CLXXVIII. AVIAN POLYNEURITIS.

FURTHER STUDIES ON THE ACTION OF VITAMIN B_1 CONCENTRATES *IN VITRO*.

BY ARNOLD PETER MEIKLEJOHN[1].

From the Department of Biochemistry, Oxford.

(*Received June 6th, 1933.*)

THE evidence from recent work has strongly suggested that vitamin B_1 is concerned with the oxidative removal of lactic acid in the brain of the pigeon. Kinnersley and Peters [1929; 1930] showed that the polyneuritis of vitamin B_1 deficiency was intimately associated with a localised accumulation of lactic acid in the brain. Gavrilescu and Peters [1931] found that the oxygen uptake of minced brain from polyneuritic birds was lower than normal in the presence of glucose, and that the addition of a vitamin B_1 concentrate *in vitro* was capable of effecting a partial reparation of this defect. More recently it has been shown [Gavrilescu *et al.*, 1932] that in the presence of lactate a similar defect in the oxidations of minced polyneuritic brain can be demonstrated, which is capable of being largely repaired by the addition of very small amounts of a vitamin B_1 concentrate *in vitro*[2]. No significant defect in the oxygen uptake of the brain was found in the absence of added substrates or in the presence of succinate. Experiments on birds recovering from polyneuritis after dosing with vitamin B_1 concentrate [Meiklejohn *et al.*, 1932] showed that there was an improvement in the oxidative behaviour of the minced brain with lactate, corresponding to the disappearance of the nervous symptoms, and with this improvement the effect of added vitamin B_1 concentrate *in vitro* diminished. It was concluded that the vitamin B_1 concentrates contain a substance capable of repairing the same defect both in the living bird and in the isolated brain, and that this defect is in the system responsible for the increased oxygen uptake of the isolated brain in the presence of added lactate. The evidence strongly suggests that the substance in the concentrates is the same chemical entity whose absence is also the cause of the symptoms of polyneuritis, that is, vitamin B_1 itself[3].

The simplest hypothesis in accord with the facts was that the defect in vitamin B_1-deficient brain lay in the oxidative removal of lactate. If this were the case the addition of vitamin B_1 concentrate, in restoring to normal the lowered oxygen uptake of the deficient brain *in vitro*, should result in an increase in the removal of lactate. This has been made the subject of an investigation, the results of which are here described. The surprising result has been obtained that there is no apparent removal of lactate corresponding to the increased oxygen uptake induced by the addition of vitamin B_1 concentrate.

[1] Senior demy of Magdalen College.

[2] It must be emphasised that this effect is catalytic. In the experiments described in the present paper 16γ of solid matter in the B_1 concentrate added is sufficient to cause an extra uptake of about 0·2 cc. or 300γ of oxygen.

[3] For further evidence of identity see Passmore *et al.* [1933].

METHOD.

The principle adopted has been to compare the oxygen uptakes of two equal portions of a homogeneous mince of avitaminous cerebrum, with and without the addition of vitamin B_1 concentrate, in the presence of similar amounts of added lactate, and to estimate afterwards the amount of lactate left by each.

The technique employed was arranged to differ as little as possible from that of previous experiments [Gavrilescu *et al.*, 1932]. The birds, in head-retraction, were guillotined in the usual manner. The cerebrums only were removed and finely minced. The mince (wet weight, 0·6 to 0·8 g.) was divided into two approximately equal portions by weighing on a rough balance. Each half was then divided between two previously weighed Barcroft-Dixon bottles of usual type, each containing exactly 3 cc. of the same solution of sodium *d*-lactate[1] in phosphate-buffered Ringer of p_H 7·4, made up as described by Gavrilescu and Peters [1931, 1]. The concentration of the lactate solution was approximately 0·006 *M* in all experiments. The exact weight of tissue in each bottle was determined by a second weighing. Glass crushers were added to break up the mince, and to each of the bottles containing one-half of the mince was added 0·1 cc. of vitamin B_1 concentrate, containing approximately 1/10th pigeon day dose and 8γ solid matter. To each of the bottles containing the other half of the mince was added 0·1 cc. of a control solution; this, and the vitamin solution were prepared as previously described [Gavrilescu and Peters, 1931, 1]. The Barcroft apparatus were set up with the usual provision for carbon dioxide absorption and were filled with oxygen as in previous experiments.

