62. The Vitamin-B₁ Sparing Action of Fat and Protein

3. The Oxidation of Pyruvate by the Tissues of Symptom-free Rats on Diets Deficient in Vitamin B₁*

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It is now well established that vitamin B₁ is concerned in the metabolism of carbohydrate. Deficiency of the vitamin results in an accumulation in the blood of lactic acid [Callazo & Morelli, 1925] and of pyruvic acid [Thompson & Johnson, 1934; 1935; Platt & Lu, 1936]. It is now known that the pyrophosphate of vitamin B₁ functions as a coenzyme in the decarboxylation of pyruvate [Lohmann & Schuster, 1937]. Studies on tissue respiration have shown that avitaminosis produces a diminished O₂ uptake in many tissues, and Peters and his co-workers at Oxford were able to demonstrate that this diminished tissue respiration in polyneuritic animals, associated with an inability to oxidize pyruvate, may be rectified by the addition of vitamin B₁ to the tissue respiring *in vitro*. They have called this phenomenon the 'catatorulin effect' [see Peters, 1936; 1940].

The role of vitamin B₁ in carbohydrate metabolism has also been demonstrated by the fact that an increase in dietary carbohydrate accentuates the symptoms of deficiency of the vitamin [e.g. Funk, 1914; Randoin & Simmonet, 1923]. It has also been shown recently that rats can thrive on a diet free from vitamin B₁ if it contains no carbohydrate. Such animals grow well and do not develop such signs of deficiency as polyneuritis or bradycardia [see preceding papers of this series: Banerji, 1940; 1941].

The work described in this paper has arisen from the last-mentioned findings. The fact that it is possible for rats to survive in the absence of the vitamin with no signs of deficiency raises the question of the state of their tissues with regard to carbohydrate metabolism. This question may be put thus: does the defect in carbohydrate oxidation, as shown for example by the catatorulin effect, persist in animals receiving no vitamin B₁, even though they are free from symptoms of deficiency? The experiments to be described show that the defect does in fact persist and that deficient animals, irrespective of whether they develop symptoms, show a definite catatorulin effect. It is clear that these results are of significance in relation to the possible mode of action of the vitamin. This point will be discussed further after we have presented our results.

EXPERIMENTAL

Young rats weighing about 50 g. were fed on the following diets:

- (1) Standard basal diet (containing about 55 % sucrose).
- (2) High protein diet (sugar-free).
- (3) High fat diet (sugar-free).
- (4) Protein-fat diet (sugar-free).

The detailed composition of the diets is shown in Table 1.

Some of the animals received in addition supplements of vitamin B₁, usually 10 µg. daily. (It has been shown that, with the standard basal diet, 8 µg. daily produce adequate growth [e.g. Yudkin, 1941].) Signs of deficiency developed only in those animals on the

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Table 1. *Composition of diets*

	Standard basal	High protein	High fat	Protein-fat
Sucrose	60	0	0	0
Fat	15	15	75	55
Light white casein	20	80	20	40
Salt mixture	5	5	5	5
Autoclaved marmite	12	12	12	12

Each animal received in addition 2 drops of cod liver oil daily.

The fat used was arachis oil in the standard basal and high protein diets, and lard in the high fat diet. Either arachis oil or lard was used in the protein-fat diet without any detectable difference in behaviour of the animals.

standard basal diet with no supplement of the vitamin. The animals were kept for 3–4 months on the diets on which they remained free of symptoms, or for 2–3 weeks, i.e. when symptoms were well developed, on the basal diet with no added vitamin. After this time, tissue respiration was studied in the Warburg manometer at 38°. Three or four animals of each group were investigated. Kidney slices were used and the determinations of O₂ uptake were done in triplicate. The decision to use kidney slices instead of the brain mince usually used by the Oxford workers was made, first because of the much greater agreement between triplicate determinations, and secondly because the brain of the rat has been shown by O'Brien & Peters [1935] to give at best only a slight catatorulin effect.* The O₂ uptake was measured in Ringer-phosphate solution at pH 7.4, in the absence or presence of pyruvate, and with or without the addition of vitamin B₁. About 20 mg. wet weight of tissue were used in each cup, which contained 2.5 ml. liquid. The quantities of pyruvate and of vitamin B₁ added were 6 mg. of the sodium salt and 3 µg. of the hydrochloride respectively. The manometers were in the bath for 15 min. for temperature equilibration before readings were begun, and thereafter readings were taken after 15 min. and then at half-hourly intervals for a total of 3½ hr. The reading for the first 15 min. was often irregular and has therefore been ignored in the results here presented. The results are expressed throughout as Q_{O₂}. The average values of the Q_{O₂} for each group of animals are shown in Figs. 1–10; more detailed protocols of individual experiments are given in the Appendix.

