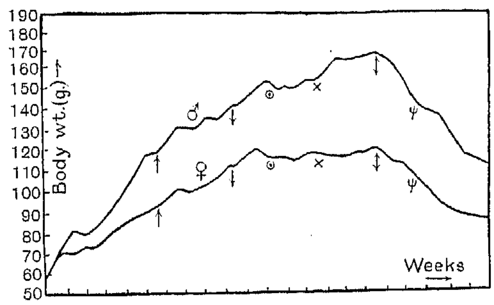

Fig. 7. Dose of vitamin B_1 = 0·075 cc. of lead filtrate throughout except as stated below; basal diet 12 from beginning of experiment; ↑ diet 24; ↓ diet 28; ⊙ restricted food intake; × food *ad libitum*; ↕ diet 45, B_1 supplement stopped; ψ diet 49 (no B_1).

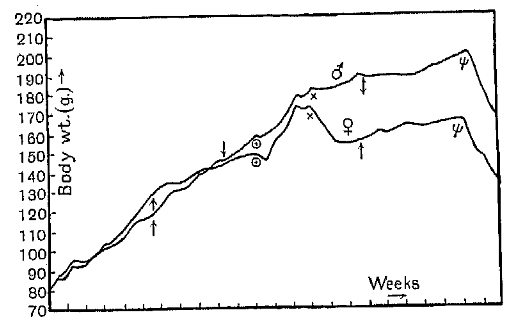

Fig. 8. Dose of vitamin B_1 = 0·075 cc. of lead filtrate throughout except as stated below; diet 13 from beginning of experiment; ↑ diet 25; diet 29; ⊙ restricted food intake; × food *ad libitum*; ↕ diet 44, B_1 supplement stopped; ψ diet 49 (no B_1).

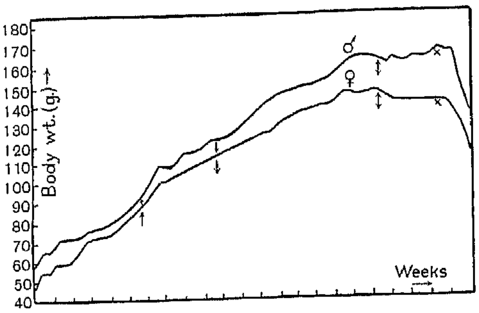

Fig. 9. Dose of vitamin B_1 = 0·075 cc. of lead filtrate throughout except as stated below; basal diet 14 from beginning of experiment; ↑ diet 26; ↓ diet 30; ↕ diet 44, B_1 supplement stopped; × diet 49 (with no B_1).

kept on a restricted amount of food for 18 days (6 g. per day for the first 3 days and 8 g. daily during the rest of the period). The changes in weight are shown in Table V.

Table V.

Rat No.	Sex	Diet	Period (days)	Initial weight (g.)	Final weight (g.)	Gain in weight in 18 days (g.)
1	♂	28	18	155	157	+ 2
2	♂	29	18	158	180	+22
3	♀	28	18	128	130	+ 2
4	♀	29	18	148	172	+24

This clearly shows that the diet containing 40 % lard promotes faster growth under the above conditions than that containing 10 % lard. Lard seems to be in this respect considerably more effective than palm kernel oil or olive oil.

Vitamin B_2, fed in the form of alkaline-autoclaved marmite, does not appear to have any effect [see also Evans and Lepkovsky, 1929].

D. The effect of the ingestion and injection of sodium lactate in vitamin B_1 deficiency.

As the accumulation of lactic acid has been stated to be concerned in the production of both avian and human "beriberi" [Peters, 1930; Hayasaka, 1930] it was considered of interest to investigate the effect of ingested sodium lactate on the rate of decline in weight and on the production of "beriberi" symptoms in vitamin B_1-deficient rats. Experiments were also carried out to observe the effect of the subcutaneous injection of sodium lactate in varying doses into deficient rats.

In this investigation the following diets were used:

Diet 21.	Sucrose	65 parts
	Palm kernel oil	10 ,,
	"Light, white casein"	20 ,,
	Salt mixture (McCollum)	5 ,,
	Cod-liver oil	2·5 ,,
	50 % alkalised marmite	10 cc.

