CLII. BIOCHEMICAL LESIONS IN VITAMIN B DEFICIENCY.

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Several attempts have been made in the past to correlate the symptoms of vitamin B deficiency with failure in the oxidations in the body. In so far as these have been made upon the tissues of polyneuritic pigeons, it is probable though not certain that the work has been actually performed upon the so-called B_1 constituent of the complex. It will not be necessary here to mention more than the salient features of the present position of this knowledge. Those who work with polyneuritic pigeons must soon become aware of the bright red appearance of the blood often present in pigeons showing symptoms. Dutcher [1918] and Findlay [1921] found a fall in catalase and glyoxalase content respectively in certain tissues of the body of pigeons deficient in vitamin B_1—the enzymic power not being restored by the addition of yeast extracts *in vitro*. Hence, vitamin B_1 could not be a co-enzyme for the systems in question. A series of further observations, Abderhalden, with Schmidt [1920], and with Wertheimer [1921] upon the oxygen uptake of tissues, Hess [1921] and Hess and Messerle [1921] upon the reducing properties of tissues for dyestuffs [see also Vasarhelyi, 1926; Roelli, 1923] led to the conception that the fault lay in some failure in tissue oxidations. Some of this earlier evidence does not bear the light of modern criticism; other experimental work has not supported it, in especial the work of Terroine and Roche [1925], of Roche [1925], and of Marrian and Drummond [1926], who obtained no evidence of interference under their conditions either with the oxidations of the animal as a whole, or of the respiration or behaviour *in vitro* to dyestuffs of tissues taken from deficient animals. Though the earlier work is not convincing as it stands, it is not clear that later researches have been performed under precisely the same conditions as those of the original workers. As stated by Gugler [1928], the possible variations in technique are very large. Further it was pointed out by Kinnersley, Peters and Reader [1928] that the symptoms of opisthotonus in the pigeon might be associated with an oxidative deficiency in some small part of the brain, insufficient in weight to be detectable in the oxidation of the tissues as a whole. In this paper it is believed that evidence has been obtained in proof of this opinion. In recent work, Kinnersley and Peters [1930] have found increased lactic acid present, especially in the lower

parts of the brains of pigeons at or approaching the stage of symptoms. This suggested some enzyme deficiency in these parts.

In the following communication it is demonstrated that birds showing symptoms of head retraction, and birds in which these symptoms are threatening, show lowered powers of oxygen uptake *in vitro* in certain parts of the brain, which are marked in the lower parts of the brain. The lowering of oxidation is not general, because the cerebellum shows no such changes. In birds which have been dosed with vitamin, but in which no rise in weight has taken place, the tissue shows a power of oxidation approximating to the normal. This shows that the depression of oxidation found in the avitaminous bird is not merely associated with the state of nutrition as judged by the weight, but is some expression of the avitaminosis.

Experimental.

The oxygen uptake of brain tissue *in vitro* has been investigated by Loebel [1925], Meyerhof and Lohmann [1926], Warburg, Posener and Negelein [1924], Holmes [1930], who have found in rat and frog brain rather large and variable oxygen uptakes, lasting for several hours, which could be maintained at approximately initial intensity for some time by addition of glucose and lactate solutions to the Ringer's solution in which the tissue was suspended. The values obtained by these authors were greater than those recorded by Abderhalden and Schmidt [1920] and Roche [1925]. The latter however worked with the whole brain, mashed and suspended in moist air. It has been shown that white and grey matter have quite different rates of oxygen uptake, Holmes for instance gives 1200 mm.^{3} per g. per hour for brain grey matter, and 300 mm.^{3} per g. per hour for white in presence of air [see also Dickens and Šimer, 1930]. For the purposes of this research there are good reasons for obtaining these measurements with the pigeon, as so much is known about the behaviour of this animal in vitamin B deficiency. It is not however practicable to obtain measurements with the cortex and white matter of the pigeon's brain separately, in fact in the case of most parts of the brain the mixture of grey and white could not be properly separated. Upon this account, our procedure has been to guillotine the pigeons as previously described [Kinnersley and Peters, 1930], remove the two halves of the brain and separate the following parts, the cerebrum, cerebellum, optic lobes and remainder. The tissue has then been finely mashed by the use of small bone spatulas, before transferring to the vessels for analysis. The brain tissue of the pigeon can be rapidly divided in this way, and it is believed to be more satisfactory than other methods for this purpose. If the operations are carried through quickly, little loss of water occurs. After shaking at 38° such brain tissue is found to be finely divided. We have tried to slice as recommended for other tissues, but have not found it satisfactory in the case of the pigeon's brain.

