LXXXI. CARBOHYDRATE METABOLISM IN BIRDS.

II. BRAIN LOCALISATION OF LACTIC ACIDOSIS IN AVITAMINOSIS B, AND ITS RELATION TO THE ORIGIN OF SYMPTOMS.

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In a recent paper [Kinnersley and Peters, 1929] it was shown that opisthotonus symptoms due to avitaminosis B_1 in pigeons were associated with an increase of lactic acid in the brain. The provisional conclusion was reached that the increased lactic acid was specifically due to lack of vitamin B_1 in the brain. If this conclusion be true, not only does it provide cogent evidence for the relation between vitamin B_1 and some phase of carbohydrate metabolism, but we feel that it is evidence of chemical change underlying the activity of the central nervous system. We have therefore analysed the problem further. It was conceivable that the changes in the brain were merely due to a general increase of lactic acid throughout the tissues. If this compound was present especially in one region of the brain, it would show that there was no direct relation between the increased blood-lactic acid and that in the brain. Further, it would be suggestive evidence for a correlation of the symptoms with a localised chemical abnormality and would indicate the portion of the brain responsible for them. Some preliminary experiments upon this question have already been published [Kinnersley and Peters, 1930].

Experimental.

The treatment of the brain tissue and the methods of analysis have been described elsewhere. No significant changes have been made. Use has been made of the "guillotine" method. The two halves of the brain have been immersed in liquid air within 5 seconds approximately, the time of immersion being stated in the tables. Freezing, though instantaneous at the cut surface, must take some seconds for completion throughout the mass. When removed from the liquid air, the brains are too hard to divide. Fortunately, we have found that thawing produces little change, if any, in brain tissue which has been frozen in liquid air. We have previously quoted two experiments in support of this [Peters, 1930]. Further experiments which illustrate the same point are shown in Table I, Exps. 1, 2 and 7. Allowing the brain tissue to thaw completely has not led to large increases in lactic acid content. In one case (Exp. 7) there would seem even to have been a decrease of the lactic acid. We do not, however, lay stress upon these changes. They indicate that brain tissue can

probably be allowed to thaw completely without much change in content of lactic acid. In practice, the required parts of the tissue have been removed before being completely thawed, to minimise any possibility of change in the abnormal tissues. About 15–20 minutes after removal from the liquid air the tissue can be cut with a knife. At this stage portions were removed with a clean, cooled scalpel and dropped into ice-cold 10 % trichloroacetic acid; any congealed blood was cleaned from the split half of the brain before selecting the parts. As the significance of the experiments depends upon comparative rather than upon absolute values, great care has been taken to work under strictly comparable conditions throughout.

Parts of the brain selected. These have been in various experiments: (1) the cerebrum, (2) the cerebellum, (3) the mid-brain with the optic lobes, and (4) the remainder, bulb and medulla. Various combinations of these have been used. By cerebrum we mean here the parts of the brain included by a horizontal cut parallel with the lower surface of the cerebral hemispheres, and in the sections of mid-brain have been included the optic lobes together with such tissues as lie in the median plane upon a horizontal line drawn approximately parallel with the lower surface of the optic lobes. The approximate weights are for the cerebrum 0·96 g., cerebellum 0·27 g., mid-brain and the remainder 0·43 g.

"*Sugar values.*" These have been obtained upon blood by the Hagedorn and Jensen method, using zinc precipitation as described by these authors. Values for "sugar" by the Hagedorn and Jensen method, obtained upon trichloroacetic acid filtrates, are usually higher than these, but are not consistently so. As shown recently by Gulland and Peters [1930], these differences are due to varying amounts of glutathione and are not so reliable as the direct zinc precipitation. In any case, values would have to be reduced by some 70 mg. per 100 cc. to obtain the true "sugar" value.

Determination of lactic acid. The following is a description of the method actually used. The samples of brain tissue were placed in tared 25 cc. centrifuge tubes containing about 5 cc. of 10 % ice-cold trichloroacetic acid to 1 g. of tissue; the tubes were quickly re-weighed to obtain the weight of the tissue. It was minced with a glass rod and left to extract in the trichloroacetic acid with the addition of an equal volume of water for 30 minutes or longer and was then centrifuged and filtered. Fresh trichloroacetic acid (2–3 cc.) was added to the residue, which was ground and allowed to stand for re-extraction for at least 30 minutes. The latter operation was repeated. Water was added and the tube centrifuged. The combined centrifugates made a total volume of about 20 cc. It was not necessary to leave the tissue to stand for 24 hours as has been claimed recently by Jungmann and Kimmelstiel [1929]. The combined centrifugates were divided into two (or in later experiments into three) parts and each of these was separately treated with 1 cc. of 10 % copper sulphate and sufficient $Ca(OH)_2$ suspension to make it alkaline (about 4 cc.). By performing these extractions separately, it has been our belief that a more proper control is made of the errors produced in the copper-lime treatment. After

standing, the mixtures were centrifuged, and the clear supernatant fluid was used for determination of lactic acid by the method of Friedemann, Cotonio and Shaffer [1927].

