A STUDY OF THE INFLUENCE OF THIAMINE AND THIAMINE PYROPHOSPHATE ON THE ANAEROBIC PYRUVATE METABOLISM OF PIG HEART MUSCLE*

by

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In this paper a study of the anaerobic metabolism of pyruvate in minced pig heart muscle in phosphate buffer at pH 6.2 is reported. The main purpose of this investigation was to gain more insight into the function of thiamine in heart muscle metabolism. In a preliminary communication[1] we already mentioned some effects of thiamine** and thiamine pyrophosphate (TPP) on the anaerobic CO_2 production from pyruvate by heart muscle. By storing Latapie mince of the left ventricle during various periods of time at + 1° C a pronounced decrease in the CO_2 production from pyruvate by a suspension of this mince occurred. The CO_2 production could, however, again be restored to the original level by adding small quantities of TPP. The TPP thus appears to be split off from its bearer protein(s) and, as determinations of TPP have shown, decomposed during storage at + 1° C, while the bearers themselves are not significantly affected by the storage. Although this TPP effect was sometimes observed in fresh mince, it was much larger in stored mince. These observations offered an opportunity to obtain more knowledge concerning those reactions of pyruvate, in which thiamine plays an essential role.

Most experiments were carried out on stored mince at pH 6.2. The buffer and the pH were rather arbitrarily chosen, as we were used to working with yeast carboxylase at this pH and a medium, consisting of sodium potassium phosphate, pH 6.2, appeared to be very suitable for obtaining the maximal effect of added TPP. Indeed the deviation from physiological conditions excludes a more or less quantitative assessment of the relative importance *in vivo* of the reactions studied under the conditions prevailing in our experiments. The work described must therefore be considered as an introduction, which will have to be followed by experiments under more physiological conditions. The determination of CO_2, as mentioned above, was completed by the determination of lactate, citrate, pyruvate, acetaldehyde, acetoin, diacetyl and acetate.

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

** In papers from this laboratory vitamin B_1 and its pyrophosphoric ester have always been denoted by aneurin and aneurinpyrophosphate (APP). Since the International Union of Chemistry has adopted the terms thiamine and thiamine pyrophosphate we shall in future write thiamine and TPP.

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EXPERIMENTAL PART

General procedure of investigation

The material used was a Latapie mince of the left ventricle of pig heart muscle. The total pig heart was removed from the animal fifteen to twenty minutes after the death of the animal at the slaughter house, and brought quickly to the laboratory in an ice-cooled vessel. As quickly as possible the left ventricle was isolated, washed to remove clotted blood, freed from fat and connective tissue and minced in a Latapie mincer. All these operations were carried out in the refrigerated room (0°). This preparation, which will further be referred to as "mince", was either used immediately ("fresh" mince) or after storage at + 1° C for different times.

The mince was always suspended in a threefold volume of 0.1 *M* Na K phosphate buffer of pH 6.2, containing 0.01 *M* $MnCl_2$, as manganous ions appeared to have a strong effect on the CO_2 production. The suspension thus contained 333 mg tissue per ml. The pH did not change by introducing the mince into the buffer. Always 1 ml of this suspension was used per Warburg vessel.

The main compartment of the Warburg vessels contained 1.0 ml tissue suspension and 0.2 ml buffer or solution of TPP in buffer, the side arm 2.5 mg (22.7 μmoles) sodium pyruvate dissolved in 0.5 ml buffer. In some experiments, *e.g.* those in which thiamine was also added vessels with two side arms were used. The quantity of pyruvate metabolized increased with increasing amounts of added pyruvate until a maximum was reached. The above-mentioned quantity of pyruvate employed was close to this maximum and yet allowed for the accurate determination of the amount of pyruvate utilized, as about one-half of the amount added remained at the end of the incubation period.

The respirometers were gassed and filled with pure nitrogen. Yellow phosphorus was always introduced into the centre well to absorb the last traces of oxygen. The carbon dioxide production was followed during 180 minutes.

