CIII. THE ACTION OF CRYSTALLINE VITAMIN B₁ ON THE TISSUE OXIDATION OF CHRONIC AVITAMINOUS PIGEON'S BRAIN.

By HÅKAN RYDIN.

From the Department of Biochemistry, Oxford.

(Received February 26th, 1935.)

GAVRILESCU AND PETERS [1931, 1, 2] showed by Barcroft's method that the oxidation *in vitro* is reduced in avitaminous pigeon's brain tissue, suspended in glucose-phosphate solution as compared with normal controls. This particularly referred to the lower parts of the brain and not to cerebellum. The reduced oxygen uptake could be restored in part by the addition of a vitamin B₁ concentrate *in vitro*. On exchanging glucose for lactate [Gavrilescu *et al.*, 1932] these effects became more marked. No significant defect in the oxygen uptake and no vitamin effect were observed in brains from normal pigeons or from avitaminous ones in absence of substrate, or in the cases where this consisted of succinate. Passmore *et al.* [1933] showed a parallelism between crystalline vitamin B₁ and the concentrate previously used. The changes in oxygen uptake so produced by vitamin B₁ do not run parallel with oxidase estimations by reduction methods. By Thunberg's methylene blue technique, Rydin [1935] was not able to show any effect of crystalline vitamin B₁ on normal or on avitaminous tissues from brain, kidneys, liver and muscle, either in presence of the substrate (lactate or succinate) or in absence of these. In order to find out whether the results obtained by the Barcroft method are specific for the avitaminosis-B₁, or whether tissue from starved animals gives the same reactions, Gavrilescu and Peters [1931, 1] carried out tissue oxidation tests in glucose-phosphate solution on brains from pigeons which had been cured of the opisthotonus symptoms with vitamin concentrate. Even before an increase of the body weight began to set in, higher values of the oxygen uptake were obtained than in the case of avitaminous birds in head retraction. Later it was shown by Meiklejohn *et al.* [1932] with addition of vitamin B₁ and further control of water intake that lactate as substrate gave the same results. The increase of the tissue oxidation in cured pigeons was parallel with the disappearance of the nervous symptoms and independent of the nutritional condition. Peters and his co-workers conclude from their investigations that the reactions obtained on avitaminous pigeon's brain are specific to avitaminosis-B₁ and independent of the nutritional condition. Further support of this view was furnished by Sinclair [1933] who showed that vitamin B₁ increases the respiratory quotient *in vitro* on avitaminous pigeons in head retraction, but does not alter the R.Q. in tests on pigeons which are fed on polished rice and show a marked loss of weight but no polyneuritic symptoms. The same conclusions were arrived at by Abderhalden and Vlassopoulos [1931] by tissue oxidation experiments with Barcroft's method on brain, liver and kidneys though their experiments are really in disagreement with those previously quoted [Gavrilescu *et al.*, 1932]. Without addition of substrate they found lower oxygen uptake for tissues from avitaminous pigeons than from starved or normal birds. The differences for brain

were not large, a decrease of -12 % for starving and -23 % for avitaminous birds as compared with the normal. [See also Galvao and Florence, 1934, and comments by Rydin.]

It has generally been concluded from the work quoted above and that of Kinnersley and Peters [1930] that there is a relation between the symptoms of head retraction and deficiency in the brain, especially the lower parts, of vitamin B₁. So far however only polyneuritic birds in head retraction have been used. It should be of interest to study the tissue oxidation in polyneuritic pigeons not showing head retraction but with other nervous symptoms such as leg weakness. These symptoms are present in pigeons which show a chronic avitaminous condition, generally when they have been fed on polished rice for a period of more than 30 days. I have therefore studied the action of crystalline vitamin B₁ on the tissue oxidation of brains from these birds, partly on the whole of the brain, partly on cerebrum and the optic lobes + "the rest" separately. The result was a significant vitamin B₁ effect in experiments with the whole of the brain and with cerebrum alone. The vitamin effect on the optic lobes + "the rest," on the other hand, was generally only slight. Also the vitamin effect during the first periods of reading up to $\frac{3}{4}$ hour was only little apparent but later on became more marked. The bearing of this work upon that of Meiklejohn, Passmore and Peters will be considered after describing the experiments.