Accuracy of the lactic acid determinations. In the case of blood, where determinations have been made upon trichloroacetic acid filtrates, duplicate values have always agreed remarkably closely, to within ± 0·01 cc. of *N*/100 iodine. This means that the values given in the tables are the average of two samples not differing from one another by more than ± 0·5 mg./100 g. tissue. For the brain tissue, all the values given in the tables are the mean of two duplicates which did not differ by more than ± 0·02 cc. (approximately ± 0·01 mg. lactic acid) and were often identical. Occasionally we have found quite wide and inexplicable variations between duplicates carried out by our method of separate precipitation at the copper-lime stage, which we are satisfied are not introduced by the reagents. In order to make clear the maximum error allowed in the calculations, it may be said that with the larger amounts of tissue, *i.e.* estimations of cerebrum and cerebellum taken together, or upon the remainder, the values given in the tables will lie within ± 2 mg./100 g. tissue of the figures actually obtained. More generally the variation is much less. But where smaller amounts of tissue only were available the error is proportionately greater.

The method of Friedemann, Cotonio and Shaffer in our hands gave a blank value upon the reagents of 0·05 to 0·06 cc. *N*/100 iodine, and a yield of 95 to 97 % when tested against recrystallised zinc lactate. Lactic acid values in the tables have not been corrected for this difference [see Friedemann, 1928].

The substance estimated. The amounts of lactic acid concerned are too small to estimate directly as the zinc salt; there is in any event doubt as to whether isolation of a zinc salt can be relied upon more than the latest oxidation methods [see Friedemann, 1928]. We have made the assumption that the substance producing aldehyde is lactic acid. Justification for this assumption can hardly be questioned in the case of the normal brains. Lactic acid itself was isolated from human brain many years ago by Thudichum [1884] and McGinty and Gesell [1925] have isolated lactic acid from the brains of dogs. It was possible, however, that the abnormal brains contained some substance other than lactic acid yielding aldehyde. It would be evidence against this if it could be shown that the aldehyde-producing substance was ether-soluble. The following experiment was therefore carried out. The brains of avitaminous pigeons were taken soon after death, extracted as usual with trichloroacetic acid, and an aliquot sample analysed after treatment with copper-lime. The remainder was then saturated with NaCl, 0·5 cc. of conc. H_2SO_4 added, and the whole extracted with ether (specially purified) in a Clausen extractor. The ether filtrates were treated with 10 cc. water, powdered talcum was added and NaOH to alkalinity, and the ether removed upon the water-bath. Portions were taken for direct estimation of lactic acid and also after copper-lime treatment. Under these conditions extraction with ether is slow.

Details of experiment. Two avitaminous brains taken; weight 4·12 g. . Trichloroacetic acid extracts analysed. (A.)

	(mg.)	(mg.)
Lactic acid present initially in A...		2·25
„ in first extraction with ether 3 hours	1·88	
„ in second extraction with ether 3 hours	0·22	
„ still present in aqueous phase after the extractions	0·14	
Total ...		2·24

In the two ether extractions therefore at least 95 % of the substance estimated was found to be ether-soluble. The excellent balance obtained shows that nothing was added in the process of extraction. This experiment makes it very probable indeed that the substance concerned in the abnormal brain is actually lactic acid [Friedemann and Kendall, 1929].

Normal brains. Table I gives the results for normal birds. Exps. 1 and 2 are the averaged results for two separate groups of five birds. The remainder represent determinations upon single birds. The lactic acid found within the time stated is approximately the same for the cerebrum, cerebellum and the rest of the brain, and amounts to some 50–60 mg. per 100 g. of tissue except in the case of the damaged brain (Exp. 7 (*a*)).

Reducing values were also obtained and showed in each case a diminution for the (*b*) sample as compared with the (*a*), but this varied from 9 to 40 mg./100 g. Throughout the work reducing values have been obtained, but they are omitted here because they show no consistency and add nothing to those previously published. The figures for brain tissue represent mg. of lactic acid per 100 g.; for blood, mg. of lactic acid and sugar per 100 cc.

