CCVI. PYRUVATE OXIDATION IN BRAIN

III. NATURE, SPECIFICITY AND COURSE OF OXIDATION CATALYSED BY VITAMIN B₁

By GRAHAM KEMP MCGOWAN AND RUDOLPH ALBERT PETERS

From the Department of Biochemistry, Oxford

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OUR interest in the aerobic oxidation of pyruvic acid¹ induced by the action of vitamin B₁ with pigeon's brain tissue, has led us to explore paths of oxidation; the object of this note is to reduce the number of possible intermediates between the acid and CO₂ + H₂O. Attempts to attack the problem directly by use of an isolated enzyme system have failed; the observations of Barron [1936] upon the "pyruvate oxidase" of Gonococci show that this activity disappears more rapidly than that of the lactate oxidase (dehydrogenase) in cold store. In brain tissues, the system seems relatively stable in presence of substrates during respiration experiments; but several attempts by varied methods to reach an active, dry preparation were unsuccessful; there were tried *inter alia* acetone, alcohol and chloroform in the cold, extraction at various reactions, and precipitation by ammonium sulphate and KCl; activity could not be restored upon addition of indophenol oxidase, cytochrome or methylene blue. Washing in distilled water causes an enormous fall in oxidation rate with pyruvate, only slightly restored by addition of the extract. It was evident that we were dealing with an enzyme system closely bound to the cell structure; and the suggestion arose that vitamin B₁ formed some kind of prosthetic group which was non-diffusible; this was consistent with the theory advanced in Part I [Peters, 1936], but especially probable when the observations of Westenbrink & Polak [1937] became available upon stability of the system in the presence and absence of vitamin B₁. More recently Lohmann's discovery [1937] that co-carboxylase is vitamin B₁ diphosphate has defined this point. From previous observations from this laboratory, the protein factor in the system must be unstable in absence of vitamin B₁, and also very susceptible to tissue poisons. It is another version of the same facts that prolonged washing of avitaminous brain gives a tissue preparation showing much less "vitamin" effect than the unwashed brain. For the investigation of the total "oxidase" system, the only course at present seems to be the use of tissues washed in cold Ringer-phosphate. Since no dry preparation can be obtained, progress can only be made by indirect methods. In Part II, the evidence from studies of R.Q. and oxygen/pyruvate ratio indicated that approximately 4 atoms were used instead of 5, if we allow for pyruvate disappearing as lactate. The CO₂ formed was 10% in excess of the theoretical for complete combustion. This indicates a loss of one molecule of pyruvic acid out of five by some channel other than complete combustion; either a part goes along an alternative path, or a coupled reaction involves a loss, or the oxidation of part is merely incomplete. The incompletely oxidized molecule gives one molecule of CO₂, and presumably remains as some two-carbon compound of which there are several obvious possibilities.

We can now inquire what is the path followed for the pyruvic acid burnt completely.

¹ Some of the experiments were mentioned in *Nature, Lond.* (1936), 138, 552.

Table I

Table II. *Effect of acetate upon the catatorulin effect*

		15 min.	15 min.	30 min.	30 min.	30 min.	Differences due to V
1259	P	1580	1302	920	720	500	245, 258, 580, 560, 675
	PV 5 γ	1825	1560	1500	1280	1175	
	P Na acet. (30 mg.)	1460	1100	932	750	610	380, 520, 548, 540, 590
	P Na acet. V	1840	1620	1480	1290	1200	

P = pyruvate. Na acet. 10 mg./ml. 0.074 *M*.

Some of the possibilities for the oxidation of pyruvate and the action of vitamin B_1 are summarized in Table I, Schemes A, B, C. In addition the action of vitamin B_1 might not be confined to pyruvate, but might be general for α -keto-acids.

Anaerobic possibilities. Schemes B and C are primarily anaerobic. *B* is essentially Krebs's scheme, where *X* is pyruvic acid or some other α -keto acid such as α -ketoglutaric acid. He himself has abandoned the idea that vitamin B_1 catalyses the reaction pyruvic acid $\rightarrow$ lactic acid + acetic acid + CO_2 , nor could we obtain evidence that it increased the anaerobic disappearance of pyruvic acid in our system. It is true that in Part II [McGowan, 1937] it was found that some of the pyruvic acid disappearing is converted into lactic acid; but this is only a small fraction. That acetic acid is an essential stage is made unlikely from the observation that even high concentrations of acetic acid have no influence upon the catatorulin effect.¹ One experiment out of three is quoted in Table II. If vitamin B_1 was acting as the diphosphate (co-carboxylase) [Lohmann, 1937], then Scheme C would include the suggestion arising from Lohmann's discovery that the preliminary stage in oxidation is conversion of pyruvic acid into acetaldehyde. It is proposed to deal with this interesting possibility later, but it must be emphasized that our experiments so far have only shown increased disappearance of pyruvic acid due to vitamin when oxygen has been present.²

Aerobic oxidation. Considering the schemes A and B we can ask two questions: (1) whether pyruvate can be replaced by other keto-acids, and (2) whether the course of oxidation follows the Toenniessen-Brinkmann scheme, or some modification of it, i.e. via succinic acid and possibly α -ketoglutaric acid. Light can be thrown upon these questions by observing whether keto-acids other than pyruvic acid show a vitamin effect and whether there is any change in the vitamin effect with pyruvate in the presence and absence of other substrates. The following substances of general interest were chosen, α -ketoglutaric acid, α -ketoadipic acid, acetoacetic acid and succinic acid. Table III gives some typical experiments.