At the end of the experiment the reactions were stopped by adding 5 ml of a 10% solution of metaphosphoric acid to the contents of the vessels. After standing for 15 minutes the contents were centrifuged and aliquots of the protein-free liquids were employed for the determination of the metabolites. When acetate had to be determined, a protein precipitating agent could not be used because of the enzymatic character of the determination. In this case 5 ml of a buffer solution of pH 7.6 were added to the contents of the vessels and after mixing thoroughly the reactions were stopped by heating at 100° for 90 seconds. After cooling the pH was 7.0–7.1, which is the right value for the determination of acetate.

Before incubation with pyruvate the mince already contained appreciable amounts of lactate and acetate while the other compounds mentioned were only present in traces or could not be detected at all.

Substances and methods of determination

Sodium pyruvate was prepared from freshly distilled pyruvic acid by the method of LIPMANN[2]; commercial sodium pyruvate from Hoffmann-La Roche was also used. The purity, checked by the carboxylase method of WARBURG, KUBOWITZ AND CHRISTIAN[3], was in both cases about 97%.

The thiamine and thiamine pyrophosphate were commercial samples obtained from Hoffmann-La Roche.

Pyruvate was determined by the method of FRIEDEMANN AND HAUGEN[4] with minor modifications, lactate according to BARKER AND SUMMERSON[5], acetaldehyde according to STOTZ[6]. Acetoin was converted into diacetyl by the method of STOTZ AND RABORG[7], which was then determined according to WHITE, KRAMPITZ AND WERKMAN[8]. Citrate was determined according to NATELSON, LUGOVOY AND PINCUS[9] with the modifications introduced by MEDUSKI[10]. Acetate was determined by the method of SOODAK AND LIPMANN*[11].

The CO_2 production was measured manometrically in a circular Warburg apparatus with

* We are much indebted to Prof. LIPMANN for a detailed description of the method and for samples of co-enzyme A, and to Dr E. P. STEYN-PARVÉ for much help with the determinations.

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32 manometers. The temperature was 38°. The vessels were shaken pivotally at a rate of 140 cycles per minute.

The tissue suspensions were run at least in duplicate, in most cases in triplicate.

RESULTS

As was already stated in our former publication[1], the CO_2 production in the absence of added pyruvate was very small and effects of the addition of TPP—if any—were smaller than the relatively large experimental error. All CO_2 produced when pyruvate was added was presumed to have been derived from the pyruvate. Subtraction of the endogeneous CO_2 production from the amount produced in the presence of pyruvate was shown not to change our results materially.

Table I shows the effect of storing at + 1° C on the quantity of pyruvate utilized, and on the amounts of CO_2 and acetoin formed. This table, moreover, indicates that the effect of storage is already nearly maximal after 24 hours and that further storage at + 1° has no considerable effect. Determinations of TPP in the mince gave a result in agreement with this observation. While the fresh mince had an average TPP content of 7 μg TPP per g of mince, this value diminished to about 3.7 in the first 24 hours of storage and remained constant for the following 120 hours of storage.

TABLE I

EFFECT OF STORING AT + 1° C ON THE PYRUVATE CONSUMPTION AND THE FORMATION OF CO_2 AND ACETOIN

Heart	Time of storing (hours)	μmoles pyruvate used	μmoles CO_2 formed	μmoles acetoin formed
41	0	11.5	8.0	3.2
	24	6.0	5.8	2.9
42	0	10.9	8.9	3.9
	72	7.0	6.1	2.5
	96	7.9	5.8	2.3
47	0	15.4	9.6	4.8
	24	12.8	7.5	3.6
52	0	10.7	9.0	4.5
	120	8.1	7.3	3.1
54	0	13.8	8.0	4.0
	48	10.2	5.9	3.0

It is evident that the decarboxylation of pyruvate to acetoin[12] is a major reaction in the system studied and that the decrease in utilization of pyruvate nearly equals the diminution of the CO_2 and acetoin production.

