39. ADENYLIC DEAMINASE ACTIVITY IN B₁-AVITAMINOSIS AND IN STARVATION

BY GWEI DJEN LU AND DOROTHY MOYLE NEEDHAM

From the Biochemical Laboratory, Cambridge

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In our previous studies of carbohydrate metabolism in vitamin B₁ deficiency [Lu, 1939] it was shown that the high pyruvate level of the blood, though running closely parallel with the bradycardia, could not be regarded as directly responsible for it. Other possible causes therefore demanded consideration.

Two series of observations led us to examine the adenylic deaminase activity of pigeon breast muscle during B₁-avitaminosis. In the first place, Birch & Mapson [1936] comparing hearts of normal and B₁-deficient rats, had reported a decrease of about 20 % in the deaminase activity of the deficient hearts; 18 hr. after administration of the vitamin, the hearts of the recovered animals showed normal activity. These authors were in fact interested in the phenomenon from the point of view of the causation of the bradycardia of deficient animals, which resembles the bradycardia caused by injection of adenylic compounds into normal animals. Secondly, Bodnar & Karell [1932], using fluoride-poisoned brei from breast muscle of pigeons deficient in the B complex, had observed greater power than normal of accumulating phosphoric esters. Similar observations were made by Nagai [1932]. Since the presence of adenylic compounds is essential for ester formation, the greater production in the deficient animals might depend upon the higher concentration of adenylic compounds, and might be regarded as an indication suggesting loss of deaminase activity when the vitamin is absent. From the point of view both of the possible connection between adenylic acid and bradycardia, and of the disturbances in deficient muscle metabolism, it seemed of interest to study deamination of adenylic acid and phosphorylation of carbohydrate in skeletal and heart muscles of deficient animals.

Changes in adenylic deaminase activity in pigeon breast muscle

In order to measure the activity of the enzyme, a small portion of breast muscle (0.5–1.0 g.) was weighed, then ground in an ice-cold mortar with 10 vol. of ice-cold *M*/10 phosphate at pH 7, a few grains of Merck's quartz being added. After standing 30 min. at 0° for extraction of the enzyme, the extract was strained through muslin. In order to obviate uncertainties due to possible synthesis of added adenylic acid to adenylypyrophosphate (owing to any carbohydrate breakdown in the extract) the extract was always dialysed at 0° against 0.5 % KCl overnight before use; no change in volume took place during this dialysis. The activity of the extracts was tested in the following way: samples containing 1.0 ml. extract, 1.0 ml. phosphate buffer (0.16 *M*, pH 6.5), 1.0 ml. adenylic acid (neutralized, and containing 3 mg. adenylic acid) and 2.5 ml. water were mixed, and incubated for 20 and 40 min. at 37°. The NH₃ formation was estimated by the distillation method of Parnas & Heller, followed by nesslerization. The dialysed extracts were diluted before use in the tests, usually 1:10. The NH₃ production observed in the tests was therefore multiplied by 100 to obtain the production per g. muscle.

Preliminary experiments showed that the optimum pH lay at 6.5, and this pH was therefore always used.

In the earlier experiments, only one test of deaminase activity was performed on each bird, which was killed at the time of the removal of the muscle. These results, while definitely lower on the average in the deficient birds than in the normal, showed considerable variation within the two groups. The method of biopsy was therefore later adopted; a piece of muscle (30–60 mg.) was removed from the breast under ether anaesthesia, and the small wound sewn up. The birds recovered well. Not more than three operations were carried out on the same bird. Control experiments showed that pieces of muscle removed at intervals of some days from a bird kept on normal diet showed no variation in deaminase content; and further that the deaminase activity was the same whether the muscle was removed under ether, or without ether when the animal was killed. The weighed fragment of muscle was in each case extracted with 100 vol. of M/10 phosphate; this extract was used in the tests without further dilution.

