VII. BRAIN RESPIRATION, A CHAIN OF REACTIONS, AS REVEALED BY EXPERIMENTS UPON THE CATATORULIN EFFECT.

By RUDOLPH ALBERT PETERS, HÅKAN RYDIN[1] and ROBERT HENRY STEWART THOMPSON[2].

From the Department of Biochemistry, Oxford.

(*Received November 2nd, 1934.*)

Previous work has shown that vitamin B_1 acts *in vitro* in respiring enzyme systems from the avitaminous pigeon's brain to induce an increased oxygen uptake [Gavrilescu *et al.*, 1932] in lactate and pyruvate solutions under defined conditions. Pyruvate was formed in lactate solutions in absence of vitamin and disappeared in its presence, suggesting that it was a normal metabolite [Peters and Sinclair, 1933; Peters and Thompson, 1934, 1]. The average value for the quotient, extra O_2 uptake in presence of vitamin/pyruvic acid disappearing, represented 2 mols. of O_2 to 1 mol. of pyruvic acid used, but careful study of this ratio showed variations sufficiently wide to lead to the tentative conclusion that pyruvate disappearance was actually an indirect effect of the added vitamin. In this paper, more evidence is produced in the same sense. The action of the vitamin depends to a marked extent upon the method of preparing the tissue; it is apparently concerned with formation of some unidentified substrate which then interacts to produce increased O_2 uptake. Hence we must visualise the extra oxygen uptake observed as being the result of a chain of chemical events in the tissue.

We have continued to use the avitaminous pigeon's brain for this work; the behaviour of mammalian brain is of course more interesting from many points of view, but use of the pigeon's brain is advantageous in view of the exact knowledge of conditions of avitaminosis and above all of the ease of removal of the brain from the soft skull. It is doubtful whether the facts given below could have been elucidated without the use of the avitaminous brain, which illustrates the advantage of material of this type in illuminating obscure phases of metabolism.

Further details of technique.

Birds used have been in acute opisthotonus symptoms; they have usually been under vitamin B_1 test for 2–8 days, so that the symptoms have appeared for a second time. Depletion is thus more complete. Unless otherwise stated the tissue has been prepared upon a warm plate at 38°; division is done with a small, blunt bone spatula (to avoid cutting and metals). Duplicates are filled alternately; the tissue in the bottles, after re-weighing, is divided with a glass crusher. It is best to allow to stand 3 mins. before making the additions of the vitamin *etc.* Observations have been made in the differential apparatus. Tissue has been put into previously tared bottles. For the rapid simultaneous handling of a large number of duplicate samples from brain tissue, this method has proved more suitable than attempts to weigh exact amounts of tissue upon a balance. All bottles have been filled with oxygen, and duplicates eva-

[1] Rockefeller Fellow. [2] Senior Demy, Magdalen College.

cuated in the order $1a$, $2a$, $3a$, $4a$, the first lot, then $1b$, $2b$, $3b$, $4b$, the second lot, a and b being duplicates, *etc.* Other details of the work have not been changed.

Unless otherwise stated, sodium pyrophosphate (at p_H 7·3) has been used throughout, made up so that an addition of 0·1–0·2 ml. of Na pyrophosphate produced a final concentration of 0·01 M. Small variations in this concentration do not seem to matter. As has been shown previously [Peters and Sinclair, 1933], this compound enhances the catatorulin effect.

Effect of temperature of preparation upon the rate of respiration[1].

The difficulty of obtaining satisfactory duplicate values for respiring brain tissue "brei" has repeatedly forced itself upon our attention. It seemed also that some general factor of unknown nature influenced the level of respiration, apart from the action of the vitamin. In particular, there was a tendency for lower values in winter. This led us to study the effect of temperature of mincing upon the subsequent rate of respiration, with remarkable results. Some of these have been recorded elsewhere [Peters and Thompson, 1934, 2].

In these experiments, equal halves of the brain of a guillotined pigeon were removed with minimum injury; without delay the halves were minced respectively for 2 mins. upon a plate cooled in ice and upon a glass hot water funnel kept warm at 38° ± 2°. In most cases right and left cerebral hemispheres were compared. The results are given in Table I, and a particularly striking case which is diagrammatic of the effect is shown in Fig. 1.

