CXXXI. PYRUVIC ACID AS AN INTERMEDIARY METABOLITE IN THE BRAIN TISSUE OF AVITAMINOUS AND NORMAL PIGEONS.

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THE recent discovery of a pyruvate reaction in lactate solutions in which avitaminous pigeon's brain has respired *in vitro* for 2 hours [Peters and Sinclair, 1933] is of interest in relation to the action of vitamin B₁, because addition of this reduces the amount of the abnormal substance (for references see Gavrilescu *et al.* [1932] and Meiklejohn *et al.* [1932]). More generally, identification of pyruvate in these circumstances lends support from an unexpected source to the recent conceptions of this substance as a normal intermediary in the metabolism of carbohydrate in the animal (see especially Embden *et al.* [1933] and Meyerhof and Kiessling [1933]). Otherwise these views depend entirely upon work with added sulphite or fluoride. There is also involved the even more fundamental problem of synthesis of carbohydrate from lactic acid, since the indication is that vitamin B₁ is related to the oxidative synthesis of lactate. With this idea in mind, we have devoted attention in the main to phenomena occurring in lactate solutions, as we are more likely so to approximate to conditions favouring the synthesis. The experiments described in this paper have been made with minced brain and in phosphate solutions to facilitate manipulations and to obtain better duplicate control. The conditions are frankly artificial and narrowed for the study of the one point, but it is most unlikely that qualitative errors have been imposed by our conditions. The presence of the substance reacting like pyruvate is equally induced by sections of avitaminous brain and was found by Sinclair (personal communication) to occur with bicarbonate solutions.

We have proved that the nitroprusside-reacting substance is actually pyruvic acid and have obtained quantitative data relating extra pyruvate disappearance in presence of vitamin to extra oxygen uptake to find out whether pyruvate disappears as a direct or indirect effect of the vitamin¹.

EXPERIMENTAL.

Pyruvic acid is known to bind bisulphite. It can be readily shown that bisulphite-binding substances (B.B.S.) (*a*) do not appear during the respiration of normal brain tissue (*b*) appear during that of polyneuritic tissue, and (*c*) disappear with addition of vitamin. Bisulphite-binding substances however do appear during the respiration of normal brain tissue in the presence of iodoacetate.

¹ A preliminary account has appeared in *J. Physiol.* **81**, 22 P.

Unless otherwise stated the minced¹ brain tissue from normal or polyneuritic pigeons (with opisthotonos symptoms) has been shaken in phosphate Ringer-lactate or pyruvate solution at p_H 7.3 [see Passmore *et al.*, 1933] in differential manometers filled with oxygen: in all cases anhydrous sodium pyrophosphate (brought to p_H 7.3 with HCl) was added in a concentration of 0.0094 *M* per bottle [Peters and Sinclair, 1933]. At the end of the respiration period (usually 2 hours) at 38° to each cooled bottle was added 0.9 ml. of 25 % trichloroacetic acid. After standing 1 hour the contents were filtered and the bottles washed out with 2 ml. of 5 % trichloroacetic acid.

Table I.

Bisulphite-binding substances (B.B.S.).

Results are expressed in ml. *N*/100 *I* per g. tissue
(1 ml. = 0.44 mg. pyruvic acid).

I. Residual.

Exp.	Initial	R.P. 2 hours	R.P. + V. 2 hours	Difference produced by vitamin
533 Normal	—	1.18	—	—
562 Avitaminous	—	1.64	1.60	0.04
663 „	—	2.08	1.69	0.39
535 „	1.17	—	—	—
564 „	—	2.80	1.74	1.06
565 „	—	1.98	1.74	0.24
Average		2.12	1.69	0.43

II. Lactate.

Exp.	Initial	L. 1 hour	L. 2 hours	L. + Iodo.	L. + Fl.
545 Normal	—	—	2.75	—	2.72
531 „	0.92	2.32	(3.30) (3.34)	—	—
533 „	—	—	2.87	—	—
534 „	—	—	3.10	—	—
541 „	—	—	3.69	7.25 (0.0018 <i>M</i>)	3.04
592 „	—	—	4.23	8.2 (0.0005 <i>M</i>)	3.8
Average			3.33	7.72	3.19

(Iodoacetic acid concentration in brackets. Fluoride 1 mg./ml. NaF (0.024 *M*).

Avitaminous.