Diet 23 was made up by incorporating 10 cc. of a vitamin B_1 preparation (lead filtrate) per 100 g. of diet 21 so that 10 g. of the diet contained 1·5–2 rat day doses.

Diet 38 was made up by incorporating 10 cc. of 50 % sodium lactate per 100 g. of diet 21.

(a) *The ingestion of sodium lactate.*

The method consisted in feeding two groups of adult rats, grown to maturity on whole wheat, brown bread and milk, on diets 21 and 38 respectively and comparing their rates of decline in weight and noting the time of onset of convulsions. Table VI summarises some of the results obtained.

The average rates at which the animals on diets 21 and 38 lost weight under the conditions studied were 8·0 and 12·5 weekly. Although this would tend to indicate a more rapid loss in weight when lactate was present

Table VI.

Rat No.	Sex	Diet No.	Period of feeding on synthetic diet (weeks)	Initial weight (g.)	Final weight (g.)	Total loss in weight (g.)	Loss in weight per week (g.)	Remarks
1	♂	21	6	131	116	15	2·5	Convulsions on 45th day
2	♂	21	6	247	165	82	13·7	Convulsions on 45th day
3	♂	21	6	221	158	63	10·5	Convulsions on 49th day
4	♂	21	6·5	165	148	17	2·6	—
5	♂	21	5·5	135	105	30	5·4	Convulsions on 44th day
6	♂	21	4·5	160	90	70	15·6	Convulsions on 29th day
7	♂	21	3	192	143	49	13·0	Had been on deficient diet for 5 weeks previously; convulsions on 56th day
8	♂	21	8	183	136	47	5·9	Convulsions on 56th day
9	♂	21	11	162	120	42	3·8	Dead on 78th day
10	♂	21	12	207	121	86	7·1	Convulsions on 85th day
11	♂	38	8	172	100	72	9·0	Convulsions on 57th day
12	♂	38	8	193	119	74	9·2	Convulsions on 57th day
13	♂	38	8	222	150	72	9·0	Convulsions on 61st day
14	♂	38	8	240	150	90	11·2	Convulsions on 61st day
15	♂	38	6	187	100	87	14·5	Convulsions on 43rd day
16	♂	38	6·5	178	120	58	9·0	—
17	♂	38	5	188	102	86	17·2	Convulsions on 43rd day
18	♂	38	3	241	178	63	21·0	Had been previously on diet 21; convulsions on 61st day

in the diet, the large individual variations among the members of each group of rats preclude too great a reliance on these figures. It has also to be noted that the presence of lactate in the diet did not hasten the appearance of the "beriberi" convulsions. But here also the values are too scattered to warrant a definite conclusion.

As the metabolism of sodium lactate was likely to be reflected in the hydrogen ion concentration of the urine, the p_H values of the urine of rats on diets 21, 23 and 38, diet 38 + vitamin B_1, and on normal stock diet were measured and are given in Table VII. The urine was collected for 5 hours over toluene.

Table VII.

Rat No.	Sex	Diet	Nature of diet	p_H of urine
1	♀	Normal stock diet	Whole wheat, brown bread and milk	6·5
2	♂	,,	,,	6·5
3	♀	23	+ vitamin B_1	6·4
4	♀	23	+ ,,	6·5
5	♂	23	+ ,,	6·5
6	♂	21	− ,,	6·2
7	♂	21	− ,,	6·5
8	♀	21	− ,,	6·4
9	♂	38	− vitamin B_1; + lactate	7·2
10	♂	38	,,	7·2
11	♀	38	,,	7·2
12	♀	38 + vitamin B_1	+ vitamin B_1; + lactate	8·6
13	♀	,,	,,	8·6
14	♂	,,	,,	8·7

These results show definitely that while the presence of sodium lactate in the vitamin B_1-deficient diet raises the p_H of the urine from approximately

6·4 to 7·2, the addition of vitamin B_1 to the lactate diet raises the p_H to 8·7. This rise in p_H occurs within 24 hours of the administration of vitamin B_1 to the basal lactate diet.

