CXXX. THE ACTION OF CRYSTALLINE VITAMIN B₁ ON THE RESPIRATION OF POLYNEURITIC TISSUES *IN VITRO*.

BY ROBERT HENRY STEWART THOMPSON¹.

From the Department of Biochemistry, Oxford.

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THE connection of vitamin B₁ with tissue oxidation has been considered for many years, and recent work has strongly suggested that this vitamin is intimately associated with the oxidation of carbohydrates. Gavrilescu and Peters [1931] showed that the oxygen uptake of minced polyneuritic pigeon's brain tissue in the presence of added glucose was significantly lower than that of the normal tissue. More recently [Gavrilescu *et al.*, 1932] this has been shown to be the case in the presence of added lactate as well, an average diminution of 33 % being found for cerebrum, and 45 % for the optic lobes; in the presence of succinate however there is no difference between the levels of oxidation of normal and avitaminous tissues, while the residual uptake, *i.e.* without added substrate, also shows no significant difference. The addition of small amounts of crystalline vitamin B₁ restores the oxygen uptake of the avitaminous tissue in the presence of lactate almost to the normal level. It is concluded that this *in vitro* effect of the vitamin on the respiration of brain tissue is specifically connected with the glucose-lactate enzyme systems, and this action is now referred to as the catarulin effect of the vitamin [Passmore *et al.* 1933].

It seems to be well established therefore that there exists a specific defect in the oxidative mechanism of the brain in B₁ avitaminosis, but with regard to the other tissues the position is not at present clear. The literature concerning the level of respiration in normal and avitaminous tissues other than brain is confused and contradictory, as may be seen from Westenbrink's review of the subject [1932, 1]. In the fifteen investigations reviewed the only positive results, as Westenbrink has pointed out, which survive statistical examination are those of Ahlgren [1923] on minced pigeon muscle and of Tsukamoto [1928] on pigeon liver slices².

Galvao and Cardoso [1932] investigated, by the methylene blue method, the reducing powers of normal and avitaminous tissues and of tissues from animals in a state of inanition. They found that the reducing power of muscle tissue from animals suffering from inanition was lower than that of the normal tissue, but that there was no lowering of the reducing power of muscles from polyneuritic animals when excessive loss of weight was prevented by forced feeding on polished rice; in the case of liver they found no difference even between normal animals and animals in a state of inanition.

¹ Senior Demy of Magdalen College.

² For further discussion of the experiments of Abderhalden and Vlassopoulos [1931] see Gavrilescu *et al.* [1932].

Westenbrink, impressed with the fact that Tsukamoto's positive results had been obtained with liver slices in contrast with the minced tissues which had been used by other workers, has recently [1932, 2] repeated the experiments of Tsukamoto, but has been unable to find any decrease in the oxygen uptake of liver slices either from pigeons or rats.

It is perhaps worthy of mention that in none of the experiments reviewed by Westenbrink, except those of Drummond and Marrian [1926] on the reducing powers of the tissues for methylene blue, nor in Westenbrink's own experiments, was any added substrate used, the tissue being placed merely in Ringer solution or in a Ringer buffered by phosphate or bicarbonate.

In view of the conflicting results obtained in the comparison of the tissue respiration of normal and avitaminous animals, the work of Peters and his colleagues on the catatorulin effect of vitamin B₁ in polyneuritic pigeon's brain tissue seems to provide a more decisive method for determining the presence of such an oxidative lesion in other avitaminous tissues. By analogy with brain, the addition of vitamin B₁ to suspensions of these tissues respiring in lactate should result in an increased oxygen uptake, if such an oxidative lesion exists. Such a procedure avoids the controversial question of the level of tissue respiration in vitamin B₁ deficiency, and also has the advantage that it is less dependent on the general state of nutrition of the animal. The following experiments therefore were primarily undertaken not in the hope of settling whether there was a specific lowering of cell-respiration in polyneuritic tissues, but to determine whether added vitamin B₁ showed any catatorulin activity in the tissues of polyneuritic pigeons other than the brain.

METHOD.

The method employed has been to kill the birds by guillotining. The organ to be investigated is then quickly dissected out and removed from the body; any surrounding connective tissue is carefully dissected away and the organ gently wiped free from blood with filter-paper. In dissecting out the kidney it has been found most convenient to pluck the back of the abdomen and then to remove the entire piece of the bony posterior abdominal wall in front of which the kidney lies; by this means the kidney can be removed with the least amount of bleeding. Muscle, heart, liver and kidney have been investigated, the muscle employed being the pectoralis major.

In all cases except where subsequently mentioned the tissues have been finely chopped up with scissors: as far as was possible to judge the tissues were minced to the same extent each time. In those experiments in which sections were used the tissue was sliced free-hand with a razor moistened with the tissue fluid only, as recommended by Harrison [1933].

