THE DISAPPEARANCE OF PYRUVIC DECARBOXYLASE AND α-KETOGLUTARIC DECARBOXYLASE FROM PIGEON MUSCLES ON THIAMINE-DEFICIENT DIETS*

by

C. H. MONFOORT

Laboratory for Physiological Chemistry, The University, Utrecht (Netherlands)

The aim of many investigations carried out in this laboratory is to find quantitative relations between the stage of thiamine deficiency and the occurrence of thiamine-deficiency symptoms on the one hand and the disappearance of thiamine pyrophosphate (TPP) and the TPP dependent enzymes from the tissues on the other hand. By determining this coenzyme and these enzymes in various tissues in the course of the time during which animals are confined to a thiamine-free diet we hope to contribute to our knowledge of the development of the symptoms and the immediate cause of death.

We have also included in this programme investigations on the so called thiamine-sparing action of fat. According to several authors, symptoms and death occur much earlier when a thiamine-free diet contains a high percentage of carbohydrate than when the carbohydrate in the diet is replaced by fat. According to EVANS AND LEPKOWSKY[1], who have coined the expression "vitamin B_1 sparing action of fat", rats on a thiamine-free high fat diet grow much better than rats on a thiamine-free high carbohydrate diet. As pointed out by WESTENBRINK[2] and by GRUBER[3] "sparing" may in this connection mean "to use less of" as well as "to dispense with".

GRUBER[4] has proved that on a diet rich in fat the organism uses up its store of TPP at a lower rate, for the amount of TPP in the tissues diminishes much more rapidly on the thiamine-free high carbohydrate diet than on the thiamine-free high fat diet (experiments with liver, cerebrum, breast muscle and heart muscle of the pigeon). Moreover GRUBER has shown that, immediately after death induced by withholding thiamine, in animals consuming the carbohydrate diet the TPP content of heart muscle and liver is lower, while the TPP content of breast muscle and cerebrum is higher than in animals receiving the fat diet. In these experiments the pigeons on the carbohydrate diet died in the mean after 16 days of thiamine deficiency, those on the fat diet after 43 days.

We ourselves[5] have reported already on our experiments on the disappearance of the pyruvic decarboxylase and the α-ketoglutaric decarboxylase** from breast muscle, heart

* This work forms part of the investigations on the metabolism and physiological action of thiamine by H. G. K. WESTENBRINK and collaborators.

** We have called the enzymes responsible for the anaerobic decarboxylation of pyruvate and α-ketoglutarate: decarboxylases. We might also have designed them by pyruvic dehydrogenase and α-ketoglutaric dehydrogenase, for the purest preparations of these enzymes prepared from animal material (pigeon breast muscle and pig heart respectively), which are homogeneous both in the electrophoretic and in the gravitational field, are active in dehydrogenation as well as in straight

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muscle and leg muscle of pigeons during the first 12 days on the same diet, free of thiamine and rich in carbohydrate, as was employed by GRUBER. We have now continued these experiments over longer periods of deficiency and have carried out similar determinations in breast muscle and heart muscle of pigeons on the diet free of thiamine and rich in fat, thus extending our own work as well as that of GRUBER (breast muscle and heart muscle belong to the two groups of organs that are distinctly different as regards the TPP content at the time of death; see above). This work has also been carried out with pigeons, as they are very suitable for forced feeding. Animals soon lose their appetite on a thiamine free diet. Therefore the difference between a carbohydrate diet and a fat diet is less distinct when the animals are allowed to eat *ad libitum*.

The pyruvic decarboxylase was determined by measuring the amount of acetoin formed from added pyruvate in a muscle homogenate under anaerobic conditions, the α-ketoglutaric decarboxylase by measuring the amount of succinic semialdehyde formed from added α-ketoglutarate under similar conditions.

EXPERIMENTAL

The normal pigeons consumed *ad libitum* a mixture of cereals (this is called diet A). The thiamine-free diets B and C contained sucrose and an isocaloric amount of pea nut oil respectively. The pigeons confined to these diets were forcibly fed through a glass tube which was introduced into the stomach. A pigeon on diet B received 2 g casein, 18 g sucrose, 0.3 g of a salt mixture, and 0.09 g of a vitamin mixture daily, a pigeon on diet C 2 g casein, 8 g pea nut oil, 0.3 g of the salt mixture and 0.09 g of the vitamin mixture. The salt mixture was composed according to JANSEN AND WESTENBRINK[6], the vitamin mixture contained all known vitamins (in the purest form obtainable) with the exception of thiamine (see GRUBER[4]).