For the normal brains in 13 out of 15 experiments (see a selection of these in Table I and Fig. 1), there was found to be a large difference between the respirations of the "warm" and "cooled" tissue in lactate solutions. This also occurred in pyruvate solutions with avitaminous brains. On the other hand the tissue in Ringer phosphate solution only (residual respiration) showed little difference. Respiration in succinate again was little affected. Among these three substrates (the only ones tried), the influence was confined to lactate and pyruvate. As in the catatorulin effect, succinate respiration is hardly influenced [*cf.* Gavrilescu *et al.*, 1932]. It might be expected that the tissue prepared "warm" would show damage to its respiration, owing to inactivation by enzymes of some essential constituent. The result is quite different. The greater respiration occurs in the tissue prepared "warm." Since there is little change in residual respiration, it is not merely an increase in tissue substrates. In older parlance, it would have been said that more lactate and pyruvate oxidases are present in the "warmed" than in the "cooled" specimen; but an increased O_2 uptake does not seem to be necessarily associated with increased disappearance of lactate [Meiklejohn, 1933]; hence it is more probable that some essential element of the carbohydrate respiration is retained in the "warmed" brain. It is to be noted that the effect is present with added pyrophosphate.

The difference in respiratory behaviour is accompanied by a visible difference in the state of the tissue; in the tissue kept "warm" the trace of blood present at the end of the period of mincing (2 mins.) is strongly reduced, whereas in that minced "cool" it is a bright red colour; it has also been our impression that the tissue kept "warm" has a slight yellowish tinge. The obvious suggestion that the effect was due to the state of the glutathione has been tested. Exp. 658 shows that the extra respiration is not restored to the "cooled" tissue by addition of glutathione.

[1] By use of the term respiration, we wish to imply that the oxygen uptake observed is a complex.

Table I. *Comparison of rates of respiration for tissue prepared under "warm" and "cool" conditions.*

Expressed as μl. O_2/g./hr. (wet weight). $2000 = Q_{O_2}$ 10.

Exp.	Substrate	Prepared	0–½	½–1	1–1½	1½–2
506	Nil	Cool	950	700	530	—
	,,	Warm	1460	930	800	—
	Lactate	Cool	2030	1510	1365	—
	,,	Warm	3060	2330	2280	—
509	Nil	Cool	940	705	585	425
	,,	Warm	1165	830	595	575
	Lactate	Cool	1950	1395	1300	990
	,,	Warm	2370	1880	1500	1380
511	Nil	Cool	655	600	394	364
	,,	Warm	830	615	525	424
	Lactate	Cool	1750	1455	1380	1260 (pp)
	,,	Warm	2255	1790	1708	1663
669	Lactate	Cool	2660	1870	1390	—
	,,	Warm	2870	2410	1790	—
	Succinate	Cool	4610	3900	2900	—
	,,	Warm	4200	3270	2470	—
670	Lactate	Cool	2270	1690	1300	1100
	,,	Warm	3100	2700	2190	1840
	Succinate	Cool	4610	3410	2420	1670
	,,	Warm	4030	3270	2440	1930

Note. The above experiments are illustrative only. 511 was made with addition of pyrophosphate (pp). In 669 and 670 the periods are 0–¼, ¼–½ hours, *etc.* In 669 and 670 concentration of succinate = 0·07 *M* (25 mg. in 3·0 ml.). Nil = Ringer phosphate only.