One rat, weighing 212 g., which was on diet 23, was injected with 2 cc. of 50 % sodium lactate. The p_H values of samples of the urine collected successively after 18 hours and 48 hours were 9·2 and 7·8. The urine collected after 7 days gave the p_H value 6·6, showing a return to the normal.

The significance of these results is discussed later.

(b) *The injection of sodium lactate.*

The results obtained by the subcutaneous injection of sodium lactate into rats on diets 21 and 23 are summarised in Table VIII. The animals on diet 21 were subjected to a fairly severe deficiency of vitamin B_1, but the deprivation was not sufficiently prolonged to allow them to go into "beriberi" convulsions spontaneously.

Table VIII.

Rat No.	Sex	Diet No.	Nature of diet	Period of feeding on synthetic diet (days)	Body weight at time of injection (g.)	Dose of 50 % sodium lactate injected (cc.)	Results
1	♂	21	– vitamin B_1	45	148	0·2	Survived
2	♀	21	– ,,	52	60	0·4	Survived
3	♀	21	– ,,	54	55	0·4	Died in 4 hours in convulsions
4	♀	21	– ,,	50	90	0·6	Survived
5	♀	21	– ,,	59	70	0·6	Died in an hour
6	♂	21	– ,,	50	95	0·8	Died in 4 hours in convulsions
7	♀	21	– ,,	58	94	0·8	Died in 1½ hours in convulsions; signs of haemorrhage
8	♀	21	– ,,	23	85	0·8	Survived; severe convulsions after 4 days
9	♂	21	– ,,	56	148	1·0	Survived
10	♂	21	– ,,	46	150	1·0	Survived
11	♀	23	+ ,,	59	174	0·8	Survived
12	♀	23	+ ,,	56	105	1·0	Survived
13	♀	23	+ ,,	49	118	1·0	Survived
14	♂	23	+ ,,	49	107	1·6	Died after 5 hours
15	♂	23	+ ,,	61	225	1·6	Survived
16	♂	23	+ ,,	50	142	1·8	Died after 6 hours
17	♀	23	+ ,,	58	122	1·8	Survived
18	♀	23	+ ,,	87	175	2·0	Survived
19	♂	23	+ ,,	49	152	2·0	Died in 3 hours
20	♀	23	+ ,,	56	150	2·0	Survived
21	♂	23	+ ,,	56	185	2·4	Survived
22	♂	23	+ ,,	49	108	2·4	Died in 3 hours
23	♂	23	+ ,,	63	225	2·4	Survived
24	♂	23	+ ,,	64	225	3·2	Convulsions; severe haemorrhage; died in 1 hour

These figures demonstrate that while a dose of about 0·8–1·0 cc. of 50 % sodium lactate per 100 g. body weight is fatal for vitamin B_1-deficient rats, animals receiving vitamin B_1 exhibit a tolerance at least one and a half times as great.

The convulsions observed in the vitamin B_1-deficient animals on injection of sodium lactate sometimes resembled typical "beriberi" convulsions. Attempts were made to find if these artificial convulsions could be cured by the administration of vitamin B_1 preparations, but death was too quick to be prevented by this means.

As the "beriberi" symptoms in pigeons have been ascribed to acidosis produced by lactic acid [Peters, 1930], it was of interest to compare the effects of the injection of similar doses of ammonium chloride, which is known to produce marked acidosis [Salkowski, 1878; Haldane, 1921], into vitamin B_1-deficient and normal rats. The quantities of ammonium chloride injected were approximately equimolecular to some of the smaller doses of sodium lactate used in the preceding set of experiments. Ammonium chloride was, however, found to be much more toxic than sodium lactate and appeared to be equally fatal to normal and deficient rats, in the doses tried (Table IX).

Table IX.