After a varying number of days on these diets, the pigeons were killed by decapitation. Immediately after death part of the large breast muscle (m. pectoralis major) and the heart were excised and cooled in an isotonic phosphate solution of pH 7.4. The preparation of the homogenates was carried out in the cold room (2° C). 2 g of the breast muscle and 2 g of the left ventricle of the heart were homogenized in 18.4 ml of 0.1 *M* potassium sodium phosphate buffer, pH 6.2, containing 0.001 *M* $MnCl_2$, with a homogenizer as described by CAMPBELL *et al.*[7]. With this homogenizer the tissues were perfectly homogenized in 1 minute. During this time the tube in which the process took place was cooled with ice and water, so that the temperature of the homogenate never increased to more than 4° C. The pH remained constant during this procedure.

2 ml samples of the homogenates were pipetted into the main compartment of cooled Warburg flasks. The center well contained a piece of phosphorus in order to remove the last traces of oxygen, still present after gassing with the purest quality of nitrogen. 2.5 mg of sodium pyruvate or 3.9 mg of sodium α-ketoglutarate were tipped in from the side bulb. After 3 hours at 37.5° C 5 ml of a 10% metaphosphoric acid solution were added to the reaction mixtures in the Warburg flasks. After centrifuging the determination of acetoin or succinic semialdehyde was carried out in the clear supernatants.

Acetoin was determined according to WHITE, KRAMPITZ AND WERKMAN[8], succinic semialdehyde in the presence of α-ketoglutarate according to a method based on the principle published by OCHOA[9] (see MONFOORT[10]). The production of CO_2 and the amounts of pyruvate and α-ketoglutarate utilized were also determined, but these data have no importance in connection with the problem discussed in this paper. Thiamine pyrophosphate was determined according to WESTENBRINK AND STEYN-PARVÉ[11]. The sodium pyruvate was a preparation purchased from Hoffmann-La Roche (97% pure),

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decarboxylation and are generally called dehydrogenases in view of the preponderance of dehydrogenation in metabolism. However, it has not yet been decided whether the same prosthetic group is responsible for both actions. REED AND DE BUSK[15] have obtained strong evidence in support of the view that in *E. coli* TPP would be responsible for anaerobic decarboxylation and lipothiamide pyrophosphate for aerobic decarboxylation and dehydrogenation. Though the animal dehydrogenase preparations mentioned above contain lipoic acid, it has not yet been demonstrated that it is combined with TPP to form lipothiamide pyrophosphate. Therefore it is possible that in muscle the same TPP molecule combined with the enzyme protein is acting under aerobic as well as under anaerobic conditions. At any rate, as these questions have not yet been decided, we believe it is advisable to speak about decarboxylases in connection with our experiments.

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the α-ketoglutaric acid a preparation from the Nutritional Biochemicals Corporation, Cleveland (at least 98 % pure). Racemic acetoin (95 % pure) was prepared according to DIELS AND STEPHAN[12], succinic semialdehyde (at least 96 % pure) according to CARRIÈRE[13].

RESULTS

Table I shows that added acetoin and succinic semialdehyde are completely recovered after 3 hours' incubation with muscle homogenates under the actual experimental conditions. Hence they must be considered to be final products under these conditions. We may therefore conclude that the rate of production of these compounds from added pyruvate and α-ketoglutarate can be regarded as a measure of the activity of pyruvic decarboxylase and α-ketoglutaric decarboxylase respectively.

TABLE I

RECOVERY OF ACETOIN (RACEMIC) AND SUCCINIC SEMIALDEHYDE (SSA) ADDED TO MUSCLE HOMOGENATES (200 mg MUSCLE) AFTER 3 HOURS' INCUBATION AT 37.5° C

Muscle	*Exp.*	*Added*				*Determined (μg)*	*Recovered (μg)*
		400 μg acetoin	*450 μg SSA*	*2 mg pyruvate*	*6 μg TPP*		
Breast	I	—	—	—	—	43	413
		+	—	—	—	456	
	II	—	—	+	—	373	408
		+	—	+	—	781	
		—	—	+	+	390	408
		+	—	+	+	798	
Heart	III	—	—	—	—	10	396
		+	—	—	—	406	
Breast	IV	—	—	—	—	0	465
		—	+	—	—	465	

In Tables II and III "severe symptoms" means that according to our experience the animals would have died in a few hours.

