CCXXXI. ON THE FUNCTION OF TORULIN. AN *IN VITRO* EFFECT OF ANTINEURITIC VITAMIN CONCENTRATES.

BY NICOLAI GAVRILESCU AND RUDOLPH ALBERT PETERS.

From the Department of Biochemistry, Oxford.

(Received October 31st, 1931.)

RECENTLY, the authors [1931, 1] have shown that there is a lowered oxygen uptake¹ as compared with the normal in pieces of minced avitaminous pigeon's brain tissue suspended in glucose-phosphate solution². The change was most marked in the optic lobes. Cure of the pigeon was accompanied by an increase in oxygen uptake, at a time when no increase in body weight occurred. This was proof that a change had occurred in the tissue as the result of introducing vitamin into the body, but not that the increase was due to the addition of vitamin as such to the tissue. Two things suggest that the changes in avitaminosis were due to the local lack of vitamin: (1) that the changes in the brain tended to be localised, and (2) that the most rapid cures of head retraction observed in this Department were induced by the injection of torulin into the cranium; in some cases indeed cure of symptoms has occurred in 30 minutes. Striking as this evidence is, it is not conclusive. The missing entity in the tissue, indicated by the localised chemical lesion, might be some hormone either made from the vitamin and brought to the brain in the circulation, or secreted as the result of a stimulus of vitamin. It has in fact been stated by Cramer [1930] that vitamin B extracts cause a rapid liberation of adrenaline from the suprarenal gland. Such an explanation might also apply to the injection experiments. There are two ways of settling the question; either it might be proved that there is less vitamin B₁ in the optic lobes, *etc.* than normal, which is not practical with the pigeon test owing to the small amounts of tissue involved,

¹ We prefer to use the expression "oxygen uptake" which exactly expresses the observations and is at present non-committal as to their meaning.

² About the time that the earlier results were communicated to the Biochemical Society, a paper appeared by Abderhalden and Vlassopoulos [1931] which we did not see until recently. The authors claim to have established a lowering of oxygen uptake in liver, brain and muscle, restored when the birds are dosed with yeast extracts. We believe that the experiments are open to certain objections in the case of the brain tissue which is the only one upon which we have worked. Rather low figures are obtained, very likely owing to the large pieces of tissue used (0.5 g.). Our own preliminary results previously reported, show not much difference in Ringer phosphate solution for oxygen uptake of cerebrum. No attempt seems to have been made to differentiate between different parts of the brain.

or it might be shown that the oxygen uptake *in vitro* is increased by the addition of vitamin concentrates. We believe that this proof has now been obtained, and present the evidence for it below [1931, 2]. If confirmed by further work, it will be the first instance in which an effect of a vitamin has been demonstrated upon a tissue *in vitro*.

EXPERIMENTAL.

The tissue. In all cases we have used birds in the standard condition of opisthotonus induced by our usual methods. Sometimes the cases had been used previously for vitamin tests. We noticed no essential difference between birds which were fresh avitaminous cases and those which had a renewed attack after previous vitamin dosing. There appears to be no point in recording details of the birds used. The effects of vitamin were observed upon the mixed optic lobes and lower parts of the brain (optic lobes/rest) and the cerebrum respectively in the presence of air and of oxygen. Doses of vitamin ranging from 0.01 to 2.0 pigeon-doses have been used, the most satisfactory amount appearing to be 0.1–0.05 pigeon-dose per 3.0 cc. of fluid, containing 0.08–0.10 g. of tissue. Since animal tissues usually contain amounts of vitamin B₁ of the order of 1–2 pigeon-doses per g. of tissue, the dose appears to be optimal if all adsorbed by the tissue. This is very likely to be the case at p_H 7.4.

