CCXXII. PYRUVIC ACID AND VITAMIN B_1 DEFICIENCY.

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The significance of pyruvic acid in carbohydrate metabolism of animal tissues has long been discussed. Since Meyerhof, Lohmann and Meier [1925] showed that out of a large number of substances investigated only pyruvic acid and lactic acid increased the oxygen uptake and carbohydrate content of muscle, pyruvic acid has been suggested as a stage in the oxidation or oxidative re-synthesis of lactic acid. Only recently, however, have Case and Cook [1931] been able to detect pyruvic acid in muscle in the absence of fixative under both aerobic and anaerobic conditions. A discussion of the possible origin and fate of this pyruvic acid is given in a recent paper by Case [1932, 2].

We have shown [Gavrilescu and the authors, 1932] that the action of vitamin B_1 concentrates *in vitro* was specifically related to the lactic oxidase system of pigeon's brain; no evidence could be obtained of interaction with the succinoxidase system. A desire to settle the point of action of the vitamin concentrates led us to the study of the behaviour of normal and avitaminous pigeons' brains in the presence of pyruvic acid. The experiments were made more interesting by the realisation that light might be thrown upon the fundamental problem of the rôle of pyruvate in carbohydrate metabolism.

Our work has elicited the unexpected fact that the oxygen uptake in the presence of pyruvate is always much more depressed than with lactate in the avitaminous brain; further, contrary to experience with lactate, this depression is not correspondingly improved by the addition of vitamin B_1 concentrates *in vitro*. Normal brains show good oxygen uptakes with pyruvate as substrate. We have not been able to establish a satisfactory explanation of these facts.

Experimental.

Method. The oxygen uptake of minced pigeon's brain has been determined as previously described [Gavrilescu *et al.*, 1932]. The estimations have all been done at 38° with 50–100 mg. of minced tissue suspended in 3·0 cc. of phosphate buffer solution. The vitamin B_1 concentrate had an activity of approximately 0·05 mg. for the bird and was the same as previously used. Vitamin B_1 deficiency was induced in the birds by our usual methods.

Pyruvic acid. The literature dealing with pyruvic acid in relation to oxidase and other biological systems is singularly silent upon the methods used to ensure the purity of the acid under experiment[1]. For our purposes freedom from the last traces of lactic acid, and of possible toxic impurities (see below) was important. We have therefore relied upon one or two redistillations of a pure commercial sample (Messrs Boots and Co.) and have in many cases used freshly distilled acid. Our samples did not crystallise, but distilled within a close range of 0·5–1·0° at pressures of 15–20 mm. Hg and were quite colourless. After keeping for 1 month the titration of one sample gave 93 % —COOH and 88 % by iodine value. Specimens of the 2:4-dinitrophenylhydrazone prepared direct from aqueous solutions gave the correct melting-point, and we have little doubt that a sufficient approach to purity was made for our purposes.

Results.

Oxygen uptake in the presence of varying concentrations of pyruvate.

Table I shows the oxygen uptake of normal and avitaminous cerebrums and optic lobes/rest in the presence of varying concentrations of pyruvate. Fig. 1 shows typical curves for normal and avitaminous cerebrums. The

Table I. *Effect of varying concentrations of pyruvate.*

Oxygen uptake in mm.³/g./hr.

Exp.	Ringer	Lactate	Pyruvate 0·007 *M*	Pyruvate 0·017 *M*	Pyruvate 0·034 *M*	Pyruvate 0·102 *M*
			Normal brains.			
			(*a*) Cerebrums.			
264	(850)	—	1750	1930	1705	(1540)
276	770	2150	2040	2120	2230	2000
278	740	1960	1980	(2350)	2300	(2050)
279	1005	1860	1710	1680	1935	1575
			(*b*) Optic lobes/rest.			
264	510	—	—	—	(1300)	1200
286	635	—	1830	2005	—	—
			Avitaminous brains.			
			(*a*) Cerebrums.			
262	775	—	1085	970	925	735
265	740	—	1010	(1080)	975	895
			(*b*) Optic lobes/rest.			
265	(660)	—	875	(870)	790	—

apparent Michaelis constant for the enzyme systems under these conditions is of the order of 0·003 *M*, both for normal and avitaminous brains. This is approximately the same as the constant previously obtained for lactate under similar conditions, if we allow for the presence of inactive lactate in the racemic mixture.