EXPERIMENTAL.

The present investigations were carried out on brain tissue with Barcroft's method under the same general test conditions as apply to other experiments in this field made in this laboratory. These conditions have been changed somewhat during the progress of the work. It is now known that the presence of Na pyrophosphate [Peters and Sinclair, 1933] and "warm" mincing [Peters *et al.*, 1935] much improve the vitamin effect and that this is always more marked in the respiration period 1-2 hours. In order to obtain some basis for comparison of this work with that of Meiklejohn *et al.*, I have not used pyrophosphate and have adopted cool conditions for mincing, because their experiments were done in colder weather. For similar reasons the cerebellum has been excluded. The pigeons were fed on polished rice and were picked out for experiments after having been on this diet for at least 30 days, showing marked leg weakness but no trace of head retraction. The birds were killed by decapitation. The brain was quickly taken out, placed on an iced plate and finely minced with a blunt bone spatula, whereupon it was transferred to the bottles. The apparatus used for the determination of the oxygen uptake was of the Barcroft-Dixon type. Into each bottle were measured 3 ml. of a solution consisting of Ringer-phosphate (p_H 7.3) together with neutralised Na lactate. The latter was added in such quantity as to make the final concentration of the Ringer-phosphate solution 0.033 *M*. The bottles were weighed before and after the addition of approximately 100 mg. of tissue. The roll of filter-paper was put in place and wetted with 2 *N* KOH, and finally 2 γ vitamin B₁ hydrochloride were added to every second bottle. (As regards the composition and preparation of the Ringer-phosphate solution as well as the preparation and administration of the vitamin solution see Passmore *et al.* [1933]). The bottles were then evacuated twice to 70 mm. Hg with subsequent refilling with oxygen. The apparatus was then transferred to the thermostat (temp. 38°) for determination of the tissue oxidation in the usual way. In the tables all the values are the mean of two determinations. The difference between these in

no case exceeded 10 %. The oxygen consumption is expressed in $\mu\text{l.}$ per g. tissue per hour. The first reading was made after 12 minutes, the second after 10 minutes and the subsequent readings at intervals of half an hour.

RESULTS.

Table I shows the action of vitamin B_1 on the tissue oxidation in experiments with the whole of the brain. In these experiments 8 pigeons were used. In all the readings the value of the figures was numerically higher for the oxygen uptake in presence of vitamin B_1 , but in some cases the effect of the vitamin was doubtful or not at all observable during the first two periods of reading ($\frac{3}{4}$ hour). The average increase of oxygen uptake due to vitamin B_1 for all the birds amounted to 17 % for the first period and to 16 % for the second period. During the third and the fourth periods the corresponding figures were 28 and 30 %. The vitamin effect set in as usual, the oxygen uptake owing to the vitamin did not decrease in proportion to the normal control but kept on a higher level during several periods. In many cases the tissue oxidation in presence of vitamin B_1 was decidedly higher than in the controls even during the first period. If the tissue oxidation in presence of vitamin is compared with that without vitamin during all the test periods, an average increase is obtained which amounts to 21 % for the addition of vitamin B_1 *in vitro* over the $1\frac{3}{4}$ hour period.