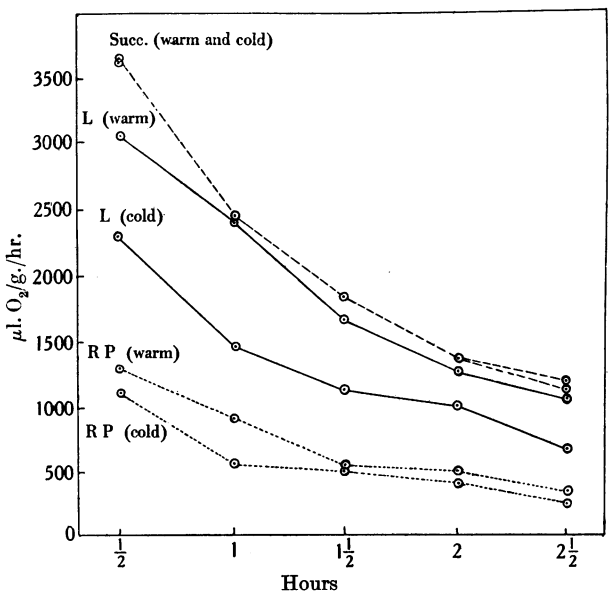

Fig. 1. Exp. 596. Basal medium Ringer phosphate, RP. Succ. = RP + Na succinate, 0·07 *M*. L = RP + Na lactate, 0·03 *M*. Pigeon's brain tissue minced "warm" and "cold". The difference in respiration rates is approximately the same for RP and Succ., but markedly different at first for L.

Exp. 658. The right and left lobes of a pigeon's brain (normal) were removed within 30 secs. of death and minced for 2 mins. upon warmed and cooled plates. Respiration μl./g./hr. Glutathione 0·009 *M*. Ringer-phosphate-pyrophosphate-lactate.

Period (hrs.) ...	¼–¾	¾–1¼	1¼–1¾
Cooled	1760	1470	1370
„ +G	1860	1560	1400
Warmed	2330	2000	1770
„ +G	2460	2160	1855

The slight effect here can be entirely explained upon the grounds of the O_2 uptake of the glutathione itself. Another possibility was tested, *viz.* that part of the effect might be due to drying of the "warmed" tissue and settling of moisture upon the "cooled," though this is really excluded by the succinate experiments; the water contents were found to be not significantly different. It is known [Kinnersley and Peters, 1929] that lactic acid production takes place with great rapidity in these brains, being complete in about 90 secs. At first it was thought that the effect was more marked in brains removed within 30 secs., but this could not be proved.

From the general relation of the catatorulin effect to pyruvate and lactate, it would be inferred that the vitamin would show greatest action upon tissue

Table II. *Avitaminous brains. Temperature effects.*

				μl./g./hr.			
Exp.	Substrate	Prepared	Vitamin	¼–¾	¾–1¼	1¼–2¼	2¼–2¾
651	Lactate	Cool	Control	1350	1080	920	980
			V	1295	1145	1050	970
			Diff.	—	65	130	—
		Warm	Control	1905	1620	1320	1095
			V	2140	1955	1730	1540
			Diff.	235	335	410	445
518	Pyruvate	Cool	Control	670	490	370	340
			V	908	686	540	510
			Diff.	238	196	170	170
		Warm	Control	935	670	470	460
			V	1080	960	800	790
			Diff.	145	290	330	330

minced "warm." The experiments, of which one example of each is given in Table II, show that this is so. In one case even there was no effect of vitamin in the "cooled" tissue.

It is especially difficult to obtain good duplicate values with the "cooled" tissue, probably because of the uneven temperatures induced, even with the best efforts to crush immediately. It is clear that scrupulous attention to minute detail is necessary to get reliable control of these enzyme systems from brain. The temperature effect certainly explains some of our puzzling difficulties in the past.

In previous work, as an experimental hypothesis, we have postulated a substrate *X* with which the vitamin interacts. We have now reached the position that the vitamin effect depends in addition upon some unknown factor present in the "warmed" tissue; it therefore depends upon the method of preparation. If the tissue minced "warm" is transferred to the cooled plate, a change in colour takes place and the tissue becomes bright red. This is followed by a fall in the lactate respiration. Hence cooling and change in the level of oxidation together

lower the respiration. On the other hand, if the "cooled" tissue is transferred after mincing to a warmed plate, change of colour indicating reduction occurs but there is no (or very little) return of the respiration rate in lactate or of increased vitamin effect, as we have found in four experiments of which Exp. 664, Table III, is one; compare with Exp. 651, Table II.

Table III.

Exp. 664. Avitaminous brain (cerebellum). Medium lactate-pyrophosphate (M) ± vitamin. Brain tissue removed within 1 min. and thoroughly minced upon cooled plate for 2 mins., colour bright red. Half transferred to warmed plate (38°), and reminced, colour dark purple. Respiration μl./g./hr.