The tissue was very finely minced as before with a bone spatula and thoroughly mixed, the mixing being continued before the withdrawal of each sample for a bottle. Samples were placed in the bottles in irregular order to avoid any possible influence of drying. Where possible determinations were made in triplicate, but the amounts of tissue are not large. A total of about 0.9 g. of minced tissue can be obtained from the cerebrum, and about 0.4 g. from the mixed optic lobes/rest. Cerebellum was not included in the study as no change in this tissue was previously observed.

The measurements. The methods used were essentially the same as previously employed; the glucose-phosphate buffered solution was made up as before; the precautions of Dixon and Elliott [1930] as to rate of shaking and absorption of CO₂ were observed as previously. The constants were checked by direct determinations under the conditions of the experiments at 38°. In the first series air was used in the bottles and in the later oxygen. The bottles were evacuated to a pressure of 710 mm. Hg before filling with oxygen and washed out once with the gas. About 20 minutes elapsed from the guillotining of the bird to placing the apparatus in the warm bath when air was used, and about 30 minutes with oxygen. It was our practice to shake for 12 minutes before taking the first reading, and to make subsequent readings every 10 minutes for 60 minutes. The results were calculated as an average for the hour of observation. With the cerebrum, the rate of oxygen uptake remained fairly constant though there was a tendency to "balling" of the tissue in some cases. This was noticeable as a lowered oxygen uptake in the first periods. In some cases allowance could be made for this by a comparison of subsequent periods

(Exps. 99, 114 and 117 have been so corrected). Both in cerebrum and optic lobes/rest, comparison of results with and without vitamin for the last 40 minutes of the periods of shaking gave substantially the same results. With the optic lobes/rest the rate of oxygen uptake fell off throughout the period of observation to an amount which varied in different experiments from 33 to 50 % of the initial rate.

The errors of the observations cannot be computed exactly. Those due to the apparatus alone are not larger than 1 %. As we have worked with a mixed tissue, we have included in the Tables all those results which could not be explained by us as due to experimental error. It appears from the figures that our results are subject in the extreme to variations of the order of ± 10 %, though many duplicate estimations are much better than this. Doubtless several of the discrepancies are due to undetected experimental error; others are probably due to unequal adsorption of vitamin upon the pieces of tissue.

Vitamin solutions. Torulin (yeast B_1) concentrates of different purity were used. The crude preparation A consisted of a 50 % alcohol concentrate of activity about 3.0 mg./pigeon-dose (1.5 B_1 units) [for details see Carter, Kinnersley and Peters, 1930]¹. Preparation B was less impure, and was an X concentrate, prepared initially from brewer's yeast, of activity 0.05 mg./day. This constitutes a purer source of vitamin B_1 than the Y concentrates, so far as other factors in the B-complex are concerned. Preparation C was one of our purest concentrates of activity 12 γ /day [see Kinnersley and Peters, 1930, 2]. The solutions were freshly prepared; two drops of water were added to the alcoholic solution (about 0.1 cc.), the alcohol present was removed by heating on the water-bath with continuous shaking; a trace of sulphuric acid ($N/20$) was then added, and the heating continued for 2 minutes. This was necessary in most cases in order to remove the last traces of barium with which it is our custom to store many of the preparations. The torulin solution was diluted to about 0.5 cc., a minimal amount of phenol red added, and the whole carefully neutralised to p_H 7.4; water was added to make the vitamin addition 0.1 cc. in each bottle. In all the later experiments, a control tube was treated similarly as to sulphuric acid, phenol red and soda and added to the control brain sample.

In the experiments in which alkali-treated vitamin was used, the procedure was the same up to the alcohol stage. After this, however, the sample was divided into two parts. One of these was neutralised approximately, the requisite amount of NaOH added and the sample heated in the water-bath in a test-tube immersed in the boiling water, with a funnel to prevent excessive concentration. After treatment with NaOH, a similar amount of cold NaOH was added to the other sample of active vitamin; the two were then neutralised in a similar way with addition of the necessary amount of phenol red. In this way the effects of excess of salt were controlled. The additions of torulin were made immediately before placing the apparatus in the bath, to avoid possible inactivation.