Table I. *Normal birds.*

Time means interval between death of the brain and immersion of the material in liquid air

Exp.	Time (secs.)	Cerebrum	Cerebellum	Rest	Notes
1	(*a*) 11	56	46	55	5 birds used. One-half (*a*) of each brain dropped into liquid air at 11 secs., and the other (*b*) at 83 secs. (*a*) and (*b*) averaged results for each set of half brains
	(*b*) 83	93	85	92	
2	(*a*) 11	52	53	58	5 birds used as in Exp. 1, but the brains allowed to thaw in air for 30 mins. longer than Exp. 1
	(*b*) 86	87	94	85	

Exp.	Time (secs.)	Cerebrum and cerebellum	Rest	Blood-lactic acid	Blood-sugar	Notes
3	5	39	43	14	251	
4	4½	56	56	17	210	
5	4	57	59·5	24·5	233	
6	4½	54	51	25	184	
7	(*a*) 5½	70*	64	33·5	221	*Brain badly damaged by knife
	(*b*)	57	53	—	—	
8	3½	54	58	13	242	"Urethanised"

Our standard technique therefore gives for normal brains similar values for lactic acid in different parts of the same brain.

Opisthotonus brains. Table II shows that in pigeons suffering from opisthotonus due to vitamin B_1 deficiency, there is an increase of lactic acid in the

lower parts of the brain as compared with the upper. The deficiency was induced by rice feeding by the usual methods employed in this laboratory.

Table II. *Opisthotonus brains. Acid mg./100 g.*

Exp.	Time (secs.)	Cerebrum	Cerebellum	Rest	Blood-lactic acid	Blood-sugar	Hours symptoms
1	6½	90	94*	124	170	164	?
2	6	65	64*	108	54	357	4
3	6	113	80*	157	—	336	6
4	4	80	85	104	31	246	1½
5	6	87·5	92	137	38	231	1½
		Cerebrum and cerebellum					
6	4–5	63		—	65	283	?
7	5	52*		86	65	405	3
8	5	75		104*	161	177	5
9	5	99		86	53	212	24†
10	7	100		123	62	262	?
11	4	103		113	66	226	2
12	4	89		98	41	270	—
			Mid-brain	Rest			
13	4	61*	52	71*	23	284	2+
14	6	98	114*	124	86	343	—
15	5	76*	105	—	52	—	4
16	4	107	127	113	84	307	—
17	3½	74	87*	98	52	231	2+

* One estimation only.

† Part of mid-brain included accidentally in cerebrum.

Last column indicates the number of hours during which symptoms had persisted.

With one exception, viz. Exp. 13, the blood-lactic acid is high and there is a general tendency for the brain-lactic acid to follow it, though this is not the absolute rule (Exps. 1 and 8). There is only one case, Exp. 9, in which the cerebrum and cerebellum contain more lactic acid than other parts of the brain; in this it was known that some of the mid-brain was accidentally included in the cerebral values. Exps. 13–17 show that as a general rule there is more lactic acid in the lowest section of the brain analysed. The localisation of this acid and its uneven distribution are suggestive evidence that the acid found does not merely accumulate from the blood stream.

In attempting to correlate the "lactic" acidosis with the origin of the symptoms, we examined the condition of the brains in birds with threatening or incompletely developed symptoms. In our previous paper [Kinnersley and Peters, 1929] we showed that rice-fed birds had a normal brain-lactic acid even after some 20 days upon the diet. In one case, however, which could be presumed to be approaching symptoms as it had been upon the diet longer than the others (27 days) we found actually a slight increase. In Table III we have collected a series of results upon birds which in our opinion were threatened with symptoms. The results are somewhat variable as would be expected. One reason for this is that in spite of an extensive experience, it is not easy to be certain in every case how near a bird is to "opisthotonus."

Table III. *Threatening opisthotonus.*

Exp.	Days on diet	Time (secs.)	Cerebrum and cerebellum	Mid-brain	Rest	Blood-lactic acid	Blood-sugar
1	20	3–4	69		79	45	279
2	24	4	—		75	44	234
3	17	5	64		72	67	246
4	22	—	41		72	67	292
5	27	7	62		97	78	269
6	30	4–5	57		73	—	—
7	32	6–7	55		63	21	242
8	12	4	66		96	87	260
9	—	5	48	52*	60*	14	244

* One estimation only.