The oxygen uptake[1] was determined in apparatus of the Barcroft type (as modified by Dixon and Elliott [1930]). The corked bottles containing Ringer's solution (3·0 cc.) were weighed before introduction of the tissue and re-weighed after placing the tissue in the fluid, from which the weight of tissue was calculated. The time from the guillotining of the bird to that of placing the apparatus in the thermostat (38° + 0·1) was 12–17 minutes, according to the number of observations in each experiment. The speed of operation was much enhanced by the use of the recent type of air-damped Sartorius balance.

The chemicals used in the research were of A.R. quality. The birds used were Homer pigeons and were fed according to the usual technique employed in this Department. The earlier experiments were done with a Ringer's solution of composition NaCl 0·9 %, KCl 0·025 %, $CaCl_2$ 0·030 %, $NaHCO_3$ 0·015 %. All others with a mixture of 80 parts Ringer with 20 parts $M/2$ acid potassium phosphate, to which was added sufficient NaOH to bring the p_H to 7·4. The precipitate which forms under these conditions was removed before use. It is realised that this mixture may not be ideal for the pigeon, and that research is needed as to the exact composition of the salts for this animal: since all the experiments were strictly comparable, it is not likely that error in the conclusions has been introduced in this manner.

Variations observed and discussion of the errors of the observations.

Despite the trouble which we have taken to work under constant conditions, we have been unable to reduce the occasional variations between duplicate samples of the same normal tissue to less than ± 5 %, though in the majority of cases duplicate observations have shown closer agreement than this. Though we have noticed that the figures of other workers have shown such occasional wide variations, we have tried to discover some of the sources of the variation. For samples of tissue from cerebellum, optic lobe and the rest of the brain, some of the variations are undoubtedly to be ascribed to the difficulty of sampling the mixed tissue: this is not so likely to apply to the cerebrum. Pieces of tissue larger than 0·12 g. tend to give low results, but there appears to be no marked difference in the behaviour of tissue which had been taken from the bird and sampled at room temperature or which had been allowed to cool in ice previously. It is very likely, though not proven here, that many of the variations are due to the failure to control the extent to which the dying cells liberate substrates other than the one studied for the enzyme systems present, or expose enzymes to the action of the substrate studied. As it was clear that the main object of our work was to reveal differences in the behaviour of normal and abnormal tissue, if such existed, we have made no attempt to do more than work under standard conditions, which would make our estimations comparable. In most of the experiments

[1] The constants of the apparatus were determined at approximately 12°, using a modification of Hoffman's method, and corrected for 38° by Warburg's formula. This method was found to be in substantial agreement with direct calibration of the apparatus at 38° by Hoffman's method.

for instance we have worked with oxygen at atmospheric pressure inside the bottles, though we are aware that the oxygen uptake can be increased under our conditions by the use of higher concentrations of oxygen. It is hoped to consider this question later. Meanwhile control experiments have shown us that our conclusions are not influenced by the pressure of oxygen employed. By working with air, we have been able to reduce substantially the time elapsing between death of the animal and the first observations, thereby minimising the possibility of other changes. For a similar reason, no attempt has been made to work with CO_2 present.

The tables show the values obtained in full, and the figures the mean of the recorded estimations, expressed in mm.^{3}/g./hour. The numbers can be readily reduced to Warburg's method of expression [1930] by taking the dry weight as approximately 20 % of the wet weight: *i.e.*

$$1000 \text{ mm.}^3\text{/g./hr.} = Q_{O_2}\ 5;\ 2000 = Q_{O_2}\ 10 \textit{ etc.}$$

Oxygen uptake of pigeon's brain in vitro *in mammalian Ringer's solution without additions.*