¹ 1 pigeon-dose = approx. 15 mg. Jansen acid clay $\equiv$ 1.5 vitamin units.

RESULTS.

The results are given in Table I, and will be considered in detail below. Before doing so mention must be made of the effect of oxygen.

Table I. *Protocols. Oxygen uptake, mm.³/g./hr.**Air. Avitaminous birds at H.R. stage.*

(a) Control and vitamin.

Exp.	Cerebrum				Optic lobe and rest (mixed)			
	Control	Vitamin	Dose added	Diff. % average	Control	Vitamin	Dose added	Diff. % average
84	{ 1230 1190 }	{ 1245 1225 }	2.0 A	+2	—	—	—	—
99	{ 1100 1090 }	{ 1090 1140 }	0.1 A	+2	{ 790 950 940 }	{ 880 940 1050 }	0.1 A	+7
100	—	—	—	—	{ 810 880 960 }	{ 840 1015 1030 }	0.1 A	+7
106	{ 925 — }	{ 1290 1270 }	0.1 A	—	{ 930 930 }	{ 1120 1050 }	0.06 A	+16
107	{ 1155 1220 1285 }	{ 1190 1240 1380 }	0.06 A	+4	{ 870 940 }	{ 890 1060 }	0.06 A	+8
108	{ 1080 1130 1130 }	{ 1150 1180 1240 }	0.1 A	+7	—	{ 1130 980 }	0.7 A	—
109	{ 1080 1125 1180 }	{ 1140 1290 1350 }	0.1 A	+11	{ 810 880 }	{ 950 1070 }	0.1 A	+20
110	{ 840 — }	{ 1090 — }	0.1 A	—	{ 745 765 795 }	{ 835 890 900 }	0.1 A	+13

(b) Control, alkali-treated and active vitamin.

Exp.	(1) Control	(2) Alkalised vitamin	(3) Active vitamin	P.D.	Diff. %		(1) Control	Alkalised vitamin	Active vitamin	P.D.	Diff. %	
					1/2	2/3					1/2	2/3
112	{ 1080 1120 }	{ 1070 1110 1130 }	—	0.1 A	Nil	—	{ 870 920 }	{ 855 950 }	—	0.1 A	+1	—
114	{ 1030 1170 }	{ 910 1080 }	{ 1214 — }	0.1 B	-10	—	—	{ 760 815 }	{ 850 865 }	0.1 B	—	+9

In presence of oxygen.

(c) Comparison of oxygen and air, together with vitamin and oxygen.

Exp.	Cerebrum				ii/iii Diff. %	Optic lobes and rest (mixed)				
	Air	(ii) O ₂	(iii) O ₂ + vit.	Dose		Air	O ₂	O ₂ + vit.	Dose	ii/iii Diff. %
117	{ 980 995 }	{ 1220 — }	{ 1470 — }	0.1 A	[+19]	810	{ 780 920 }	{ 930 1000 }	0.1 A	+15
118	{ 955 1015 }	{ 1330 1360 }	{ 1520 1580 }	0.1 A	+15	810	{ 905 965 }	{ 920 1150 }	0.1 A	+10
119	{ 1070 1150 }	{ 1470 1550 }	{ 1645 1700 }	0.1 A	+10	1135	{ 1040 1190 }	{ 1240 1275 }	0.1 A	+13
120	{ 890 975 }	{ 1250 1320 }	{ 1220 1470 }	0.09 B	+5	1030	{ 1140 1170 }	{ 1330 1460 }	0.08 B	+21
123	{ 1120 1130 }	{ 1200 1500 }	{ 1480 1620 }	0.11 B	+15	805	{ 1020 1030 }	—	—	—

Table I (*cont.*)

(d) Active, alkali-treated vitamin and controls in presence of oxygen.