[1] Since the completion of this work, the papers by Case [1932, 1, 2] have appeared. The paper by Wendel [1932] also appeared while this was in progress.

A factor, however, appears which was not present in our previous experiments with succinate and lactate, namely inhibition with higher concentrations. This effect was obtained with our most highly purified specimens, and in our opinion cannot be due to a toxic impurity. Bernheim [1928] showed that pyruvic acid in the same concentrations had an inhibitory effect on the lactic acid dehydrases of yeast. We have found no evidence of pyruvic acid inhibiting the oxygen uptake in the presence of lactate at concentrations below 0·034 *M*. As a result of this inhibitory effect there is no broad optimum for maximum oxygen uptakes. In one experiment the maximum was at 0·007 *M*, and the inhibition started at 0·017 *M*. In another there was no inhibition with 0·03 *M* pyruvic acid. We have worked with 0·007 *M* and 0·034 *M* pyruvate, and although we cannot be certain that these will give maximum uptakes, they will not be far from the optimum.

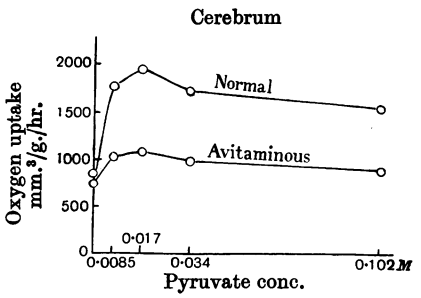

Fig. 1.

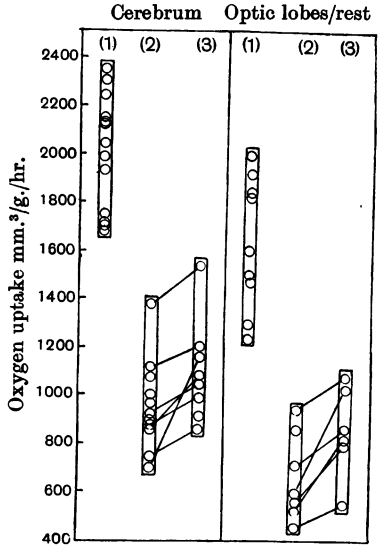

Fig. 2.

Fig. 1. Oxygen uptake of cerebrum from pigeon, normal and avitaminous, in relation to concentration of pyruvate.

Fig. 2. Oxygen uptake of pigeon's brains, (1) normal, (2) avitaminous, (3) avitaminous + vitamin B_1 concentrate.

Oxygen uptake for normal and avitaminous brains.

(1) *Comparisons with lactate and pyruvate as substrates.* Fig. 2 shows all the results obtained with normals using pyruvate as substrate. Most of the figures are in Table I and the averages are in Table II. Table III contains all the results for avitaminous brains and the effects of addition of vitamin B_1 concentrates *in vitro*.

It will be seen that the normal brains take up as much oxygen in the presence of pyruvic acid as in the presence of lactic: indeed the averages are

Table II. *Pyruvate results.*

Normals.

Oxygen uptake in mm.³/g./hr.

	Lactate	Pyruvate 0·007 *M*	Pyruvate 0·017 *M*	Pyruvate 0·034 *M*
Average cerebrum	1620	1870	2010	1895
Average optic lobes/rest	1380	1840	1800	1480

Table III.

Oxygen uptake in mm.³/g./hr.

Exp.	Lactate	Pyruvate Concentration *M*	Pyruvate Control	Pyruvate Vitamin	Increase %
		Avitaminous birds.			
		(*a*) Cerebrums.			
238	—	0·034	730	870	19
241	(1360)	0·034	900	995	10
245	1160	0·034	705	(1165)	66
249	1375	0·034	890	915	2
266	—	0·034	900	1050	17
300	1430	0·017	1380	(1550)	12
301	1290	0·017	(1340)	1600	19
266	—	0·017	885	1090	23
262	—	0·017	970	—	—
265	—	0·017	(1080)	—	—
267	1200	0·007	920	1210	30
281	(1150)	0·007	(1125)	1210	8
262	—	0·007	1085	—	—
265	—	0·007	1010	—	—
		Average	990	1160	20
		(*b*) Optic lobes/rest.			
245	—	0·034	600	(1040)	70
248	—	0·034	460	560	22
249	—	0·034	570	810	42
262	—	0·034	530	830	57
300	1130	0·017	(955)	8090	14
301	(980)	0·017	770	1140	48
280	—	0·007	720	875	21
265	—	0·007	875	—	—
		Average	610	910	40