Columns 1, 2 and 3 of Table II show the influence of added TPP on the amounts of pyruvate used and of acetoin and CO_2 formed by stored mince. It is demonstrated by the figures in Tables I and II that the enzyme responsible for the formation of acetoin and CO_2 from pyruvate according to equation (1) is partly decomposed during storage at + 1° C and can be restored to its initial activity by adding TPP.

$$2CH_3C(=O)-COO^- + 2H^+ \rightarrow CH_3C(=O)-CHOH-CH_3 + 2CO_2 \qquad (1)$$

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TABLE II

INFLUENCE OF THE ADDITION OF TPP TO A SUSPENSION OF MINCE STORED AT + 1° C ON THE AMOUNTS OF PYRUVATE METABOLIZED AND VARIOUS METABOLITES FORMED

Exp. No.	1 μmoles pyruvate		2 μmoles CO_2		3 μmoles acetoin		4 μmoles lactate		5 = 1–4 μmoles "net pyruvate"	
	control	5 μg TPP added	control	5 μg TPP added	control	5 μg TPP added	control	5 μg TPP added	control	5 μg TPP added
106	— 6.95	—10.56	+6.07	+ 8.48	+2.50	+3.92	+1.20	+1.08	— 5.75	— 9.48
114	—12.77	—14.50	+7.52	+ 9.75	+3.56	+5.50	+2.03	+0.56	—10.74	—13.94
115	— 8.21	—12.53	+6.86	+ 9.52	+3.10	+5.52	+1.91	—0.56	— 6.30	—13.09
117	—11.50	—15.64	+8.50	+11.93	+3.29	+6.71	+1.70	+1.17	— 9.80	—14.47
123	—10.41	—12.20	+7.66	+ 8.77	+3.64	+4.58	+0.53	—1.27	— 9.88	—13.47
124	—10.36	—13.32	+7.57	+ 8.95	+3.15	+4.02	+1.39	—0.67	— 9.02	—13.99
125	— 9.57	—12.37	+7.05	+ 8.50	+3.00	+4.45	+1.10	—0.81	— 8.47	—13.18
126	— 8.24	—10.79	+7.21	+ 8.14	+3.07	+4.11	+1.08	+1.76	— 7.16	— 9.03
127	— 8.37	—11.98	+6.86	+ 8.50	+2.89	+4.45	+2.10	+0.07	— 6.17	—11.91
129	— 7.50	—11.38	+4.32	+ 7.09	+1.54	+3.67	—0.07	—0.79	— 7.57	—12.17
133	— 8.13	—11.77	+7.31	+ 9.09	+3.08	+4.12	+2.72	+1.12	— 5.41	—10.65
136	—10.22	—12.64	+5.93	+ 7.84	+2.99	+4.36	—0.63	—0.87	—10.85	—13.51
P-value of difference	< 0.001		< 0.001		< 0.001		< 0.01		< 0.001	

From the figures representing the pyruvate consumption and the CO_2 production it is clear, however, that this reaction is not the only one occurring. The possibility that acetoin is only a transitory stage and not a final product was ruled out by experiments in which acetoin was added to the suspension solely or simultaneously with pyruvate. A typical experiment is shown in Table III. It demonstrates that acetoin is not decomposed under the prevailing conditions.

TABLE III

STABILITY OF ACETOIN IN A SUSPENSION OF FRESH MINCE IN THE ABSENCE AND THE PRESENCE OF PYRUVATE AND TPP

	No pyruvate no TPP		No pyruvate 5 μg TPP		22.7 μmoles pyruvate no TPP		22.7 μmoles pyruvate 5 μg TPP	
μmoles acetoin added	—	4.54	—	4.54	—	4.54	—	4.54
μmoles acetoin determined after incubation	0.24	4.77	0.22	4.69	4.46	8.78	4.67	8.96
pCt of added acetoin recovered	—	100	—	98	—	95	—	94

To estimate the fate of the pyruvate utilized the amount reduced to lactate must be known. Therefore lactate determinations were carried out. As the amount of lactate already present is rather large (about 3 mg lactic acid per g of mince) and the change in the lactate content amounts to only 10 to 20% of this amount, the figure for the quantity of pyruvate reduced to lactate or lactate oxidized to pyruvate contains a rather large error as it is obtained by subtracting two large figures. In Table II the changes in the lactate content are given together with the amounts of pyruvate used and CO_2 and acetoin formed by stored mince, without and with added TPP. This table shows clearly that in the presence of added TPP less lactate is formed or more is consumed than in its absence. This effect is highly significant ($P < 0.01$). It will be discussed below.