Condition		¼–¾	¾–1¼	1¼–3¾
Cooled	M	1050	675	645
	M+V	1275	960	915
Cooled, then warmed	M	1190	865	795
	M+V	1355	1025	1000

In effect then a mild degree of cold destroys an essential factor, irreversibly. This may be by physical separation, which is rather the opinion of the Warburg school as to the difference in glycolysis *etc.* between sections and tissue pulp. A more attractive view is that there is irreversible oxidation of some essential co-enzyme, when the action of the dehydrogenases is depressed by the cool conditions.

Evidently determinations of Q_{O_2} in tissue "brei" must be influenced by these findings. In previous papers from this laboratory (except the last) the mincing was carried out at room temperature, and the values must be a mixture of the "cool" and "warm" values. The conclusions are not influenced, as they are based upon comparative work.

The catatorulin effect as a chain of reactions.

Until now the simple hypothesis that the vitamin acted upon a substance X with the direct result of increased oxygen uptake was tenable. A new experiment of different type disproves this simple view.

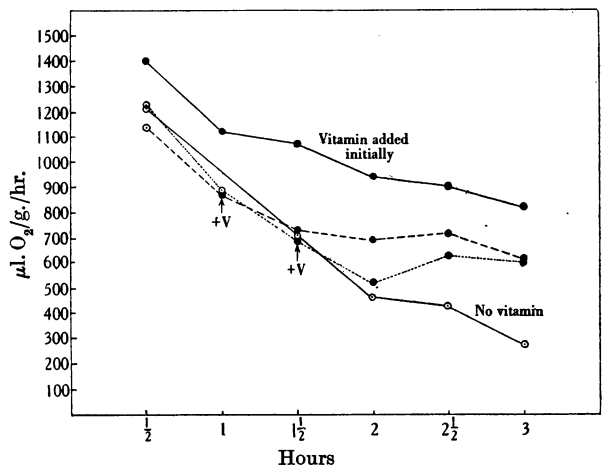

Fig. 2. Exp. 614. o No vitamin. • Vitamin present. Arrow indicates time of addition of vitamin. Avitaminous brain as usual.

Table IV.

Exp. 614. Vitamin added after commencing respiration.

Hours ...	0–½	½–1	1–1½	1½–2	2–2½	2½–3
A. Control	1215	—	710	460	430	270
B. Vitamin	1400	1120	1070	940	900	820
C. Vit. at 1 hr.	1140	870*	722	690	715	610
D. Vit. at 1½ hrs.	1225	885	690*	515	640	600

Note. All contained initially Ringer-phosphate-pyrophosphate and lactate. Vitamin was added to B before commencing the experiment, and to C and D at the times indicated by the asterisks. Dixon-Keilin apparatus used.

It has been realised for a long time in this laboratory that vitamin added at some interval after the start of respiration checks the fall in respiration but produces no rise. In unpublished work, Meiklejohn, Passmore and Peters found a tendency for the rate to be stabilised by added vitamin.

A more recent experiment shows this clearly.

In these tissue "brei" from the avitaminous bird, lactate, lactate + pyrophosphate, lactate + pyrophosphate + vitamin have increasing effects in mitigating the fall in respiration, as well as in increasing the initial rate when added initially.

Of the possible interpretations of these facts we were most attracted by the view that the "stabilised" respiration was due to the formation of some substance during the first 1–2 hours of respiration, which was subsequently oxidised. This was tested in the following way; avitaminous brain tissue was allowed to respire for periods up to 2¼ hours in Ringer-phosphate-pyrophosphate ± vitamin B_1, after which lactate was added. Exps. 644 (lactate) and 645 (pyruvate) given in Table V, and 645 (lactate) in Fig. 3 illustrate the results obtained. Delayed

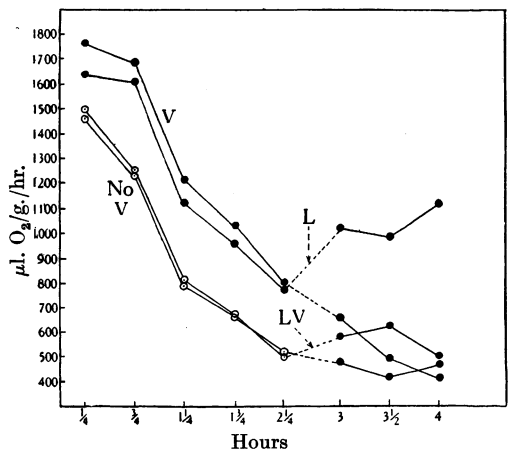

Fig. 3. *Delayed substrate experiment.* 0–2¼ hours' respiration in Ringer-phosphate with and without vitamin. At 2¼ hours, additions of L and LV made to two bottles, and of V to one of remaining bottles. Rise is large only when L is added to tissue previously treated with V. L = lactate. V = vitamin 2γ. • Vitamin present. o No vitamin present. Tissue weights, 340–360 mg. Pyruvate and lactate both used, for pyruvate see Exp. 645, Table V.