With α -ketoglutaric acid, Table III (*a*), there is an increased oxygen uptake with normal pigeon's brain. Using 20 mg. in absence of pyruvic acid there is no

¹ Extra O_2 uptake induced by vitamin B_1 .

² Except in rat cerebrum.

Table III. *Pigeon's brain; oxygen uptake with α -keto-acids*

In all experiments the concentration of pyruvate used was 0.020 *M*.

In each group of experiments, the concentration of vitamin was the same, but the amount added is only given with the first experiment in which it is used.

(a) α -Ketoglutaric acid (α -COGI) (5 experiments quoted out of 10). O_2 uptake μ l./g.

Exp. no.	Condition	Addition	Concentration	0- $\frac{1}{4}$	$\frac{1}{4}$ - $\frac{1}{2}$	$\frac{1}{2}$ -1	1-1 $\frac{1}{2}$	1 $\frac{1}{2}$ -2 hr.	V differences
1079	Normal	Nil	—	1510	1065	900	627	440	—
		α -COGI	0.045 <i>M</i>	1925	1415	1155	887	705	—
		α -COGI	0.109 <i>M</i>	1920	1580	1231	924	652	—
		Pyr.	—	3545	3125	2695	2290	1805	—
		Pyr. + α -COGI	(0.045 <i>M</i>)	3210	2955	2710	2200	1702	—
1077	Normal	Nil	—	1410	1145	865	604	468	—
		α -COGI	0.0048 <i>M</i>	1710	1360	1130	831	686	—
		α -COGI	0.0097 <i>M</i>	1740	1401	1060	845	715	—
		α -COGI	0.029 <i>M</i>	1845	1410	1160	963	772	—
		α -COGI	0.048 <i>M</i>	2000	1492	1242	1002	800	—
1081	Avit.	Nil	—	(1620)	(1235)	(928)	(731)	497	—
		V (3.5 γ)	—	1607	1345	1000	771	571	—
		α -COGI	0.045 <i>M</i>	1945	1715	1226	971	805	—
		α -COGI + V	0.045 <i>M</i>	1700	1605	1157	970	(902)	—
1085	Avit.	Nil	—	1780	1435	1053	780	580	—
		V (3.5 γ)	—	1790	1448	1140	880	580	—
		α -COGI	0.045 <i>M</i>	2160	1715	1315	1070	700	—
		Same + V	—	(2153)	(1730)	1450	1266	935	—
1082	Avit.	Pyr.	—	1970	1623	1192	870	540	873, 1020, 990
		Pyr. V	—	2560	2270	2065	1890	1530	—
		α -COGI Pyr.	0.045 <i>M</i>	2365	1890	1525	1090	875	795, 975, 905
		α -COGI Pyr.V	0.045 <i>M</i>	2750	(2605)	2320	2065	1780	—

(b) α -Keto adipic acid (3 experiments quoted out of 7) (α -keto adipic 0.052 *M*)

Exp. no.	Condition	Addition	0- $\frac{1}{4}$	$\frac{1}{4}$ - $\frac{1}{2}$	$\frac{1}{2}$ -1	1-1 $\frac{1}{2}$	1 $\frac{1}{2}$ -2	2-2 $\frac{1}{2}$	V differences
1094	Normal	Nil	1492	1129	890	620	468	332	—
		α -CO-adip.	1435	1025	865	627	459	357	—
		Pyr.	3230	2815	2395	2030	1675	1430	—
		Pyr. + α -CO-adip.	3080	2746	2330	1950	1465	1355	—
1097	Avit.	Nil	1430	990	795	600	—	—	—
		V. 3 γ	1595	1240	973	697	—	—	—
		α -CO-adip.	1234	960	687	560	—	—	—
		Same + V	1375	1087	842	678	—	—	—
1101	Avit.	Pyr.	2600	2080	1630	1302	894	—	725, 810, 788,
		Pyr. V	3110	2805	2440	2090	1670	—	771
		Pyr. + α -CO-adip.	2440	2030	1635	1240	834	—	415, 455, 610,
		Pyr. + α -CO-adip. + V	2850	2450	2190	1855	1460	—	626