For convenience and to lessen confusion, an augmentation of respiration produced by vitamin B₁ will be referred to as indicating the presence of the 'specific metabolic defect'. This term, we feel, conveys best the impression of the oxidative disability found in deficient tissues.

RESULTS

Effect of vitamin B₁ in vitro on respiration

(a) *Standard basal diet.* The addition of vitamin B₁ to respiring kidney slices of polyneuritic rats resulted in a considerable and rapidly increasing augmentation of respiration, especially in the presence of pyruvate (Fig. 1). The consumption of 10 µg. of the vitamin daily by animals on the same diet resulted in an almost complete suppression of this effect (Fig. 2). A slight increase due to the added vitamin was observed towards the end of the experiment, probably owing to the gradual loss of vitamin from the tissues during this time. Peters & Rossiter [1939] found a somewhat higher catatorulin effect (some 30% increase in O₂ uptake during the 2nd hr.) in kidneys from normal rats.

(b) *High protein diet.* Kidney slices from animals on this diet, with no supplement of vitamin B₁, showed in the presence of pyruvate a decided and increasing metabolic defect, somewhat lower than that shown by the polyneuritic animals (Fig. 3). In the

* In special conditions, it is possible to demonstrate an increased respiration in rat's brain with vitamin B₁ [Barron, Goldinger, Lipton & Lyman, 1941b].

absence of pyruvate, the vitamin produced much less effect with this group of animals than with the polyneuritic group. Supplementing the diet with $10\mu\text{g}$. of the vitamin daily resulted in a complete abolition of the *in vitro* effect of added vitamin (Fig. 4).

Fig. 1 A

Fig. 1 B

Fig. 1. Standard basal diet, no supplement.

A = rate of respiration at hourly intervals.

----- Curve O = in the absence of added substrate (= residual respiration).

..... P = in presence of pyruvate.

B = % increase in respiration due to added vitamin B₁.

----- B₁ = in presence of vitamin B₁.

..... P + B₁ = in presence of pyruvate and vitamin B₁.

Fig. 2 A

Fig. 2 B

Fig. 2. Standard basal diet + $10\mu\text{g}$. vitamin B₁ daily. Key: as in Fig. 1.

(c) *High fat diet.* A high fat diet with no added vitamin B₁ resulted in a definite catatorulin effect in the kidney, though this was lower than with either of the two

previous diets (Fig. 5). The increase in O₂ uptake was produced by the vitamin both in the absence and presence of pyruvate. Animals receiving the high fat diet with the daily supplement of vitamin B₁ behaved quite differently from animals on the other two

Fig. 3 A

Fig. 3 B

Fig. 3. High protein diet, no supplement. Key: as in Fig. 1.

Fig. 4 A

Fig. 4 B

Fig. 4. High protein diet + 10 μg. vitamin B₁ daily. Key: as in Fig. 1.

diets in respect to the effect of vitamin B₁ *in vitro* on the O₂ uptake in the presence of pyruvate (Fig. 6). They showed a distinct metabolic defect, which, although lower than for animals receiving no vitamin B₁, reached a maximum of about 70 % after 2 hr. On the other hand, as with the other two diets, the O₂ uptake in the absence of pyruvate was very little increased by the vitamin.