Exp.	Cerebrum					Optic lobes and rest (mixed)						Remarks	
	(1)	(2)	(3)	Dose P.D.	Diff. %		(1)	(2)	(3)	Dose	Diff. %		
	Control	Alkalised vitamin	Active vitamin		1/2	2/3	Control	Alkalised vitamin	Active vitamin		1/2		2/3
124	—	1530 1720 1810	1600 1660 1870	0.1 B	—	+2	—	745 972	1090 1130	0.1 B	—	+28	2 <i>N</i> NaOH 20 min.
125	—	1330 1495 1660	1720 1730 1750	0.07 B	—	+16	—	1005 1180	1030 1250	0.07 B	—	+5	0.5 <i>N</i> NaOH 45 min.
126	1130 1210 1280	—	1190 1220 1325	0.01 B	+2	—	752 760	—	810 915	0.01 B	+13	—	
127	—	1470 1790	1500 1750 1790	0.05 B	—	+3	—	1210 1350	1340 —	0.05 B	—	Nil	0.5 <i>N</i> NaOH 30 min.
128	—	1610 1670 1690	1690 1720 1820	0.05 B	—	+5	—	1130 1260	995 1210	0.01 B	-8	—	0.5 <i>N</i> NaOH 30 min.

Active, alkali-treated vitamin, etc. (*continued*).

Exp.	Cerebrum					Optic lobes and rest (mixed)						Remarks	
	(1)	(2)	(3)	Dose P.D.	Diff. %		(1)	(2)	(3)	Dose P.D.	Diff. %		
	Control	Alkalised vitamin	Active vitamin		1/3	2/3	Control	Alkalised vitamin	Active vitamin		1/3		2/3
129	—	1510 1550 1580	1340 1350 1720	0.05 B	—	—9	—	1070 1045	1345 1380	0.05 B	—	+30	0.5 N NaOH 30 min.
130	1705 1825	1710 1760	1920 1935	0.01 B	+10	+11	—	1120 1160	1080 1160	0.01 B	—	+2	0.5 N NaOH 30 min.
131	1175 1185	1330 1400	1455 1500	0.05 B	+25	+7	—	925 930	980 1280	0.05 B	—	+23	2 N NaOH 60 min.
133	1190 1270	1390 1420	1440 1490	0.10 B	+19	+4	—	890 915	1040 1070	0.10 B	—	+20	2 N NaOH 60 min.
138	1550 —	— —	1560 1550	0.1 C	—	Nil	1125 1180	—	980 1135	0.01 C	-10	—	
139	1340 —	— —	1410 1460	0.1 B	+7	—	1030 895	—	875 1110	0.01 B	+3	—	
150	1200 1240	—	1300 1380 1425	0.10 B	+17	—	910 940	—	900 975	0.05 B	+1	—	
151	1410 1490 1510	—	1490 1520 1600	0.10 C	+14	—	805 920	—	970 1140	0.1 C	+16	—	
153	1280 1380	1190 1220	1220 1260	0.05 C	-7	-4	—	1240 1330	1390 1570	0.05 C	—	+15	2 N NaOH 30 min.
154	1640 1700	1500 1560	1530 —	0.05 C	-9	Nil	—	1170 1280	1400 1400	0.05 C	—	+14	2 N NaOH 30 min.
164	—	—	—	—	—	—	1150 1225	—	1262 1304 1340	0.1 C	+10	—	Brains of 2 birds mixed
165	—	—	—	—	—	—	1120 1120 1190 1175	—	1204 1340 1220 1200	0.1 C	+6	—	Brains of 2 birds mixed
184	1302 1430	—	1240 1425	0.1 C	-25	—	1202 1225	—	1280 1382	0.1 C	+10	—	
185	—	—	—	0.1 C	—	—	990 1130 1152	—	1240 1310	0.1 C	+16	—	

Comparison of the results with oxygen and air respectively.