The oxygen uptake was measured in bottles of the Barcroft-Dixon type containing 3 ml. of Ringer-phosphate solution at p_H 7.3 (made up according to the usual formula used in this laboratory) to which neutralised sodium lactate, prepared from recrystallised zinc lactate, had been previously added in the amount required to make the final concentration of lactate 0.033 *M*. To the bottles containing vitamin 2.8 ml. of Ringer-phosphate-lactate solution were added before the addition of the tissue; the subsequent addition of a further 0.2 ml. of the medium containing 1–2 γ of the crystalline vitamin [Kinnersley *et al.* 1933] bringing the final volume up to 3 ml. The bottles were weighed before and after the addition of the tissue; after the second weighing the tissue

was finely broken up with a flat-ended glass rod and the vitamin added to those bottles where required. Rolls of filter-paper moistened with 2*N* KOH were used to absorb the CO₂. The bottles were then twice evacuated to 70 mm. and filled with oxygen. The temperature of the bath was 38°.

All the avitaminous birds, which were fed according to the usual procedure adopted in this laboratory, showed well-marked symptoms of head-retraction at the time of use. The amounts of tissue employed in each case varied with the different tissues on account of differences in their rates of oxygen consumption: the approximate average amounts per bottle were 70 mg. for kidney, 100 mg. for muscle and liver, and 130 mg. for heart. All the values given are the averages of duplicate estimations, the agreement being in nearly every case within 7 %.

RESULTS.

It was found that with avitaminous kidney the addition of the vitamin produced in all cases a large and striking increase in the oxygen uptake measured over four successive half-hour periods, but with muscle, heart and liver the effect when present was always small and not actually statistically significant.

The results obtained with avitaminous muscle, heart and liver, with and without the addition of vitamin are shown in Table I.

The oxygen uptakes are expressed in microlitres of oxygen per g. of tissue per hour over four successive half-hour periods: this method of expression has been used in order to render the results more comparable with those of other workers on this problem; for those who prefer the modern nomenclature $2000\mu\text{l.}/\text{g.}/\text{hour} = Q_{O_2} \cdot 10$ approximately. It can be seen from this table that the oxygen uptake of these organs is little affected by the addition of the vitamin: in the muscle and heart experiments the bottles containing vitamin had in some cases a lower uptake than the controls, and in those cases where the vitamin did bring about an increase, this increase was always small, reckoned either as the excess amount of oxygen taken up in $\mu\text{l.}$ per g. of tissue per hour or as the percentage rise over the control, and was not statistically significant¹. In the case of the liver the addition of the vitamin produced in every case a small increase in the oxygen uptake, but here also though the actual amount of oxygen taken up was greater than in the case of muscle or heart, the percentage increase was of the same order and was again not statistically significant except possibly for the fourth half-hour period. Since the vitamin effect, when present, was in every case so slight, and in view of the fact that statistical examination of the results shows that the increase is definitely not significant, it was thought unnecessary to do more than five of these experiments on each tissue. Four additional experiments were done using slices of liver, but no effect was obtained by this method.

Table II shows the results obtained with avitaminous kidney with and without the addition of vitamin. As in the previous experiments the amount of vitamin added was 1–2 γ per bottle. It may be seen that with kidney the addition of the vitamin produced in every case a large increase in the oxygen uptake, obviously of quite a different order from the effects obtained with the other tissues, amounting in the fourth half-hour period to 885 $\mu\text{l.}$, or 58 % of the oxygen uptake without added vitamin.

¹ The significance of the results obtained in this research was determined by means of Fisher's "t" test.

Table I. *Avitaminous heart, muscle and liver.*O₂ uptake (μ l. O₂/g./hour).*Heart.*

Exp.	Periods (hours)			
	0- $\frac{1}{2}$	$\frac{1}{2}$ -1	1-1 $\frac{1}{2}$	1 $\frac{1}{2}$ -2
8. L.	850	430	345	255
L. + V.	835	460	412	265
11. L.	725	447	277	182
L. + V.	772	464	274	189
12. L.	837	475	252	193
L. + V.	872	506	351	202
14. L.	715	341	178	165
L. + V.	804	379	213	158
37. L.	705	335	179	156
L. + V.	695	405	214	167
Average L.	765	405	245	190
L. + V.	795	445	295	195
Average increase	+30	+40	+50	+5

Muscle.

5. L.	2555	794	257	—
L. + V.	2410	937	305	—
35. L.	750	415	294	232
L. + V.	770	460	262	179
37. L.	780	300	146	76
L. + V.	700	310	140	116
38. L.	1415	480	213	118
L. + V.	1535	510	222	138
39. L.	545	193	114	95
L. + V.	515	202	154	105
Average L.	1210	435	205	130
L. + V.	1190	485	215	135
Average increase	-20	+50	+10	+5

Liver.