In some preliminary experiments performed by one of us in collaboration with Mr R. B. Fisher (April, 1930), the value for the oxygen uptake of normal pigeon's brain tissue in unbuffered mammalian Ringer's solution was found to be in general agreement with those of others. The oxygen uptake was followed for several hours after death and was found to be fairly constant for 30–90 mins., tending to show a slight diminution from 90–120 mins. The values for cerebrum (1000–1380) mm.^{3}/g./hr. were greater than those for the midbrain and optic lobes (1040), and these again greater than for the "rest" (730–930). This difference has been found to persist throughout the experiments. It is not certain that it is due only to the relative proportions of white and grey matter. Comparison of the normal and avitaminous birds revealed little difference between the two, except an indication of a fall for the optic lobes. As the object of our work was to determine the differences between the normal and abnormal, we restricted the subsequent periods of observation to 30–90 minutes after death.

Oxygen uptake of pigeon's brain tissue in vitro *in phosphate-Ringer with addition of glucose (0·25 %).*

In these experiments, the following technique was adopted uniformly. The apparatus containing the samples of tissue was placed in the bath 12–17 minutes after death. It was then shaken for 10 minutes with the taps open, after which the taps were closed. Readings were then made over a period of 60 minutes and the oxygen uptake for the sample considered to be the average uptake of oxygen during the period in question. In the majority of experiments the readings during the hour were consistent; there was a tendency in some experiments with the avitaminous "lower parts" for a slight fall towards

the end of the hour period. Though there was only a small difference between the normal and avitaminous birds for the oxygen uptake in the preliminary experiments, in which Ringer's solution alone was used, the difference is accentuated in the presence of glucose. Table I shows the actual values obtained in the various experiments. In Fig. 1 there is shown upon the chart

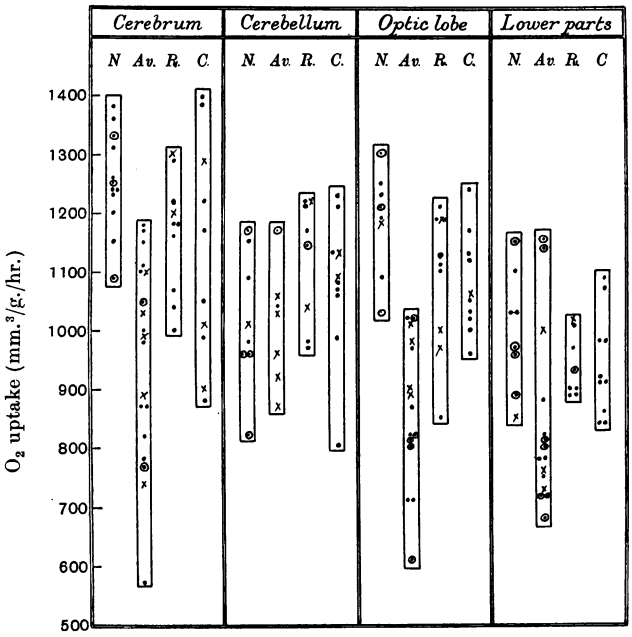

Fig. 1. Oxygen uptake *in vitro* of pigeon's brain (mm.^{3}/g./hr.). *N.* = normal; *Av.* = avitaminous; *R.* = rice fed; *C.* = cured avitaminous. • Average of two estimations agreeing within ±5 %. ⊙ Single determination. × Average of two estimations, not agreeing with ±5 %.

the average figures in the experiments. It will be seen that the values for optic lobe, cerebrum and the lower parts are reduced for the avitaminous birds; for cerebrum and optic lobes by some 20 % and for lower parts by 20 %, on the average. The values are about the same however for cerebellum. Though there appears to be no doubt about the conclusions for optic lobe and cerebrum, it was possible that the value for the lower parts might not have been significant. The matter has been kindly examined for us by Dr R. A. Fisher (Harpenden) by statistical methods. He has tested normal *v.* avitaminous for cerebrum and for lower parts and regards the difference for cerebrum as clearly significant: for lower parts he regards the difference as also significant, there being little probability of getting so large a deviation by chance. The figures prove that there is a difference in certain oxidation systems between normal and avitaminous brains, especially marked in cerebrum and optic lobes, and not present in the cerebellum. Examination of the chart will show that there was a marked tendency for bad duplicates in the samples from avitaminous brain. This must surely be significant and seems