addition of lactate (or pyruvate) shows a comparatively small increase in respiration with tissue incubated without vitamin, but a large increase with tissue incubated in the presence of vitamin.

Table V. *Delayed type experiments.*

Exps. 644 and 645. Avitaminous brain (cerebellum), prepared warm. Medium (M) Ringer-phosphate + sodium pyrophosphate (p_H 7·3). Vitamin 2γ in Ringer-phosphate-pyrophosphate. After shaking for 2¼ hours bottles were removed, additions made, bottles re-filled with oxygen, and replaced in bath. Time 5–10 mins. Readings commenced after a further 12 minutes' shaking. Solutions adjusted to make final volume 3·0 ml. Lactate added in 0·1 ml. to make 0·033 *M*.

Exp. 644.		Respiration μl./g./hr.									
	Tissue weight	Hours					Addi-	Hours			
	mg.	0–¼	¼–¾	¾–1¼	1¼–1¾	1¾–2¼	tions	2¾–3	3–3½	3½–4	4–4½
M only	156	1240	910	755	535	490	LV	545	515	515	525
	184	1310	1045	760	585	460	V	365	375	280	240
M + V	147	1465	1225	1015	800	650	L	855	915	810	800
	194	1515	1270	1090	885	745	Nil	555	425	430	365
Exp. 645.		Pyruvate (for lactate see Fig. 3).									
M only	325	1420	1105	745	610	465	PV	440	460	495	—
	267	1300	1050	680	535	420	V	420	360	395	—
M + V	346	1760	1620	1225	1025	821	P	940	955	990	—
	308	1710	1590	1660	980	820	Nil	560	510	430	—

Differences in respiration after the additions.

644	LV–V	180	140	234	285				
	L–Nil	300	491	380	437				
	Diff.	120	351	146	152				
645	LV–V	107	208	42	—	PV–V	20	100	100
	L–Nil	365	492	700	—	P–Nil	380	445	460
	Diff.	258	284	660	—	Diff.	360	345	360

Note. To get satisfactory results, this experiment requires larger amounts of tissue than usual. It is better to use two brains, or, if unavailable, one cerebrum distributed in four bottles. In the latter and more usual case, duplicate estimations are not possible. The experiment was sometimes done in the Dixon-Keilin apparatus, but it is quite effective in the usual type of bottle. The differences in respiration are very large; the larger amounts of tissue complicate the issue with vitamin at the beginning owing to the larger amount of residual lactate present, but in most cases the rise is beyond experimental error.

This experiment has been repeated many times (25) with essentially the same results, though the rise is variable. Pyruvate can be substituted for lactate, and pyrophosphate when present in the incubation period enhances the effect.

In several of the experiments, the rise upon addition of lactate is proportional to the value for the respiration immediately before making the addition. In others the rise for the vitamin-treated samples is sufficiently large to exclude this interpretation. Hence something is present in the vitamin-treated sample which develops an oxygen uptake only when treated with substrate.

In the "delayed substrate" experiments the respiration increases upon adding lactate. Hence, the vitamin cannot be merely preventing the disintegration of the respiratory system [*cf.* Peters and Sinclair, 1933]. But there must be an accompanying inactivation of some essential co-enzyme, since the respiration after 2 hours never recovers to more than 1200–1400 μl., a value considerably less than the initial respiratory value.

A new hypothesis which embraces these further facts is as follows:

Stage 1 Stage 2
$X \longrightarrow Y$ + Pyruvate or lactate and O_2 = Products of oxidation and oxidative synthesis
↑
Vit. B_1
Pyrophosphate

Stage 1 requires little if any extra O_2, and is therefore likely to be a "dehydrogenase" stage. Stage 2 needs the presence of the co-enzyme injured by oxidation. *X* must be regarded as a precursor of *Y*.