Table II shows the decline of pyruvic decarboxylase activity of breast muscle and heart muscle in the course of thiamine deprivation.

Breast muscle. During the first 12 days of deficiency the difference between the carbohydrate diet and the fat diet is not significant. As on the average the pigeons live much longer on the latter diet than on the former it might be expected, presuming decarboxylase activity would continue to be lost on the fat diet, that the final decrease on this diet would be much greater than that on the carbohydrate diet. This was indeed observed. Hence the severe symptoms immediately preceding death occur at a much higher pyruvic decarboxylase activity of the breast muscle on the carbohydrate diet than on the fat diet.

Heart muscle. Now we see that there is a significant difference between the effects of the diets after 12 days: the activity of the pyruvic decarboxylase has diminished much more on the carbohydrate diet than on the fat diet. So the decline on the fat diet is slower, but as the deficiency takes more time to develop on this diet, there is no more

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a significant difference between the pyruvic decarboxylase activities of heart muscle on both diets when the pigeons have incurred severe symptoms.

TABLE II

ACETOIN PRODUCTION IN MUSCLE HOMOGENATES

Diet A = mixture of cereals; Diet B = carbohydrate diet, free of thiamine; Diet C = fat diet, free of thiamine. All standard deviations mentioned are standard deviations of the means (standard errors). P-values calculated according to WILCOXON[14].

Diet	*Conditions of pigeons*	*Number of pigeons*	*6 µg TPP added*	*µM acetoin produced in 3 h*		*Indices for P at foot of table*
				Breast muscle	*Heart muscle*	
A	normal	8	—	4.3 ± 0.2	2.3 ± 0.1	1^-
			+	5.2 ± 0.2	3.6 ± 0.1	1^+
B	4 d. def., no symptoms	8	—	3.5 ± 0.3	1.8 ± 0.1	2^-
			+	4.8 ± 0.4	3.8 ± 0.1	2^+
	12 d. def., no symptoms	10	—	2.4 ± 0.2	0.3 ± 0.02	3^-
			+	5.0 ± 0.4	3.2 ± 0.2	3^+
	18 d. def. in mean (11 d. to 25 d.), severe symptoms	13	—	1.7 ± 0.3	0.2 ± 0.03	4^-
	20 d. def. in mean (17, 18 and 26 d. def.), severe symptoms	3	—	1.6 ± 0.2	0.1 ± 0.02	5^-
			+	3.6 ± 0.2	1.8 ± 0.3	5^+
C	12 d. def., no symptoms	10	—	2.7 ± 0.2	1.1 ± 0.1	6^-
			+	4.5 ± 0.3	3.1 ± 0.2	6^+
	26 d. def., no symptoms	10	—	0.8 ± 0.1	0.3 ± 0.03	7^-
			+	2.9 ± 0.3	2.3 ± 0.1	7^+
	33 d. def. in mean (29 d. to 37 d.), severe symptoms	9	—	0.4 ± 0.03	0.1 ± 0.02	8^-
	51 d. def. in mean (44, 44 and 48 d. def., severe symptoms; 59 and 60 d. def., no symptoms)	5	—	0.3 ± 0.02	0.1 ± 0.02	9^-
			+	2.7 ± 0.2	1.5 ± 0.2	9^+

	Breast muscle	Heart muscle
	$P_{(1^- - 2^-)} = 0.01$; $P_{(1^- - 3^-)} < 0.001$;	$P_{(1^- - 2^-)} = 0.02$; $P_{(1^- - 3^-)} < 0.001$;
	$P_{(1^- - 4^-)} < 0.001$; $P_{(1^- - 6^-)} < 0.001$;	$P_{(1^- - 4^-)} < 0.001$; $P_{(1^- - 6^-)} < 0.001$;
	$P_{(1^- - 7^-)} < 0.001$; $P_{(1^- - 8^-)} < 0.001$.	$P_{(1^- - 7^-)} < 0.001$; $P_{(1^- - 8^-)} < 0.001$.
	$P_{(1^+ - 2^+)} > 0.1$; $P_{(1^+ - 3^+)} > 0.1$;	$P_{(1^+ - 2^+)} > 0.1$; $P_{(1^+ - 3^+)} > 0.1$;
	$P_{(1^+ - 5^+)} < 0.001$; $P_{(1^+ - 6^+)} > 0.1$;	$P_{(1^+ - 5^+)} < 0.001$; $P_{(1^+ - 6^+)} > 0.1$;
	$P_{(1^+ - 7^+)} < 0.001$; $P_{(1^+ - 9^+)} < 0.001$.	$P_{(1^+ - 7^+)} < 0.001$; $P_{(1^+ - 9^+)} < 0.001$.
	$P_{(3^- - 4^-)} < 0.001$; $P_{(4^- - 5^-)} > 0.1$;	$P_{(3^- - 4^-)} > 0.1$; $P_{(4^- - 5^-)} > 0.1$;
	$P_{(6^- - 7^-)} < 0.001$; $P_{(7^- - 8^-)} < 0.001$;	$P_{(6^- - 7^-)} < 0.001$; $P_{(7^- - 8^-)} = 0.004$;
	$P_{(8^- - 9^-)} > 0.1$; $P_{(4^- - 8^-)} < 0.001$.	$P_{(8^- - 9^-)} > 0.1$; $P_{(4^- - 8^-)} > 0.1$.