Table I. *Cerebrum and the lower parts mixed.*

Exp.	Periods (hours)				Loss of weight %	Symptoms
	0- $\frac{1}{4}$	$\frac{1}{4}$ - $\frac{3}{4}$	$\frac{3}{4}$ -1 $\frac{1}{4}$	1 $\frac{1}{4}$ -1 $\frac{3}{4}$		
1 L	3330	2450	1920	1510	38.5	No bird in head retraction, none could fly
L + V	3360	2820	2270	1850		Leg weakness not very apparent for the rest nothing remarkable
2 L	1180	830	650	590	42.5	Severe leg weakness. Bad walk. Rugged
L + V	1340	900	690	650		
3 L	2200	1750	1300	1030	43.5	Moderate leg weakness and ataxic walk
L + V	3020	2500	1910	1540		
4 L	2190	1570	1190	870	29.1	„ „
L + V	2640	2110	1810	1400		
5 L	2050	1800	1450	1160	48.9	Moderate leg weakness. Very ataxic walk. Rugged
L + V	2330	2030	1700	1430		
6 L	1690	1780	1260	1090	55.1	Severe leg weakness. Cannot walk more than a few steps. Rugged
L + V	1820	1870	1670	1260		
7 L	2450	1980	1450	1370	56.2	„ „
L + V	2980	2040	1820	1750		
8 L	2210	1740	1420	1040	29.7	Leg weakness not very apparent. Very ataxic walk
L + V	2640	1920	1780	1350		
Av. L	2160	1740	1330	1080	42.9	
L + V	2520	2020	1700	1400		
Diff. $\mu\text{l. O}_2$ %	360	280	370	320	—	
	16.7	16.1	27.8	29.6		

Table II illustrates the effect of vitamin B_1 on the oxygen uptake in different parts of the brain, and shows a comparison between the effect on cerebrum on the one hand, and on the optic lobes + the rest on the other. Fifteen pigeons were used in these experiments. For the cerebrum, the vitamin effect was approximately the same as that obtained for experiments on the whole brain.

The average vitamin action on the tissue oxidation during the first period was small but later on showed a great increase amounting to 35.5 % during the fourth period. The average vitamin effect for all the periods was 18 %. It varied however very considerably for the different pigeons. For the optic lobes + "the rest," the vitamin effect was considerably less. The highest average vitamin effect amounted to 11.7 % for period 3. The tests showed great individual variations. Thus pigeons 10 and 13 showed a large vitamin effect even on these parts of the brain. The increase of the tissue oxidation in presence of vitamin B₁ was statistically significant for the last two periods, both for the cerebrum and the lower parts.

Table II. *Cerebrum and the lower parts separately.*

O ₂ uptake in μl./g./hr.										
Period (hours)							Loss of weight %	Temp. of the bird	Symptoms No bird in head retraction, none could fly	
Exp.	Cerebrum			Optic lobes + "the rest"						
	1-2	2-1½	1½-1¼	1-2	2-1½	1¼-1¼				
9	L	2010	1400	1130	1560	1380	910	46.6	39.4°	Moderate leg weakness. Moderately ataxic walk
	L + V	2300	1770	1690	1670	1310	1080			
10	L	1900	1800	1240	1020	620	600	42.3	39.6°	" "
	L + V	2020	1570	1440	1400	990	810			
11	L	2000	1380	820	1460	1230	750	44.8	39.1°	" "
	L + V	2280	1980	1330	1550	1400	900			
12	L	1960	1690	1050	1520	1320	790	29.3	38.9°	" "
	L + V	2060	1890	1230	1560	1280	820			
13	L	2100	1600	1230	1400	1020	1060	40.0	39.4°	Moderate leg weakness. Very ataxic walk
	L + V	2160	1860	1550	1870	1540	1420			
14	L	1310	980	610	1370	980	680	51.3	38.6°	Severe leg weakness. Cannot walk more than a few steps. General condition bad. Rugged
	L + V	1760	1560	1070	1440	1160	810			
15	L	2560	2080	1440	1710	1480	1150	41.9	38.5°	Moderate leg weakness and moderately ataxic walk
	L + V	2730	2350	1950	1940	1610	1160			
16	L	1490	1320	1120	1180	900	820	47.6	39.9°	" "
	L + V	1690	1310	1210	1210	1010	860			
17	L	2190	1550	1490	1340	1270	1120	46.4	38.8°	Moderate leg weakness. Very ataxic walk. Rugged
	L + V	2500	1980	1820	1610	1470	1230			
18	L	1830	1510	1120	1550	1260	1020	51.1	38.4°	" "
	L + V	2390	2180	1850	1530	1300	990			
19	L	1450	1060	940	1100	900	780	31.7	39.3°	" "
	L + V	1770	1390	1200	1080	930	830			
20	L	1350	1020	820	1000	870	600	39.3	39.1°	Severe leg weakness. Very ataxic walk
	L + V	1480	1240	980	1010	930	730			
21	L	1050	660	610	1140	710	640	34.0	38.4°	" "
	L + V	1330	830	820	1130	800	690			
22	L	1780	1560	1040	1470	1030	830	48.7	38.2°	" "
	L + V	2270	2030	1840	1510	1130	940			
23	L	2230	1690	1450	1890	1670	1320	26.2	39.9°	Leg weakness not very apparent. Rather good walk, not ataxic
	L + V	2450	1930	1770	2010	1700	1310			
Av.	L	1810	1420	1080	1380	1110	870	—	39.0°	
	L + V	2080	1730	1450	1500	1240	970			
Diff.	μl. O ₂ %	270 14.9	310 21.8	370 34.3	120 8.3	130 11.7	100 11.5			