The deficient diet usually consisted of thoroughly washed polished rice plus one drop of radiostoleum per day. The control birds received the same, together with 15 I.U. of vitamin B₁ each per day. When the deficient birds were to be recovered, they were usually put on to full stock diet (mixed pigeon corn) as well as receiving the amounts of B₁ recorded. The controls were put on to stock diet at the same time. In some cases, the effect of giving B₁ injections to the deficient birds, while continuing the rice diet, was tried. In some later experiments, as is pointed out in the appropriate places, a modified Carter diet was used. This consisted of 15 % Glaxo, 75 % rice starch, 5 % fresh butter and 5 % salts. It was supplemented by 0.5 g. autoclaved marmite, and one drop of radiostoleum every second or third day. This diet is not readily eaten by pigeons; but if they are provided with no other food, and if they receive B₁, they will accept it after a few days. All the birds in an experiment were therefore given B₁, until they were eating the diet well and maintaining weight. Then B₁ was removed from the diet of those destined for deficiency. The degree of this deficiency was judged (a) by onset of head retraction; this occurred fairly regularly in the earlier experiments, but much more variably later; (b) by heart rate; this symptom also is well known to be very unreliable in pigeons; (c) by the great loss of weight and weakness of the birds. With regard to the heart rate, Dr L. J. Harris, to whom we are indebted for much valuable advice, informed us that using the modified Carter diet he has succeeded in getting the heart rate reliably down, and up again with B₁; on this diet he found head retraction (which is probably a symptom of chronic B₁ deficiency) much rarer; the birds may die from heart failure in a shorter time and before head retraction comes on [Harris, 1939].

The curves in Figs. 1 and 2 show the results of the earlier series of experiments. It can be observed that the normals form a group with a considerably higher average than the deficient birds. The results for the recovered birds show an even wider scatter, and their average is intermediate between that of the other two groups.

In the later work, a series of experiments was done on each bird, muscle being removed at different stages of deficiency or during recovery.

Since B₁-avitaminosis leads to loss of appetite, great reduction in food intake and very serious wasting of the muscle, it was necessary to find out whether low diet plus B₁ led to change in deaminase activity of the muscle. Control birds were therefore used, paired with the deficient birds, so that each control received only as much food as the deficient partner had eaten on the previous day. The controls received 15 I.U. B₁ by injection per day. The importance of paired

feeding is now becoming generally recognized in nutritional investigations [cf. McDonald & McHenry, 1939; Follis *et al.* 1940].

Fig. 1.

Fig. 1. All birds on polished rice diet. — Normal. ---- Deficient.

Fig. 2.

Pigeon
no.

Notes on deficient birds

- 16 Marked head retraction.
- 17 Marked head retraction. Heart rate 140.
- 18 Head retraction. Heart rate 195.
- 31 Marked head retraction.
- 32 Marked head retraction.

Fig. 2. All birds on polished rice diet till markedly deficient. Transferred to stock diet when B_1 was administered.

Pigeon
no.

Notes on recovered birds.

- 28 Heart rate 240. 1 mg. B_1 per day given for 2 days and killed on the 3rd day. Heart rate 480.
- 29 Heart rate 245. 4 doses of B_1 given. Heart rate rose to 420 on the 2nd day; on the 5th day it was 220. Killed 5th day.
- 30 Heart rate 175. 1 dose of 0.3 mg. B_1 . Killed next day. Heart rate 300.
- 19 Severe head retraction. 0.3 mg. B_1 per day for 4 days. Killed 5th day. Heart rate 285.
- 23 Received 2 mg. B_1 . Killed next day. Heart rate 240.
- 24 Heart rate 240. Received 1 mg. B_1 each day for 5 days. Killed 6th day. Heart rate 450.
- 34 Head retraction. 0.3 mg. B_1 each day for 2 days. Killed 3rd day. Heart rate 280.
- 35 Received 0.3 mg. B_1 . Interval of 1 day. Then 1 mg. a day for 2 days. Killed next day. Heart rate 180.
- 33 Marked head retraction. 1 mg. B_1 per day for 2 days. Killed 3rd day. Heart rate 310.
- 36 Heart rate 154. 0.3 mg. B_1 . Interval of 2 days. Then 1 mg. per day for 4 days. Killed next day. Heart rate 430.

The results show in every case a marked fall in deaminase activity in the same bird when examined before and after production of avitaminosis (Figs. 3-8 and 10-11). In every case of semi-starvation plus B_1 administration, a marked fall was also found; this fall was not so great as in the deficient birds but neither

Fig. 3. No. 38.

Fig. 4. No. 42.

Fig. 5. No. 43.

Fig. 6. No. 44.

Fig. 7.

Fig. 8.

In Figs. 3-11 — indicates body weight; --- indicates deamination rate;
H.R. indicates heart rate.

was the loss of weight so great, in spite of the fact that the food intake was the same (Figs. 7-11).