It was stated previously that the uptake of oxygen could be increased by performing the experiments with oxygen in the bottle instead of air, but that our general conclusions were not influenced by this. In Table II we have recorded the average results from several recent experiments together with those from our previous paper. It will be seen that the new ones are quite in agreement with the old. The conclusion that there is lowered oxygen uptake in glucose-phosphate solutions in the avitaminous brains is made more certain by these results. The increase in oxygen uptake for oxygen as against air with

Table II. *Comparison of oxygen uptake in presence of air and oxygen.*(a) Different birds. mm.³/g./hour*.

Tissue		Air	Limits	Oxygen	Limits	Increase
Cerebrum	N.	1315	(1000-1600)	1810	(1470-2220)	+ 38 %
	Av.	1030	(880-1210)	1341	(980-1670)	+ 32 %
Optic lobes/rest	N.	1100	(960-1280)	1480	(1300-1600)	+ 38 %
	Av.	880	(805-1135)	1055	(980-1670)	+ 16 %

N. = Normal. Av. = A vitaminous.

(b) Comparisons upon tissue from same bird (avitaminous).

	Cerebrum		OL/LP	
	Air	O ₂	Air	O ₂
(1)	990	1220	810	850
(2)	980	1340	810	930
(3)	1110	1510	1135	1120
(4)	880	1280	1030	1150
(5)	1050	1350	805	1030

* 1000 mm.³/g./hr. = QO₂ (Warburg) 5·0 approx.

normal brains is rather variable, amounting on the average to + 38 % for cerebrum and + 32 % for the rest. This would be interpreted easily upon the usual view that the presence of oxygen activated underlying pieces of the tissue not otherwise reached at the pressure of oxygen in air, if it were not for the results with the avitaminous tissue. With the avitaminous cerebrum, increased oxygen pressure increased the uptake by 30 %. With the avitaminous mixed optic lobes and lower parts, however, the uptake was not increased on the average more than some 20 %, and in several cases which are given in abstract in Table II a comparison of the tissue with and without oxygen shows no difference. Since there is no apparent difference in the state of division of the tissue for normal and avitaminous brains, doubt is thrown upon the simple explanation that the increases observed in these minced brain samples are due merely to increased penetration of oxygen. As previously mentioned, at the conclusion of the experiment the tissue is mostly in the form of a fine suspension. The matter merits further attention. In the series of experiments which follow, the effects of vitamin concentrates have been followed both in the presence of air and of oxygen; rather similar results have been obtained with each.

Effects of vitamin concentrates.

In the protocols given in Table I, we have given the experiments as they were obtained, and have included all those in which no obvious experimental error could be detected. In this way the whole evidence upon which conclusions are based is before the reader. It will be recalled that previously we reached the conclusion that there are differences between the cerebrum and the rest of the brain in avitaminosis. Kinnersley and Peters [1930, 1] advanced the view that the changes in lactic acid metabolism commenced in the lower parts of the brain and gradually spread to the cerebrum, and in our previous paper we concluded that the two parts of the brain were not always equally affected. In this work too, we have found marked differences between the behaviour of the two parts, and are therefore considering them below as separate tissues.

Mixed optic lobes and the rest.