Table I. *Values for oxygen uptake of*

N. = normal; *Av.* = avitaminous; *R.* = rice fed;

Cerebrum				Cerebellum			
N.	*Av.*	*R.*	*C.*	*N.*	*Av.*	*R.*	*C.*
1410 (1)	1000 (4)	1260 (6)	1100 (53)	1170 (3)	1170 (11)	1210 (15)	1070 (53)
1360	970	1330	930			1240	1120
1090 (9)	770 (8)	1200 (15)	980 (52)	820 (9)	1000 (14)	1230 (70)	790 (57)
1330 (10)	1050 (11)	1170	1000	960 (12)	740	1120	810
1250 (12)	1110 (14)	1030 (70)	1030 (63)	960 (10)	970 (52)	1140 (73)	1090 (63)
1360 (13)	950	970	880	1090 (13)	1150	1340 (83)	1060
1360	1170 (27)	1270 (73)	910 (65)	1100	1030 (55)	1100	1050 (65)
1250 (16)	1180	1140	860	1200 (16)	920	980 (72)	1080
1220	870 (52)	1420 (83)	1240 (63)	1100	990 (56)	970	1210 (69)
1300 (62)	870	1190	1200	940 (62)	850	990 (54)	1050
1320	790 (52)	1120 (72)	1010 (71)	1090	1120 (78)	1100	990 (71)
1210 (20)	1010	1020	1100	990 (86)	950	990 (58)	1000
1320	1150 (55)	1160 (54)	1460 (75)	970	1150 (80)	970	1160 (75)
1260 (21)	1050	1160	1340		1020	1210 (15)	1100
1220	860 (56)	990 (58)	1190 (76)			1240	1180 (76)
1250 (88)	890	1090	1050				980
1240	520 (61)	1250 (44)	1360 (77)				1200 (77)
1190 (89)	500	1190	1420				1220
1120	640 (64)	1200 (15)	1230 (96)				1250 (96)
1180 (90)	850	1170	1360				1220
1220	1210 (66)		1080 (51)				1000 (51)
	990		1140				1080
	1140 (78)						
	1170						
	1050 (80)						
	940						
	1200 (84)						
	1160						
	840 (87)						
	810						
	780 (91)						
	790						
Average:							
1260	946	1162	1113	1032	1004	1122	1082

to indicate, as do the results for the different parts, an uneven distribution of certain catalysts.

The differences observed between normal and avitaminous brains are not of importance so far as the phenomena of avitaminosis B_1 are concerned, unless it is found that such differences are not merely due to the lowered state of nutrition—we purposely avoid the use of the word inanition. An avitaminous bird under consideration has lost some 40 % of its normal weight, if

pigeon's brain in vitro (*mm.*^{3}/*g./hr.*).

C. = cured avitaminous. Figures in brackets are exp. no.

Optic lobe				Lower parts			
N.	*Av.*	*R.*	*C.*	*N.*	*Av.*	*R.*	*C.*
1300 1270 (1)	710 720 (4)	870 840 (6)	1070 970 (53)	890 (3)	680 (18)	930 (15)	940 895 (53)
1270 1230 (3)	860 890 (8)	1250 1130 (15)	910 1010 (57)	1150 (9)	810 (11)	860 920 (70)	860 820 (57)
1300 (9)	610 (11)	1030 910 (70)	950 1040 (63)	970 (10)	790 740 (14)	1090 950 (73)	970 870 (63)
1210 (10)	820 990 (14)	1090 1110 (73)	990 1130 (65)	960 (12)	880 880 (27)	870 930 (83)	860 820 (65)
1030 (12)	810 830 (27)	1230 1110 (83)	990 1080 (69)	1020 1050 (13)	800 770 (52)	900 900 (72)	1000 970 (69)
1050 1310 (13)	1140 920 (52)	1170 1250 (72)	1190 1060 (71)	1030 (16)	830 690 (55)	910 870 (58)	820 910 (71)
1200 1180 (66)	830 910 (55)	1100 900 (58)	1190 1120 (75)	780 920 (62)	780 690 (56)	940 1000 (54)	1060 910 (75)
1050 1130 (62)	710 710 (56)	1080 1160 (54)	1210 1274 (76)	1090 1110 (86)	720 (61)	990 1040 (44)	1110 1080 (76)
1050 1240 (86) 1140	800 (61)	980 1080 (44)	1470 1100 (77)		720 (64)		1080 1070 (77)
	820 (64)	1250 1130 (15)	1050 1060 (96)		800 (66)		930 890 (96)
	810 (66)		910 1060 (51)		1160 830 (78)		780 860 (51)
	930 1000 (78)				790 780 (80)		
	1030 1010 (80)				1140 (84)		
	1020 (84)						
Average:							
1185	864	1083	1083	997	814	940	929