Exp.	L. 1 hour	L. + V. 1 hour	Difference	L. 2 hours	L. + V. 2 hours	Difference	L. + Fl. 2 hours
535	6.88	5.5	1.38	10.2	7.14	3.06	5.8
539	—	—	—	9.37	5.63	3.74	—
537	5.96	3.78	2.18	7.16	3.93	3.23	—
551	6.6	5.4	1.20	8.95	6.58	2.37	—
553	—	—	—	8.85	6.41	2.44	—
554	—	—	—	9.30	5.23	4.07	—
557	—	—	—	9.88	6.03	3.85	—
558	—	—	—	10.0	5.10	4.90	—
560	—	—	—	11.10	5.33	5.77	—
569	—	—	—	7.45	4.69	2.76	—
580	—	—	—	9.8	5.28	4.52	5.33
Average	6.48	4.89	1.59	9.28	5.58	3.70	5.56

¹ In unpublished results we have found that a large quantitative difference may be induced in respiration by variations in temperature of mincing. The data as to temperature have not been included in this paper, because no qualitative effect seems to have been produced upon the quotient studied.

The combined filtrates were treated with *N* NaOH to bring to p_H 2.0 and made up with water to a final volume of 15 ml. for each bottle containing originally 3 ml. of Ringer *plus* brain tissue. 5 ml. of this solution were then treated with 0.5 ml. of *M* NaHSO₃ and allowed to stand for 15 min. [Clift and Cook, 1932]. Excess bisulphite was removed with *N*/10 and then *N*/100 iodine; 5 ml. of saturated NaHCO₃ were added and the liberated bisulphite was titrated with *N*/100 iodine. The results are given in Tables I and I A.

Table I A.

Abstract of results (averages).

	Initial	R.P. 2 hours	R.P. + V. 2 hours	Diff.	L. 1 hour	L. + V. 1 hour	Diff.	
Residual.								
Normal (1)	—	1·18	—	—	—	—	—	
Avitaminous (5)	1·17	2·12	1·69	0·43	—	—	—	
Lactate.								
Normal (6)	0·92	—	—	—	2·32	—	—	
Avitaminous (11)	—	—	—	—	6·48	4·89	1·59	
	L. 2 hours	L. + V. 2 hours	Diff.	L. + Fl. 2 hours	L. + Fl. + V. 2 hours	Diff.	L. + Iodo.	L. + Gl. + Iodo.
Lactate.								
Normal (6)	3·33	—	—	3·19	—	—	7·72	3·18
Avitaminous (11)	9·28	5·58	3·70	5·56	3·90	1·66	—	5·67

Results in Table I are the mean of duplicate estimations; they agree within ± 2 % for L. (avit.), (± 0.01 ml. iodine for the equivalent of 1.0 ml. bottle contents). For smaller amounts of pyruvate the error is proportionately greater. V. = in all cases, 2 γ crystalline vitamin B₁ hydrochloride per Barcroft bottle [Kinnersley *et al.*, 1933].

Substances combining with bisulphite are present in very small amounts in normal brain and increase slightly during respiration in lactate (not more than 0.4 mg. per g.). The amounts formed with avitaminous brain are of a different order (ten times as much); they decrease 39 % with added vitamin.

A comparison of experiments in Ringer-phosphate only, with those in lactate show a slight increase of B.B.S. with lactate in normal, but a large increase for avitaminous brains. Hence lactate is essential for the formation of the B.B.S. which are decreased in amount by added vitamin. Before proceeding further, it is necessary to know how much of the B.B.S. is really pyruvic acid. The method of Clift and Cook [1932] of heating in alkali to remove substances other than pyruvic acid did not prove satisfactory in our hands, and could not be so certain as isolation of the 2:4-dinitrophenylhydrazone [Case, 1932].

Case's method involves the following steps:

1. Addition of 2:4-dinitrophenylhydrazine to the trichloroacetic acid solution.
2. Extraction of mixed hydrazine and hydrazones with ethyl acetate.
3. Neutralisation with CaCO₃.
4. Evaporation and extraction with toluene.
5. Extraction with Na₂CO₃.
6. Acidification and extraction with ethyl acetate.
7. Evaporation to dryness.
8. Estimation in solution in alcoholic potash.

This has been modified as follows. Stage 1. Same; 5 ml. 1 % hydrazine in 2*N* HCl added to 20.0 ml. trichloroacetic acid solution.