The oxygen uptake was measured over a period of 3 hours at 38°. In every case each apparatus was levelled-off once in the course of the experiment. After the last reading the bottles were detached and their contents treated as described below.

Discussion of method.

The method of dividing the minced tissue into two halves and comparing the behaviour of each requires that the mince shall be of a homogeneous nature. For this reason only the cerebrums have been used. On account of the small size of the pigeon's brain it has been necessary to utilise the whole of the minced cerebrum to obtain a sufficient difference in the oxygen uptake between the control and vitamin-treated samples of the mince. Unfortunately this has made it impossible to perform any further estimations on the same tissue other than the simple comparison here described.

d-Lactate has been used to reduce the total amount of lactate present to the lowest limit compatible with a reasonable oxygen uptake. For the same reason each half of the mince has been divided between only two Barcroft bottles. From this point of view it would have been more convenient to include the whole of each half of the mince in only one bottle; but dividing the tissue has two advantages, it reduces the amount of tissue in each bottle to a safe limit (100 to 200 mg.), and provides a check on the oxygen uptakes by determining each in duplicate.

[1] The sodium *d*-lactate used was freshly prepared before each experiment from a recrystallised specimen of zinc sarcolactate by the method of Meyerhof and Lohmann [1926]. The zinc sarcolactate was prepared, with the kind assistance of Mr R. B. Fisher, from horseflesh by a modification of Fletcher and Hopkins's method [1907]. The recrystallised specimen was dried to constant weight at 120° and stored over $CaCl_2$ in a vacuum desiccator. A weighed sample gave a zinc oxide yield of 99·5 % of the theoretical.

In practice it has not been found possible to divide each half of the mince into two exactly equal samples, but the oxygen uptake of each sample has always been found to be proportional to its weight, both in the control and vitamin-treated samples [see Dixon and Elliott, 1930]. The oxygen uptake per g. of tissue has been calculated from the uptake of each sample. In ten cases, from the experiments quoted in this paper, the difference between these figures from two samples of the same half of the mince has been less than 5 %, and in the remaining four less than 10 %. This is an indication of the homogeneous nature of the mince.

The following are the results of three experiments in illustration of this. They have been especially selected for the inequality of tissue between the two samples. In most experiments the division has been more equal.

	Avitaminous cerebrum	
Exp. No.	Wet weight of tissue mg.	Oxygen uptake, per g. tissue in 3 hours, in the presence of 0·006 *M* sodium *d*-lactate, cc.
6	108	3·35
	207	3·38
8	206	2·71
	147	2·69
10	127	2·96
	209	2·96

In theory at least the uneven division of each half of the mince requires that both the oxygen uptake and the lactate removal of each separate sample shall be directly proportional to its mass, if the method is to be acceptable. With regard to the oxygen uptake this is shown to be the case. But the problem of lactate removal must be considered. A large lactate removal, not accompanied by an uptake of oxygen and not proportional to the amount of tissue present, might theoretically effect the validity of the results. In practice, however, the uneven division of the tissue is roughly of the same order for each of the halves of the mince whose lactate removal is compared. This should largely compensate for the error introduced by such an improbable contingency.

The calibration constants of the Barcroft apparatus, for converting scale divisions into mm.^{3} of gas absorbed, were determined under the exact conditions of the experiments as in previous work [Gavrilescu *et al.*, 1932]. The constants so determined have a probable error of less than $\pm$ 1 %. This was confirmed by redetermining the constant of each apparatus during the course of the research.