It is generally accepted that lactate formation is a blind alley of metabolism, viz. that lactate can be only formed and metabolized via pyruvate. We have considered this assumption to be true in our case. *A change in lactate content was not observed when pyruvate was omitted from the suspension.* By subtracting the amount of lactate formed from the amount of pyruvate consumed, we obtain therefore a quantity stating the amount of pyruvate used for other reactions than reduction to lactate. According to OLSON, PEARSON, MILLER AND STARE[13] this quantity is called "net pyruvate". From Table II it is clear that the amount of "net pyruvate" used is larger than the amount which would be necessary to form the quantities of acetoin measured. Table IV gives the amounts of "net pyruvate" and of CO_2, remaining after subtracting the amounts involved in the formation of acetoin.

We see that in the absence of added TPP more CO_2 is formed than corresponds with the amount of acetoin formed, while in the presence of added TPP there is a deficit

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TABLE IV

AMOUNTS OF "NET PYRUVATE" USED AND CO_2 FORMED IN REACTIONS OTHER THAN DECARBOXYLATION TO ACETOIN

Exp. No.	μmoles "net pyruvate"		μmoles CO_2	
	control	5 μg TPP added	control	5 μg TPP added
106	— 0.75	— 1.64	+ 1.07	+ 0.64
114	— 3.62	— 2.94	+ 0.40	— 1.25
115	— 0.10	— 2.05	+ 0.66	— 1.52
117	— 3.22	— 1.05	+ 1.92	— 1.49
123	— 2.60	— 4.31	+ 0.38	— 0.39
124	— 2.72	— 5.95	+ 1.27	+ 0.91
125	— 2.47	— 4.28	+ 1.05	— 0.40
126	— 1.02	— 0.81	+ 1.07	— 0.08
127	— 0.45	— 3.01	+ 1.08	— 0.40
129	— 4.49	— 4.83	+ 1.24	— 0.25
133	+ 0.75	— 2.41	+ 1.15	+ 0.85
136	— 4.87	— 4.79	— 0.05	— 0.88
P-value of difference	not significant		< 0.001	

of CO_2 as compared to acetoin. The increase in CO_2 consumption upon addition of TPP is indeed highly significant ($P < 0.001$). Therefore we are compelled to assume that besides acetoin formation two other reactions are also responsible for the total "net pyruvate":

(1) a reaction in which CO_2 is produced, probably oxidative decarboxylation leading to acetate and CO_2; (2) a reaction in which CO_2 is utilized together with pyruvate (or lactate, see below).

Hence, including also the conversion of pyruvate into lactate, we presume that the pyruvate is metabolized in all along four pathways.

If the four proposed reactions are further assumed to be the only ones occurring, the amounts of pyruvate used for each can be calculated for all experiments, while their interrelationship can be established by the calculation of correlation coefficients concerning the various reactions. The distribution of pyruvate over the four reactions and the correlation coefficients with their P-values are given in Tables V and VI.

Tables IV and V show that the CO_2 fixation is significantly increased by added TPP. The figures in Table VI show that a very strong negative correlation exists between the fixation reaction and the reduction of pyruvate to lactate. This fact indicates that the observed effect of TPP on the reaction pyruvate $\rightleftharpoons$ lactate might be a secondary effect, depending upon a primary effect on the fixation reaction, both reactions being linked in some way or another (see Discussion).

In some experiments determinations of acetate were carried out. They showed that the mince already contained acetate while the addition of pyruvate led in some cases to the formation, in other cases to the utilization of acetate. Addition of TPP always caused a highly significant decrease in acetate formation or increase in acetate consumption. A connection of this acetate consumption with the fixation of CO_2 to pyruvate seems possible; but it is still uncertain which product might be formed as no citrate could be detected.