we interpret its normal weight as the maximum weight reached by the bird feeding naturally upon a mixed corn diet. A method of control which has been used with success has been to feed a bird upon yeast extract together with the amount of polished rice consumed daily by a bird feeding naturally upon polished rice alone [Kon and Drummond, 1927]. Under these conditions the curves for weight loss of two such animals run closely parallel. In the circumstances of our experiments it appeared that the best control would be

a bird which had lost some 40 % of its weight, but in which deficiency of vitamin B_1 had been excluded[1]. It has been recently shown [Carter, Kinnersley and Peters, 1930], that birds at the minimum weight reached upon polished rice do not increase in weight when given a daily dose of our charcoal concentrates. We have therefore obtained the oxygen uptake for the different

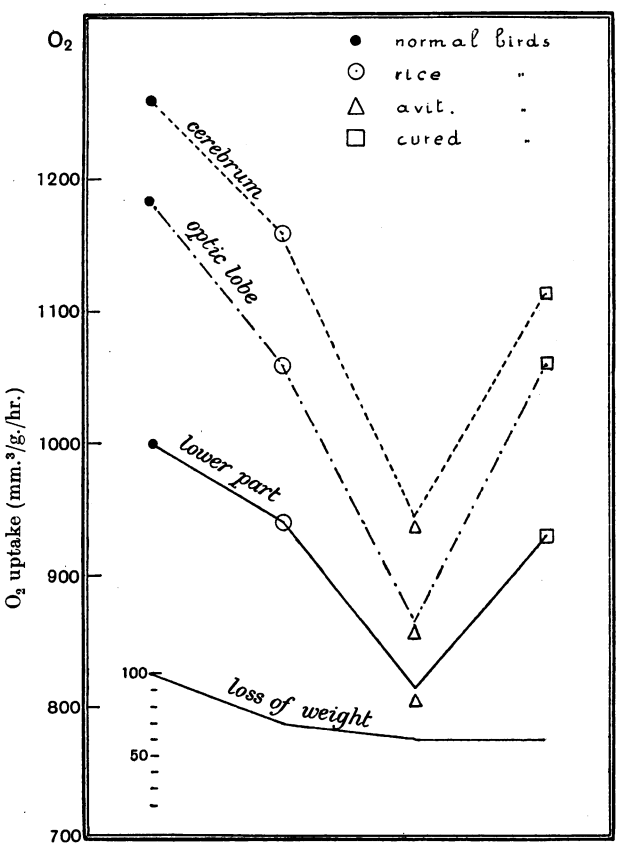

Fig. 2. Diagrammatic comparison of average oxygen uptake of different parts of the brain (pigeon) with the fall in weight at different states of avitaminosis. Ordinates, average oxygen uptake in mm.^{3}/g./hr. Abscissae, periods in the feeding at which the average uptakes were observed. The interval between the first and second points represents 20–30 days; that between third and fourth points 24–72 hours approximately. The diagram includes a curve for the average weight at the same periods, showing that the average oxygen uptakes do not follow the fall in weight in the avitaminous periods.

parts of the brain in (*a*) birds which have been fed upon polished rice until the weight has dropped to some 40 % of the normal, to which doses of vitamin

[1] Throughout this paper we have made the assumption that the changes induced in the oxidations are produced by the vitamin B_1 in the extracts which cures the pigeon. This is justified in the present state of knowledge, because in some cases cures were induced with vitamin B_1 extracts of the *X* type (Appendix) [see Carter, Kinnersley and Peters, 1930]. It should always however be remembered that until a pure preparation of vitamin B_1 is obtainable, any conclusion of this nature can only be provisional.

have been in addition administered (rice in Table I), and (*b*) birds which have been fed upon rice until symptoms appeared and which have then been cured by addition of vitamin (cured in Table I). In some cases the vitamin has been administered for several days before carrying out the experiment.