A casual glance at the protocols for this part of the work shows that in spite of variations the general trend of the results is towards an increase in the presence of vitamin. The results are shown diagrammatically in Fig. 1. Out of 19 experiments, 6 in air, and 13 in oxygen, 10 show a clear increase for the vitamin-treated tissue in the sense that there is no overlap in the figures; 7 of these 10 are in the presence of oxygen¹. We have examined and excluded the possibility that some particular use of the same bottles for the same parts of the experiment might have led to the result, as also that the order in which the tissue was transferred to the bottles might produce the effects found. The other possibility that the results might be the consequence of the method of determining constants has also been eliminated by finding these directly at 38°, which led to a slight constant correction. The problem therefore narrows down to the following question; what are the chances that we have made favourable selections in these 10 experiments? To take a concrete case, Exp. 119, we might suppose that the possible errors in sampling this particular tissue lay between 1000 and 1300, and that by chance we had selected our samples to give the apparent increase for vitamin. The matter is one of statistics, and the probability against this favourable selection is very great. The mean percentage difference is 10 % approx. It can be calculated that a mean percentage difference of 4.8 % would be likely to occur by chance only once in 100 times. We feel therefore that the results constitute proof that the addition of vitamin concentrates increases the oxygen uptake. The effect is catalytic: where the preparations B and C have been used, a larger weight of oxygen is taken up by the vitamin-treated tissue than the weight of solid added. As an example with the purest C, 0.5 γ added to 3.0 cc. of the solution increased the average O₂ uptake by 15 γ oxygen. Controls showed that no increase took place in absence

¹ The remainder show an average increase except Exp. 138, which cannot be explained and was performed with 1/100 pigeon-dose of vitamin, a rather small amount.

of tissue. The average increase of 12 % is about half that of the lowered oxygen uptake previously recorded.

Fig. 1. Chart showing results for mixed optic lobes/rest with and without vitamin. $\times$ + vitamin concentrates, $\circ$ control. Ordinates O_2 mm.³/g./hr. Experiments arranged along abscissae in increasing order of basal oxygen uptake. Other results for Exps. 99, 100, 107, 117, 118, 150, 165, 139, etc., grouped together and shown on right of chart.

The next question, whether the increase is due to the vitamin itself or to some other base in the concentrates, cannot be settled absolutely in the absence of pure vitamin B_1 . Concentrates of quite different purity give the same result. Treatment with alkali is known to inactivate vitamin B_1 . It has been shown previously that 1 hour's heating at the temperature of the boiling water-bath with 0.5 *N* NaOH will inactivate a concentrate of 0.06 mg. activity. This probably varies for the preparation, and requires reinvestigation [Kinnersley and Peters, 1928]. In the experiments below, it appeared from pigeon tests that 30 minutes' heating at the temperature of the boiling water-bath with approx. 0.5 *N* NaOH was not enough to inactivate preparation B, but that 30 minutes with 2*N* NaOH was enough. We have compared the effect of alkali-treated concentrates with untreated controls, containing a similar amount of salt. In Table III the results of this treatment have been collected for the oxygen experiments and arranged in order of alkali treatment. 0.5 *N* NaOH for 30 minutes seems to be marginal, whereas 2*N* NaOH for 30 minutes seems to be sufficient to inactivate the factor concerned. Such a treatment was found by animal tests to reduce the activity to less than 10 % of the original. The

Table III. *Oxygen uptake (mm.³/g./hr.) for mixed optic lobes/rest with active and alkali-treated vitamin B concentrates.*

Exp. No.	Dose added	Preparation	Vitamin		Difference	Alkali treatment at 100° approx.
			Alkali-treated	Active		
127	0.05	B	1210 } 1350 }	1340 } — }	[? nil]	0.5 N 30 min.
129	0.05	B	1070 } 1045 }	1345 } 1380 }	+ 30 %	0.5 N 30 „
125	0.07	B	1005 } 1180 }	1030 } 1250 }	+ 5 %	0.5 N 45 „
124	0.10	B	745 } 972 }	1090 } 1130 }	+ 28 %	2N 20 „
153	0.05	C	1240 } 1330 }	1390 } 1570 }	+ 15 %	2N 30 „
154	0.05	C	1170 } 1280 }	1400 } 1400 }	+ 14 %	2N 30 „
131	0.05	B	925 } 930 }	980 } 1280 }	[+ 23 %]	2N 60 „
133	0.10	B	890 } 915 }	1040 } 1070 }	+ 20 %	2N 60 „