It is quite possible that *Y* is merely an active state of *X*, stabilised by vitamin B_1-pyrophosphate, or indeed that the vitamin merely prevents the relapse of *Y* into an inactive state.

If this hypothesis is correct, we should find marked differences in the responses to lactate of "warmed" and "cooled" tissue after the incubation period, without much effect previous to lactate addition. Exps. 660 and 661 in Table VI

Table VI. *Delayed addition of substrate; "warm" and "cooled" tissue.*

Exp.	Period (hrs.) ...		0–¼	¼–¾	¾–1¼	1¼–2¼	2¼–2¾ ↓	3–3¼	3¼–3¾	3¾–4¼	4¼–4¾
660	Cooled	Rpp	1175	835	700	500	380	760	560	450	410
		„ +V	1280	915	730	540	385	785	750	690	630
							Diff.	25	190	240	220
	Warm	Rpp	1755	1260	970	730	495 ↓	950	750	650	550
		„ +V	2040	1430	1110	760	540	1245	1255	1185	1145
							Diff.	295	505	535	595
661	Cooled	Rpp	1520	1024	845	695	525 ↓	805	600	515	445
		„ +V	1325	1040	920	850	560	1100	885	815	780
							Diff.	295	285	300	335
	Warm	Rpp	1790	1605	1085	840	610 ↓	910	700	630	530
		„ +V	2080	1790	1365	1015	690	1240	1240	1225	1155
							Diff.	330	540	595	625

Note. Rpp = Ringer-phosphate-pyrophosphate medium. V = 2γ vitamin B_1. Lactate added at the arrow (0·1 ml. to make 0·033 *M*). In each of these experiments separate halves of the cerebrum were removed as quickly as possible with minimum injury and minced for 2 mins. respectively on "warmed" and "cooled" plates; colour of "warmed" tissue was purple and of "cooled" bright red. Addition of vitamin was made after weighing and subdivision of the tissue.

show that this is so (two similar experiments are not quoted). It is remarkable how much the delayed vitamin effect is increased in the "warmed" tissue. The initial vitamin effect in the "warmed" is accounted for by the presence of residual lactate.

The nature of X[1] *and* Y. Upon the present hypothesis, a substance should behave as *Y* if it interacts immediately upon addition of lactate to produce a rise in respiration; it cannot be α-glycerophosphate, because this was shown to be independently additive in oxygen uptake experiments by Peters and Sinclair. The "delayed" experiment does not decide whether vitamin B_1 is essential for stage 2.

A substance will substitute for *X* if it causes a delayed increase in respiration when lactate + V are present, or increases the rise upon lactate + vitamin addition in the "delayed" experiment.

Several obvious brain constituents have been tested in the attempt to identify the unknowns. So far no effect of the required type has been seen with creatine, creatinine, inositol, galactose, xanthine or crude hexosediphos-

[1] A possible alternative hypothesis. We have given what appears to be the simplest interpretation of our facts. It is just possible that the facts can be explained without recourse to the "indirect" oxidation view. If the vitamin in oxidising the residual lactate maintains the stability of the essential factor, we can then explain the delayed type of experiment as being merely the result of better protection of the essential factor from irreversible destruction. In this case, there would be no need to postulate the substance *X*.

phate (candiolin). Several experiments with extracts of brain have given suggestive, but not decisive, results. Some *X* appeared to be present in a neutralised trichloroacetic extract of hen's or pigeon's brain.

Discussion.

These experiments upon brain tissue depleted of vitamin B_1 demonstrate that oxidations (in the sense of oxygen uptake) with added lactate or pyruvate are secondary to a preparative stage, which is presumably one of H transfer. They seem fundamental, because the oxidation system of lactate is involved. As has been previously suspected, tissue respiration involves an organised chain of reactions, even in a "brei" of this nature.