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TABLE V

DISTRIBUTION OF PYRUVATE ON THE VARIOUS REACTIONS

μmoles pyruvate metabolized along the different pathways

Exp. No.	Decarboxylation to acetoin		Reduction		"Oxidative decarboxylation"		"CO_2-fixation"	
	control	5 μg TPP added	control	5 μg TPP added	control	5 μg TPP added	control	5 μg TPP added
106	5.00	7.84	1.20	1.08	0.95	1.14	— 0.11	0.49
114	7.12	11.00	2.03	0.56	2.04	0.84	1.65	1.98
115	6.20	11.04	1.91	— 0.56	0.45	— 0.68	— 0.22	0.83
117	6.58	13.42	1.70	1.17	2.54	— 0.24	0.64	1.26
123	7.28	9.16	0.53	— 1.27	1.49	1.98	1.11	2.36
124	6.30	8.04	1.39	— 0.67	1.95	1.95	0.69	2.87
125	6.00	8.90	1.10	— 0.81	1.74	1.94	0.70	2.35
126	6.14	8.22	1.08	1.76	1.03	0.34	— 0.03	0.43
127	5.78	8.90	2.10	0.07	0.77	1.30	— 0.32	1.71
129	3.08	7.34	— 0.07	— 0.79	2.85	2.86	1.62	1.99
133	6.16	8.24	2.72	1.12	0.11	2.17	— 1.03	0.20
136	5.98	8.72	— 0.63	— 0.87	2.42	1.95	2.48	2.87
P-value of difference	< 0.001		< 0.01		not significant		< 0.001	

TABLE VI

CORRELATION COEFFICIENTS OF THE DIFFERENT REACTIONS

The correlations have been calculated for the figures represented in Table V. The P-value of a correlation coefficient is stated between brackets only if the latter is significant

Control

	Reduction	Oxid. decarb.	CO_2-fixation
Decarboxylation to acetoin	+ 0.356	— 0.630 (P < 0.05)	— 0.152
Reduction	—	— 0.461	— 0.796 (P < 0.01)
Oxid. decarboxylation	—	—	+ 0.643 (P < 0.05)

TPP added

	Reduction	Oxid. decarb.	CO_2-fixation
Decarboxylation to acetoin	+ 0.184	— 0.830 (P < 0.01)	— 0.193
Reduction	—	+ 0.133	— 0.840 (P < 0.01)
Oxid. decarboxylation	—	—	+ 0.436

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TABLE

INFLUENCE OF THIAMINE AND TPP ON THE PYRUVATE

Exp. No.	μmoles pyruvate				μmoles CO_2			
	1 No addit.	2 5 μg TPP	3 6 μg thiam.	4 5 μg TPP 6 μg thiam.	5 No addit.	6 5 μg TPP	7 6 μg thiam.	8 5 μg TPP 6 μg thiam.
100	—14.92	—15.15	—12.15	—17.70	+ 5.20	+ 5.14	+ 4.50	+ 5.41
103	—11.52	—11.54	—10.31	—12.52	+ 4.07	+ 4.25	+ 4.07	+ 4.43
105	—10.89	—11.20	—11.20	—12.13	+ 4.52	+ 4.84	+ 4.52	+ 4.91
109	— 9.95	—10.44	— 9.85	—13.14	+ 5.00	+ 5.15	+ 4.91	+ 5.73
113	—15.36	—14.57	—15.43	—16.54	+ 4.89	+ 4.91	+ 4.89	+ 5.27
132	—10.68	—12.33	—10.58	—13.52	+ 4.57	+ 5.09	+ 4.73	+ 5.43
134	— 8.85	— 8.85	— 8.70	—10.89	+ 4.61	+ 5.07	+ 5.05	+ 5.55
135	—13.78	—14.77	—13.69	—15.29	+ 4.09	+ 4.34	+ 4.18	+ 4.48
signif. P-val.		$4-3 < 0.01$ $4-2 < 0.01$				$6-5 < 0.02$ $8-6 < 0.01$ $8-7 < 0.001$		

In our former paper we reported some effects of thiamine and TPP on the CO_2 production from pyruvate by fresh mince. While the addition of thiamine and TPP separately had no significant effect on the CO_2 production, simultaneous addition of small amounts of both compounds resulted in a significant increase of CO_2. The addition of thiamine could not be replaced by increasing the amount of TPP. The results of seven experiments in which pyruvate and acetoin were also determined are given in Table VII.