Rat No.	Sex	Diet	Period on synthetic diet (days)	Body weight at time of injection (g.)	Dose of 22·2 % sol. of NH_4Cl injected (cc.)	Results
1	♂	21	56	90	0·9	Died in 15 minutes
2	♂	21	49	80	0·45	Died in 15 minutes
3	♀	21	—	100	0·9	Died in 10 minutes
4	♀	21	—	147	0·9	Died in 15 minutes
5	♂	23	56	198	1·8	Died in 5 minutes (severe haemorrhage)
6	♂	23	56	200	0·9	Died in 20 minutes
7	♀	23	57	170	0·9	Died in 15 minutes

E. The oxidative mechanism of tissues in vitamin B_1-deficiency.

Suggestions regarding the impairment of the oxidative apparatus in "vitamin B" deficiency are numerous. Drummond and Marrian [1926, where the literature up to 1926 is quoted], from a series of experiments carried out with rats, found, however, no experimental support for the theory. The question has been very recently revived by Abderhalden and Vlassopoulos [1931] working with the liver, muscle and brain tissues of pigeons.

The present work was undertaken in view of the statement of Peters [1930] regarding an increase in the production of lactic acid in vitamin B_1 deficiency in the pigeon and its concentration in the brain, and of the recent experiments of Roche [1931], which also tend to show an accumulation of three-carbon bodies in the blood of avitaminous pigeons. It was considered, firstly, that if in vitamin B_1 deficiency the specific mechanism concerned in the removal of lactic acid was involved, its impairment might not be revealed by the technique employed by Drummond and Marrian, who used succinate as the metabolite in their anaerobic reduction experiments with methylene blue. The succinodehydrogenase system might be unimpaired in vitamin B_1 deficiency, while the lactic dehydrogenase system might be damaged. Secondly, the experiments on the direct oxygen uptake of tissues might not lead to any definite conclusions on this question, because the lactic-oxidising system, while being qualitatively important, might not be quantitatively so great in relation to the total uptake that its impairment would cause a markedly diminished oxygen consumption. This diminution would have to be sufficiently large to be outside the error caused by the fortuitous presence of various metabolites in the particular tissue examined at the moment of killing the animal. As in such experiments the oxygen uptakes of the tissues of different animals have to be compared, the variation introduced by this factor is likely to be large. A more correct idea of the condition of the lactic-oxidising system would be obtained by measuring the increased oxygen uptake due to added lactate, though there might not be a strictly proportional relation between the two owing to the competition of different substrates being involved in the question. Thirdly, since Kinnersley and Peters [1930] have shown that lactic acid accumulates chiefly in the brain, it was considered possible that while other "beriberi" tissues might not show any marked difference in oxygen consumption from normal tissues, the brain might reveal the difference. The liver might also be concerned in this question, as it apparently plays an important part in lactic acid metabolism [Cori and Cori, 1929].

In the following experiments, therefore, which were carried out throughout with the brain and liver tissues of rats, the significant values are considered to be those that were obtained after the addition of sodium lactate as substrate, both in the anaerobic experiments with methylene blue and the experiments on direct oxygen consumption.

(1) *Experiments with Thunberg's methylene blue technique.*

The Thunberg tubes were arranged according to the following general scheme.

	Tubes without lactate cc.	Tubes with lactate cc.
Phosphate buffer (p_H 7·4)	2·0	2·0
Methylene blue (1/5000)	1·0	1·0
Na lactate ($M/60$)	—	0·5
Distilled water	2·0	1·5
Tissue suspension in phosphate buffer	1·0	1·0

Temp. of bath 37°.

The results of certain typical experiments with liver and brain tissues of normal and vitamin B_1-deficient animals are shown in Table X.

Figures of a similar order were obtained in other experiments. Thus, with 0·148 g. of the brain tissue of one deficient rat the time of reduction of methylene blue in presence of sodium lactate was 4·1 minutes, the reduction time without lactate being 18·2 minutes, showing a fair degree of efficiency of the mechanism concerned in the oxidation of lactic acid.

Though both the liver and brain tissues of normal animals reduce methylene blue more rapidly than the corresponding tissues of vitamin B_1-deficient

Table X.

Liver tissue.