The results of these experiments are also shown in Table I and Fig. 1, and the averages in Fig. 2. As would be expected the cerebellum shows no change. In the cerebrum, optic lobes and lower parts however dosing with the vitamin has given a rise of approximately 20 % in the rate of oxygen uptake. This proves conclusively that the lowered oxygen uptake is not a question of general inanition but is specifically associated with some entity in the vitamin extracts, which if not identical with vitamin B_1 must accompany it rather closely[1].

In grouping together the results for the avitaminous birds in head retraction and cured birds above, we have purposely overlooked certain minor differences which may influence the values. These are the length of time during which symptoms have persisted, and, in the case of the cured and rice-fed birds, the time elapsing between giving the dose and killing the bird. These points are partly discussed in the Appendix to this paper in which the protocols of the experiments are also given.

In drawing these conclusions as to the significance of the differences between the normal and avitaminous birds, we have considered the possibility that the water content of the brains might be different. This matter was extensively investigated by Roche [1925], who showed only very small differences, not amounting to more than about 2 %. In control experiments of our own we have obtained similar results.

The significance of the changes.

We feel that these results, together with the observations upon lactic acid in the brain [Kinnersley and Peters, 1930], show finally that the symptoms are central in origin in the polyneuritic pigeon. We have evidence of chemical change in the central nervous system on the one hand, and on the other find that the opisthotonus is relieved most quickly by injection of vitamin B_1 concentrates into the pigeon's cranium. From a theoretical point of view the changes might be due either to some general interference with the circulation in the brain, or to actual alterations in the tissue. The fact that the cerebellum does not appear to share in the general fall in oxidations (or in the changes in lactic acid) suggests that we are not dealing with a general failure of circulation. Though the condition of opisthotonus once established is progressive, it is wrong to consider such animals as moribund in the sense that no conclusions drawn at this time can be considered as valid indicators of vitamin B_1

[1] Reference to Fig. 2 shows that the oxygen uptake for the "cured" and "rice-fed" birds dosed with vitamin B_1 concentrates does not rise to the normal value completely. Though this may represent some change, we do not consider that the difference is sufficiently established by our experiments to warrant any far-reaching conclusions.

deficiency. Such birds show such a good blood pressure in many cases that the blood in the severed carotid arteries will spurt for some distance after the use of the guillotine. This leads to the conclusion that the differences in oxidations are not due to the lowered body weight or to failure of circulation. The localised nature of the trouble as well as of the increased lactic acid suggests that the differences are due to lack of some entity in the part affected. Kinnersley and Peters drew the conclusion that there was some change in the tissues of the brain, concomitant with the appearance of the symptoms, which led to accumulation of lactic acid first in the parts of the brain other than cerebrum and cerebellum and gradually involved the cerebrum. This picture seems to be in general true for the oxygen uptake (see Appendix). The other possibility that the depression of oxidation is due to some circulating toxic substance seems unlikely, both because such effects might be expected to be more evenly distributed, and also because, under the conditions of our experiments, the concentration of any such toxic stuffs in the tissues should be rapidly lowered by diffusion into the large volume of Ringer's solution. It seems therefore that the cause of the tissue changes can be one of three possibilities, a lowered content of either (*a*) certain enzymes (oxidases), or (*b*) certain essential substrates other than glucose, or (*c*) certain co-enzymes. The presence of the missing entity is dependent upon the presence of vitamin, as it is restored to the tissue by the cure of the bird. It is hoped that further work will decide whether it is the vitamin itself, or whether the vitamin is an intermediary in the production of some other substance.