Subsequent treatment for the estimation of lactate.

Two methods have been employed for the precipitation of the proteins. At first trichloroacetic acid was used as a precipitant. Since it seemed possible that the results might be affected by the nature of the reagent employed, some further experiments were performed using Schenk's method, as described by Lehnartz [1928][1].

[1] In particular reference should be made to the claim of Lehnartz that the presence of trichloroacetic acid interferes with the estimation of lactate solutions and produces variable yields. This applies to a distillation method of lactate analysis. This has not been confirmed in this research in which the method of Friedemann and Kendall [1929] has been used. The agreement between several estimations on the same lactate solutions has been very nearly as good as in experiments where Schenk's method has been employed. (Within 0·02 cc. *N*/200 iodine on an average, as compared with an average variation of 0·01 cc. with Schenk's method.) Such improvement as there has been with the adoption of Schenk's method is thought to be due solely to increased skill in performing the estimations.

A. *Trichloroacetic acid method.* The procedure adopted was essentially the same as that of other workers [*e.g.* Kinnersley and Peters, 1930; Fisher, 1931; Ashford and Holmes, 1931]. The contents of each Barcroft bottle were treated with 0·5 cc. 20 % trichloroacetic acid immediately after the last reading of the oxygen uptake. The bottles were left in cold store overnight. The contents of the two control bottles were decanted into the same centrifuge-tube, and the bottles were washed out with three lots of 3 cc. of water. After centrifuging, the clear solution was poured off through a small filter-paper into a 200 cc. flask. The tissue in the tube was ground up with a glass rod in 0·5 cc. 20 % trichloroacetic acid and left to stand with frequent stirring for half an hour. 6 cc. of water were added with stirring, the whole was centrifuged, and the washings were poured off through the filter into the flask. The whole operation was repeated. The filter was then washed through with three or four lots of water. The bottles to which vitamin B_1 concentrate had been added were treated in exactly the same manner. To each flask was added 1 cc. 10 % copper sulphate solution and 1 cc. 20 % lime suspension (sufficient to make the solution alkaline). Both were made up to the mark, and left for an hour before filtering through small creased filter-papers. The first few cc. of the filtrates were rejected. Two solutions were thus obtained containing the lactate left by equal amounts of cerebrum, which had been placed in equal quantities of the same lactate solution, and treated with and without vitamin B_1 concentrate respectively.

B. *Schenk's method.* After the last reading 5 cc. 3 % $HgCl_2$ and 4 cc. 2 % HCl were added to each bottle. After standing overnight the bottles were washed out and their contents centrifuged as in the trichloroacetic acid experiments. The centrifugates were poured off into boiling-tubes, and the tissue left behind was twice ground up with glass rods and washed with 6 cc. of water. After centrifuging the washings were added to the boiling-tubes. To each tube about 0·5 cc. 20 % NaOH was added, thus bringing the p_H of the solutions to about 2. After treatment with H_2S for an hour and filtering into a vacuum flask, the filter was carefully washed, and air was drawn through the filtrate for several hours. The solution was then washed out into a 200 cc. flask, 3 drops of phenol red solution were added, and the solution was roughly neutralised with 20 % NaOH (about 0·8 cc.). Copper-lime treatment was carried out as before.

Percentage recovery of methods. Two previously estimated lactate solutions were treated by the above procedures. The Schenk method gave a recovery of 100 % in both cases. The trichloroacetic acid method gave yields of 94 and 95 %. The loss may arise in the initial filtration after removal of the precipitated proteins. No correction has been applied to the results for this loss, as it is not sufficient to affect them.