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TABLE III

SUCCINIC SEMIALDEHYDE (SSA) PRODUCTION IN MUSCLE HOMOGENATES

Diet A = mixture of cereals; Diet B = carbohydrate diet, free of thiamine; Diet C = fat diet; free of thiamine. All standard deviations mentioned are standard deviations of the means (standard errors). P-values calculated according to WILCOXON[14].

Diet	*Condition of pigeons*	*Number of pigeons*	*6 μg TPP added*	*μM SSA produced in 3 h*		*Indices for P at foot of table*
				Breast muscle	*Heart muscle*	
A	normal	7	—	7.3 ± 0.5	5.8 ± 0.4	1^-
			+	9.0 ± 0.4	6.3 ± 0.3	1^+
B	4 d. def., no symptoms	9	—	4.6 ± 0.4	6.0 ± 0.5	2^-
			+	9.2 ± 0.3	7.0 ± 0.3	2^+
	12 d. def., no symptoms	9	—	4.8 ± 0.4	4.5 ± 0.1	3^-
			+	8.8 ± 0.4	5.8 ± 0.3	3^+
	18 d. def. in mean (11 d. to 25 d.), severe symptoms	13	—	4.7 ± 0.2	3.0 ± 0.2	4^-
	20 d. def. in mean (17, 18 and 26 d. def.), severe symptoms	3	—	5.4 ± 0.3	3.2 ± 0.6	5^-
			+	8.1 ± 0.5	6.5 ± 0.5	5^+
C	12 d. def., no symptoms	10	—	6.0 ± 0.4	6.2 ± 0.4	6^-
			+	9.2 ± 0.4	6.7 ± 0.3	6^+
	26 d. def., no symptoms	10	—	3.0 ± 0.4	4.1 ± 0.2	7^-
			+	7.1 ± 0.5	5.5 ± 0.3	7^+
	33 d. def. in mean (29 d. to 37 d.), severe symptoms	9	—	2.9 ± 0.1	4.4 ± 0.2	8^-
	51 d. def. in mean (44, 44 and 48 d. def., severe symptoms; 59 and 60 d. def., no symptoms	5	—	2.3 ± 0.2	3.2 ± 0.1	9^-
			+	4.6 ± 0.3	4.5 ± 0.4	9^+

Breast muscle	Heart muscle
$P(_1{}^-{}_2{}^-) < 0.001$; $P(_2{}^-{}_3{}^-) > 0.1$;	$P(_1{}^-{}_2{}^-) > 0.1$; $P(_1{}^-{}_3{}^-) < 0.001$;
$P(_2{}^-{}_4{}^-) > 0.1$; $P(_1{}^-{}_6{}^-) = 0.02$;	$P(_1{}^-{}_4{}^-) < 0.001$; $P(_1{}^-{}_6{}^-) > 0.1$;
$P(_1{}^-{}_7{}^-) < 0.001$; $P(_1{}^-{}_8{}^-) < 0.001$.	$P(_1{}^-{}_7{}^-) < 0.001$; $P(_1{}^-{}_8{}^-) < 0.001$.
$P(_1{}^+{}_2{}^+) > 0.1$; $P(_1{}^+{}_3{}^+) > 0.1$;	$P(_1{}^+{}_2{}^+) > 0.1$; $P(_1{}^+{}_3{}^+) = 0.09$;
$P(_1{}^+{}_5{}^+) = 0.07$; $P(_1{}^+{}_6{}^+) > 0.1$;	$P(_1{}^+{}_5{}^+) > 0.1$; $P(_1{}^+{}_6{}^+) > 0.1$;
$P(_1{}^+{}_7{}^+) = 0.01$; $P(_1{}^+{}_9{}^+) < 0.001$.	$P(_1{}^+{}_7{}^+) = 0.01$; $P(_1{}^+{}_9{}^+) < 0.001$.
$P(_3{}^-{}_4{}^-) > 0.1$; $P(_4{}^-{}_5{}^-) > 0.1$;	$P(_3{}^-{}_4{}^-) < 0.001$; $P(_4{}^-{}_5{}^-) > 0.1$;
$P(_6{}^-{}_7{}^-) < 0.001$; $P(_7{}^-{}_8{}^-) > 0.1$;	$P(_6{}^-{}_7{}^-) < 0.001$; $P(_7{}^-{}_8{}^-) > 0.1$;
$P(_8{}^-{}_9{}^-) = 0.02$; $P(_4{}^-{}_8{}^-) < 0.001$.	$P(_8{}^-{}_9{}^-) < 0.001$; $P(_8{}^-{}_4{}^-) < 0.001$.