This suggests that the loss of deaminase activity depends rather on the starvation condition than on the absence of B_1 . The results of experiments on recovery substantiate this view (Figs. 3-6, 9, 10). For although return of deaminase activity is sometimes seen in these experiments after administration

Fig. 9.

Fig. 10.

Fig. 11.

of B_1 , it occurs to a marked extent only when the animal is rapidly recovering weight. If for example B_1 is given but the rice diet is continued, there is usually a rise in heart rate and general improvement in the condition of the animal; but after an initial rise the weight may remain stationary or fall slightly again. If, on the other hand, the administration of B_1 is accompanied by a change to the stock diet, there is rapid increase in weight, which may return to normal in about 4 days. It is only under the latter conditions that marked recovery of deaminase activity has been observed, and even under these conditions there

were cases where the deaminase activity continued to fall while the muscle weight was being rapidly regained. This question is further discussed later.

In a few cases the B₁ content of the muscle and liver tissue was estimated by the thiochrome method.

	Liver μg. B ₁ per g.	Muscle μg. B ₁ per g.
No. 49 (killed in deficient state)	0.06	<0.03
51 (semi-starved but receiving B ₁)	2.3	1.1
56 (semi-starved but receiving B ₁ ; last dose day before death)	3.4	2.5

These results show that, in spite of the great wastage of tissue going on, the animals receiving B₁ retain it in their organs.

Changes in phosphorylation activity in pigeon breast muscle

The results of Bodnar & Karell [1932] on increased esterification of phosphate in B₁-deficient muscle were confirmed. We used the same conditions as theirs, except that the samples were kept at 37° for 40 min. instead of for some hours at room temperature. 1 g. brei was treated with 0.5 ml. 1 % Na₂HPO₄; 0.5 ml. 2 % NaF and 15 mg. glycogen. The mean of eight experiments on normal birds gave a value of 0.72 mg. P esterified in 40 min. (limits 0.4–1.0); while the mean for nine deficient birds was 0.98 mg. (limits 0.7–1.3). These differences are not so large or so constant as those found by Bodnar & Karell—their averages were 0.70 mg. for normal and 1.25 mg. for deficient animals.

In the brei inorganic P is disappearing, both by the Parnas esterification of glycogen with formation of hexosemonophosphate, and the 'coupled' esterification via adenylic acid with formation of hexosediphosphate. Some experiments were done to see whether the phosphorylase system showed different activities in the two cases. Muscle extract was made by crushing the muscle with an equal volume of ice-cold water; in order to obtain esterification by the phosphorylase system alone two methods were adopted:

(1) The extract was allowed to stand 24 hr. before use.

(2) The fresh extract was incubated with *M*/250 iodoacetic acid for 15 min. before addition of substrate, to bring about inhibition of the 'coupled' mechanism.

In both cases, to 1 ml. of extract were added 0.5 ml. 0.33 *M* phosphate at pH 7.0 and 15 mg. glycogen.

The average result for five extracts from normal birds by the first method was 0.58 mg. P esterified per ml. in 45 min.; for six deficient birds the average was 1.05 mg. By the second method the average for four normal birds was 0.66 mg., for five deficient birds 0.89 mg.

It thus appears that phosphorylase activity is enhanced to some extent in the B₁-deficient animals. In this connexion it is interesting that although muscular weakness and dystrophy exist also in vitamin E deficiency, the phosphate esterification is there distinctly decreased [Lu *et al.* 1940].

Adenylic deaminase and vitamin B₁ in vitro

Before it became likely that the connexion between the enzyme and the vitamin was an indirect one, we made some tests to find whether addition of the vitamin or cocarboxylase to the deficient extract would restore the deaminase activity to normal. But although we tried many experimental variations, we were never able to observe any such activating effect.

Changes in deaminase activity in rat leg muscle

Similar experiments to those on the pigeon breast muscle were carried out on rat leg muscle. In each case all the muscles of one leg were removed, chopped and well mixed, before a portion was weighed out. The results are given in Table 1.