Lactate determinations. The method of Friedemann, Cotonio and Shaffer [1927], as modified by Friedemann and Kendall [1929], was employed. The apparatus was of usual type, with the exception of the absorption tower. This was made according to a design devised by Mr R. B. Fisher. It consisted of a narrow-bored tube containing a single tier of large glass beads retained by a constriction at the bottom. The tower was designed to take only 2 cc. of bisulphite solution, instead of the usual 10 cc. The solution employed was correspondingly more concentrated (4 %). By this method it was possible to wash out the bisulphite completely with only a small volume of water, and so to reduce the bulk of solution to be titrated at the finish. $N/200$ $KMnO_4$ was used for the oxidation, Na_2HPO_4 to liberate the bound bisulphite and $N/200$ iodine for the final titration. The iodine solution was made up fresh every day from a stock $N/10$ solution, which was checked at intervals against standard

thiosulphate. A battery of three apparatus was constantly employed, each estimation being performed in triplicate on aliquot samples, containing 0·2 to 0·5 mg. of lactic acid. When two solutions were compared (control and vitamin-treated), the estimation of one was performed directly after that of the other, and as nearly as possible under the same conditions. The blank titration of each apparatus was frequently checked, and the percentage yield of the estimation was determined at intervals on a standard zinc lactate solution. The method gave constant yields of 92 ± 1 %, although the conditions of Friedemann and Kendall for a maximum yield have been observed throughout. A correction has been applied to all experimental results to bring these values up to 100 %.

Results.

1. *Control and vitamin-treated avitaminous cerebrum in the presence of added lactate: oxygen uptake and lactate removal compared.*

Table I.

	I Wet weight of tissue mg.		II Oxygen uptake in 3 hours cc.		III Lactate recovered after 3 hours mg. of lactic acid	
Exp. No.	C. Control	V. Vitamin-treated	C. Control	V. Vitamin-treated	C. Control	V. Vitamin-treated
Trichloroacetic acid experiments:						
1	406	391	1·12	1·34	3·39	3·48
3	362	344	1·06	1·27	2·46	2·46
5	389	410	1·27	1·58	2·08	2·10
6	316	318	1·06	1·23	2·78	2·87
Schenk experiments:						
7	226	226	0·46	0·67	1·71	1·81
9	408	397	0·95	1·14	2·97	2·97
12	381	393	1·07	1·38	2·80	2·52
14	387	390	0·97	1·23	3·39	3·19

Table II.

	I Observed lactate difference mg. lactic acid C.–V. in Col. III, Table I		II Observed extra oxygen uptake cc. V.–C. in Col. II, Table I	III Expected lactate difference. "Lactic acid equivalent" of extra oxygen mg.	
Exp. No.		x			y
1	−0·09	±0·10	0·22	0·30	±0·05
3	0	0·08	0·21	0·28	0·05
5	−0·02	0·10	0·31	0·41	0·07
6	−0·09	0·10	0·17	0·23	0·06
7	−0·10	0·05	0·21	0·28	0·03
9	0	0·02	0·19	0·25	0·06
12	+0·28	0·02	0·31	0·41	0·06
14	+0·20	0·04	0·26	0·35	0·07
Average	+0·02	±0·06		+0·31	±0·06

Cols. x and y represent the estimated maximum experimental errors in the figures in Cols. I and II respectively (see text).

The experimental results are given in Table I.

In Table II these results are compared. Col. I (of Table II) gives the observed differences in lactate left by the control and vitamin-treated halves of the mince. The differences in oxygen uptake are given in Col. II; these figures represent the extra oxygen uptake due to the addition of vitamin B_1 concentrate. Previous work has suggested that this extra uptake is concerned with the oxidative removal of lactate. The least amount of lactate that this extra oxygen could remove is the amount that it would completely oxidise, and this is given in Col. III. (1 cc. of oxygen would completely oxidise 1·34 mg. of lactic acid.)

The maximum probable experimental error in these results is given in Cols. x and y. This has been calculated as follows.

Errors. The error in weighing the tissue is negligible. An air-damped Sartorius balance was used throughout which is sensitive to 0·1 mg.

