CCLXII. THE EFFECT OF VITAMIN B₁ UPON THE RESPIRATORY QUOTIENT OF BRAIN TISSUE.

BY HUGH MACDONALD SINCLAIR¹.

From the Department of Biochemistry, Oxford.

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GAVRILESCU AND PETERS have shown [1931, 1] that minced polyneuritic pigeon's brain tissue, suspended in glucose-phosphate solution, gives a lowered oxygen uptake as compared with the normal. Later they found [1931, 2] that this deficiency was partially restored *in vitro* by adding vitamin B₁ concentrates in very small amount. The effect was catalytic. Peters and his colleagues [Gavrilescu *et al.*, 1932] showed that the effect was enhanced in lactate solution; but since the avitaminous tissue oxidised succinate normally, the cytochrome system was not impaired. They concluded that vitamin B₁ was specifically concerned with carbohydrate metabolism.

The simplest explanation was that vitamin B₁ was a catalyst essential for the oxidative removal of lactic acid. It is not, however, the lactic oxidase coenzyme described by Banga *et al.* [1932], neither can preparations of the latter give the *in vitro* effect of vitamin B₁ [Boyland, 1933, using the Thunberg technique; Peters and Sinclair, unpublished, using measurement of oxygen uptake]. Also, Meiklejohn [1933] could find no extra disappearance of lactic acid corresponding to the extra respiration produced by the addition of the concentrates to the polyneuritic tissue. It was therefore important to study the R.Q. of the tissue under these conditions. A preliminary account of these experiments has already appeared [Sinclair, 1933].

The respiratory quotient of brain tissue from various animals has usually been found to be nearly unity. Loebel [1925] found that the R.Q. of excised rat brain was 0.99 in fructose, 0.92 in glucose and 0.86 in Ringer solution. Dickens and Greville [1933] reported that the R.Q. of brain cortex of rats and rabbits, buffered either with phosphate or bicarbonate, was unity whether glucose, fructose or no substrate was present. Ashford and Holmes [1931] found that the R.Q. of chopped rabbit brain, in Ringer-phosphate (apparently unbuffered), was 0.88 and rose to unity when lactate was added. Himwich and Nahum [1929] analysed the gases in the blood entering and leaving the brains of dogs and obtained values of unity under a variety of conditions. Himwich and Fazikas [1932] stated that minced tissue of rats, in glucose-phosphate solution, gave the following values: cortex 0.99, stem 0.93, cerebellum 0.89, medulla 0.89. All this work has emphasised that the metabolism of mammalian brain under a variety of conditions is essentially that of carbohydrate.

Very recently Himwich *et al.* [1933] have reported observations on the R.Q. of the cerebral cortex of three pigeons and one dog suffering from polyneuritis. The values obtained with the avian tissue, using the Warburg apparatus, were 0.76, 0.86 and 0.66, but it is not stated in what medium the determinations were made. Himwich assumes, apparently on no evidence, that the R.Q. of

¹ Senior Demy of Magdalen College.

normal avian cortex would be unity and believes that his results indicate a diminished ability of the brain to oxidise lactic acid. Meiklejohn [1933] could demonstrate no inability of the brain to oxidise lactic acid in avian polyneuritis.

Many authors have studied the R.Q. of polyneuritic animals, with very conflicting results. Ramoino [1915] found that when four pigeons were fed on polished rice, their weight and R.Q. fell progressively although their oxygen uptake was not decreased. On feeding an extract of the rice-polishings, their weight and R.Q. increased, and they became very active. The lowest value of the R.Q. was 0.35, and he concluded that the low values were due to incomplete oxidation processes producing toxic substances. Caridroit [1922] reported that the R.Q. of three pigeons fed on a vitamin B-deficient diet started at 0.9, fell to 0.7 and then rose to 0.8 at the time of symptoms. Anderson and Kulp [1922] did careful work on six hens and concluded that there was no noticeable change in R.Q. in vitamin starvation. They concluded that Ramoino's results were due to leaks in his apparatus. Magne and Simonnet [1922] rightly believed that their experiments were too few to analyse the metabolic disturbance in polyneuritis. Nevertheless they concluded that the R.Q. of pigeons was the same in polyneuritis as in fasting. They found that intravenous injection of glucose raised the R.Q. of a fasting pigeon but produced no significant rise in a polyneuritic pigeon, whence they concluded that the latter was "un animal alimenté" but unable to utilise carbohydrates. In contrast to this, Mattill [1923] found no difference in the basal quotients of normal and avitaminous rats and concluded that vitamin B was not specifically related to the metabolism of carbohydrate since in both groups of animals the R.Q. rose after administration of sucrose or glucose. Gerstenberger and Burhans [1922] believed that studies of the R.Q. showed that polyneuritic pigeons could burn carbohydrates completely; van Berkhout [1926] found a decreased production of CO_2 . Jansen and Mangkoewinoto [1920] generally found a fall in R.Q. in paddy-birds. Okada and Sakurai [1926] and Fleming [1923] reported that the R.Q. was unaltered in human beriberi, and Lawrow and Matzko [1926] actually found a rise in R.Q. and in oxygen consumption in avitaminous hens.