From these results we can only conclude that in these experiments the addition of TPP significantly influences the formation of CO_2 as well as that of acetoin, but the effect on pyruvate utilization did not appear to be significant. Thiamine alone had no influence at all. Simultaneous addition of both compounds, however, caused a highly significant increase of the amounts of pyruvate used and CO_2 and acetoin formed as compared to TPP added alone. As only a small number of lactate determinations were carried out, conclusions concerning the effect of the simultaneous addition of both compounds on the different reactions cannot yet be drawn. It is clear, however, that the effect which was first observed when measuring CO_2 production can be even more convincingly demonstrated by determination of pyruvate and acetoin.

DISCUSSION

The evidence leading to the assumption of four reactions of pyruvate under the prevailing experimental conditions has already been discussed. It might be added that two of these reactions, the conversion to lactate and to acetoin, were established by direct evidence, while the other two reactions, the "oxidative decarboxylation" and the CO_2-fixation, had to be derived in an indirect way from the determination of various metabolites.

The second decarboxylation reaction is a reaction producing CO_2 from pyruvate, while the other product is still uncertain. It is not acetaldehyde, but might be acetate.

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VII

METABOLISM BY A SUSPENSION OF FRESH MINCE

μmoles acetoin				μmoles lactate			
9 No addit.	10 5 μg TPP	11 6 μg thiam.	12 5 μg TPP 6 μg thiam.	13 No addit.	14 5 μg TPP	15 6 μg thiam.	16 5 μg TPP 6 μg thiam.
+4.74	+4.88	+4.01	+5.56				
+3.16	+3.40	+3.31	+3.74				
+3.93	+4.21	+3.94	+4.97				
+5.05	+5.00	+4.04	+5.84				
+4.52	+5.09	+5.33	+5.73				
+4.75	+5.60	+4.45	+5.96				
				+1.67	+0.78	+1.67	+1.67
+4.03	+4.44	+4.04	+5.03	+0.89	−0.78	+2.89	+2.11
10 − 9 = 0.02 12 − 11 < 0.001 12 − 10 < 0.001							

As, however, acetate is consumed in our experiments we cannot decide this question before more knowledge is gained concerning acetate metabolism under the prevailing conditions.

The product of the reaction in which pyruvate and CO_2 are utilized is also unknown. It is not oxaloacetate, which is very unstable and would have been decomposed again. As acetate is utilized, citrate determinations were carried out, but with negative result.

The correlation coefficients show some interesting features. The second decarboxylation reaction has a strong negative correlation with the formation of acetoin, in the absence and in the presence of TPP. Perhaps this correlation might suggest that a two-carbon compound is formed, which—depending upon conditions as yet unknown—can either condense to acetoin or form acetate. Whether acetoin is a physiologically occurring intermediate or more or less an artefact, whose presence is perhaps due to a lack of some necessary factor, is not yet clear.

The highly significant linkage between the conversion of lactate to pyruvate and the fixation reaction deserves special comment. As the conversion of lactate to pyruvate is catalyzed by an enzyme which can cooperate with either DPN or TPN[14], this reaction might perhaps be a source of the TPN-H_2 necessary for the fixation of CO_2 to pyruvate by the "malic enzyme" as discovered in pigeon liver by MEHLER, KORNBERG, GRISOLIA AND OCHOA[14, 15]. Whether a similar enzyme operates in pig heart muscle remains, however, to be demonstrated. It also seems possible that lactate rather than pyruvate is the direct precursor of the fixation product by yielding on oxidation some form of "active pyruvate".

Addition of TPP has no significant influence on the "oxidative decarboxylation". This does not mean that this reaction is not catalyzed by a TPP bearing enzyme. It might be that TPP is not split off from this enzyme during storage. Even if the same intermediate is formed as in the decarboxylation to acetoin, which is strongly TPP-dependent, addition of TPP would not necessarily enhance the "oxidative" decarboxylation, as the formation of "acetate" might already proceed at the maximal rate.