(d) *Protein-fat diet.* Rats on this diet, as on the high fat and high protein diets, survived in apparently perfect health for many months. Two animals receiving this diet with no added supplement of vitamin B₁ were used for the investigation of tissue respiration. The results obtained were roughly intermediate between those obtained with

Fig. 5 A

Fig. 5 B

Fig. 5. High fat diet, no supplement. Key: as in Fig. 1.

Fig. 6 A

Fig. 6 B

Fig. 6. High fat diet + 10 µg. vitamin B₁ daily. Key: as in Fig. 1.

the high protein and high fat diets. A moderate and gradually increasing augmented respiration was produced by the addition of the vitamin in the presence of pyruvate, and a smaller effect was produced in its absence (Fig. 7).

Absence of relationship between extent of the 'specific metabolic defect' and the development of other symptoms of deficiency

It will be seen that the extent of the metabolic defect was greater with the deficient animals receiving the standard basal diet than with those receiving a diet containing no

Fig. 7 A

Fig. 7 B

Fig. 7. Fat-protein diet, no supplement. Key: as in Fig. 1.

Fig. 8 A

Fig. 8 B

Fig. 8. Standard basal diet + 2 μ g. vitamin B₁ daily. Key: as in Fig. 1.

carbohydrate. It might thus be argued that the development of the symptoms which occurs only in the former group is associated with this larger 'specific metabolic defect'.

In order to test this, some rats were fed with the standard basal diet with daily supplements of $2\mu\text{g.}$ of the vitamin. After 6–8 weeks, these animals developed severe polyneuritis and, had they not been killed for the experiment, would have soon died. The results show that the increase in respiration produced by vitamin B_1 was appreciably less

Hr.
Fig. 9 A

Hr.
Fig. 9 B

Fig. 9. Standard basal diet + $8\mu\text{g.}$ vitamin B_1 daily. Key: as in Fig. 1.

Hr.
Fig. 10 A

Hr.
Fig. 10 B

Fig. 10. Standard basal diet + $27\mu\text{g.}$ vitamin B_1 daily. Key: as in Fig. 1.

in these animals than in animals receiving no dietary supplement (Fig. 8). Comparing these results with those from deficient but symptomless animals receiving the carbohydrate-free diets, the increase in the absence of pyruvate was higher than that in the high protein animals, about equal to that in the 'high fat' animals and lower than that in the 'protein-fat' animals. In the presence of pyruvate the order of increased respiration was reversed—the animals on the standard basal diet + $2\mu\text{g.}$ vitamin B_1 daily showed

a catatorulin effect lower than that of the high protein animals, equal to that of the high fat animals and greater than that of the protein-fat animals.

The main fact which emerges from these findings is that an animal may have a moderate metabolic defect and die of polyneuritis (on the standard basal diet), or it may have a greater metabolic defect and yet grow well and remain quite free from symptoms (on the carbohydrate-free diets).

Effect of other factors on respiration

Since the main object of these investigations was to study the effect of vitamin B₁ on tissue respiration in animals on different diets, we do not propose to do more than mention other and incidental findings, although many of them appear of interest.

(a) *Effect of pyruvate in vitro on respiration.* Whether pyruvate produced an increased O₂ uptake in kidney slices depended on the composition of the diet of the animals, on the addition or otherwise of dietary vitamin B₁ and on the presence or absence of vitamin B₁ in the Warburg cup during the measurement of the respiration. These findings are summarized in Table 2.

Table 2. *Effect of pyruvate on respiration*

	Diet			
	Standard basal	High fat	High protein	Protein-fat
1. Animals receiving no supplement of vitamin B ₁ :				
Effect of pyruvate in absence of vitamin B ₁	-	-	-	+
Effect of pyruvate in presence of vitamin B ₁	+	-	+	+
2. Animals receiving dietary supplement of vitamin B ₁ (10 µg. daily):				
Effect of pyruvate in absence of vitamin B ₁	+	+	+	Not studied
Effect of pyruvate in presence of vitamin B ₁	+	+	+	Not studied

+ indicates an increased respiration in the presence of pyruvate; - indicates no effect.

Some of these results are rather surprising; for example with no dietary vitamin B₁, only animals on the protein-fat diet show an increased respiration with pyruvate. It would, however, be unnecessarily speculative to discuss the possible reasons for these results until further experimental data are available.