Note. In a recent communication Vøgt-Møller [1931] has raised the question whether the symptoms of vitamin B_1 deficiency are due to the accumulation of methylglyoxal in the polyneuritic animal. The evidence for this is based upon the fact that liver extracts from avitaminous animals convert hexosephosphate to methylglyoxal, and not to lactic acid. While this is an interesting observation, in conformity with Findlay [1921], it would be dangerous to believe that lactic acid cannot accumulate in the avitaminous bird, until evidence to the contrary is produced. There is evidence now in the literature for the presence of increased lactic acid in the blood in birds [Collazo and Morelli, 1925; Kinnersley and Peters, 1930] and in man [Hayasaka, 1929], in the brain of the pigeon [Kinnersley and Peters, 1930] and in the tissues of the pigeon [Fisher, 1931]. In view of the possibility that methylglyoxal initially present in a trichloroacetic acid extract might be converted to lactic acid during the process of estimation, we have examined fresh trichloroacetic acid extracts of brain, blood and other tissues of the polyneuritic pigeon for the presence of methylglyoxal by the use of dinitrophenylhydrazine, but have not detected its presence. It can therefore be stated that any amounts of this substance, if present, can only be small; the upper limit would be set by the precipitation reaction with the reagent at about 1 mg. per 100 g. tissue. Since in the work of Kermack, Lambie and Slater [1927] it was found to be necessary to inject amounts of 0·7 g. of methylglyoxal per kg. into animals in order to produce symptoms, it seems unlikely that such small amounts even if present could be responsible for symptoms. As we have evidence of the presence of increased lactic acid, it therefore seems to be unnecessary at present to postulate that the effects are due to some other substance, the presence of which has not yet been demonstrated in the affected part.

SUMMARY.

1. Brain tissue from polyneuritic pigeons (vitamin B_1 deficiency) shows *in vitro* a lowered power of oxygen uptake in the presence of glucose as substrate. This is the case when symptoms are prolonged in all parts of the brain except the cerebellum.

2. Control birds of equal weight to the avitaminous, but previously dosed with vitamin B_1, and birds which have been cured by dosing with vitamin B_1 do not show the same depression of oxidation, which is therefore associated with the specific deficiency.

3. The symptoms of opisthotonus are associated with chemical changes in certain parts of the brain and are central rather than peripheral in origin.

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APPENDIX.

In Tables II, III, IV are recorded certain of the essential points from the protocols of the experiments, especial attention being drawn to points of interest in connection with the symptoms. In the references to dosing with vitamin B_1, it will be noted that cures have been effected with both the so-called *X* and *Y* preparations [Carter, Kinnersley and Peters, 1930]. The *X* preparations are relatively more free from vitamin B_5 than the *Y* preparations.

Table II. *Cured avitaminous birds (C. in Table I).*

Exp. No.	Bird No.	Sex	Days on diet	Loss of weight* %	Date of head retraction 1	Date killed 2	Weight on date 1 g.	Weight on date 2 g.	Lactic acid in blood at death mg./100 cc.	Remarks
51	753	—	22	36	Feb. 10	Feb. 10	172	172	—	Killed 5 hours after vitamin injection. 8 pigeon doses (*Y* preparation). 11.15 a.m., 12.15 p.m., no better. 2 p.m., symptoms cured. 4.15 p.m., practically well except that (1) vision was not completely restored, (2) not able to fly properly
57	456	—	45	47	„ 16	„ 17	280	264	71	7·5 pigeon dose (*X* preparation) injected on Feb. 16. Very fit when killed on Feb. 17
63	701	—	23	37	„ 18	„ 20	240	245	24	Feb. 18 and 19. 3 doses by mouth (*Y* preparation). When killed on 20th still showed weakness in wings, though head retraction completely disappeared on Feb. 19
65	412	♂	35	41	„ 17	„ 23	246	243	45	Feb. 17. 5 doses (*Y* preparation); Feb. 18, 19, 20, 21, 22, 3 doses daily by mouth. Altogether the bird received 24 day doses; bird rapidly recovered from symptoms and seemed well, but even when killed on 23rd, it still showed slight incoordination and difficulty in alighting properly
69	895	♀	22	29	„ 25	„ 26	167	173	34	5 doses (*Y* preparation) given 24 hours previous to death
71	635	♂	39	43	„ 25	„ 27	223	224	26	Feb. 25. 5 doses *Y*, Feb. 26, 3 doses
75	774	♂	37	51	Mar. 2	Mar. 3	258	233	—	Mar. 2. 15 doses (*X* preparation). 3rd flies fairly well. Kept in a dark box for 20 mins. before killing
76	476	♂	28	30	„ 3	„ 4	294	293	—	Mar. 3. 10 doses *X* preparation
77	870	—	32	49	„ 3	„ 4	205	210	—	Mar. 3. 10 doses *X* preparation
96	901	—	28	39	„ 26	„ 27	217	210	—	Mar. 26. 6 doses *X* preparation

* Loss of weight at time of symptoms. In some cases the lactic acid in the blood is not completely normal. This is probably connected with the amount of exercise, though from the results of Fisher [1931] it is likely that the high lactic acid represents a lingering vitamin B deficiency in other parts of the body. The doses were given by mouth, unless otherwise stated.