EXPERIMENTAL.

The method of Dixon and Keilin [1933] has been used for the determinations, since this is better than the methods of Dickens and Šimer [1930; 1931, 1] and of Warburg [1924].

The determinations were made over a period of 1 to 2 hours upon minced brain tissue in 3.0 cc. mammalian Ringer solution buffered with $M/10 \text{ KH}_2\text{PO}_4$, p_{H} 7.3, and containing sodium lactate (0.033 M) (as described by Passmore *et al.* [1933]). The side-bulbs contained 0.3 cc. 2*N* HCl, and the bottom taps 40 % KOH.

The birds were killed by decapitation with a guillotine. The skull was opened, and the brain removed, freed as far as possible from membranes and blood and finely minced with a bone spatula. The tissue was then divided into as nearly equal portions as possible and placed in the previously filled and tared bottles. After re-weighing, the tissue was finely divided with a flat-ended glass rod¹, the rolls of filter-paper and the glass rods were inserted, and the apparatus assembled and placed in the bath at 38°. 50 to 55 minutes elapsed from the time of death until this stage was reached. Oxygen was passed for 10 minutes through the apparatus while shaking

¹ It is found that if care is taken in breaking up the tissue with the glass rod, no tissue is removed from the bottle, and the amount of medium lost is very small and equal to the amount lost from the control bottle.

by the method described by Dixon and Keilin. The gas was then shut off, the side-taps closed and the apparatus shaken for a further 3 minutes. The taps were then closed and the acid tipped into the left-hand bottles. The remaining procedure was as described by Dixon and Keilin.

In the early experiments, mercury was used to displace the KOH but was found to be unsatisfactory as it sometimes shook over into the medium. In most of the experiments glass rods have been used (see Dixon and Keilin). The apparatus have not been shaken at the speed recommended by Dixon and Elliott [1930], because at that speed the tissue becomes washed up and stuck upon the sides of the bottles, and there is danger of the acid not reaching and killing it immediately. A speed of about 70 complete revolutions per minute has been used.

The constants of the apparatus were calculated from the formula given by Dixon and Keilin. When oxygen and phosphate buffer have been used instead of CO₂ and bicarbonate, the changes in pressure have not been large, and so paraffin, coloured with Sudan III, has been used in the manometers.

All determinations, except where stated, have been done in duplicate and a few in triplicate or quadruplicate. When the duplicates have not agreed to within $\pm 5\%$, the average value has been bracketed. I have found that the duplicates agree better for the measurement of Q_{O_2} than for R.Q. This may be due to the tap-grease (made from lard, bees-wax and olive oil) dissolving CO₂. This would, of course, affect the R.Q. but not the Q_{O_2} . The error from this source can only be small and does not apply to the determinations made in presence of CO₂.

Throughout, precisely the same additions have been made to the left-hand bottle as to the right-hand, but it was found impossible to get the weights of tissue identical. In all experiments, the amounts used have been between 90 and 200 mg., and the amounts in any pair of bottles have always agreed to within $\pm 5\%$, usually considerably less. The differences in bound CO₂ will therefore not be significant.

The birds used in nearly all the experiments have been either Blue Chequer or Blue Bar Homer pigeons. About equal numbers of each sex have been used, but as neither the type of bird nor the sex appears to make any significant difference to the results details have been omitted. In most of the experiments, cerebrums only have been used. Although the lesion in polyneuritis is more marked in the lower parts of the brain [Kinnersley and Peters, 1930], the cerebrum provides more tissue, a higher Q_{O_2} and a more homogeneous mince. Varying amounts of white matter are unavoidably included, but two experiments have shown that the R.Q. of the cerebrum is not very different from that of the lower parts of the brain.

RESULTS.

Table I shows some results obtained with normal pigeons [fed on mixed corn, as described by Kinnersley *et al.*, 1928].

Table I. *Normal tissue in phosphate-buffered lactate.*

Exp.	Tissue	Time (mins.)	Q_{O_2}	R.Q.		
2. iii. 33	Whole brain (except cerebellum)	60	—	0.89*		
3. iii. 33	"	60	—	0.90†		
6. iii. 33	"	60	—	0.86		
13. iii. 33	"	60	8.63*	0.88†		
28. iii. 33	"	60	7.35†	0.83†		
26. iv. 33	{ Cerebrum	45	(8.60)	0.93		
	{ Optic lobes and mid-brain			5.75		
22. iv. 33	Cerebrum	60	(10.73)	0.87		
15. viii. 33	"	90	10.73	0.99†	10.53	0.96†
16. viii. 33	"	60	9.05	0.83	9.80†	0.90†
18. viii. 33	"	90	9.45†	0.88†	8.60	0.90

* Quadruplicate.