(b) *Effect of dietary vitamin B₁ on respiration.* In both this and the subsequent section, a comparison is made between the respiratory activities of the various groups of animals. It is clear that, with the small number of animals investigated in each group, apparent differences in behaviour between the groups must be considered carefully before deciding that they are significant. On the other hand, within any one group, differences produced in the tissue respiration of individual animals by the addition of vitamin B₁ or pyruvate, as described in the foregoing sections, are of much more obvious significance.

The question whether tissues from deficient animals, in the absence of any addition *in vitro*, show a lower respiration than tissues from normal animals has been the subject of many investigations. On the one hand, several workers have claimed that they have observed such a decreased metabolism in deficient tissues [e.g. Abderhalden & Schmidt, 1920; Abderhalden & Wertheimer, 1921; Abderhalden & Vlassopoulos, 1931; Tsukamoto, 1928; Sherman & Elvehjem, 1936]. Westenbrink [1932*a*], however, has shown that the results of Abderhalden and his co-workers are not statistically significant. Several investigators, too, have been unable to find any difference between normal and deficient tissues [e.g. Drummond & Marrian, 1926; Westenbrink, 1932*b*]. These divergent findings may be explained in part by the fact that the differences between normal and deficient tissues are frequently small, and also by the fact that the different tissues used by different workers are not equally affected by the deficiency. It is well known for example that the liver of deficient animals of many species shows no catatorulin effect, although in special conditions an effect may be demonstrable [Barron *et al.* 1941*a*].

The results of the present investigation suggest that a third factor which may determine a reduced respiration in the tissues of deficient animals is the composition of the diet. Kidney slices from rats reared on the carbohydrate-free diets show no decreased residual respiration if dietary vitamin B₁ is withheld, whereas those from rats receiving the standard diet do show this. It may be that this diminution in respiration is associated with the presence of symptoms of deficiency in the latter animals. This is supported by the fact that a supplement of 2 μ g. of the vitamin to animals receiving the standard basal diet, which does not prevent the development of polyneuritis, also results in a decreased residual respiration compared with the symptom-free animals receiving 10 μ g. daily.

Most workers are agreed that, in the presence of glucose, lactate or pyruvate, there is a lower respiration by deficient tissues. This has been shown, for example, in pigeon's brain [Gavrilescu & Peters, 1931], in pigeon's kidney [Thompson, 1934] and in chicken's brain, heart and kidney [Sherman & Elvehjem, 1936]. In the present paper, it is clearly seen that kidney slices from deficient rats, whatever the composition of the diet, show a lower respiratory rate with pyruvate than tissues from animals receiving the same diet to which vitamin B₁ has been added.

This effect of dietary vitamin B₁ was studied a little more closely with animals receiving the standard basal diet. In addition to the daily supplements of 2 and 10 μ g. already described, some animals were given 8 μ g. and others 27 μ g. (Figs. 9, 10). On the whole, the rate of oxidation of pyruvate, both in the presence and absence of vitamin B₁ was progressively higher with increasing supplements, except that with 27 μ g. the rate was not appreciably higher than with 10 μ g.

(c) *Effect of the composition of the diet on respiration.* A comparison of the rates of respiration measured in similar conditions (presence or absence of pyruvate or vitamin B₁) of kidney slices of animals on the different diets reveals certain consistent differences. Thus, the substitution of the sucrose of the standard basal diet by protein did not appear substantially to alter the respiration. On the other hand, substitution with fat results in a higher initial respiration, which rapidly falls to values often considerably lower than those for animals receiving the standard basal or the high protein diets. If the sucrose is replaced partly by fat and partly by protein, the respiratory values observed are, as might be expected, intermediate between the two other values. This effect of dietary fat on kidney respiration contrasts sharply with the absence of effect on liver respiration, where it has been shown that diets containing large quantities of fat or protein do not change the O₂ uptake of rat's liver slices [Yudkin, 1942].

DISCUSSION

Effect of vitamin B₁ in vitro on respiration

The average increase in respiration over the 3 hr. period of study is summarized in Table 3.