2. Same; at least 3 extractions, and often more.
3. Successive extractions with alkaline phosphate, 1/3 vol. The first extraction with saturated alkaline phosphate (Na), and subsequent with $M/15$.
4. Acidification with HCl and extraction with ethyl acetate.
5. Repeat stage 3.
6. Repeat stage 4, making acid to p_H 2.5 approx.
7. As Case.
8. As Case.

We found that our extracts did not give good results by Case's method unmodified. Since the pure hydrazone of pyruvic acid was found to be completely extracted by alkaline phosphate we proceeded as in stages 3 and 4, continuing the extraction with alkaline phosphate until fresh extracts were colourless, or showed only a faint brown-yellow colour, due to traces of hydrazine. With this exception other stages were repeated until colourless. The trace of hydrazine carried over into 4 is removed almost completely in 5 and 6. The combined ethyl acetate solutions from 2-3 bottles (equivalent to 200-300 mg. tissue) are conveniently made up to 5-6 ml. 1-2 ml. (Ostwald pipette) can be evaporated in a small measuring flask to 0.1 ml., cooled and made up to the mark with the alcoholic potash. Standards containing 0.5 mg. per 1.0 ml. ethyl acetate have been used for comparison ($\equiv$ 0.15 mg. pyruvic acid). The 0.1 ml. ethyl acetate left assists solution in the alcoholic potash and minimises the risk of hydrolysing the hydrazone. It appears to have no influence upon the determination provided that the estimation is performed reasonably soon.

The method has the great advantage that heating is always avoided up to stage 6, and if necessary after this by evaporation *in vacuo*. Hence unstable hydrazones such as that of oxaloacetic acid [Clift and Cook, 1932] should be detected more easily. Stages 2-6 for 4 estimations take approximately 4 hours.

Stage 7 reaches a high accuracy with 0.5 mg. or even less of hydrazone. Stages 2-6 give a good recovery of pyruvic acid 2:4-dinitrophenylhydrazone added at stage 2 to the combined ethyl acetate extracts. Examples are given in Table II.

Table II. *Recoveries.*

1. Pyruvic acid 2:4-dinitrophenylhydrazone added at stage 2. Final ethyl acetate phase made up to 6.0 ml. approx. and 2.0 ml. taken for analysis in all except *c* (3.0 ml.). 1.0 mg. $\equiv$ 0.328 mg. pyruvic acid.

	Added mg.	Found mg.	Corr. mg.
(a)	1.0	1.02	1.0
(b)	1.0	1.02	1.0
(c)	1.0	1.03	1.0
(d)	1.5	1.49	1.48

2. Pyruvic acid added at stage 1.

	Added mg.	Found mg.	Corr. mg.
(e)	0.492	0.494	0.488
(f)	0.492	0.496	0.490
(g)	0.984	0.984	0.981
(h)	0.984	0.960	0.957

As stated previously a trace of hydrazine accompanies the hydrazone, making a difference of approx. 0.01 mg. hydrazone (0.003 mg. pyruvic acid) per 1.0 ml. of final phase taken. This can be allowed for as a blank, if desired. Our slight modification is presumably more specific in its extraction than the Na_2CO_3 stage, but we have not investigated this.

Table III gives the results of estimations by the modified Case 2:4-dinitrophenylhydrazone method upon solutions from some of the experiments given in Table I. The experiments are given in order and probably increase in accuracy;

Table III. *Substrate lactate. Pyruvic acid, estimated by 2:4-dinitrophenylhydrazine method. mg./g.*

Exp.	L.	L. + V.	L. - (L. + V.)	L. %*	L. + V. %*	Diff. O ₂ uptake	μl./ mg. P.A.	Remarks
554	3.32	1.66	1.66	81	72	1345	815	M.P. hydrazone low, 193°
557	2.90	1.68	1.22	67	63	962	790	M.P. 216°
558	3.67	1.57	2.10	84	69	1010	480	M.P. low, 204°
560	3.30	1.37	1.93	68	58	1115	580	(No pyrophosphate)
569	3.31	1.79	1.52	102	88	1162	765	
580	3.88	1.89	1.99	90	82	1020	515	
Average	3.40	1.66	1.73	82	72	1102	657	

* % of B.B.S. estimated as pyruvic acid 2:4-dinitrophenylhydrazone.