At first sight it may seem that Exps. 10, 13 and 17 contradict the hypothesis previously advanced in this laboratory that head retraction is associated with biochemical changes in the optic lobe + "the rest." If it were certain that these birds were showing no head retraction, then the hypothesis would have to be modified. However, in the case of the results for the optic lobe + "the rest" upon the pigeons

with chronic leg weakness, 11–17 birds showed no larger vitamin effects during the first hour period than those which have been found for normals [Gavrilescu *et al.*, 1932]; differences between L and L + V are almost within the experimental error. It seems possible therefore that the exceptions were actually latent cases of head retraction. With weak birds, it is admittedly difficult to decide whether muscular weakness is not masking any opisthotonus. The preponderance of evidence in Table II is that the leg weakness is accompanied by negligible deficiency in vitamin B₁ in the optic lobes + “the rest,” but by some demonstrable deficiency in the cerebrum. In my experiments during 1 hour period this may be reckoned as 17 %, whereas in the experiments of Meiklejohn *et al.* for cerebrum of pigeons with head retraction, which seem to be the most nearly comparable, it was 28 %. The cerebral deficiency seems therefore to be less for the chronic birds.

DISCUSSION.

Previous work on the oxygen uptake of avitaminous pigeon's brain carried out for the purpose of clearing up the correlation between avitaminosis-B₁ and inanition, shows that these phenomena are independent of each other. The reduced tissue oxidation and the vitamin B₁ effect on avitaminous pigeon's brains seem to be directly correlated with the head retraction and independent of the nutritional condition. In my experiments on brains from avitaminous pigeons showing leg weakness but no head retraction, a clear vitamin effect was present in tests on the whole of the brain and on the cerebrum, but generally only a slight effect was observed on the lower parts (the optic lobes + “the rest”). The experiments consequently show that vitamin B₁ can increase the tissue oxidation *in vitro* even in brains from avitaminous pigeons, from which head retraction is absent but which have nervous symptoms in the form of leg weakness. So far as comparison can be made with tests in the laboratory carried out upon a different batch of pigeons at a different time, the vitamin B₁ deficiency is rather less for the cerebrum in “leg weakness” than in the “opisthotonus” cases examined. The “leg weakness” is not correlated with deficiency of vitamin B₁ in the optic lobes + lower parts, but there arises from my work the possible new point that the chronic symptoms may be partly central in origin. There is a high correlation between lowered vitamin B₁ in the cerebrum and these symptoms.

SUMMARY.

1. The action of crystalline vitamin B₁ on the tissue oxidation of brains from chronic avitaminous pigeons showing leg weakness but no head retraction was studied by Barcroft's method in the presence of lactate and Ringer-phosphate solution.
2. Vitamin B₁ evidently increased the tissue oxidation in experiments on the whole of the brain and on cerebrum alone but generally only brought about a slight increase with the lower parts of the brain (the optic lobes + “the rest”).
3. When compared with the results previously obtained in this laboratory from pigeons with head retraction, the vitamin effect was less marked. This particularly refers to the lower parts of the brain where the pigeons in head retraction showed a pronounced increase of the oxygen uptake with addition of vitamin *in vitro*.

I am very deeply indebted to Prof. Peters for his great interest and advice during the course of this work.

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