		Time of decoloration without lactate (mins.)		Time of decoloration with lactate (mins.)	
Unwashed liver tissue (0·15 g.)	Normal	5·5 5·75 5·7	5·65	5·0 4·05 4·15	4·4
	Vitamin B_1-deficient	11·6 9·25 10·0	10·28	6·6 7·2 7·2	7·0
Washed liver tissue* (0·15 g.)	Normal	Slight decoloration in 3 hours		35·2 40·25	37·72
	Vitamin B_1-deficient	,,		49·0 46·0	47·5

Brain tissue.

Washed brain tissue† (0·14 g.)	Normal	26·1 24·0 24·4	24·8	6·1 5·6 5·9	5·87
	Vitamin B_1-deficient	37·25 38·0 37·5	37·58	7·0 6·9 6·8	6·9

* Washed twice with 100 cc. of Ringer-Locke solution per 1·5 g. of tissue. Too much washing apparently causes a large loss of lactic dehydrogenase.
† Washed once with 100 cc. Ringer-Locke solution per 1·4 g. tissue.

animals in the absence of sodium lactate (Table X), no valid conclusion regarding the oxidative mechanism can be drawn from these figures, as has been pointed out earlier. More significant values are obtained by calculating the ratio ($a:b$) of the time of reduction of methylene blue without lactate to the time of reduction with lactate in each case (Table XI).

Table XI.

	$a:b$
Liver tissue of normal rat	1·30
,, deficient rat	1·47
Brain tissue of normal rat	4·22
,, deficient rat	5·44
,, ,,	4·50

These ratios indicate that the acceleration of the reduction of methylene blue due to added lactate by normal and deficient liver and brain tissues is approximately of the same order for each type of tissue. The variations fall within the limits of error of the technique.

(2) *Experiments on the oxygen consumption with the Barcroft apparatus.*

These experiments were carried out with the chopped whole brain tissue (unwashed).

The general arrangement of the cups is shown in Table XII. Temp. 38°.

Table XII.

	Without lactate		With lactate	
	Left-hand cup cc.	Right-hand cup cc.	Left-hand cup cc.	Right-hand cup cc.
Phosphate buffer (p_H 7·2)	1·0	1·0	1·0	1·0
Sodium lactate ($M/10$)	—	—	—	0·5
Ringer solution	1·5	0·5	1·5	0·5
Distilled water	0·5	0·5	0·5	—
Brain tissue suspension in phosphate buffer	—	1·0	—	1·0

Some results are shown in Figs. 10 and 11 which represent extreme values. Thus in one case the rate of oxygen consumption of the normal tissue was

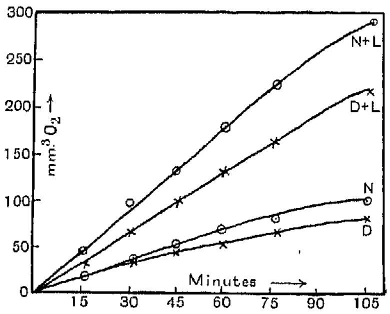

Fig. 10.

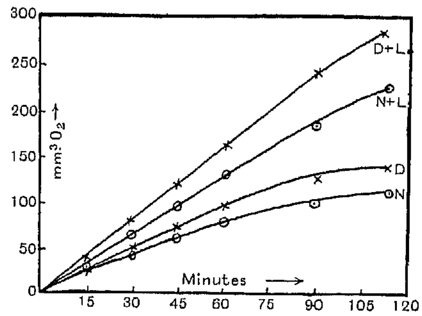

Fig. 11.

Fig. 10. Oxygen consumption of 0·1 g. brain tissue.
×—×—× Tissue of deficient animal in convulsions (D).
⊙—⊙—⊙ Tissue of normal animal (N).
D + L stands for "beriberi" tissue with added lactate.
N + L stands for normal tissue with added lactate.

Fig. 11. Oxygen consumption of 0·13 g. brain tissue.
×—×—× Tissue of deficient animal (D).
⊙—⊙—⊙ Tissue of normal animal (N).
D + L stands for "beriberi" tissue with added lactate.
N + L stands for normal tissue with added lactate.

somewhat greater than that of the "beriberi" tissue, while in the other case the situation was reversed, though this latter case was somewhat exceptional.