Note to Table II.

Of the above, the following gave oxygen uptakes below the average for the group of cured avitaminous birds. It is interesting to note that in practically all cases, some abnormality was still noticeable in the behaviour of the bird.

No.	Time after dose	Oxygen uptake: Cerebrum	Optic lobe	Lower part	Remarks
51	5 hours	Average	Low	Low	Vision not restored
57	24 hours	Average	Average	Low	Very fit
63	2 days	Low	Low	Average	Wings still weak
65	6 days	Low	High	Low	Some incoordination still present
71	2 days	Average	High	Low	No observation recorded

Table III. *Avitaminous birds with symptoms (Av. in Table I).*

Exp. No.	Bird No.	Days on diet	Weight loss %	Hours symptoms	Oxygen uptake*			Remarks
					Cerebrum	Optic lobe	Lower parts	
4	339	19	34	6	Average	Low	—	—
14	367	26	41	2	High	Average	Low	—
27	333	18	—	6	High	Average	Average	—
55	644	28	38	—	High	Average	Low	Emprosthotonus and weak
56	632	28	36	30	Low	Low	Low	Emprosthotonus for 30 mins. only. Symptoms threatening for 3 hours previously
74	920	19	34	—	High (1210)	High (1120)	High (1150)	Symptoms threatening only. O_2 uptake, mm.³/g./hr.
84	415	21	30	2	High	High	High	Symptoms threatening only
87	980	24	34	30	Low	—	—	—

* In relation to average for avitaminous birds.

Notes. In this table are included those birds from Table I (avitaminous column) of which the time of onset of symptoms was known. The others were mostly birds which had developed symptoms overnight. In addition, experiment 74 has been added. With exception of 74 and 84, it will be seen that all birds showed a lower oxygen uptake in some part of the brain. In Exps. 14, 27, 55, a lowered oxygen uptake in optic lobe and lower parts was associated with a comparatively normal cerebrum value. This suggests that we are dealing with the same distribution as the lactic acid (Kinnersley and Peters). The lesion seems to commence in the part of brain other than cerebrum-cerebellum, and to extend to the cerebrum as symptoms are continued.

Exp. 74 shows that a bird with threatening symptoms may still show high oxygen uptake. Exp. 84 constitutes an exception in that it showed high oxidation together with symptoms. The interpretation is probably that the bird normally has a higher oxygen uptake than the usual normal.

Table IV. *Rice-fed birds (R. in Table I).*

Mostly dosed in addition with vitamin some hours or days before use.

Exp. No.	Bird No.	Sex	Days on diet	Weight loss %	Date of head retraction 1	Date killed 2	Weight on date 1	Weight on date 2	Lactic acid blood	Remarks
15	447*	—	23	—	—	—	—	—	—	Rice diet (not dosed with vitamin B_1)
44	382	—	30	39	Feb. 4	Feb. 4	290	290	—	Vision good at time of death
53	442	♂	23	32	,, 11	,, 11	265	265	26	10 doses B_1 (*X* preparation) given 90 mins. previous to death
54	732	♂	24	32	,, 11	,, 12	276	275	25	10 doses B_1 (*Y* preparation) on Feb. 11
58	655	♂	29	35	,, 16	,, 17	234	230	36	8 doses B_1 (*Y* preparation) on Feb. 16, 24 hours before death
70	628	♀	38	44	,, 23	,, 27	—	—	—	4 doses B_1 daily Feb. 23–27
72	722	♂	40	44	,, 22	,, 28	272	284	—	4 doses B_1 daily Feb. 24–27
73	814	♀	33	35	,, 22	Mar. 2	214	204	—	Feb. 22, 0·5 g. marmite +4 doses B_1 (*Y* preparation); Feb. 24–28, 4 doses B_1 daily
83	781	♀	41	40	,, 28	,, 4	283	260	—	Feb. 25, Mar. 3 and 4, 1 g. marmite

* Other data unfortunately missing.