Table II further shows the effect of adding TPP *in vitro*. A small effect is already observed in the case of normal muscles. There are two possible explanations for this, *viz.*

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a small part of the pyruvic decarboxylase is present in the tissue *in situ* as the apoenzyme, even when the body is abundantly supplied with thiamine, or part of the prosthetic group is split off in the course of the preparation of the homogenate. We believe the latter to be the most probable.

It further appears that in both muscles and on both diets the apoenzyme is maintained during the first 12 days of thiamine deficiency, for the same level is reached upon adding TPP to the homogenates of the muscles of the 12 days' deficient pigeons as in the case of homogenates of the muscles of normal pigeons. When the deficiency lasts longer the apoenzyme is partly lost, for in the later stages addition of TPP does not restore the pyruvic decarboxylase activity to its maximal level.

The data concerning the α-ketoglutaric decarboxylase activity of the muscle homogenates are assembled in Table III.

Breast muscle. We see that on the carbohydrate diet the activity declines during the first 4 days of deficiency, but then remains practically constant until the occurrence of severe symptoms, which would have been followed by death in a couple of hours. The activity is then still rather high.

The decline on the fat diet is smaller in the first 12 days, but it gradually increases as thiamine deprivation is prolonged. In the last stage of deficiency the α-ketoglutaric decarboxylase activity is significantly lower on the fat diet than on the carbohydrate diet.

Heart muscle. Now we see that the activity remains constant for 4 days on the carbohydrate diet; after 12 days on this diet the activity has significantly decreased, and this decrease continues until severe symptoms are observed.

On the fat diet, however, the activity has not yet diminished after 12 days; after 26 days a significant decrease is observed. The activity remains more or less on this level until severe symptoms are observed, though there is some tendency to decrease still further with prolonged time of survival. In most cases the α-ketoglutaric activity is higher at death on the fat diet than on the carbohydrate diet.

On the carbohydrate diet the apo-α-ketoglutaric decarboxylase appears to be maintained both in breast muscle and in heart muscle until the last stage of deficiency (on the average 18 days).

On the fat diet, however, on which the time of survival is much longer, there is a definite decrease of the amount of apoenzyme in the stages when symptoms occur (compare the activities of normal and deficient homogenates in the presence of added TPP).

To compare the decrease of pyruvic decarboxylase and α-ketoglutaric decarboxylase activities with progressive deficiency Figs. 1 and 2 may be consulted. In these figures the activities, expressed in percentages of the activity of the homogenates of the normal muscles, are plotted against the period of thiamine deprivation. These figures show that under similar conditions, *viz.* carbohydrate or fat diet, both in breast- and heart muscle a much higher percentage of active α-ketoglutaric decarboxylase remains in the tissue as compared to pyruvic decarboxylase. Thus the former enzyme always appears to lose its prosthetic group less readily than the latter, with one exception only: in the breast muscle during the first 4 days of deficiency on the carbonhydrate diet; in that case the breast muscle loses its α-ketoglutaric decarboxylase activity with an extreme rapidity.

As mentioned above the pigeons with severe symptoms were in a condition from which it could be predicted that they would die within a few hours. In general death was much more acute on the carbohydrate diet than on the fat diet, and on the latter

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temporary remissions were often observed. But we had sufficient experience with thiamine-deficient pigeons to be able also to predict the rapid approach of death in the case of the fat-fed pigeons. Therefore we may conclude that both enzymes never disappear completely from the muscles examined.