Addition of lactate as substrate resulted in a very marked increase in the rate of oxygen uptake of both the normal and "beriberi" tissues. The orders of increase, however, show some variations, and it appears that this technique would not reveal any but a considerable damage to the lactic-oxidising mechanism in vitamin B_1 deficiency, unless a statistical analysis of a very large number of results were made.

The cytochrome content of normal and "beriberi" tissues.

The brain, liver, kidney and heart muscle tissues of normal rats and vitamin B_1-deficient rats in convulsions (which were nearly killed by chloroform and then bled to death) were thoroughly washed and examined for the cytochrome bands in the usual way with the microspectroscope, after the addition of a trace of sodium hydrosulphite, if necessary. All the cytochrome bands were equally prominent in the corresponding tissues of normal and avitaminous animals. There was, apparently, no diminution of the cytochrome content in vitamin B_1 deficiency.

Discussion.

The experiments described in Section A offer no support to the contention of Randoin and Simonnet [1924] that the requirement of vitamin B_1 has a relation to the carbohydrate content of the diet of pigeons. On the other hand, it has to be pointed out that these experiments on the relation between vitamin B_1 and the protein/carbohydrate ratio of the diet constitute no evidence against the theory that vitamin B_1 plays some part in carbohydrate metabolism, as it is quite possible that the vitamin is required also for the degradation of the glucogenic residue of the protein molecule. The non-dependence of the requirement of vitamin B_1 on the nature of the dietary carbohydrate (glucose, fructose, sucrose and maltose) is not probably of much significance, because presumably all these carbohydrates yield the same three-carbon compounds by degradation and, if vitamin B_1 is concerned in the metabolism of the latter [Peters, 1930; Vogt-Møller, 1931; Roche, 1931] it is quite reasonable that the requirement of vitamin B_1 should be independent of the nature of the carbohydrate. The decline in weight of animals receiving galactose as the sole source of carbohydrate is probably to be related to its inefficient utilisation and its low renal threshold. Its absorption through the intestinal wall is known to be rapid [Cori, 1925; McCance and Madders, 1930]. The lactose diet had a somewhat deleterious effect in that it tended to produce diarrhoea.

The experiments on the relation between vitamin B_1 and dietary fat indicate that all fats are not of equal value so far as the sparing action on vitamin B_1 is concerned. Thus lard is apparently more efficient in this respect than palm kernel oil and olive oil. It is, therefore, not clear how far the sparing action is the effect of fat *per se*. While the observations of Evans and Lepkovsky [1929] on this subject might be interpreted to mean that the vitamin plays some rôle in carbohydrate metabolism, other possible explanations might also be suggested. Thus, assuming that the vitamin acts only by stimulating the appetite in some unknown way, it is possible that in Evans and Lepkovsky's experiments, if the limiting factor in the growth of the deficient rats were calorie intake, the rats receiving fat-rich diets would grow better because of the relatively much greater calorie intake. The observation mentioned in this paper, that rats receiving restricted amounts of a diet containing 40 % lard grew better than those receiving the same quantities of a diet containing 10 % lard when both groups were given a sub-optimum dose of vitamin B_1, might also be interpreted in a similar manner. The phenomenon of refection reminds one also of a possible function of the intestinal bacteria of rats subsisting on high-fat diets. It may be mentioned incidentally that the high-fat diets did not exert any deleterious effect on the animals [see also Osborne and Mendel, 1924], except that they were greasy looking and somewhat sluggish.