higher. On the other hand the uptake of the avitaminous brains is very much lower than for lactate, *e.g.* Exp. 245; residual oxygen uptake 665; pyruvate 705; lactate 1160. It might be considered from these results that depressed oxidation with pyruvate represents a more fundamental lesion than that with lactate. The effect of addition of vitamin B_1 concentrates, however, does not confirm this.

(2) *Addition of vitamin B_1 concentrates.* Reference to Table III and Fig. 2 shows that the effect of the concentrate is very variable. Often the increase can be entirely explained by the effect on the residual uptake. Often, especially in the optic lobes/rest, the effects are large, but in no case does the concentrate ever raise the uptake above 1140 mm.³/g./hr., where the minimum normal

figure is 1550, and the average 1850. The vitamin acting *in vitro* is unable to restore the oxidising powers to anything approaching normal with pyruvate as substrate. This suggests that the change is not connected with the deficiency of vitamin B_1 directly. If this interpretation of the results is correct, it raises two possibilities. The failure of the avitaminous brain to take up oxygen in the presence of pyruvic acid might be due either to changes in the brain tissue secondary to the vitamin B_1 deficiency, or to the absence from the avitaminous minced brain of some necessary somatic factor (supplied by the blood stream).

Birds killed with iodoacetic acid.

With regard to the first possibility, the only change of which we know, apart from changes in oxidation, is the increase in lactate in avitaminous as compared with normal brains. If lactate and pyruvate were both oxidised at some common centre, it might be that the increased concentration of lactate blocked the pyruvate. This we have tried to test by killing birds with iodoacetate (IAA), which is known to inhibit lactic acid production in these brains. Kinnersley and Peters [1930] for instance found in normals killed with IAA 20–30 mg./100 g. of lactic acid and in avitaminous 30–60 mg.[1] This would be quite different from the normal 100–150 mg./100 g. and avitaminous 150–250 mg./100 g. The dose of IAA used for killing the birds was 75 mg./1000 g. body weight. For safety we have used IAA in the bottles during the experiments in a concentration 0·1 mg. per 1 cc. of Ringer as suggested by Fisher [1931]. This has some effect on oxidation both with pyruvic acid and lactic acid, as the poison is not absolutely defined in its action [Lohmann, 1931], and with increased concentrations glyoxylase systems are affected [Dudley, 1931]. The decrease is shown in Table IV. With normal brains the pyruvate and lactate

Table IV. *Iodoacetic acid experiments.*

Oxygen uptake in mm.^{3}/g./hr.

	Cerebrums			Optic lobes/rest		
Exp.	Ringer	Lactate	Pyruvate	Ringer	Lactate	Pyruvate
			(*a*) *Normal brains.*			
289	460	(1150)	1135	380	1125	(1120)
293	(440)	(1380)	1300	385	945	1100
296	550	1610	1165	515	1405	1095
299	500	1390	1240	(425)	1060	1070
			(*b*) *Avitaminous brains.*			
288	490	(1030)	(775)	—	560	(510)
294	505	970	(670)	—	650	450
297	530	1160	900	320	780	550
298	430	1000	670	390	750	580

oxygen uptakes are almost identical. The extra oxygen uptake for pyruvate has disappeared. In one experiment, No. 296, however, the pyruvate oxidations are much more markedly depressed than the lactate. There is an indication that the IAA has a possible specific inhibitory effect on pyruvic acid

[1] The cerebrum generally gave the lower figures.

oxidations, and this must moderate any conclusions drawn from these experiments. In the avitaminous brains there is still the increased depression of oxygen uptake in the presence of pyruvate and there is no marked improvement on vitamin addition. Even with the lowered lactic acid content of the IAA brains the pyruvate is less efficient. Hence there is no indication that the changes in pyruvate behaviour are due to blockage by lactate.