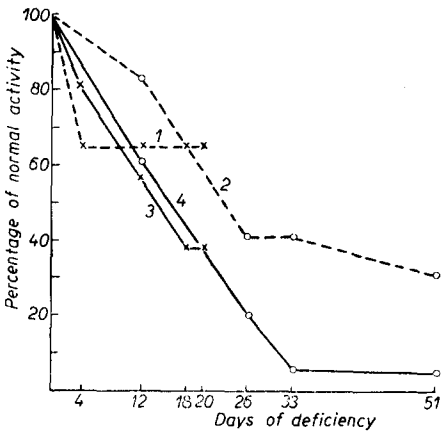

Fig. 1. Percentual decrease of the enzyme activities in breast muscle. 1. α-Keto-glutaric decarboxylase, carbohydrate diet; 2. α-Ketoglutaric decarboxylase, fat diet; 3. Pyruvic decarboxylase, carbohydrate diet; 4. Pyruvic decarboxylase, fat diet.

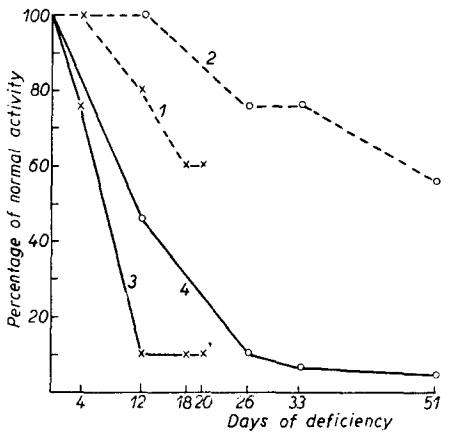

Fig. 2. Percentual decrease of the enzyme activities in heart muscle. 1. α-Keto-glutaric decarboxylase, carbohydrate diet; 2. α-Ketoglutaric decarboxylase, fat diet; 3. Pyruvic decarboxylase, carbohydrate diet; 4. Pyruvic decarboxylase, fat diet.

TABLE IV

THIAMINE PYROPHOSPHATE CONTENT OF MUSCLES

Diet A = mixture of cereals; Diet B = carbohydrate diet, free of thiamine; Diet C = fat diet, free of thiamine. "Standard errors".

Diet	*Condition of pigeons*	*Breast muscle*		*Heart muscle*	
		Number of pigeons	*μg TPP per g*	*Number of pigeons*	*μg TPP per g*
A	normal	20	7.3 ± 0.1	20	6.3 ± 0.2
B	4 d. def., no symptoms	21	4.9 ± 0.2	19	3.9 ± 0.1
	12 d. def., no symptoms	24	3.2 ± 0.1	20	1.7 ± 0.1
	18 d. def. in mean (11 d. to 25 d.), severe symptoms	13	2.7 ± 0.1	13	1.4 ± 0.06
C	12 d. def., no symptoms	10	4.9 ± 0.1	10	3.7 ± 0.1
	26 d. def., no symptoms	10	1.7 ± 0.08	10	1.6 ± 0.02
	33 d. def. in mean (29 d. to 37 d.), severe symptoms	9	1.8 ± 0.1	9	1.7 ± 0.1

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Table IV summarizes the results of all TPP determinations carried out during this investigation. In general they confirm GRUBER's results. Furthermore the remarkable fact that the decrease of the TPP content in the later stages of deficiency is only very small or nil (comp.: carbohydrate diet, pigeons after 12 days of deficiency without symptoms and pigeons with severe symptoms; fat diet, pigeons after 26 days of deficiency without symptoms and pigeons with severe symptoms) permits the following conclusion. Presuming that enzymes with TPP as a prosthetic group, other than the two enzymes studied, occur in the normal muscles in not too small amounts these must also lose their prosthetic group to a large extent long before the symptoms occur, for otherwise the TPP content of the muscles would still decrease considerably in the last stages.

DISCUSSION

According to GRUBER[4] TPP disappears less rapidly from the tissues, when the thiamine-free diet contains fat instead of carbohydrate. Actually, this diminished rate of utilization of TPP is not due to an effect of the fat in the diet, but to the absence of carbohydrate. This appears from an experiment by the same author[3], in which he administered various amounts of carbohydrate, but no fat. The feeding of a small amount of carbohydrate provoked a less rapid disappearance of TPP than the feeding of a larger amount. Hence in the discussion of our experiments the presence or the absence of carbohydrate in the diet should be emphasized and not the presence of fat.