The results are within normal limits for cerebrum and cerebellum, but raised for the lower part of the brain. If we exclude the two experiments showing a value for the lactic acid in the "rest" as high as 97 mg., the remaining values all lie under 80 mg. per 100 g. Practically all therefore are below those of Table II. Exps. 7 and 8 are within the normal limits for lactic acid in blood and brain. It is very probable that we had here selected birds which were not sufficiently advanced to show the brain condition. This is supported by the low blood-lactic acid. The facts are therefore that in the avitaminous bird, lactic acid begins to increase in the lower parts of the brain before the symptoms of opisthotonus appear. When it reaches a certain value (under the conditions of our experiments some 80 mg. per 100 g.) opisthotonus tends to arise.

The presence of opisthotonus is associated with an increase in the amount of lactic acid present; such increases are again unevenly distributed. A rise in lactic acid usually takes place in all parts of the brain, but this is most marked in the lower regions. The rise in the cerebellum is not more than that in the cerebrum. In the experiments in which the mid-brain and "rest" have been analysed separately, there tends to be more in the "rest," but this is not without exception, though not too much weight can be attached to such differences as appear owing to the experimental error involved in handling these small amounts of tissue. Together with the uneven increases in the brain, there occurs an increase in lactic acid in the blood. The increases in brain-lactic acid, however, do not follow the increases in blood-lactic acid directly. This suggests that the two are really independent, which would also follow from the uneven distribution of the lactic acid in the brain. Before, however, being able to decide that this is so, another possibility must be considered. It has been suggested to us that the uneven distribution of lactic acid might be merely the result of some structural peculiarities in the brain tissue, which lead to irregular adsorption from an increased blood-lactic acid, or to irregular elimination of lactic acid from the tissue. As we have had no success with attempts to raise the blood-lactic acid by intravenous injections of lactic

solutions in normal, unanaesthetised birds, we have had recourse to the expedient of flying birds round a room for periods of 30 minutes, and then taking the brains for analysis (Table IV).

Table IV. *Exercised birds.*

Exp.	Time (secs.)	Cerebrum and cerebellum	Rest	Blood-lactic acid	Blood-sugar
1	5	62	60	80	237
2	4–5	71·4	73	50	237
3	4	93*	89*	121	184
4	3½	73	79·5	126	219
5	4	81	83	86·5	295

* Poor duplicates.

Table IV brings out several points. Exercise increases the blood-lactic acid as would be expected, bringing it in fact to a level even above the amount usually found in avitaminosis. Together with this increase, when the bird is exhausted (Exps. 3, 4 and 5) there appears in the brain an amount of lactic acid as high as that in the bird with symptoms of threatening opisthotonus; but it is evenly distributed. The lactic acid in the brain does not increase above 90 mg. even with enormously high blood-lactic acid. The localised nature of the chemical lesion in vitamin B_1 deficiency responsible for the appearance of increased lactic acid in certain parts of the brain lends support to the conclusions of Fisher and Peters [1930] that the condition is not caused by impairment of brain circulation. It is associated with the initiation of symptoms of a peculiar type which are cured by administration of vitamin B_1, concurrently with the return of brain-lactic acid to a more normal value. In our opinion the results indicate that there is some missing factor in the tissue. Since this change is ameliorated by giving vitamin B_1 (most speedily of all by the intracranial injection of torulin [Peters, 1930]), lack of vitamin B_1 in the tissue leads to the accumulation of lactic acid in that tissue. This appears to be therefore a proof of the connection of vitamin B_1 with the intermediary metabolism of carbohydrates, a view which has been often mooted [Braddon and Cooper, 1914; Funk and v. Schönborn, 1914; Randoin and Lecoq, 1927].

We are upon less certain ground when we attempt to guess the exact function of torulin from the presence of the excess lactic acid. There is no evidence that the glycolytic power of the tissue is impaired, or that the powers of synthesis of the lactic acid precursor are diminished. In experiments upon birds showing symptoms, we have found that the brain-lactic acid maximum, after allowing the tissue to stand for 90 seconds before freezing, was in one case for cerebrum and cerebellum 142 mg. and for the remainder 150 mg., the blood-lactic acid being 56 mg., and in another case for cerebrum, etc. 178 mg., and for the rest 230 mg. The lactic acid maxima are much greater than normal and are not necessarily different for different parts of the brain in the deficient birds. There must be therefore more precursor in these brains than in the normal. This is curious and as yet unexplained. Insulin lowers the lactic acid

maximum for brain and vitamin B_1 deficiency raises it. These facts must be reconciled with any general theory of the condition. The evidence against the view that the increased lactic acid is due to an enhanced rate of glycolysis which has been presented elsewhere is still valid. Upon these grounds we think that vitamin B_1 is concerned with the oxidative removal of lactic acid, though admitting that a substantive proof of this is at present lacking.