Concentrating our attention on the catatorulin effect proper, that is, the increased O₂ uptake induced by vitamin B₁ in the presence of pyruvate, the main result of our experiments is that kidney slices from animals receiving no vitamin B₁ in their diet always showed a definite catatorulin effect. The extent of the augmentation in respiration depended on the diet, but it was always considerable and showed a typical increase during the course of the experiment. A daily intake of 10 μ g. vitamin B₁ entirely or almost entirely eliminated this effect with the standard basal or high protein diet but in the case of the high fat diet it only reduced it by about one-half. (The effect of dietary intake of vitamin B₁ with the protein-fat diet was not studied.)

The proportionate decrease in the metabolic defect with increasing amounts of dietary vitamin B₁, as shown in the series of animals receiving the basal diet, is interesting. Particularly is it interesting to find that the defect was still present to some extent in

Table 3. *Effect of vitamin B₁ in vitro on respiration*

Diet	Presence or absence of symptoms	Daily supplements of vitamin B ₁ μ g.	% increase in O ₂ uptake due to addition of vitamin B ₁	
			In absence of pyruvate	In presence of pyruvate
Basal	+	0	68	127
	+	2	23	50
	-	8	13	22
	-	10	7	8
	-	27	4	-1
High protein	-	0	10	75
	-	10	-1	-1
High fat	-	0	21	50
	-	10	9	23
Protein-fat	-	0	37	38

rats receiving 8 and 10 μ g. on this diet, for the condition of these rats, as far as could be seen from weight increase and lack of symptoms, was quite as good as that of animals which received 27 μ g. of the vitamin.

We do not know the reason for the persistence of the moderate metabolic defect in animals on a high fat diet receiving 10 μ g. of vitamin B₁. It may well be that higher doses of the vitamin with this diet would eliminate the defect. Studies now being made on the stores of the vitamin in the tissues of animals on the various diets may throw some light on these points. However, the point which we wish to emphasize is that a considerable catatorulin effect is shown by the kidneys of all animals deprived of the vitamin, whatever the composition of the diet, and occurs irrespective of the presence or absence of signs of deficiency. (We may perhaps reiterate that only animals receiving the standard basal diet with no added vitamin B₁ or with 2 μ g. daily develop these signs.) This observation clearly has an important bearing on theories of the mode of action of the vitamin.

Role of vitamin B₁

Let us first of all clarify the position with regard to the catatorulin effect in different tissues. For reasons which we have already stated, we chose the kidney for our experiments. Most stress, however, has been laid by other workers—notably Peters and the Oxford school—on the catatorulin effect in the brain of deficient animals. We hope for this reason to repeat this work with deficient pigeons whose brain, unlike that of rats, shows a good catatorulin effect. In our opinion, however, the emphasis on the brain as the site of the biochemical lesion has tended to give a false orientation towards the problem of vitamin B₁ deficiency.

The view of those who regard the brain lesion as of fundamental importance in the causation of the symptoms has perhaps been stated best by Peters [1940] who writes: 'It has now been proved that the nervous symptoms are due to an actual deficiency of vitamin in the brain tissue, which leads to failure of proper functioning on the part of an essential enzyme system concerned with the intermediate metabolism of carbohydrate'. We wish, however, to draw attention to the following points.

(1) The catatorulin effect in deficient animals is not confined to the brain. It has, for example, been demonstrated in the kidney of the rat, pigeon and chicken, and in the heart muscle of the chicken [Thompson, 1934; Sherman & Elvehjem, 1936; this work]. In fact, 11 years ago Fisher [1931], working in Peters's laboratory, stated: 'There is a definite general defect in lactic acid metabolism in the polyneuritic pigeon, in addition to the local defect described by Kinnersley and Peters'.

(2) The brain of the polyneuritic rat sometimes shows no catatorulin effect [O'Brien & Peters, 1935].

(3) The defect in carbohydrate metabolism in the brain can at most account for changes

in the central nervous system, such as convulsions. The peripheral neuritis found in most deficient animals must have a different cause, as of course must the oedema found in some deficient animals.