Table 1. *Change in deaminase activity of normal and deficient rat leg muscle*

	No.	Heart rate	Deamination mg. NH_3N set free by 1 g. muscle in 40 min. at 37°
Normal	II	—	2.24
	XIII	534	2.85
	XIV	500	2.25
	XV	540	3.60
	XVI	520	3.70
	XVII	540	3.35
	XVIII	570	3.63
	XXVII	490	2.17
	XXVIII	520	2.37
		Average	2.90
Deficient	III	—	3.36
	IV	—	2.35
	V	375	2.34
	VI	300	2.09
	VII	385	2.27
	VIII	320	2.03
	XIX	—	2.12
	XX	—	2.40
		Average	2.37
Recovered	IX	475	2.85
	X	—	1.95
	XI	490	1.89
	XII	530	3.03
		Average	2.43

It is obvious that the decrease in deaminase activity is not nearly so marked as in pigeon muscle; on the average it does not exceed 20 %. It is to be observed that even this small difference between the normal and the deficient depends on the high values in rats nos. XV–XVIII, all of which belonged to one group.

Adenylic deaminase activity in rat heart muscle

In a number of the rat experiments just described, the deaminase activity of the hearts was also tested, and to our surprise, no significant difference between the three groups could be detected. A series of experiments was therefore carried out, using similar conditions to those of Birch & Mapson [1936], to whom we are indebted for information about their experimental details. The heart was weighed and ground with quartz and 10 vol. of ice-cold water in a cooled mortar. After extracting at 0° for 30 min., the extract was strained off through muslin and allowed to stand at room temperature for 2 hr. in order that any glycolysis in the extract might be completed. 1 ml. of the extract was then mixed with 1.2 ml. *M*/6 phosphate buffer pH 7.0, 0.2 ml. neutralized solution containing 6 mg. adenylic acid, and 3.6 ml. water. The samples were incubated at 37° for 20 and 45 min. (Table 2). The difference is only 7 %.

Table 2. *Changes in deaminase activity of normal and deficient rat heart muscle*

	No.	Heart rate	Deamination mg. $\text{NH}_3\text{-N}$ set free by 1 g. heart in 45 min. at 37°
Normal	33	550	0.68
	34	515	0.62
	42	500	0.73
	43	520	0.71
	46	480	0.64
	47	500	0.62
	54	510	0.52
	53	480	0.60
		Average	0.64
Deficient	35	410	0.64
	36	390	—
	37	380	0.77
	38	390	0.65
	39	314	0.65
	40	130	0.74
	41	220	0.73
	44	320	0.66
	45	350	0.70
	55	280	0.68
	52	300	0.76
		Average	0.69

The effect of ions on the adenylic deaminase

While this work was in progress a paper by Conway & Cooke [1939] appeared in which the powerful effect of certain anions, notably phosphate and carbonate, in inhibiting the activity of the deaminase at low concentrations of substrate was described. It seemed necessary, therefore, especially as our substrate concentrations were low (0.06 %), to include examination in citrate buffer in determining the activity of the enzyme. Extra samples were set up in which the $M/30$ phosphate buffer was replaced by $M/20$ citrate. With pigeon muscle it was found that deamination is about 3.5 times as fast in citrate buffer under our conditions; with rat muscle, about 8.5 times as fast. The effect of the citrate buffer appeared to the same extent with both normal and deficient muscle extracts. Conway & Cooke suggest that the effect of the phosphate ion may be explained by assuming adsorption of the anion on the enzyme with exclusion of the adenylic acid, which would agree with the diminished inhibitory effect found by them with increasing substrate concentration in rabbit muscle extract. Similar results were obtained by us on rat muscle extract. In citrate buffer, using 1 : 1250 dilution of muscle extract, at 0.06 % adenylic acid concentration, 9.28 mg./g. $\text{NH}_3\text{-N}$ were set free in 10 min.; with 10 times the substrate concentration, 0.6 %, the NH_3 formation was approximately doubled—21.0 mg./g. In phosphate buffer, on the other hand, increasing the substrate concentration ten-fold led to a nearly twenty-fold increase in deamination. At 0.06 % adenylic acid, the $\text{NH}_3\text{-N}$ set free was 1.1 mg./g. in 10 min.; at 0.6 % substrate, 18.65 mg./g.

It may be noticed that in citrate buffer, where presumably the enzyme is maximally active, there is little difference between the deamination rates of pigeon and rat muscles; in the rat muscle extract the enzyme shows a greater degree of inhibition by phosphate.

The rates of deamination in rat heart muscle were also compared in phosphate and citrate buffers. Very little difference was found.