Exp. 590. Samples of avitaminous cerebrum in bottles as follows: 4 contained L. only, 4 L. + V. All were treated similarly, but two of each were removed from the bath after the preliminary shaking period, cooled and treated with trichloroacetic acid. The remainder were shaken 1 hour.

	min.	Pyruvic acid		% as pyruvic acid	Weight tissue	- P.A./g.	Diff. P.A.	O ₂ /g.
		As B.B.S.	As 2:4- dinitro.					
L.	12	0.185	0.075	41	0.174	0.43	—	—
L.	60	0.502	0.398	78	0.205	1.91	1.48	1710
L. + V.	12	0.157	0.068	50	0.215	0.32	—	—
L. + V.	60	0.290	0.218	72	0.194	1.08	0.76	2070

Diff. P.A. = 1.48 - 0.76 = 0.72 mg.
Diff. O₂ = 360 μl.
O₂/P.A. = 500.

Note. Single estimations only were performed in these experiments owing to the small amount of pyruvic acid present.

in all cases L. and L. + V. have been extracted side by side. The last experiment (590) is given separately; an attempt was made to estimate the influence of the initial period upon the pyruvic acid disappearance, when there is no estimation of oxygen. The ratio + O₂/- pyruvic acid is within the limit for the others. Pyruvic acid evidently forms a large proportion of the B.B.S. and disappears in presence of added vitamin. Some 20 % of B.B.S. are not pyruvic acid, and this proportion increases as the result of the vitamin action. From this it may be inferred that one of the substances into which pyruvic acid is transformed is a non-acidic substance containing an aldehyde or ketone group.

In Exps. 551, 557, after one or two further purifications by repetition of stages 5 and 6, pure pyruvic acid hydrazone separated upon evaporation of the ethyl acetate phase, M.P. 216°, which showed no alteration in a mixed M.P. Traces of other hydrazones which give a red colour with alcoholic potash often accompany the pyruvic acid hydrazone, giving this a red tint and a lower M.P.; the amount of such substances is small, so that there seems no doubt that the main substance concerned is pyruvic acid.

Pyruvate as substrate.

The conclusion that the vitamin catalyses disappearance of pyruvate as well as increased O₂ uptake can be confirmed by substitution of pyruvate for lactate in the respiring system. This is in striking contrast to lactate, extra disappearance of which was not shown to take place in presence of vitamin [Meiklejohn, 1933].

In the experiments recorded in Table IV (A, B) the amounts of pyruvate present in the Ringer-phosphate solution were 5.0 mg. approx. (0.019 M).

Table IV. (A) *Pyruvate, titrated as B.B.S.*

(Data for sum of duplicate bottles.)

Exp.	P.					P. + V.			
	Initial pyruvate mg.	Weight tissue g.	Pyruvate 2 hours mg.	Pyruvate (mg.) dis- appearing	O ₂ uptake per g. 2 hours	Weight tissue g.	Pyruvate 2 hours mg.	Pyruvate mg./g.	O ₂ uptake per g. 2 hours
				per g.	μl.				μl.
562	10.1	0.2302	8.9	5.2	1220	0.2192	8.1	9.15	2450
563	[9.91]	0.220	8.9	[4.6]	1270	0.1944	8.7	[6.25]	2290
564	10.86	0.2370	9.55	5.52	1350	0.1787	9.6	7.06	2050
565	[10.36]	0.1983	9.45	[4.6]	2180	0.1589	9.2	[7.3]	3165
567	11.5	0.1966	9.95	7.9	1700	0.2112	9.4	9.95	2540
582	10.3	0.1975	9.8	2.53	1635	0.1850	9.45	4.6	2855
588	11.95	0.1664	11.55	2.4	2170	0.1697	10.83	6.6	3595
590	11.95	0.1971	11.17	3.95	1785	0.2049	10.3	8.05	2870
567*	11.5	0.2025	10.2	6.42	1275	0.2233	9.2	10.3	2850

* With lactate present also.

(B) *Pyruvate, estimated by hydrazone method**.

Exp.	P.		P. + V.	
	Pyruvate 2 hours mg.	% B.B.S.	Pyruvate 2 hours mg.	% B.B.S.
563	8.4	94.5	8.15	94
564	9.2	96	8.4†	87.5†
565	8.65	91.5	8.25	90
567	9.37	94	9.3	99
567‡	9.75	95.5	8.9	97
582	9.08	92.5	8.56	90.5
588	10.7	92.5	10.11	93.5

* Mean of duplicate observations agreeing within 2 %, in exps. 567, 582, 588. Remainder single observations.