The lactate recovered from each sample of minced tissue is calculated from at least three separate lactate estimations in each case. No single estimation in the experiments quoted differed from the mean by more than 0·03 cc. $N/200$ iodine. The maximum errors in Col. x are obtained by taking the outside figures of each group of three estimations.

Each reading of the Barcroft apparatus is taken as being correct to within one scale division (about 3 mm.^{3}). The final reading in all cases would then be correct to within ± 1 %. The calibration constant of each apparatus has a probable error of ± 1 %. The maximum error in the calculated oxygen uptakes is therefore ± 2 %, and in the difference between two such estimations ± 4 %. The "extra oxygen uptake" was 25 % of the control uptake on the average, and so the average maximum error in the "lactic acid equivalent" of the extra uptakes is ± 16 %. Calculated separately, the estimated maximum errors in these figures vary from ± 9 to ± 25 %, and are given in Col. y. It must be emphasised that these are maximum errors. It is not likely that the actual errors have ever approached these figures.

Reference to Col. I of Table I shows that in some experiments there is an appreciable difference in the amount of tissue between the control and vitamin-treated portions of the mince. The difference is never more than 5 %. Nevertheless this means that the observed extra oxygen uptake and difference in lactate removed is not an exact measure of the influence of the added vitamin B_1 concentrate.

A 5 % excess of tissue in the control sample, for instance, will be sufficient to increase appreciably the oxygen uptake and the lactate removed above the figures that would be given by an amount of tissue exactly equal to the vitamin-treated sample. It will also increase the amount of lactate at the beginning of the experiment by virtue of the lactate preformed in the excess tissue. The error introduced by this difference in tissue may be accurately estimated by the following considerations. The oxygen uptake of the excess tissue will be proportional to its mass, and can be calculated from the uptake of the control sample.

Table II.

Figures corrected as described in the text.

Exp.	Observed lactic acid difference mg.	Expected lactic acid difference mg.
1	−0·05	+0·35
3	+0·04	+0·35
5	−0·07	+0·30
6	−0·09	+0·22
7	−0·10	+0·28
9	+0·02	+0·29
12	+0·26	+0·37
14	+0·19	+0·34
Average	+0·03	+0·31

The lactate removed by the excess tissue can be calculated from the results given in the next section. 1 g. of tissue has been found to remove about 4 mg. of lactic acid in 3 hours under these conditions. The preformed lactate in the excess tissue will increase the lactate at the start of the experiment by about 2 mg. per g. When the experimental results are corrected by these values, Table II then reads as above (p. 1315). These figures are subject to the same possible experimental errors as are the uncorrected figures.

It will be seen that the general nature of the results cannot be affected by any small differences that have occurred between the amounts of tissue in the control and vitamin-treated halves of the mince.

Reference to Col. I, Table II, shows that these experiments have failed to demonstrate any marked disappearance of lactate corresponding to the extra oxygen uptake induced by the addition of vitamin B_1 concentrate to minced avitaminous cerebrum. In eight experiments, only two have shown any greater removal of lactate by the vitamin-treated tissue as compared with the control, and in these the increase in lactate removed is less than the least amount that could be expected if the extra oxygen uptake induced by the vitamin B_1 concentrate were due to the oxidative removal of lactate. The differences in lactate removal between the control and vitamin-treated samples are of the order that might be expected from a slightly uneven distribution of enzymes in the minced tissue.

Four other experiments have been performed, which have not been included in the results given. They have been rejected on various grounds, chiefly because they were found to be susceptible to a rather wide margin of possible experimental error. In so far as any conclusions may be drawn from them they are in agreement with the foregoing results, and offer no evidence of any contrary behaviour.