	NH ₃ -N mg./g.	
	In phosphate	In citrate
Normal heart	0.68	0.74
Deficient heart	0.62	0.58

The bulk of the heart consists, of course, of ventricle. According to Conway & Cooke [1939] ventricular tissue in the rabbit contains an adenosine deaminase but little if any true adenylic deaminase, although the latter is present in the auricular tissue. The adenosine deaminase was found by them not to be inhibited by phosphate. The fact therefore that the rat heart shows little difference of activity in different buffers suggests that here too an adenosine deaminase may be concerned. Conway & Cooke propose two methods whereby it may be tested whether adenylic acid is directly deaminated, or only after initial dephosphorylation.

(1) Adenylic deaminase of animal tissues seems to be specific for muscle adenylic acid, but if the deamination is indirect, by means of adenosine deaminase, the rates of deamination of yeast and muscle adenylic acids will be the same (assuming the rates of dephosphorylation to be the same). This method was tried on rat heart, but it was found that yeast adenylic acid was practically untouched. This result is not conclusive for the presence of true adenylic deaminase, as it may only mean that the rates of dephosphorylation differ in this case.

(2) The NH₃ set free after addition of adenosine to saturate the adenosine deaminase may be compared with the amounts set free after addition of adenosine and adenylic acid together.

The following experiment was performed. The extract was made as usual from normal hearts. The adenosine solution contained 12.5 mg./ml.; the adenylic acid solution 30 mg./ml.; the buffer was *M*/6 phosphate, pH 7.0.

	1	2	3	4	5
Extract	1.0	1.0	1.0	1.0	1.0
Buffer	0.6	0.6	0.6	0.6	0.6
Adenylic acid	0.2	0	0	0.2	0.2
Adenosine	0	0.4	0.8	0.4	0.8
NH ₃ formation, mg. N/g. tissue in 20 min.	0.029	0.057	0.054	0.054	0.054

These results show that adenosine deaminase is present and saturated with substrate under the conditions used, for doubling the concentration of adenosine leads to no increase in NH₃ formation. That a separate enzyme for deamination of adenylic acid is absent, is shown by the fact that addition of adenylic acid to the system has no effect on the liberation of NH₃.

DISCUSSION

In this work it has been established that during the inanition accompanying vitamin B₁ deficiency in the pigeon, skeletal muscle suffers a loss in adenylic deaminase which may be as great as 75 %. In two or three cases it was even 90%. That the changes cannot be due to changes in water content of the muscle is shown by the results of Roche [1924], who found the water content of normal pigeon breast muscle to be 74.8 %, of avitaminotic birds (on polished rice diet) 73.6 % and of birds in inanition 75.3 %.

The facts that injection of vitamin B₁ leads to no rapid increase in deaminase activity, that the fall occurs on a low diet even if vitamin B₁ is injected and

that vitamin B₁ has no activating effect on the enzyme of the deficient extract *in vitro*, all indicate that the vitamin is not playing here any sort of coenzyme role, as its pyrophosphate does in the oxidation of pyruvic acid.

It may be interesting to compare the effect of vitamin B₁ deficiency on deamination with its effect on respiration. Roche [1924] found that respiration of the isolated muscle tissue was the same in normal, deficient and starved birds, and he did not observe any fluctuations due to rice in the crop, etc. Galvao & Pereira [1937] found that breast muscle tissue from vitamin B₁-deficient pigeons may have a very low respiration, even after administration of B₁ to the bird. On the other hand, the respiration of the muscle from the recovered animals might rise to normal, and this return seemed to be correlated with the ingestion of food, or with the fact that the birds, in becoming deficient, had not lost excessively in weight. The suggestion is that the intensity of tissue respiration observed depends not only on the presence of the vitamin, but also on availability of substrate.

The same conclusion is reached by Lipschitz *et al.* [1938] in their work on the pyruvate oxidation by brain, kidney and liver, in vitamin B₁-deficient chicks. They found that the ability of kidney, liver and brain tissue from polyneuritic birds to oxidise added pyruvate was impaired. The feeding of glucose to the deficient birds led to a rise in the power of the liver to remove pyruvate, though normal values were not reached. The liver and kidney tissue from fasted birds also showed a decreased power to remove pyruvate, and the oral administration of glucose to the fasted birds led to restoration of the ability of the liver tissue to remove pyruvate. Thus it seemed that both with polyneuritic and fasted birds, the provision of some other substrates (derived from glucose) was involved in the pyruvate removal. In the fasted birds, the provision of these substrates seemed to be the only factor necessary; with the polyneuritic birds, complete restoration of the power to remove pyruvate was not attained with glucose only.