† Some known to have been lost.

‡ With lactate present also.

Table IV (B) shows that there is much more satisfactory agreement between the B.B.S. by titration and the hydrazone estimations than for lactate. A summary of the essential differences is given in Table V.

Table V. *Substrate pyruvate. The relation between pyruvate disappearing and O₂ uptake due to added vitamin B₁ (differences only).*

Exp.	Pyruvate mg./g.	O ₂ uptake ml./g.	O ₂ /P.A.
562	3.72	1230	330
563	1.55	1020	658
564	1.45	700	482
565	2.53	985	389
567	1.93	840	435*
582	1.95	1220	625
588	3.95	1425	361
590	3.95	1085	275
Average	2.63	1063	443

* With lactate also present 380.

A point needing discussion arises in connection with some of the data in Table III. If the amounts of tissue are approximately equal the initial amount of pyruvate need not be known; if not, error may be introduced. Expressed mathematically:

If c = initial concentration of pyruvate,

a_1, a_2 = amounts present after time t , in pyruvate and pyruvate-vitamin respectively,

b_1, b_2 = weights of tissue (mg.),

k = constant,

d = difference wanted, all being in suitable units,

we have made the general assumption that

$$(c - a_1) = kb_1, \text{ etc.} \quad \dots\dots(1),$$

$$(c - a_1) \frac{100}{b_1} \text{ and } (c - a_2) \frac{100}{b_2} = \text{disappearance of pyruvic acid per 100 mg. of tissue for P. and P. + V.}$$

respectively.

$$\text{Hence} \quad d = \frac{100}{b_1 \cdot b_2} [c(b_2 - b_1) + a_2 b_1 - a_1 b_2] \quad \dots\dots(2).$$

Unless $b_1 = b_2$, it is necessary to know c .

In most experiments c is known; in Exps. 563, 566, its value was doubtful owing to changes which pyruvic acid appears to undergo at slightly alkaline reaction. Clift and Cook [1932] heat in alkaline solution to remove bisulphite-binding compounds other than pyruvic acid, and we have found that over-neutralisation with NaOH has little effect upon the pyruvate provided that the solution is dilute. But direct addition of N NaOH to pyruvic acid, a procedure adopted in some experiments to minimise volume, may be attended by 50 % changes in bisulphite-binding power. This was not realised at first.

In Exps. 563 and 565, where low values were obtained for the titration of bisulphite-binding substances, we have substituted for c by use of the equation

$$(c - a_1) = k_1 b_1 \quad \dots\dots(3).$$

Other known errors in estimating the final quotient lie in the initial 12 minute period in which pyruvic acid disappears and the oxygen uptake is not estimated. In general this will not be serious, as it affects both P. and P. + V., and the oxygen uptakes differ less at first than afterwards.

The extra amounts of pyruvic acid disappearing are of the same order though larger than those with lactate, though the extra oxygen uptakes are very similar in the two sets of experiments, *i.e.* the catatorulin effect is not changed by the substrate. Addition of lactate to pyruvate also caused no change in behaviour in Exp. 567. The consistent vitamin effect with pyrophosphate present is in striking contrast to the earlier work of Meiklejohn *et al.* [1932], who found in absence of pyrophosphate an abnormally lowered respiration with pyruvate and a most variable vitamin effect. From this it was concluded that pyruvate was not a stage in the oxidation of lactic acid, and that another factor was needed to secure pyruvate oxidation. That the failure to interact was not due to blockage by excess lactate in the avitaminous brains was proved by the use of iodoacetic acid. The lowered respiration has been a constant feature of these experiments, but the consistency of the catatorulin effects indicates that pyrophosphate takes the place of the missing factor.