The curves in Figs. 1 and 2 representing the change of pyruvic decarboxylase and α-ketoglutaric decarboxylase of breast muscle and heart muscle of pigeons on the fat diet, are thus characteristic for diets containing no carbohydrate. As these figures show, the phenomena appear to be much more complicated than one could expect from the results of the determination of total TPP, as carried out by GRUBER. During the first 18 or 20 days there is not much difference in breast muscle between the rates of inactivation of pyruvic decarboxylase on both diets, but the difference is very pronounced in the case of heart muscle (after 12 days of thiamine deprivation on the carbohydrate diet the pyruvic decarboxylase activity has already decreased to 10% and on the diet free of carbohydrate to only 45% of the normal value). The difference between the effects of the two diets is very remarkable for the α-ketoglutaric decarboxylase: in breast muscle the inactivation is much more pronounced on the carbohydrate diet than on the diet containing no carbohydrate in the first 4 days; during the following days, however, no further inactivation takes place on the former diet, while the inactivation gradually proceeds on the latter. In heart muscle the activity remains constant during the first 4 days on the carbohydrate diet and during the first 12 days on the diet containing no carbohydrate. After these initial periods the decrease sets in at about equal rates on both diets.

Altogether we see that the determination of total TPP, which is not only the prosthetic group of the two enzymes studied, but very probably also that of other enzymes, gives only a very incomplete picture of thiamine metabolism during thiamine deprivation. However, summarizing various conclusions from Tables II and III, to be found in the Experimental Part, we see that at death both kinds of muscle tend to show the same differences between their enzyme activities on the two diets as between their total TPP contents, as described by GRUBER. This is schematically shown in Table V.

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TABLE V

TPP CONTENTS AND ENZYME ACTIVITIES AT DEATH

	Breast muscle	*Heart muscle*
TPP content at death	on diet B > on diet C	on diet B < on diet C
Pyruvic decarb. activity at death	on diet B > on diet C	on diet B = on diet C
α-Ketoglutaric decarb. activity at death	on diet B > on diet C	on diet B < on diet C

As we have seen, the decarboxylases can be easily determined in muscles, as acetoin and succinic semialdehyde appear to be final products under anaerobic conditions. We have obtained evidence that it is not so easy in other tissues. If the conception of REED AND DEBUSK that the TPP responsible for anaerobic decarboxyoation is also responsible for the oxidation of pyruvate and α-ketoglutarate by artificial electron acceptors as ferricyanide also applies to the animal enzymes, these decarboxylases may possibly be determined in other tissues by measuring the rate of oxidation of these substrates by ferricyanide.

However this may be, in our opinion the results of this investigation indicate, that the determination, during the progress of thiamine deficiency, of all enzymes which have TPP or a TPP-lipoic acid complex as prosthetic group in the largest possible number of tissues promises to give much more insight into the origin of the symptoms of thiamine avitaminosis, which finally depend on a derangement of metabolism.

SUMMARY

In homogenates of breast muscle and heart muscle acetoin and succinic semialdehyde are final products of the anaerobic metabolism of pyruvate and α-ketoglutarate respectively. Therefore the pyruvic and α-ketoglutaric decarboxylase activities of these muscles could be measured by determining the rates of production of these compounds from pyruvate and α-ketoglutarate added to the homogenates.

During thiamine deprivation both activities decreased according to the curves shown in Figs. 1 and 2. Pronounced differences exist between both kinds of muscle regarding the rates of decline of the activities as well as the activities remaining at death. The presence or absence of carbohydrate in the thiamine-free diet also has a marked influence on both phenomena.

In general the α-ketoglutaric decarboxylase activity decreases less rapidly than the pyruvic decarboxylase activity.

At death the pyruvic decarboxylase activity of breast muscle is much higher on the carbohydrate diet than on the fat diet, while in this regard no difference appeared to exist in heart muscle. Regarding the α-ketoglutaric decarboxylase activity at death, this is also higher on the carbohydrate diet than on the fat diet in breast muscle, while in most cases the reverse was observed in heart muscle.

As compared to the normal activity of breast muscle or heart muscle the final percentage decrease of the pyruvic decarboxylase activity is always much greater than that of the α-ketoglutaric decarboxylase activity, but the former also is never completely lost.