Note. The above experiments in oxygen. Exp. 114 (air) gives a similar result. Exps. 128 and 130 not included, in which heating was for 30 min. with 0.5 N NaOH, and only 1/100 dose used.

inactivating effect of alkali therefore is roughly the same upon vitamin B₁ and the factor considered. Here again 0.01 pigeon-dose seems to be marginal. Taken together with the fact that our purest concentrates show the effect, we have reached the best proof at present available that the oxidation factor concerned is vitamin B₁. The necessary concentration so far as can be judged from this limited series of experiments lies between 0.1 and 0.01 pigeon-dose, the latter being marginal. With preparation C, this means a dilution in 3.0 cc. of about 1 in 30,000,000.

The cerebrum.

We have mentioned previously our conviction that the condition in this tissue is different. To the eye, the minces from the two tissues appear quite dissimilar. It has been steadily forced upon us during the course of this work that the behaviour towards vitamin concentrates is also different, a conclusion quite at variance with our preconceptions. The effects of different concentrates do not appear to be identical, nor does the inactivation follow the same course, though there is ample proof that the addition of certain concentrates improves the uptake of oxygen.

Out of 6 completed experiments in air, 2 showed no overlap between the figures (Nos. 99 and 108). The remainder only showed a small increase upon the average. For experiments in oxygen, out of 13 completed experiments, 6 (Nos. 118, 119, 130, 131, 133, 150) showed an increase such that there was no overlap in the duplicate observations (in some cases triplicate). Of the remainder, 4 showed increases upon averages of different degree. Three gave a

decrease. This we cannot explain at present, but it is noteworthy that the two significantly negative results were obtained with the purest preparation (90 A). There is a definite tendency (see also 138) for this preparation to be less effective than the others in increasing oxygen uptake in the cerebrum. Before discussing the possible meaning of this, we can consider the results for activated and inactivated vitamin. In the experiments with each bird, the vitamin solution was the same throughout; hence any variations in the inactivation should affect both cerebrum and the rest equally. It is noticeable that there are cases in which the difference for active and inactive vitamin is definite for the optic lobes, *etc.*, but is not so for the cerebrum. This is illustrated by Exps. 124, 129, 133, 153 and 154. It appears to be more difficult to inactivate the preparation for cerebrum than for the rest. From each set of experiments therefore we reach the conclusion that in the cerebrum the lowered oxygen uptake is due to causes other than vitamin B₁ deficiency alone. It is possible that the vitamin is unstable in the presence of factors sometimes associated with the cerebral tissue, but in view of the apparent difficulty of inactivating the tissue for the cerebral factor, a more attractive hypothesis is that the cerebral tissue is suffering from an additional deficiency of some other factor. This would be consistent with the very irregular action of our purest preparation, which for instance contains very little vitamin B₄. Such speculations must be rather random in the absence of work upon the more obvious points which require an answer. Much might be learnt for instance from a comparison of the effect of vitamin upon the tissue in the presence and absence of glucose. This should settle how far the effect was exerted upon the residual oxygen uptake. Our present conclusion must be that vitamin B₁ is less consistently effective in catalysing an increased oxygen uptake in the cerebrum than in the other parts of the brain. This confirms the opinion reached previously that the condition in the two parts of the avitaminous brain is different [see Kinnersley and Peters, 1930, 1] and ourselves [1931, 1, Appendix], and that the optic lobes and lower parts are more directly connected with the vitamin B₁ deficiency as such.

Normal brains and washed tissues.

The behaviour of normal brain tissue and of washed tissues to vitamin concentrates does not influence the particular facts under discussion in this paper, though it might have some bearing upon their interpretation. The few experiments, which we have made up to the present, do not justify us in drawing any definite conclusions.

DISCUSSION.