30. L.	1105	800	597	442
L. + V.	1185	868	667	506
34. L.	1265	890	650	—
L. + V.	1290	890	660	—
35. L.	2200	1330	905	675
L. + V.	2210	1340	1020	760
37. L.	1465	1090	715	585
L. + V.	1765	1205	830	708
38. L.	1870	1230	900	600
L. + V.	1960	1280	980	715
Average L.	1580	1070	755	575
L. + V.	1680	1120	830	670
Average increase	+100	+50	+75	+95

Table II. *Avitaminous kidney.*

		O ₂ uptake (μ l./g./hour).			
		Periods (hours)			
Exp.		0- $\frac{1}{2}$	$\frac{1}{2}$ -1	1-1 $\frac{1}{2}$	1 $\frac{1}{2}$ -2
11.	L.	2120	2060	1845	1485
	L. + V.	2335	2500	2385	2220
12.	L.	2100	2075	1760	1525
	L. + V.	2450	2480	2405	2340
14.	L.	2355	2275	1885	1555
	L. + V.	2735	2935	2735	2660
19.	L.	2265	2120	1790	1585
	L. + V.	2820	2780	2790	2630
24.	L.	1870	1875	1725	1475
	L. + V.	2340	2140	2490	2215
34.	L.	2365	2130	1840	1575
	L. + V.	2630	2650	2515	2450
Average	L.	2180	2090	1810	1535
	L. + V.	2550	2580	2550	2420
Increase (μ l. O ₂)		370	490	740	885
% increase		17	23	41	58

It is also noteworthy that whereas the respiration of the avitaminous kidney *in vitro* falls off steadily, the addition of the vitamin maintains the respiration at an almost constant level. In view of this catatorulin effect of the vitamin on the kidney as well as on the brain it is of interest to compare the rates of respiration of normal and avitaminous kidney tissue in the presence of lactate. The results obtained with normal kidney without added vitamin are shown in Table III.

Table III. *Normal kidney.*

		O ₂ uptake (μ l./g./hour).			
		Periods (hours)			
Exp.		0- $\frac{1}{2}$	$\frac{1}{2}$ -1	1-1 $\frac{1}{2}$	1 $\frac{1}{2}$ -2
13		3120	3455	3085	3225
17		2440	2510	2425	2485
18		2830	2865	2680	2860
27		2470	2460	2775	2265
28		3075	3145	2965	3150
43		3570	3345	3475	3265
Average		2920	2965	2900	2875

It will be seen that the respiration of normal kidney, like that of the avitaminous kidney in the presence of added vitamin, maintains itself at a fairly constant level (Fig. 1). Statistical examination of these figures also shows that the level of oxygen uptake of the avitaminous kidney is significantly lower than that of the normal, while Table III shows that the lowered level of respiration of the avitaminous kidney is appreciably restored by the addition of the vitamin. Four experiments were done to determine whether added vitamin had any effect on the respiration of normal kidney in the presence of lactate; it was found that there was no effect.

Fig. 1. Mean rate of oxygen uptake of minced kidney in presence of lactate.

N = normal; A = avitaminous; V = avitaminous + vitamin.

DISCUSSION.

It seems justifiable to conclude from the results of this research that the addition of vitamin B₁ to muscle, heart or liver tissue from polyneuritic pigeons has no significant effect on the oxygen uptake *in vitro*, but that it shows a marked catatorulin effect on polyneuritic kidney respiring in the presence of lactate.

As already pointed out the primary object of this research was to investigate, not the rate of respiration of normal and avitaminous tissues, but the catatorulin effect of the vitamin in polyneuritic tissues other than brain. But in view of the discovery of this striking effect on polyneuritic kidney, it seemed essential to determine whether the extra oxygen uptake produced by the added vitamin was a restoration of a lowered level of respiration to the normal level. The results show that, as in the case of brain, the respiration of the polyneuritic kidney *in vitro* in the presence of added lactate is markedly lower than that of the normal tissue, the lowering becoming more pronounced in the third and fourth half-hour periods. There exists therefore in the kidneys of polyneuritic pigeons in opisthotonus some oxidative lesion which is capable of being largely repaired *in vitro* by the addition of vitamin B₁. No experiments have yet been done to decide the stage in avitaminosis at which the kidney lesion commences.

It is possible that this kidney lesion may be connected in some way with the oedema which is sometimes present in polyneuritic birds, but it is certain that other factors must also be concerned, since no obvious oedema was present in any of the birds used during this research.

SUMMARY.

1. The respiration of minced muscle, heart, liver and kidney tissues from polyneuritic pigeons has been studied in the presence of lactate with and without the addition of small amounts of vitamin B₁.

2. The addition of the vitamin to polyneuritic muscle, heart or liver has no significant effect on the oxygen uptake of these tissues *in vitro*, but in the case of polyneuritic kidney the addition of the vitamin brings about a large and statistically significant increase in respiration.

3. The respiration of the polyneuritic kidney in the presence of lactate is significantly lower than that of normal kidney; the addition of the vitamin largely restores this defect.

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