In the case of deamination it seems that the explanation of the loss of deaminase activity is not loss of coenzyme (see above) or lack of metabolites, for in all cases the activity is tested in greatly diluted dialysed extracts; the suggestion arises that the enzyme is actually lost from the tissue. In the great loss of material that goes on from the muscle, all the protein fractions may not be involved in exact proportion to their concentration in the muscle; it would not be surprising if, in the substance broken down and removed, some enzymes were involved more than others. That some enzymes escape destruction to a disproportionate degree is suggested by Nagai's finding [1932] that glycerophosphatase activity is greater by about 20 %, and hexosediphosphatase activity by about 40 % in the deficient muscle. But phosphate metabolism in a muscle *brei* is so complex that a number of factors may be influencing the accumulation of phosphate in these cases just as they may be influencing esterification of phosphate in the phosphorylation experiments mentioned earlier.

If the protein of the deaminase enzyme has to be resynthesised after recovery, the conditions governing its reappearance in full activity may be very complex. The muscle might increase greatly in weight and in protein content, without proportionate formation of the enzyme, and this state of affairs might account for the cases (such as 54 and 55, Fig. 10), where, after administration of the vitamin and stock diet, there is rapid increase in weight accompanied by still further fall in deaminase activity.

The function of the adenylic and adenosine deaminases in general, and their relation to the regulation of heart beat in particular, are obscure. Their function, especially that of the very active adenylic deaminase in skeletal muscle, has

often been regarded as one of detoxication, and Birch & Mapson [1936] regarded even a 20 % decrease in the activity of the heart enzyme as a possible explanation of the bradycardia of B_1 deficiency. As we have found, however, this fall in heart deaminase activity does not always accompany B_1 -avitaminosis, even when the bradycardia is as well marked as usual; and, moreover, the heart rate may return to normal while the deaminase of the skeletal muscles does not do so (see e.g. Figs. 3 and 4).

Conway & Cooke [1939] have pointed out that in spite of the deaminases of the tissues, an adenine nucleotide (other than muscle adenylic acid) exists in blood plasma, to the extent of about 5–10 mg./100 ml.; red blood cells contain adenylypyrophosphate and adenosine, and are freely permeable to the adenylic acid which is probably formed there. There is no evidence as to the concentrations of adenylic acid compounds normally reaching the sensitive sites of the heart, or what increase in concentration is necessary at these sites for the effects of bradycardia to be produced.

Conway & Cooke have remarked that the effective deamination rate in skeletal muscle is 500–1000 times that in any other tissue. NH_3 is also formed in skeletal muscle from adenylic acid 20 times as fast as it is from adenosine by the tissues with the most active adenosinase (appendix, jejunum). It seems that considerable quantities of adenine nucleotides must escape from the intestine into the blood; and if this is the case it becomes difficult to regard the skeletal deaminase as a mechanism to protect the rest of the body from effects of adenylic acid. If the adenylic deaminase has some function within the muscle itself, this is at present unknown.

SUMMARY

1. Muscle tissue of animals in vitamin B_1 deficiency shows a diminution of the activity of adenylic deaminase. This is especially marked in pigeon breast muscle, less so in rat leg muscle. Differences between these animals in the absolute levels of their deaminase activities may be largely due to ionic inhibition effects as discussed in the text. Rat heart muscle deaminates adenylic acid indirectly. The latter is first dephosphorylated, and then NH_3 formation is caused by action of adenosine deaminase. No difference was observed in rate of NH_3 liberation between normal and deficient heart muscles.

2. Decrease in the adenylic deaminase activity is accompanied by some increase in phosphorylase activity in the deficient muscle.

3. Paired feeding experiments with inanition unaccompanied by vitamin B_1 deficiency show, however, that the diminution of deaminase activity is primarily connected with the starvation, and only secondarily with vitamin deficiency. The return to normal is slow, requires normal food intake and cannot be brought about by vitamin B_1 alone.

4. Bradycardia, on the other hand, gives place rapidly to normal heart rate as a result of administration of B_1 alone. It seems unlikely, therefore, that the bradycardia is due to accumulation of adenylic acid following the decreased deaminase activity.

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