There is one possible source of fallacy in the conclusions from these results which requires discussion. The production, or failure of oxidation, of some substance estimating as lactate in the vitamin B_1-deficient tissue, in the presence of added vitamin B_1 concentrate, might be sufficient to mask an actual increased oxidation of lactate. The possibility of lactate oxidation in the brain sparing other oxidations has received consideration from other workers. The method of Friedemann and Kendall reduces the number of substances which are estimated as lactate to very few. If the results were due to such a substance it would be necessary that enough should accumulate in 1 g. of tissue to be estimated as about 0·9 mg. of lactic acid. From the figures given by Friedemann, Cotonio and Shaffer this would require 4·5 mg. of cystine or 9 mg. of malic or glyceric acid. Most other substances would need to accumulate in much greater quantities. It is thought improbable that an increased lactate oxidation of the order that the added concentrate might induce could spare the removal of other substrates to this extent.

This investigation has been somewhat handicapped by the small size of the pigeon's brain. From the point of view of the estimations it would have been an advantage to perform large-scale experiments using several g. of minced cerebrum from a number of birds. Such a method has not been adopted for several reasons. In the first place it seemed possible that a mixture of tissue from several birds might introduce other variable factors, secondly it was desired to keep this research in line with previous work by using the same technique, and lastly it was impossible to rely on obtaining several birds in head-retraction at the same time. The experiments have therefore been performed on single cerebrums. The expected difference in lactate removal that has been investigated is admittedly small, and for this reason considerable care has been taken to define the accuracy of the results. It has been shown that the probable maximum errors are insufficient to affect the general nature of the results.

2. *The removal of lactate by avitaminous cerebrum.*

As a subsidiary problem the removal of lactate by the minced avitaminous cerebrum has also been studied in the absence of added vitamin B_1 concentrate.

Table III. *Minced cerebrum of polyneuritic pigeon incubated for 3 hours in the presence of added lactate.*

All lactate figures given in terms of mg. of lactic acid. Lactate preformed in the tissue taken as 2 mg. per g. of tissue. Schenk's method used in the extraction of lactate.

Exp. No.	I Wet weight of tissue g.	II Estimated lactate at start	III Lactate recovered after 3 hours	IV Estimated lactate removed in 3 hours	V Oxygen uptake in 3 hours cc.	VI "Lactic acid equivalent" of oxygen uptake
7	0·23	2·42	1·71	0·71	0·46	0·60
8	0·35	4·61	3·06	1·55	0·95	1·27
9	0·41	4·71	2·97	1·74	0·95	1·27
10	0·34	4·57	2·90	1·67	0·99	1·32
11	0·38	4·48	2·92	1·56	1·08	1·44
12	0·38	4·50	2·80	1·70	1·07	1·43
13	0·30	4·90	3·68	1·22	0·72	0·96
14	0·39	5·08	3·39	1·69	0·98	1·31

The experimental results are given in Table III. The table has been compiled from the figures of control samples of cerebrum in experiments set up primarily for the investigation of the problem discussed in the previous section. Exactly the same methods have been employed as have been previously described. Schenk's method of protein precipitation was used. The lactate solution added to the tissue was previously accurately estimated. In calculating the amount of lactate at the start of the experiment allowance must be made for the lactate preformed in the tissue itself. Unfortunately there was never sufficient tissue left over after filling the Barcroft bottles to provide enough for an accurate estimation of the preformed lactate.

Kinnersley and Peters [1929; 1930] have shown that the amount of lactate in avitaminous pigeon's brain 1 minute after death is about 1·5 mg. per g. of tissue on the average. Thereafter little further increased formation of lactate seems to occur in the tissue, even after incubation for 1 hour in Ringer at 38°. The highest figure found by them for avitaminous brain was 2·3 mg. per g.

As a safe figure the total amount of lactate provided by the tissue itself can be taken as 2 mg. per g. (with a possible variation of 0·5 mg.). In Col. II of Table III an allowance for this amount of preformed lactate has been made in the estimated total lactate at the start of the experiment.

Col. III gives the lactate recovered after incubating the tissue for 3 hours in oxygen. This is calculated from the mean of at least three lactate estimations in each case.