As described in Section D the appearance of symptoms was not markedly hastened by the presence of sodium lactate in a vitamin B_1-deficient diet. The absorption of sodium lactate is known to be fairly rapid [Cori, 1930] and the change in p_H of the urine indicates that the ingested sodium lactate was being metabolised. The expectation that sodium lactate might precipitate the appearance of symptoms in deficient rats was based on the observation of Kinnersley and Peters [1930] regarding the accumulation of lactic acid in the brain and on the evidence presented by McGinty [1929] to show that brain tissue normally removes some of the lactic acid from circulation. The p_H (8·4) of the urine of rats receiving the lactate diet *plus* vitamin B_1, which was higher than the p_H (7·2) of the urine of rats receiving the lactate diet *minus* vitamin B_1, might be interpreted to indicate a greater efficiency of the animals receiving vitamin B_1 in metabolising ingested lactate. This phenomenon might, however, also be ascribed to the diminished consumption of the lactate diet in vitamin B_1 deficiency. How far the ability to dispose of lactic acid is affected in vitamin B_1 deficiency is at present under investigation in experiments both *in vivo* and *in vitro*.

The injection experiments with sodium lactate showed a definitely decreased tolerance to lactate in vitamin B_1 deficiency. It is possible, however, that this was due to the comparatively weak condition of the deficient rats. The convulsions induced by the injection of lactate sometimes resembled "beriberi" convulsions, but insulin convulsions are also similar in appearance.

The significant evidence brought forward by Kinnersley and Peters [1929, 1930] regarding an increase in blood-lactic acid and a localised lactic acidosis in the brain finds support in the recent experiments of Roche [1931] on the blood of deficient pigeons, and of Hayasaka [1930], who observed that sodium lactate, injected intravenously into normal and cured "beriberi" human subjects, disappeared faster than that injected into "beriberi" subjects. As has been indicated earlier, the evidence for the depressed oxidation of various tissues of deficient pigeons [Abderhalden and Vlassopoulos, 1931], and for the diminished respiration of the blood of "beriberi" subjects [Kimura, 1930], might be misleading unless the differences are of a relatively high order, and unless a very large number of measurements are taken. In a recent note, which appeared after the present work had been completed, Gavrilescu and Peters [1931] have recorded the interesting observation that the lowering of the oxygen consumption of the brain tissue of avitaminous pigeons was confined especially to certain parts of the brain. If such be the case, it might have escaped detection in the experiments described in Section E, which were carried out with the whole brain tissue of rats. These experiments on the increased oxidation due to added lactate indicate, however, that the mechanism concerned in the oxidative removal of the particular metabolite, lactate, is not seriously damaged in vitamin B_1 deficiency. While it is possible that a statistical analysis of a large number of measurements might indicate a small amount of injury to this mechanism, it is considered probable that, so far as the lactic acid observed to accumulate by Kinnersley and Peters is concerned, it is the mechanism of its "synthetic" removal which is damaged rather than that of its oxidative removal. Holmes and Ashford [1930] have demonstrated the existence of a Meyerhof quotient for the brain, though at the moment no information is available as to the fate of the lactic acid which is removed but not oxidised by brain tissue. In fact, our knowledge about the steps by which the lactic acid in muscle is resynthesised into glycogen, is also very meagre [Long and Grant, 1930]. Investigation is in progress to test the accuracy of the above suggestion.

Summary.

1. The requirement of vitamin B_1 for young growing rats, receiving a sub-optimum dose of vitamin B_1, was found to be independent of the protein/carbohydrate ratio of the diet.

2. The requirement of vitamin B_1 under the same conditions was likewise independent of the nature of carbohydrate in the diet, glucose, fructose, sucrose or maltose. With galactose and lactose as sole sources of carbohydrate in the diet the animals were found to decline in weight.

3. The relation of the requirement of vitamin B_1 to the dietary fat has been investigated. While palm kernel oil and olive oil had little sparing action on the vitamin, lard appeared to have a definite effect.

4. The ingestion of sodium lactate did not appreciably hasten the appearance of symptoms in vitamin B_1-deficient rats. The p_H of the urine of animals receiving lactate *plus* vitamin B_1 was higher than that of animals receiving lactate *minus* vitamin B_1. The lethal dose of injected sodium lactate was lower for deficient animals than for normal ones.

5. The cytochrome content of the liver, kidney, brain and heart muscle tissues of rats was not diminished in vitamin B_1 deficiency. The mechanism for the oxidation of lactic acid by the liver and brain tissues of deficient rats appeared to remain fairly efficient.

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