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The influence of TPP on the conversion of lactate to pyruvate cannot be a direct effect as it is universally known that this reaction is catalyzed by a dehydrogenase acting with the pyridine nucleotides. The positive correlation of this reaction with the CO_2 fixation, as discussed above, indicates that it will probably be the latter reaction which is primarily enhanced by adding TPP.

The effect of the simultaneous addition of thiamine and TPP to fresh mince is very interesting, because it is the first known effect of thiamine on animal tissues which is not caused by its conversion to TPP. Whether any physiological importance should be attached to this effect cannot yet be decided. As was already stated[1] the effect cannot be explained by inhibition by thiamine of phosphatase action on TPP. In our opinion even a tentative explanation of this effect can be better postponed until more experimental results are known.

SUMMARY

1. By storing Latapie mince of the left ventricle of pig heart muscle at + 1° C for one or more days a partial inactivation of some enzymes concerned with anaerobic pyruvate metabolism occurs. The initial metabolic activity can be restored by adding small amounts of thiamine pyrophosphate (TPP) to the stored mince suspended in phosphate buffer of p_H 6.2. Obviously TPP is partly split off from its bearers and decomposed by tissue phosphatases during storage. These phenomena offered a possibility of studying some aspects of the rôle of TPP in pyruvate metabolism.

2. In a suspension of the mince in phosphate buffer at p_H 6.2 pyruvate undergoes at least four anaerobic reactions, which are mentioned below. While the evidence for the first two of these reactions (a and b) is direct and straight-forward, the other two reactions (c and d) had to be derived from indirect evidence, which was, however, statistically highly significant. These reactions are:

a. direct decarboxylation to acetoin and CO_2;

b. reduction to lactate;

c. decarboxylation to another product, probably oxidative decarboxylation to acetate;

d. fixation of carbon dioxide to pyruvate or lactate.

3. By investigating the distribution of pyruvate over these reactions in a suspension of stored mince without and with the addition of TPP and calculating the correlation coefficients between the amounts of pyruvate metabolized along the various pathways conclusions could be drawn regarding the interrelations of the different reactions and the effect of TPP on those reactions.

4. Highly significant negative correlations were established between reactions a and c, and between reactions b and d.

5. TPP has a significant effect on the formation of acetoin, on the conversion of lactate to pyruvate and on the CO_2-fixation. It appears that the effect on the conversion of lactate to pyruvate is only a corollary of the effect of TPP on the fixation of carbon dioxide.

6. Simultaneous addition of small amounts of thiamine and TPP to a suspension of fresh mince has a significant effect on the utilization of pyruvate and the formation of acetoin and CO_2, as compared to TPP added alone.

RÉSUMÉ

1. Lorsqu'une pâte Latapie de ventricule gauche de coeur de Porc est conservée quelques jours à + 1° C, certains enzymes agissant sur le métabolisme anaérobie de l'acide pyruvique, sont partiellement désactivés. L'activité originale peut être restaurée plus ou moins complètement par addition de petites quantités de pyrophosphate de thiamine (TPP) à une suspension de pâte conservée dans un tampon phosphate de p_H 6.2. Il est probable que le TPP est mis en liberté et décomposé par les enzymes tissulaires pendant la conservation. Ces phénomènes nous ont permis d'étudier quelques aspects du rôle du TPP dans le métabolisme de l'acide pyruvique.

2. Quatre réactions anaérobies au moins ont lieu dans une pâte suspendue dans un tampon-phosphate de p_H 6.2. Nous avons pu démontrer directement les deux premières (a et b), tandis que les deux autres (c et d) ont été déduites indirectement, mais avec une grande probabilité statistique. Ces réactions sont les suivantes:

a. décarboxylation directe avec formation d'acétoïne et de CO_2;

b. réduction en acide lactique;

c. décarboxylation avec formation d'une autre substance, probablement décarboxylation oxydative en acide acétique.

d. fixation de CO_2 sur l'acide pyruvique ou lactique.