Col. IV gives the estimated lactate removed (Cols. II–III).

For the purposes of comparison the total oxygen uptake is given in Col. V, and in Col. VI the "lactic acid equivalent" of the oxygen uptake (that is, the amount of lactic acid that it would completely oxidise).

It will be seen that the total oxygen uptake would be only sufficient completely to oxidise from 75 to 90 % of the lactate apparently disappearing. If the preformed lactate in the tissue is taken as 1·5 mg. per g. instead of 2 mg., the oxygen taken up could completely oxidise 85 to 100 % of the disappearing lactate.

An important factor in these experiments is the efficiency of the extraction of the lactate from the tissue. If the Schenk precipitate retained a significant amount of lactate, there would be an exaggeration of the apparent lactate removed.

Previously estimated lactate solutions have therefore been added to minced brain under the conditions of an actual experiment and the mixture treated for lactate estimations as described previously. The following were the results obtained.

	Tissue added g.	Lactate added mg. of lactic acid	Lactate recovered mg. of lactic acid
Normal cerebrum	0·11	3·60	3·71
,, ,,	0·10	1·42	1·48
Avitaminous cerebrum	0·10	1·83	2·02

The lactate recovered is greater than the lactate added by an amount of the same order as the probable preformed lactate in the tissue.

The experimental results of this section cannot therefore be attributed to experimental loss of lactate during the process of extraction.

In view of the absence of direct determinations of the lactate preformed in the tissue, too much stress should not be laid on the figures given in Table III. Nevertheless these experiments show beyond doubt that minced vitamin B_1-deficient cerebrum can remove a considerable quantity of lactate (1·40 mg./g. of tissue/hr. on the average). This would require for its complete oxidation at least the whole oxygen taken up by the tissue.

This presents the same problem as the work of Ashford and Holmes [1931] on rabbit's brain, and, in the same way, suggests a removal of lactate by some path other than direct oxidation.

The large removal of lactate seems to make it still more probable that there is no defect in the removal of lactate by the minced vitamin B_1-deficient brain[1].

Discussion.

The results of this research indicate that the nature of the lesion in vitamin B_1-deficient brain is less simple than previous evidence suggested. The lesion results in a lowered oxygen uptake of the minced brain in the presence of lactate. The addition of vitamin B_1 concentrate to the minced brain largely restores the lowered uptake and yet causes no corresponding increase in the removal of lactate. The vitamin B_1 concentrate must be concerned with the oxidation of some substance other than lactate itself, although its action is in

[1] The investigation of the removal of lactate by the normal brain is not part of the object of this research. Two preliminary experiments however suggest that it is of the same order as in the vitamin B_1-deficient brain. The results of these experiments are given below.

	Normal minced cerebrum				Lactic acid equivalent of oxygen uptakes (mg.)	
	Duration of incubation in oxygen	Wet weight of tissue mg.	*d*-Lactic acid added mg.	Lactic acid removed mg.	Total uptake	Extra uptake due to lactate addition
Bird A:						
Sample I	90 mins.	120	3·60	0·35	0·47	0·18
,, II	90 ,,	130	3·60	0·28	0·48	0·18
Bird B:						
Sample I	120 mins.	100	1·42	0·42	0·45	0·22
,, II	120 ,,	100	1·42	0·32	0·40	0·19

some way dependent on the presence of lactate[1]. The evidence of previous work can leave little doubt that vitamin B_1 concentrate specifically restores the oxidation that is defective in the minced vitamin B_1-deficient brain[2]. If this evidence is accepted, it must be concluded that this defect in oxidation does not interfere with the removal of lactate itself in the minced brain. On the other hand the accumulation of lactate in the living brain in vitamin B_1 deficiency [Kinnersley and Peters, 1929; 1930] suggests that the removal of lactate is defective *in vivo*. It has been suggested previously that lactate may be removed in the brain by some path other than direct oxidation. The lesion in vitamin B_1-deficient brain may affect an oxidation at some essential stage in the metabolism of lactate subsequent to its initial removal. This might result in the accumulation of lactate in the living brain; while under the artificial conditions of minced brain *in vitro*, it might have no influence on the initial removal of lactate, but result instead in the continuous formation and accumulation of the product of lactate whose oxidation is impaired by the lesion. The evidence can be interpreted in other ways, but it is thought that this forms the most simple explanation at the present time.