Pyruvic acid is the first organic substance which has been proved to disappear *in vitro* as the result of action of vitamin B₁, this change accompanying the extra oxygen uptake. Much of the evidence presented up to this point is consistent with the idea that the vitamin acts directly upon the pyruvate. We hesitate to take this at its face value. In spite of the constancy of oxygen uptake, there is not much agreement between the O₂/P.A. values. Though the errors involved in the estimation of small differences admittedly reach a high value in Table V, the variations appear to be outside the limits of error. It must be remembered that in the earlier experiments, the vitamin effect failed with pyruvate in tissue which reacted in presence of lactate. Further we have found in a few experiments at p_H 6.6 that pyruvate functions in place of pyro-

phosphate in inducing good oxygen uptake with vitamin, but the disappearance of pyruvate and hence the ratio is again variable (Table VI). (*Cf.* Peters and Sinclair [1933] for behaviour at p_H 6.6.)

Table VI. *Respiration in pyruvic acid at p_H 6.6 (estimated as B.B.S.).*

Exp.	P.					P. + V.				
	Initial* pyruvate mg.	Weight* tissue g.	Pyruvate* (2 hours) mg.	Pyruvate dis- appearing mg. per g.	O ₂ uptake μ l. per g.	Weight* tissue g.	Pyruvate* (2 hours) mg.	Pyruvate dis- appearing mg. per g.	O ₂ uptake μ l. per g.	
563 (b)	[9.81]	0.2454	8.7	[4.6]	1060	0.1991	8.5	[6.5]	1820	
570 (a)	11.95	0.2194	10.5	6.6	1410	0.1949	9.9	10.5	2425	
572 (a)	11.5	0.2633	10.4	4.2	1270	0.2519	9.7	7.15	1800	
(b)	11.5	0.1785	10.4	6.15	1215	0.1972	10.1	7.1	1715	
575 (a)†	[5.1]	0.1866	4.35	[4.6]	1220	0.1866	4.15	[5.0]	1505	
(b)†	—	0.1858	4.15	[4.6]	1165	0.2001	3.96	[5.8]	1600	

Summary.

Exp.	Pyruvate mg./g.	O ₂ μ l./g.	O ₂ /P.A.
563 (b)	- 1.9	+ 760	400
570 (a)	- 3.9	+ 1015	260
572 (a)	- 3.0	+ 530	180
(b)	- 1.0	+ 500	500
575 (a)†	- 0.4	+ 285	700
(b)†	- 1.2	+ 435	360

* Expressed per sum of duplicate bottles.

† Lactate also present.

(a) Without pyrophosphate.

(b) With pyrophosphate.

The balance of the evidence inclines to the view that pyruvate disappearance is really consequent upon some other change, and that the vitamin catalyses some other reaction. The sum total of these reactions indicates that about two molecules O₂ are taken up for one molecule of pyruvic acid disappearing; the average ratio for the lactate experiments is 660 μ l./mg., for pyruvate 450 μ l./mg.; calculated for quotient 2 mol. O₂/1 mol. P.A. = 506.

According to Sinclair [1933] the R.Q. for the extra oxygen uptake due to vitamin in lactate-phosphate solution is 1.6. Hence the average for lactate (660 = 2.5 mol. O₂ approx.) should give 3.75 mol. CO₂ per 1 mol. pyruvic acid disappearing.

For complete oxidation we should have

and R.Q. = 1.2. There is therefore evidence that more CO₂ is being evolved than is required for complete combustion of the pyruvic acid, which indicates entry of some other substance into the oxidation system.

We have the curious paradox that at p_H 7.3 pyrophosphate seems to be needed for pyruvate extra respiration, whereas at p_H 6.6 pyrophosphate is required for lactate extra respiration; at this p_H however pyruvate will function in place of pyrophosphate in restoring catatorulin effect in the presence of lactate. In this complex effect, there appears to be no co-operation of α -glycerophosphate [Peters and Sinclair, 1933], which might have been expected upon the Meyerhof scheme. It is possible that both pyruvate and pyrophosphate are acting as H acceptors at p_H 6.6.

The substance formed from pyruvate in the avitaminous brain is not yet known. Since Meiklejohn [1933] showed that there was no change in lactate accompanying the extra O_2 uptake, and since there is some variation in the ratio O_2 /P.A., it seems to be unlikely that lactate is the main substance. In any case, such a conversion involves a reduction.