We are aware for instance that the appearance of the acid might be secondary to some change in p_H of the tissue induced by lack of vitamin, or that the function of vitamin B_1 might even be to stimulate the production of some missing hormone, which then activates the tissue. In the latter case it seems to be more difficult to explain the localisation.

It must be emphasised that the figures in the above tables, though strictly comparable, do not represent the initial state of the "resting" brain-lactic acid.

Further considerations. In the above experiments, the use of anaesthetics or similar substances has been avoided in order to minimise any complication produced by their results. It must not be forgotten that substances like urethane are well known to disturb surface reactions, and that they inhibit the action of the cell dehydrogenases [Keilin, 1929]. However, since Cobet [1929], in work which only came to our notice when the major part of this research was completed, has found that brain-lactic acid in deep urethane anaesthesia is very low, we have carried out a few experiments upon this point. Cobet found that in the case of rabbits the resting values for lactic acid (by a colorimetric method) were 10–12 mg./100 g., increasing very much upon injury. The pigeons employed by us were deeply urethanised. After having been left undisturbed for periods of 30 minutes, the birds were guillotined and the samples of tissue frozen as usual. Table V gives the results.

Table V. *Lactic acid in brains of "urethanised" animals.*

Exp.	Condition	Time (secs.)	Cerebrum and cerebellum		Rest	Blood-lactic acid	Blood-sugar
1	Normal	3½	54		58	13	242
2	,,	3½	46		60	18	237
3	,,	3½	80		73	11	228
4	,,	3½	52		68	13	274
5	Opisthotonus	5–6	82		101	40	249
			Cerebrum	Cerebellum			
6	,,	4	64	80	87	14	253
7	,,	4	72	62	91	26	261

Our "urethane" results in no case approach those of Cobet. It seems to be impossible even with rapid work in pigeons to reach his low limits, though the degree of injury was certainly no more in our experiments. Inspection of Table V shows that the normal urethanised pigeons show most inconsistent values for brain-lactic acid, but have a low blood-lactic acid. Results for avitaminous birds are the same as usual.

Iodoacetic acid. In a recent publication Lundsgaard [1930] has shown that animals poisoned with monoiodoacetic acid die in *rigor* without showing any rise of lactic acid in the muscles. The muscle is fixed as it were in the resting state [see also Meyerhof, 1930]. Table VI shows the amounts of lactic acid found in pigeons killed by intravenous injections of the poison. Injections were made into the wing vein and varied from 50 to 100 mg. of the compound. Brains were removed in the usual manner and immersed in liquid air as soon as possible after the death of the animal, which took place at times varying from 2 to 15 minutes after injection. The time elapsing between injection and death apparently did not influence results.

Table VI. *Pigeons killed by iodoacetic acid.*

Exp.	Condition	Time of death (mins.)	Cerebrum and cerebellum	Rest	Blood- lactic acid	Blood- glucose
1	Normal	4	24·5	24·7	7·9	217
2	,,	8	29·5	29·5	6·6	214
	,,	2	27·5	35	—	—
3	H.R.	5	34	47	26·6	214
4	,,	15	36	56·5	81	231
5	,,	7½	53	75	81	272

These results are of great interest, being the lowest which we have obtained for normal and avitaminous pigeons' brains. It will be noticed that the "opisthotonus" birds all show higher lactic acid, and that in these the lower part of the brain is again 13–22 mg. higher than the values for the cerebrum and cerebellum. These amounts bear roughly the same relation to one another as do those of Tables I and II. Until more evidence is forthcoming as to the exact action of monoiodoacetic acid, we cannot draw conclusions as to whether the experiments of Table VI represent the lactic acid content of the tissues during life. But the results constitute the strongest additional support for the views put forward in the earlier part of this paper, and exclude the possibility that the high lactic acid of the avitaminous brain is due to increased rate of glycolysis.

Relation of the symptoms to the presence of lactic acid.