It is concluded from this research that the lesion in vitamin B_1-deficient brain affects an oxidase system that is associated with lactate, but is not concerned with the removal of lactate itself in isolated brain tissue.

Summary.

1. Previous work has shown that the oxygen uptake of minced pigeon's brain in the presence of lactate in vitamin B_1 deficiency is lower than the normal. The addition *in vitro* of vitamin B_1 concentrate, in small amounts, largely restores the defect.

2. It is here shown that the addition *in vitro* of small amounts of vitamin B_1 concentrate, while increasing the oxygen uptake of the avitaminous cerebrum in the presence of added lactate causes no significant increase in the amount of lactate removed.

3. The minced cerebrum of the vitamin B_1-deficient pigeon readily removes lactate *in vitro*. The amount removed in 3 hours is sufficient to require at least the total oxygen taken up by the tissue for its complete oxidation.

I wish to express my most sincere thanks to Prof. Peters for his constant encouragement and advice during the course of this research. I also wish to thank Mr R. B. Fisher for his advice on the method of performing the lactic acid estimations and for his assistance in the preparation of the specimen of *d*-lactate used. I am also indebted to Mr Kinnersley for supplying the vitamin concentrate.

[1] In previous experiments [Gavrilescu *et al.*, 1932] it was shown that the addition of vitamin B_1 concentrate *in vitro* increased the oxygen uptake of vitamin B_1-deficient cerebrum by 145 mm.^{3}/g./hr. on the average in the absence of added substrates, and 340 mm.^{3}/g./hr. in the presence of added lactate. The small increase in the absence of added substrates was attributed to the presence of lactate preformed in the tissue itself.

[2] The addition of vitamin B_1 concentrate to the avitaminous brain in the presence of succinate, and to the normal brain with lactate, causes no significant increase in the oxygen uptake [Gavrilescu *et al.*, 1932]. Furthermore in birds recovering from polyneuritis the effect of the concentrate in the presence of lactate decreases as the oxidative behaviour of the brain with lactate improves [Meiklejohn *et al.*, 1932].

REFERENCES.

Ashford and Holmes (1931). *Biochem. J.* **25**, 2028.
Dixon and Elliott (1930). *Biochem. J.* **24**, 820.
Fisher (1931). *Biochem. J.* **25**, 1410.
Fletcher and Hopkins (1907). *J. Physiol.* **35**, 247.
Friedemann, Cotonio and Shaffer (1927). *J. Biol. Chem.* **73**, 335.
—— and Kendall (1929). *J. Biol. Chem.* **82**, 23.
Gavrilescu and Peters (1931, 1). *Biochem. J.* **25**, 1397.
—— —— (1931, 2). *Biochem. J.* **25**, 2150.
—— Meiklejohn, Passmore and Peters (1932). *Proc. Roy. Soc. Lond.* B **110**, 431.
Kinnersley and Peters (1929). *Biochem. J.* **23**, 1126.
—— —— (1930). *Biochem. J.* **24**, 711.
Lehnartz (1928). *Z. physiol. Chem.* **179**, 1.
Meiklejohn, Passmore and Peters (1932). *Proc. Roy. Soc. Lond.* B **111**, 391.
Meyerhof and Lohmann (1926). *Biochem. Z.* **171**, 421.
Passmore, Peters and Sinclair (1933). *Biochem. J.* **27**, 842.