3. Nous avons étudié la répartition de l'acide pyruvique parmi ces réactions dans une suspension

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de pâte conservée avec et sans addition de TPP et calculé les rapports entre les quantités d'acide pyruvique transformées suivant ces diverses voies. De cette manière nous avons pu tirer des conclusions sur la relation qui pourrait exister entre lesdites réactions et sur l'action du TPP.

4. Nous avons constaté une correlation négative entre les réactions a et c et entre les réactions b et d, qui pouvait être mise en évidence très nettement par la statistique.

5. Le TPP a une action sur la formation d'acétoïne, sur la transformation d'acide lactique en acide pyruvique et sur la fixation de CO_2. Ces effets pouvaient aussi être mis en évidence par la statistique. L'action sur la transformation de l'acide lactique en acide pyruvique ne semble être qu'une conséquence de l'effet sur la fixation de CO_2.

6. L'addition simultanée de petites quantités de thiamine et de TPP à une suspension de pâte fraîche exerce une action plus importante sur la consommation d'acide pyruvique et sur la formation d'acétoïne et de CO_2 que l'addition de TPP tout seul. Cette différence entre l'action de TPP + thiamine et de TPP seul pouvait aussi être mise en évidence par la statistique.

ZUSAMMENFASSUNG

1. Wenn Latapiebrei des linken Ventrikels von Schweineherzmuskel einige Tage bei + 1° C gelagert wird, tritt eine teilweise Inaktivierung einiger, beim anaeroben Umsatz der Brenztraubensäure wirksamer, Enzyme auf. Die ursprüngliche Stoffwechselaktivität kann mehr oder weniger vollständig wiederhergestellt werden, wenn einer Suspension des gelagerten Breis in Phosphatpuffer von p_H 6.2 kleine Mengen Thiaminpyrophosphat (TPP) zugefügt werden. TPP wird anscheinend von seinen "Trägern" abgespalten und während der Lagerung von Gewebephosphatasen zersetzt. Diese Phänomene boten eine Möglichkeit, einige Aspekte der Rolle von TPP im Brenztraubensäurestoffwechsel zu untersuchen.

2. In einer Suspension des Breis in Phosphatpuffer von p_H 6.2 treten mindestens vier anaerobe Reaktionen der Brenztraubensäure auf, die unten erwähnt werden. Während der Beweis für die Existenz der beiden ersten Reaktionen (a und b) direkt erbracht werden konnte, mussten die beiden anderen Reaktionen (c und d) durch indirekte Beweisführung, jedoch mit sehr grosser statistischen Wahrscheinlichkeit, abgeleitet werden. Die Reaktionen sind die folgenden:

a. anoxydative Decarboxylierung zu CO_2 und Azetoin;

b. Reduktion zu Milchsäure;

c. Decarboxylierung zu einem anderen Produkt, vermutlich oxydative Decarboxylierung zu Essigsäure;

d. CO_2-Bindung an Brenztrauben- oder Milchsäure.

3. Die Verteilung der Brenztraubensäure auf diese Reaktionen in einer Suspension von gelagertem Brei ohne und mit Zufügung von TPP wurde untersucht und die Korrelationskoëffizienten zwischen den Mengen Brenztraubensäure, die auf den verschiedenen Wegen umgesetzt wurden, wurden berechnet. Auf diese Weise konnten Schlüsse über den eventuellen Zusammenhang der verschiedenen Reaktionen und über den Effekt von TPP auf diese Reaktionen gezogen werden.

4. Eine sehr signifikante negative Korrelation wurde zwischen den Reaktionen a und c und zwischen den Reaktionen b und d festgestellt.

5. TPP hat einen signifikanten Effekt auf die Bildung von Azetoin, auf den Umsatz von Milch- zu Brenztraubensäure und auf die CO_2-Bindung. Die Wirkung auf den Umsatz von Milch- zu Brenztraubensäure ist anscheinend nur eine Folge des Effekts auf die CO_2-Fixierung.

6. Gleichzeitige Zufügung kleiner Mengen von Thiamin und TPP zu einer Suspension von frischem Brei hat auf den Brenztraubensäureverbrauch und auf die Bildung von Azetoin und CO_2 einen im Vergleich zur Zugabe von TPP allein signifikanten Effekt.

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