The apoenzymes are completely maintained in the muscles during most of the period of deficiency; they are only partly lost in the last stages, with the exception of the α-ketoglutaric decarboxylase on the carbohydrate diet.

RÉSUMÉ

Les produits finaux du métabolisme anaérobie du pyruvate et de l'α-cétoglutarate dans des homogénats de muscle pectoral ou de muscle cardiaque sont respectivement l'acétoine et la semialdéhyde succinique. Par conséquent on peut mesurer les activités pyruvique et α-cétoglutarique décarboxylasiques de ces muscles en déterminant les vitesses d'apparition de ces corps à partir du pyruvate et de l'α-cétoglutarate ajoutés aux homogénats.

La diminution des deux activités au cours d'une carence en thiamine est représentée par les Figs. 1 et 2. Les vitesses de diminution des activités ainsi que les activités résiduelles au moment

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de la mort sont très différentes selon le muscle envisagé. La présence ou l'absence de glucides dans le régime sans thiamine influe également beaucoup sur ces deux phénomènes.

En général l'activité décarboxylasique vis à vis de l'α-cétoglutarate diminue moins rapidement que l'activité vis à vis du pyruvate.

Au moment de la mort, l'activité de la pyruvique décarboxylase du muscle pectoral est beaucoup plus élevée pour un régime glucidique que pour un régime lipidique; cette différence ne s'observe pas dans le cas du muscle cardiaque.

L'activité de l'α-cétoglutarique décarboxylase varie de la même façon en fonction du régime dans le muscle pectoral, alors que, dans la plupart des cas, elle varie en sens inverse dans le cas du muscle cardiaque.

Le pourcentage final de diminution d'activité, par rapport à l'activité normale, de la pyruvique décarboxylase est toujours beaucoup plus grand que celui de l'α-cétoglutarique décarboxylase; cependant la perte d'activité du premier enzyme n'est jamais totale.

Les apoenzymes persistent dans les muscles pendant la plus grande partie de la carence; ils ne sont perdus que partiellement dans les derniers stades, sauf dans le cas de l'α-cétoglutarique décarboxylase et d'un régime glucidique.

ZUSAMMENFASSUNG

In Homogenaten des Brust- und Herzmuskels sind Acetoin und Bernsteinsäurehalbaldehyd Endprodukte des anaeroben Stoffwechsels der Brenztraubensäure bezw. der α-Ketoglutarsäure. Daher konnten die Aktivitäten der Brenztraubensäure- und der α-Ketoglutarsäure-Decarboxylase gemessen werden, indem die Geschwindigkeit der Produktion dieser Verbindungen nach Zusatz von Pyruvat und α-Ketoglutarat zu den Homogenaten bestimmt wurde.

Bei Vitamin B_1 Mangel nahmen die Aktivitäten entsprechend den Kurven der Fig. 1 und 2 ab. Es bestehen ausgesprochene Unterschiede zwischen den beiden Muskelarten, wenn der Geschwindigkeitsabfall der Aktivitäten und die Aktivitätsreste beim Tod betrachtet werden. Die Gegenwart oder Abwesenheit von Kohlehydrat bei Vitamin B_1 freier Diät hat einen merkbaren Einfluss auf beide Phänomene.

Im allgemeinen fiel die α-Ketoglutarsäure-Decarboxylase-Aktivität weniger schnell ab, als die der Brenztraubensäure-Decarboxylase.

Beim Tod ist im Brustmuskel die Pyruvat-Decarboxylase Aktivität nach Kohlehydratdiät viel höher als nach Fettdiät, während im Herzmuskel keine solche Verschiedenheit zu bestehen scheint. Ebenso ist im Brustmuskel die α-Ketoglutarat-Decarboxylase Aktivität beim Tod nach Kohlehydratdiät grösser als nach Fettdiät, während in vielen Fällen im Herzmuskel das umgekehrte beobachtet wurde.

Verglichen mit der normalen Aktivität von Brust- und Herzmuskel ist der finale prozentuale Abfall der Pyruvat-Decarboxylase Aktivität immer viel grösser, als der der α-Ketoglutarsäure-Decarboxylase, aber auch die erstere geht niemals vollständig verloren.

Die Apoenzyme der Muskeln bleiben während des grössten Teiles der Mangelperioden vollständig erhalten. Ein teilweiser Verlust tritt allein in den letzten Stadien ein, mit Ausnahme der α-Ketoglutarsäure-Decarboxylase bei Kohlehydratdiät.

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