If the experiments in Tables II and III are examined, it will be noticed that in practically every case in which the cerebrum and cerebellum were separately analysed, the values were identical within the limits of experimental error. They were also always lower than the value for the rest of the brain. In Exp. 2, Table II, the values for cerebrum and cerebellum are actually within normal limits. In the experiments of Table III the fact that often immediately before symptoms appear the values for cerebrum and cerebellum analysed together are within normal limits indicates that the cerebellum is sometimes normal (so far as lactic acid is concerned). This suggests that the symptoms are associated directly with the presence of lactic acid in the lower parts of the brain. Certain other facts support this. Fisher and one of us in

another connection have repeatedly removed the cerebrum and basal ganglia under ether anaethesia in avitaminous birds. As soon as the bird recovers from the anaesthetic, the symptoms reappear. They cannot be due to release of cerebral control, because it is well known that decerebration alone makes little difference to the appearance of a pigeon. This confines the problem to the lower part of the brain, and to the cerebellum. Now the removal of the latter induces a similar condition to the "opisthotonus" of avitaminosis. The matter has been lately studied carefully by Bremer and Ley [1927].

Symptoms are induced by removal of the cerebellum, and hence the problem of their origin in avitaminosis reduces either to (1) increased stimulation of the mid-brain, etc., or (2) to release of cerebellar control. Since we have found cases in which symptoms are present with a practically normal "cerebellar"-lactic acid, any possible release of cerebellar control cannot take place by a lactic acid block in the cerebellum. So the symptoms must be associated with increased lactic acid in the lower parts of the brain. The condition produced would appear to be the "brain" analogue of *rigor* in the muscle. Activity of the brain tissue concerned produces lactic acid which then tends to accumulate because it is incompletely removed. When a certain concentration is reached, opisthotonus appears. This would be consistent with the common experience that symptoms can be readily induced by exercise when avitaminosis is sufficiently advanced. It is of course impossible upon the evidence here presented to decide whether the lactic acid acts by inhibition or by stimulation.

Visual inspection of the parts especially affected shows that they appear white, whereas the cerebrum appears grey. This difference in appearance may possibly underly the special susceptibility of the lower parts of the pigeon's brain to lactic acidosis. We do not feel that there is at present any justification for thinking that vitamin B_1 deficiency affects conducting rather than cell tissue, because Dr Carleton[1] finds that the whitish-looking mid-brain tissue contains a large proportion of cells. The matter evidently warrants further investigation.

Discussion.

We recognise that the view which has been advanced as to the origin of these symptoms in avitaminosis does not appear to be consistent with our own experiments in Table IV upon birds exhausted by exercise. These certainly show that lactic acid can accumulate without inducing the syndrome in question, but it is felt that a general condition of this nature cannot fairly be compared with a localised affection.

It must be remembered that ether anaesthesia in the avitaminous bird will abolish the symptoms, without altering the changes in the lactic acid. While the condition here investigated is a concomitant of lactic acid accumulation, this is certainly not the case with other similar symptoms. Insulin

[1] Personal communication.

convulsions in the pigeon (as has been shown by Holmes and Holmes for the rabbit) are associated with a low brain-lactic acid not varying in amount in different parts of the brain. It is impossible to bring into one scheme all the various conditions (apart from surgical lesions) which will induce this condition in the pigeon, without having recourse to some conception such as that of the "Meyerhof cycle" [1926] for carbohydrate metabolism. With the idea, however, that maintenance of the activity of the tissue depends upon a cyclical chemical process, it becomes possible to understand how changes of varying type may result in the same interference with function. This matter has been discussed elsewhere [Peters, 1930].

In our previous paper, we drew attention to the discrepancy between our results and the earlier view of Holmes and Holmes [1925, 1926 and 1927] that lactic acid in the brain arises immediately from the blood-sugar. Since then these authors have kindly drawn our attention to a later publication [1928] in which this view is to some extent modified. We feel that the above results can only be interpreted upon the view that there is a precursor of lactic acid in the tissue itself, however much this may in turn be in equilibrium with the blood-sugar.

SUMMARY.

1. The increased lactic acid found in the brains of pigeons showing symptoms of opisthotonus due to avitaminosis B_1 is localised especially in the lower parts of the brain.

2. In the period of rice feeding during which symptoms are threatening, lactic acid is increased only in the lower parts of the brain.

3. The localised character of the chemical lesion proves that vitamin B_1 is associated with the intermediary metabolism of carbohydrate.

4. Symptoms are due to the accumulation of lactic acid in the lower parts of the brain.

5. The lowest lactic acid found in the brain of a normal pigeon was 24 mg. per 100 g. of tissue, after death by injection of monoiodoacetic acid.

6. Severe exercise may cause a rise in the lactic acid content of the brain, which is distributed evenly over the tissue.

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