The simplest explanation of the accumulation of pyruvic acid in the avitaminous tissue and its disappearance in presence of vitamin B_1 is that pyruvic acid is a normal intermediary stage in some phase of carbohydrate metabolism. This is in essence the Embden-Meyerhof view. The phenomena embodied in the new anaerobic fermentation schemes evidently take some part in aerobic respiration too; according to Meyerhof and Kiessling [1933] fluoride should interfere with the appearance of pyruvic acid from phosphoglycerate, and iodoacetate with its disappearance. Consistently with this, we find that no increased pyruvate appears in normal brain tissue respiring in lactate, though a large increase occurs in the presence of iodoacetate (see Exps. 541 and 592, Tables I and VII). Fluoride (Exps. 535, 580, Table I) much reduces the pyruvate formed

Table VII.

Normal brain.

Respiration (μ l./g.)	Substrate	(Hours)				Total (2 hours)
		0- $\frac{1}{2}$	$\frac{1}{2}$ -1	1-1 $\frac{1}{2}$	1 $\frac{1}{2}$ -2	
	L.	2660	2360	1900	1430	4170
	L. + iodo.	2460	1580	870	340	2625
	L. + fluoride	1470	1310	715	520	2005

Pyruvate estimations.

Substrate	B.B.S.	2:4-dinitro.	%	Tissue weight	- P.A./g.	O_2
L.	0.315	0.255	81	0.170	1.5	4170
L. + iodoacetate	0.665	0.512	77	0.185	2.77	2625
L. + fluoride	0.132	—	—	0.0792	1.33	2005

L. - L. + iodoacetate. Ratio O_2 /P.A. = 825.

by avitaminous brain in lactate solutions. Both poisons reduce the O_2 uptake. Other known origins for pyruvate can probably be dismissed with exception of oxaloacetic acid [Krebs, 1933]. Origin from alanine seems mainly confined to kidney [Krebs, 1932], and that from lactic acid [Banga *et al.* 1932; Barron and Hastings, 1933] is not blocked by fluoride (personal communication from Dr E. Boyland). The appearance of pyruvate during respiration of brain tissue *in vitro* and its inhibition by fluoride provide evidence of the re-entry of lactate into some fermentation system; consideration of this must be postponed until we have more precise data of the action of these poisons upon the cataturulin effect.

Note. The figures throughout are given in terms of tissue wet weight, and not as Q_{O_2} , because there seems to be no advantage in the use of this symbol in this comparative work. The values can be readily translated by considering the dry weight to be 20 % of the wet weight values. $Q_{O_2} = 10 \equiv 2000$ ml./g.

SUMMARY.

1. Disappearance of pyruvic acid and of bisulphite-binding substances accompanies the extra oxygen uptake induced by the action of crystalline vitamin B_1 in pigeon's brain tissue respiring *in vitro*. Estimations have been made by the method of Clift and Cook and by a modified Case method.

2. Pyruvate also disappears when substituted for lactate.
3. The average value for the quotient extra O_2 uptake/pyruvic acid disappearing approximates to 506, representing 2 mols. O_2 taken up to 1 mol. pyruvic acid disappearing, but there are wide variations, which lead to the belief that disappearance of pyruvate is an indirect effect of vitamin action.
4. The phenomena are consistent with the view that pyruvic acid is a normal intermediary in the metabolism of pigeon's brain tissue. In agreement with the Embden-Meyerhof fermentation scheme, it accumulates with respiring normal brain tissue in presence of iodoacetate and not of fluoride.

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Note added in proof by R. A. P. In a recent paper [Birch and Mann (1934), *Biochem. J.* **28**, 622] it is stated that we assumed that the oxidation affected was that of lactic acid. Reference to the papers in question, particularly Gavrilescu *et al.* [1932, p. 445], will convince that we were pedantically careful to write oxygen uptake in presence of lactate. In regard to the later criticism of the conclusions drawn from the previous pyruvate experiments, these authors seem to have overlooked the important point that some of the experiments then performed showed only trifling increases with pyruvate and vitamin as compared with pyruvate alone. Later in their paper, they refer to our original statement that 1/10 pigeon dose produced a maximum increase. Unfortunately this has not been corrected in print, though a correction can be inferred from reference to Passmore *et al.* [1933], where about 1/2 pigeon dose produced a nearly maximum effect. The crude preparation originally used was found upon subsequent test to contain more vitamin than had been supposed.

The melting-points in the above paper are corrected. There is some doubt in the literature as to the exact melting point of pyruvic acid 2:4-dinitrohydrazone. The most usual figure obtained is 216° . We have, however, had in our hands a specimen which melted at 218° . It is hoped that sufficient of the compound from the lactate experiments may become available for further confirmation by analysis.