† Triplicate.

‡ Single determination.

With addition of
2γ vitamin B₁
per 3.0 cc.

Table II shows results obtained with tissue from birds killed while in opisthotonus due to deficiency of vitamin B₁. The birds had been fed for about 30 days on polished rice by the usual methods used in this laboratory, and the precautions of Kinnersley *et al.* [1928] were observed. The vitamin preparation used was a crystalline concentrate (No. 64-19 of Kinnersley *et al.* [1933]). The bottles to which it was to be added received only 2.0 cc. of the ordinary medium. After the tissue had been broken up with the glass rod, the vitamin was added made up in 1.0 cc. of the medium. (The addition was made to both left- and right-hand bottles.) The amount used (1 to 2 γ per 3.0 cc.; 2.2 γ =1.0 International unit) was such as would give an almost maximum catatorulin effect [Passmore *et al.*, 1933].

Table II. *Avitaminous cerebrums in lactate.*

Exp.	Time (mins.)	Q_{O_2}		R.Q.		R.Q. of extra respira- tion	Vitamin conc. (γ /3.0 cc.)
		Control	Vitamin	Control	Vitamin		
10. v. 33	60	5.50	6.15*	0.74	0.84*	1.7	2.2
11. v. 33	130	(8.70)	11.00	0.65	0.88*	1.3	2.2
6. vi. 33	63	5.80	7.18	0.62	0.89	2.0	2.0
9. vi. 33	60	7.50	8.85*	0.74	—	—	1.0
12. vi. 33	120	4.35	5.58	0.69	0.87	1.5	2.0
16. vi. 33	60	5.25*	7.80	0.58*	0.77	1.2	2.0
19. vi. 33*	60	5.35	6.55	0.71	0.93	1.9	2.0
12. vii. 33	120	4.23	6.20	0.68	(0.91)	1.4	2.0

* Single determination.

Table III shows some results obtained with birds fed on polished rice for varying periods. In some cases, symptoms were threatening; in others, the bird was very active and apparently in good health. It will be seen that in some cases there is a slight rise in the R.Q. and Q_{O_2} , in others no effect.

Table III. *Cerebrums of rice-fed birds in lactate.*

Exp.	Time (mins.)	Q_{O_2}		R.Q.		No. of days on diet	% loss in wt. on diet
		Control	Vitamin	Control	Vitamin		
27. iii. 33	60	5.95*	—	0.67	—	19	—
20. vi. 33†	65	9.05	—	0.86	—	20	—
3. vii. 33*	60	9.20	10.15	0.81	0.87	26	35.6
6. vii. 33*	60	9.00	9.40	0.88	0.75	22	33.2
7. vii. 33*	60	8.00	7.80	0.99	1.09	23	32.3
10. vii. 33	60	8.98	9.10*	0.76	(0.83)	26	36.5
11. vii. 33	60	8.15*	8.08	0.81*	1.01	27	37.0
13. vii. 33	90	7.28	8.03	0.92	0.91	21	26.6

Vitamin concentration, 1 γ /3.0 cc. in Exp. 6. vii. 33; 2 γ /3.0 cc. in the remainder.

* Single determination.

† Triplicate.

Determinations in CO₂-bicarbonate.

Experiments in a medium containing $M/10$ KH₂PO₄ cannot be regarded as being very physiological. Yet Dickens and Šimer [1931, 2] found that the respiration of normal tissue with glucose as substrate was affected neither qualitatively nor quantitatively by substituting CO₂-bicarbonate for phosphate. Ashford and Holmes [1931], however, found that the oxygen uptake of chopped rabbit brain, both in presence and absence of lactate, was larger in CO₂-bicarbonate than in phosphate, and the value of the "Meyerhof quotient" also de-

pended upon the buffer used. Meyerhof [1925] found that phosphate was necessary for the resynthesis of lactic acid in muscle; and Meyerhof *et al.* [1925] showed that there were quantitative differences in respiration in presence of sodium or of potassium phosphate. It was therefore important to study the respiration of the avitaminous brain in CO₂-bicarbonate, with and without added phosphate.