(4) The results reported in the present paper show that tissues from deficient animals may have a defective carbohydrate metabolism even though the animal itself is free from symptoms.

It seems to us much more likely that the ill-effects of deficiency are to be explained rather by the presence of some toxic substance produced during an incomplete or perverted carbohydrate metabolism than by the inability *per se* to carry out a metabolic process which, according to the theory, is essential for normal physiological function. The work described in this paper makes it clear that the metabolic disability is present in any animal deprived of the vitamin but that symptoms appear only when carbohydrate is fed. That is, it is possible for the animal to thrive even though the biochemical lesion is present: if however the animal is forced to attempt the metabolism of dietary carbohydrate, this lesion will lead to the accumulation of some toxic product which is responsible for the symptoms.

Our general conclusion, therefore, is that the explanation of the signs of deficiency of vitamin B₁ is likely to be found in the once widely held intoxication theory. What the toxic product is—whether it is methyl glyoxal as suggested by Vogt-Møller [1931], or adenylic acid as suggested by Birch & Mapson [1936], or some as yet unidentified product—we cannot from our experiments presume to judge.

SUMMARY

Earlier work has shown that rats can thrive on a diet free from vitamin B₁ if it contains a large proportion of fat or of protein. Experiments have now been carried out to determine whether the absence of the more obvious signs of deficiency, such as loss of weight, polyneuritis and bradycardia, extends to the defect in carbohydrate metabolism studied by the Peters school. The respiration of kidney slices from rats on different diets was determined in the presence and absence of pyruvate and the effect observed of the addition *in vitro* of vitamin B₁. It was found that the 'specific metabolic defect', that is the defective oxidation of pyruvate which is restored by the addition of vitamin B₁ to the respiring tissue, could be demonstrated in all animals deprived of the vitamin, whatever the composition of the diet and irrespective of the presence or absence of other signs of deficiency. The bearing of this on current theories of the mode of action of vitamin B₁ is discussed.