Analysis of the behaviour of the minced optic lobes and rest of the pigeon's brain may seem a devious way to search for the nature of vitamin B₁ deficiency, and a test for vitamin B₁. We have been led to it by failures to demonstrate the action of vitamin concentrates upon more obvious tissues with lactic acid

systems, following the work upon the lower parts of the brain [Kinnersley and Peters, 1930, 1]. It may be hoped that these experiments will lead to some easier test for vitamin B₁, which may be specific enough to settle some of the present confusion, inevitable as long as we are unable to assess the precise influence of other factors of the complex upon vitamin B₁ tests. It is possible, though we think unlikely in view of what has been said earlier, that these reactivations are not due to the vitamin. The proof that a catalytic effect is involved decides between the three possibilities discussed in our earlier paper. It is not clear of course what oxidase system is involved, but it is hoped that experiments at present in progress will decide this point.

Accepting the view provisionally that the vitamin B₁ is itself concerned, it may be inferred that it takes part as such in tissue metabolism without the intermediary action of an internal secretion; its well-known presence in tissues is therefore not an accident of storage.

So far as we are aware, this appears to be the first experimental support for a direct relation between a local biochemical lesion and an abnormal condition of the central nervous system. The argument can be briefly stated. Injection of torulin into the cranium very rapidly relieves opisthotonus symptoms; addition of the same *in vitro* to the optic lobes/rest of the brain restores in part the lowered power of oxygen uptake present in avitaminosis. With the same part of the brain is associated especially the increase in lactic acid found by Kinnersley and Peters [1930, 1]; vitamin rapidly induces an increased rate of disappearance of lactic acid in the tissues of the avitaminous animal [Fisher, 1931]. Realising that there is still much that is not clear, the lesion seems evidently to affect the tissue metabolism of carbohydrate, a view which has been often mooted. Perhaps for the first time, we obtain here experimental grounds for belief in the great importance of the antineuritic vitamin B₁ as such in the normal functioning of the central nervous system.

CONCLUSIONS.

1. The oxygen uptake of finely minced pigeon's brain tissue (glucose-phosphate solution) is increased on an average some 40 % by performing the estimations in oxygen instead of air, in the case of cerebrum and the mixed optic lobes and parts below from the normal brain. With the avitaminous (B₁) brain, less or even no increase takes place with the mixed optic lobes and lower parts.

2. In the presence of oxygen the lowered oxygen uptakes previously observed in the brain in avitaminosis B₁ are more marked.

3. Partial restoration of oxygen uptake *in vitro* is observed upon addition of vitamin B₁ concentrates to the mixed optic lobes and lower parts of the avitaminous pigeon.

4. This action of the concentrates follows the vitamin B₁ and is inactivated by treatment with alkali as is vitamin B₁. The action is catalytic.

5. The effects are not always obtained with the cerebral tissue, which behaves differently.

6. Vitamin B₁ is directly concerned with the oxidative metabolism of the lower parts of the pigeon's brain.

We are indebted to the Government Grant Committee of the Royal Society and to the Medical Research Council for grants towards this work. We are also grateful to Mr H. W. Kinnersley for the concentrates used, to Mr Meiklejohn for help in some of the experiments, and to Mr Yates of the Statistical Department, Harpenden, for aid in the analysis of the figures.

REFERENCES.

- Abderhalden and Vlassopoulos (1931). *Pflüger's Arch.* **226**, 808.
Carter, Kinnersley and Peters (1930). *Biochem. J.* **24**, 1832.
Cramer (1930). Personal communication.
Dixon and Elliott (1930). *Biochem. J.* **24**, 820.
Fisher (1931). *Biochem. J.* **25**, 1410.
Gavrilescu and Peters (1931, 1). *Biochem. J.* **25**, 1397.
——— (1931, 2). *J. Physiol., Proc. Physiol. Soc.* (In press.)
Kinnersley and Peters (1928). *Biochem. J.* **22**, 419.
——— (1930, 1). *Biochem. J.* **24**, 711.
——— (1930, 2). *Biochem. J.* **24**, 1856.