The oxygen uptake in the presence of pyruvic acid of the brains of birds cured by dosing with concentrates.

The authors [1932] had previously investigated the oxygen uptake of cured avitaminous birds' brains in the presence of lactate. Table V gives figures for pyruvate and lactate for two groups of birds both dosed with 8–12 pigeon

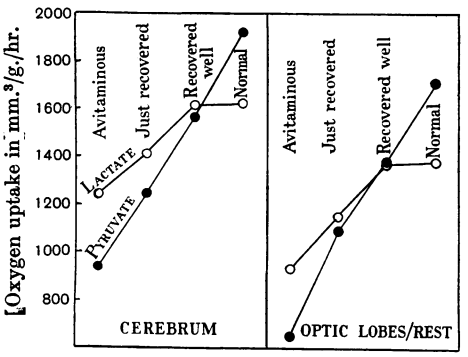

Fig. 3. Chart showing oxygen uptake (average) in relation to condition of bird, avitaminous, just recovered, recovered well, normal. ● pyruvate, ○ lactate as substrate.

Table V. *Dosed birds.*

Oxygen uptake in mm.³/g./hr.

	Cerebrums				Optic lobes/rest	
	Lactate	Pyruvate			Lactate	Pyruvate
Exp.		Concentration *M*	Control	Vitamin		
		(*a*) *Birds just out of symptoms.*				
291	1430	0·017	(1200)	(1235)	995	890
290	1325	0·017	1280	(1290)	1395	1085
274	1350	0·007	1320	1790	1225	1320
284	1380	0·017	(1220)	(1270)	1270	1040
273	1520	0·007	(1355)	1600	(1220)	1100
285	(1430)	0·017	(1100)	1510	1005	1005
Average	1410		1245	1450	1140	1070
		(*b*) *Birds well recovered.*				
283	1665	0·017	1580	(1770)	(1380)	(1410)
292	1540	0·017	1500	—	(1295)	(1285)
272	(1480)	0·007	1405	(1570)	(1350)	—
277	1685	0·007	(1730)	1720	1420	—
282	1660	0·017	1780	1600	1380	(1475)
275	1650	0·007	(1400)	1790	1405	1270
Average	1615		1565	1690	1370	1380

doses (approximately vitamin B_1 units). Group (*a*) had only just recovered from head retraction and were very weak. Group (*b*) had had a longer time for recovery. The averages for lactate and pyruvate for each group and for normals and avitaminous are shown in Fig. 3. There is a recovery of oxygen uptake in the presence of pyruvate, but it is not so complete as for lactate. No relationship has been found between the degree of recovery with the two substrates in individual birds. We feel that these experiments indicate that the fundamental lesion associated with the nervous symptoms is concerned with the lactate, and that the change in behaviour to pyruvate is in some way secondary.

Discussion.

These experiments make it difficult to believe that pyruvic acid is a normal stage in oxidation of lactic acid, unless it be assumed, as suggested by Neuberg [1927] that pyruvic acid formed in the tissues molecule by molecule is constitutionally different from added pyruvic acid. In this respect, we are in agreement with Case and Cook [1931] and Case [1932, 1, 2].

We have thought that for the normal oxygen uptake of pyruvate to be regained by avitaminous tissue a somatic factor might be required, whose co-operation with the vitamin is needed for normal oxidations in the presence of pyruvic acid. We cannot say whether such a factor is needed to remove a toxic substance in the tissues or to provide a co-ferment. A few experiments with addition of serum have given very varied and inconclusive results.

Summary.

1. Normal pigeon's brain when minced gives a large oxygen uptake in the presence of pyruvic acid.

2. The avitaminous pigeon's brain gives a very low oxygen uptake in the presence of pyruvic acid, which is not restored by addition of vitamin B_1 concentrate *in vitro*. These same concentrates are capable of restoring lowered oxygen uptake with lactate as substrate.

3. The defect in oxidation is not associated with the high lactic acid content of the avitaminous brains.

4. There is no indication that pyruvic acid is a stage in the oxidation of lactic acid in the pigeon's brain.

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