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APPENDIX

		Q_{O_2} in presence of											
		O			Vitamin B ₁			Pyruvate			Pyruvate and vitamin B ₁		
Hour of observation ...	Diet	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Exp. no.													
Standard basal, no supplement	1	10.0	6.5	4.0	21.1	14.4	9.5	11.0	6.7	4.3	14.6	11.3	8.4
	2	14.0	9.3	6.2	16.8	11.9	7.3	9.5	6.8	3.7	14.8	14.4	12.8
	3	10.9	7.2	5.0	16.1	13.8	11.2	9.8	6.7	3.4	23.1	22.4	18.5
	4	—	—	—	—	—	—	10.6	7.3	4.7	19.6	16.8	11.4
	Mean	11.6	7.6	5.1	18.0	13.4	9.4	10.1	6.7	3.8	17.5	16.0	13.3
Standard basal + 10 µg. vitamin B ₁ daily	5	13.1	10.7	8.4	12.9	11.2	9.2	20.6	15.6	11.0	20.4	16.1	11.9
	6	16.3	13.5	11.0	16.5	13.6	11.0	33.5	29.7	25.0	33.6	29.7	24.6
	7	17.7	12.3	10.7	19.3	15.0	12.9	29.7	25.3	18.0	30.5	30.2	27.7
	Mean	15.7	12.2	10.0	16.3	13.3	11.0	27.9	23.5	18.0	28.1	25.3	21.4
High protein, no supplement	8	17.4	12.6	9.4	20.0	15.0	11.5	18.7	10.8	7.0	23.9	22.4	16.3
	9	16.9	11.6	9.7	19.6	14.3	9.9	17.9	13.2	8.6	26.5	23.3	20.8
	10	18.9	13.9	10.3	19.7	15.3	11.7	18.5	10.2	5.1	25.1	22.4	17.0
	11	16.1	13.2	11.0	16.3	13.1	11.2	15.9	11.7	7.0	20.3	20.0	15.0
	Mean	17.3	12.8	10.1	18.8	14.4	11.0	17.7	11.5	6.9	24.0	22.0	17.2
High protein + 10 µg. vitamin B ₁ daily	12	17.3	13.3	10.7	16.6	12.3	10.7	24.1	20.3	13.4	23.9	19.8	13.5
	13	18.4	12.7	10.9	18.2	12.3	10.5	33.7	28.3	24.1	32.9	27.7	23.7
	14	20.0	14.1	11.0	20.2	14.0	11.2	28.6	24.4	20.6	28.8	24.5	21.2
	Mean	18.5	13.4	10.8	18.3	12.9	10.8	28.8	24.4	19.4	28.5	24.0	19.5
High fat, no supplement	15	17.0	5.8	3.1	19.1	7.9	5.1	16.0	6.0	3.2	20.4	8.9	6.1
	16	18.2	7.6	4.7	19.4	10.6	6.2	15.3	7.3	4.2	20.5	12.2	7.5
	17	15.2	8.0	5.4	18.4	10.1	6.5	13.5	6.0	3.0	19.5	10.4	6.5
	18	—	—	—	—	—	—	14.4	7.1	3.7	21.9	10.7	5.5
	19	—	—	—	—	—	—	14.6	6.7	4.3	19.5	9.8	5.3
	Mean	16.8	7.1	4.4	18.9	9.5	5.9	14.9	6.4	3.5	20.1	10.5	6.7
High fat + 10 µg. vitamin B ₁ daily	20	14.6	7.6	4.8	17.9	8.2	5.2	27.6	12.8	6.3	30.1	17.3	8.8
	21	14.9	6.5	3.8	14.5	6.5	4.0	24.6	10.3	5.4	26.9	14.4	6.4
	22	14.4	7.1	3.5	14.6	8.0	4.9	25.3	10.1	5.2	29.1	15.3	7.5
	Mean	14.6	7.1	4.0	15.7	7.6	4.7	25.8	11.0	5.6	28.7	15.7	7.6
Protein-fat, no supplement	23	15.2	9.2	6.6	22.0	15.7	8.9	27.2	15.2	7.2	35.4	24.1	15.2
	24	18.3	11.7	8.3	21.1	14.7	11.0	26.6	15.7	8.8	28.8	20.8	14.6
	Mean	16.7	10.4	7.4	21.6	15.3	10.0	26.9	15.5	8.0	32.1	22.5	14.9
Standard basal + 2 µg. vitamin B ₁ daily	25	14.9	9.4	5.0	17.4	10.8	6.2	16.6	10.7	7.2	21.9	17.0	13.0
	26	15.6	12.5	8.3	16.5	14.3	10.4	23.8	15.7	8.4	28.5	22.3	15.7
	27	13.4	8.4	5.3	16.3	13.3	9.6	13.9	7.9	4.7	19.3	15.7	11.8
	Mean	14.7	10.1	6.2	16.7	12.8	8.7	18.2	11.5	6.9	23.2	18.3	13.5
Standard basal + 8 µg. vitamin B ₁ daily	28	16.3	11.6	9.3	16.3	11.7	9.8	16.1	11.4	9.1	16.4	11.8	9.5
	29	17.0	14.4	11.5	18.8	17.1	14.2	16.2	12.2	7.5	21.3	19.6	15.9
	30	15.3	13.5	11.2	19.8	16.1	12.4	25.0	23.5	20.0	26.3	26.0	25.3
	Mean	16.2	13.1	10.7	18.3	15.0	12.1	19.1	15.7	12.2	21.3	19.1	16.9
Standard basal + 27 µg. vitamin B ₁ daily	31	20.4	13.1	10.6	22.6	14.2	10.8	32.9	29.3	23.6	31.4	29.2	23.2
	32	15.9	10.6	9.9	15.9	10.5	10.0	21.2	15.5	11.5	20.6	15.8	12.5
	33	14.0	8.6	6.1	14.2	8.9	6.4	22.7	18.0	14.5	22.3	18.1	14.3
	34	—	—	—	—	—	—	29.7	29.0	24.3	31.3	29.2	25.2
	Mean	16.8	10.8	8.5	17.6	11.2	8.7	25.6	20.9	16.5	24.8	21.0	16.7