(c) Aceto-acetic acid (3 out of 6 experiments quoted)

Exp. no.	Condition	Addition	Concentration	0- $\frac{1}{4}$	$\frac{1}{4}$ - $\frac{1}{2}$	$\frac{1}{2}$ -1	1-1 $\frac{1}{2}$	1 $\frac{1}{2}$ -2	V differences
1104	Avit.	Nil	—	1370	1130	866	620	—	390
		3 γ V	—	1805	1440	1245	1010	—	—
		Ac. ac. acid	(0.042 <i>M</i>)	1590	1130	1037	756	—	344
		—	—	1760	1550	1350	1100	—	—
		Pyr + Ac. ac.	(0.042 <i>M</i>)	1730	1240	1055	732	645	410, 430, 490, 593,
		Same + V	—	2140	1670	1545	1325	1213	568
1105		Pyr.	—	1410	1085	807	585	400	549
		Pyr. V	—	1770	1580	1430	1084	949	—
		Pyr. + Ac. ac.	(0.035 <i>M</i>)	(1635)	(1325)	(1055)	682	556	528
		Same + V	—	1500	1710	1500	1120	1084	—
1106		Pyr.	—	1917	1490	1110	840	—	—
		Same + V	—	2315	2195	1870	1552	—	—
		Pyr. + Ac. ac.	0.075 <i>M</i>	(1810)	(1430)	(1210)	880	—	—
		Same + V	—	2215	1975	1795	1500	—	—

vitamin effect in Exp. 1081, but an apparent slight one in Exp. 1085. More work would be needed to decide whether this is significant in view of the small uptakes involved, but it is clearly of a different order from that with pyruvic acid; the effect was not noticeable for an hour, which suggests that, if real, it is secondary to some other change. With pyruvic acid present (Exp. 1082), there is no significant increase or decrease of the vitamin effect (Fig. 1.)

α -Ketoadipic acid, Table III (b), caused no increased or decreased oxygen uptake either alone or together with pyruvic acid. The only exception to this statement, a decrease during the first half hour, is probably insignificant. In any event there is no large change.

Acetoacetic acid, Table III (c), produced a slightly increased oxygen uptake by itself, but this was no evidence of an interaction with pyruvic acid or with vitamin.

These experiments show that the vitamin B₁ effect is not general for keto-acids or even α -keto-acids; at present the only substance known to react is pyruvic acid. Since there is no increased catatorulin effect with pyruvate + other acids, it is unlikely that any interaction with these substances is concerned (i.e. Scheme B is excluded). Further, since they fail to decrease the catatorulin effect in presence of pyruvate, even when present in high concentration, they cannot be intermediates in the oxidation.

Fig. 1. Ordinates represent oxygen uptake for period of experiment 1-2 hr. Figure at top of rectangle is the amount of extra oxygen uptake due to added vitamin.

Succinic acid. The possibility that pyruvic acid can be oxidized via succinic acid has been so often raised [Toenniessen & Brinkmann, 1930; Elliott & Schroeder, 1934; Weil-Malherbe, 1937; Krebs, 1936; 1937] that it was naturally considered

here. Earlier work from this laboratory had shown of course a marked independence of succinic dehydrogenase and vitamin-reacting systems. If one stage in the oxidation of pyruvate is via succinate, then saturation of the succinic dehydrogenase present by succinate should lead to diminution of the total amount of extra oxygen uptake due to the vitamin. Experiments showed that this was not the case. Fig. 1 records the essential data for 1 experiment out of 4. Within the limits of error, the O_2 uptake due to vitamin was the same in presence and absence of succinate. The conclusion is that the vitamin B_1 -catalysed pyruvate oxidation does not take the course suggested by the Toenniessen-Brinkmann scheme or by other modifications of the scheme involving succinic or α -ketoglutaric acids. The path is at present undefined. Of the possible Schemes A or C, A seems the more probable, but experiments are now in progress in an attempt to decide whether Scheme C with the intermediate acetaldehyde (Lohmann) is possible.

Experimental

α -Ketoglutaric acid and α -ketoadipic acid were prepared by the methods of Blaise & Gault [1911]: acetoacetic acid was made from the purified methyl ester [Ljunggren, 1924]. The remaining experimental details have not varied significantly from previous technique; Na and K pyruvate mixture $0.020 \pm 0.004 M$ has been prepared by neutralization of the diluted pure acids with a mixture of $N NaOH$ and KOH with Na and K in proportions present in the Ringer-phosphate solution pH 7.3; respirometer bottles have contained 3.0 ml. fluid.

SUMMARY

The "pyruvate oxidase" system in pigeon's brain of which vitamin B_1 is a constituent is not a general α -keto-oxidase. The path of oxidation does not appear to include succinic, α -ketoglutaric or acetoacetic acid, and so differs from other suggested modes of degradation of pyruvic acid.

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