We have been led by another path to the technique of Quastel and Wheatley [1932] upon which they base the conclusion that narcotics influence the oxidation of lactate rather than succinate. This conclusion is strikingly coincident with the independent finding of Gavrilescu *et al.* [1932], that the nervous symptoms in avitaminosis are related specifically to a lactate rather than a succinate lesion. The finding of Gavrilescu *et al.* can however be demonstrated upon freshly removed tissue. The technique of Quastel and Wheatley [1932] involves a preliminary autoxidation of the brain tissue for periods up to 3 hours, after which the substrate is added. It seems clear now that the subsequent reaction of the tissue to substrate will depend upon some action of vitamin B_1 (and possibly other factors) in the preparative stage.

Since the narcotic (or poison) is added during the preparative stage, the narcotic may well be influencing this preliminary stage, and no conclusions can really be drawn about the effect upon the oxidative stage. This is borne out by experiments upon the effect of fluoride and iodoacetate in the delayed substrate experiment in an accompanying paper [Peters *et al.*, 1935]. Hence the interpretation of Quastel and Wheatley might require modification to the extent that narcotics influence a constituent in the "coupled" oxidation of lactate[1]. That the "coupled" oxidation of lactate might be so influenced at a dehydrogenase stage by narcotics is satisfactory because it brings these effects into line with a generalisation of Keilin [1929] that narcotics in general interfere with the reduction of cytochrome by dehydrogenases.

Much has been made of the very real difference between the behaviours of tissue sections and "brei" in many types of experiment. The writers suggest that in sections the co-enzyme present especially in "warm" minced brain is better protected from irreversible oxidation by association with an active dehydrogenase system, which is more complete.

In regard to the specific application to vitamin B_1 problems, the possible interpretations discussed by Meiklejohn of his failure to correlate extra oxygen uptake of the vitamin with increased disappearance of lactate were three. Of these the most acceptable seems to be that vitamin catalyses the coupled oxidation in presence of lactate of some other substance. It is proved here that pyruvate can replace lactate; presumably the removal of "avitaminous" pyruvate is secondary to the action of vitamin B_1. Unfortunately, we cannot yet settle whether pyruvate or lactate is immediately concerned in the interaction. We must further point out that the proof that vitamin B_1 directly catalyses meta-

[1] It is possible that ignorance of these facts is the source of controversy between Bülow and Holmes and Quastel and colleagues; but see a recent paper by Quastel and Wheatley published while this paper was in course of preparation.

bolism of some carbohydrate constituent is still lacking in absence of knowledge as to the exact nature of *Y*, though there is abundance of evidence as to the indirect connection with carbohydrate metabolites.

SUMMARY AND CONCLUSIONS.

1. As compared with tissue prepared "cold," pigeon's brain tissue prepared "warm" shows marked differences in oxygen uptake. A higher respiration is found in lactate and pyruvate solutions with the "warmed" tissue, whereas only slight differences occur in Ringer phosphate and succinate solutions.

2. The catatorulin effect of vitamin B_1 is most marked with tissue prepared "warm."

3. Vitamin B_1 interacts with some unknown substrate to produce a substance which increases the oxygen uptake in the presence of lactate or pyruvate. The catatorulin effect therefore depends upon a chain of tissue reactions.

4. Sodium pyrophosphate influences the interaction with the unknown substrate.

5. The following hypothesis for the oxidation system is suggested:

Stage 1 Stage 2
$X \longrightarrow Y$ + Pyruvate or lactate and oxygen = Products of oxidation and oxidative synthesis
↑
Vitamin B_1. Pyrophosphate
(depressed by fluoride or iodoacetate)

We are indebted to the Medical Research Council for grants in aid of this work and to Messrs H. W. Kinnersley and J. R. O'Brien for their help in preparation of vitamin B_1.

REFERENCES.

Gavrilescu, Meiklejohn, Passmore and Peters (1932). *Proc. Roy. Soc. Lond.* B **110**, 431.
Keilin (1929). *Proc. Roy. Soc. Lond.* B **104**, 206.
Kinnersley and Peters (1929). *Biochem. J.* **23**, 1126.
Meiklejohn (1933). *Biochem. J.* **27**, 1310.
Peters and Sinclair (1933). *Biochem. J.* **27**, 1910.
—— and Thompson (1934, 1). *Biochem. J.* **28**, 916.
—— —— (1934, 2). *J. Soc. Chem. Ind.* **53**, 453.
—— Rydin and Thompson (1935). *Biochem. J.* **29**, 63.
Quastel and Wheatley (1932). *Biochem. J.* **26**, 725.