The experiments have been performed in Ringer-lactate (as above) with 0.025 *M* sodium bicarbonate. The final solution when saturated with CO₂ had *p*_H 7.3, but practically no calcium was precipitated. In the experiments with phosphate, sodium dihydrogen phosphate was added to make the final concentration *M*/45. This was brought to *p*_H 7.3 with 20 % NaOH. Gas mixture composed of O₂ + 5 % CO₂ was used, and the medium was saturated with this before being added to the bottles. The manometers were filled with Clerici fluid. Variations in the tonicity of the various media have not been controlled, since Gavrilesco and Peters, in unpublished observations, found that large variations produced no effect upon the respiration.

Tables IV and V show the results obtained with tissue from normal and avitaminous birds respectively.

Table IV. *Normal tissue in CO₂-bicarbonate with lactate.*

Exp.	Tissue	Time (mins.)	Medium	Q _{O₂}	R.Q.	Q _M ^{0.2}
12. ix. 33	Cerebrum	120	Lactate	5.90	0.83	-1.1
13. ix. 33	Whole brain (except cerebellum)	120	Lactate	6.03*	0.81†	-1.2†
15. ix. 33	,,	120	Lactate	5.55	0.79	-0.8
			Lactate + phosphate	6.15	0.90	(-1.1)
18. ix. 33	,,	120	Lactate	6.53	0.82	-1.2
			Lactate + phosphate	6.50‡	0.93‡	(-1.7)‡

* Quadruplicate.

† Triplicate.

‡ Single determination.

Table V. *Avitaminous tissue in CO₂-bicarbonate with lactate.*

Exp.	Time (mins.)	Medium	Q _{O₂}	R.Q.	Q _M ^{0.2}	R.Q. of extra respira- tion
19. ix. 33	120	Lactate	4.98	0.71	-0.5	—
		Lactate + vitamin B ₁	6.20	0.81	-0.8	1.2
21. ix. 33	109	Lactate	5.68	0.72	-0.4	—
		Lactate + vitamin B ₁	6.45	0.80	-0.7	1.5
16. ix. 33	110	Lactate + phosphate	3.70*	—	—	—
		Lactate + phosphate + vitamin B ₁	5.28	0.91	(-1.0)	2.0
20. ix. 33	120	Lactate + phosphate	4.38*	0.75*	(-0.8)	—
		Lactate + phosphate + vitamin B ₁	5.65	0.88	(-1.3)	1.5

Tissue. Whole brain except cerebellum.

Vitamin B₁, 2γ/3.0 cc.

* Single determination.

Table VI gives the average values obtained in the whole series of experiments. After the mean value for each series is given the standard deviation, $\sqrt{\frac{(\bar{x}-x)^2}{n-1}}$; “*t*” is the difference between two means divided by the estimated standard error of this difference; and “*P*,” the probability of falling outside the range $\pm t$, is derived from the Table given by Fisher [1932].

Table VI. *Mean values $\pm$ standard deviation.*

A. Phosphate:	R.Q. $\pm \sigma$	"t"	"P"	R.Q. of extra respiration
Normal brain (except cerebellum)	0.87 $\pm$ 0.05	1.22	0.23	—
Normal cerebrum	0.90 $\pm$ 0.06			
Normal cerebrum + vitamin B ₁	0.92 $\pm$ 0.03			
"Rice-fed" cerebrum	0.86 $\pm$ 0.09	1.03	0.33	—
"Rice-fed" cerebrum + vitamin B ₁	0.91 $\pm$ 0.11			
Avitaminous cerebrum	0.68 $\pm$ 0.06	71	0	1.6 $\pm$ 0.3
Avitaminous cerebrum + vitamin B ₁	0.86 $\pm$ 0.06			
B. CO ₂ -bicarbonate:	R.Q. $\pm \sigma$	Q _M ^(b)		R.Q. of extra respiration
Normal brain (except cerebellum)	0.81 $\pm$ 0.02	-1.1		—
Ditto + phosphate	0.91 $\pm$ 0.05	(-1.3)		—
Avitaminous brain (except cerebellum)	0.71 $\pm$ 0.02	-0.45	}	1.4 $\pm$ 0.2
Ditto + vitamin B ₁	0.81 $\pm$ 0.02	-0.75		
Ditto + phosphate	0.75	(-0.8)	}	1.8 $\pm$ 0.4
Ditto + phosphate + vitamin B ₁	0.90 $\pm$ 0.05	(-1.15)		

In calculating the statistics, certain points arise. The standard deviation (σ) has been calculated from the whole set of values in each series and not from the average values (mean of duplicates, *etc.*) given in Tables I to V. In two experiments, only half the usual concentration of vitamin was used (1 γ instead of 2 γ), but as already stated 1 γ is known to give a nearly maximum cataturulin effect. In the case of the birds fed for different periods on polished rice, but not in opisthotonus, the standard deviation is very large because the birds were in varying degrees of vitamin deficiency. The experiments performed with bicarbonate buffer have been too few to permit of convincing conclusions being drawn.

Fisher's method of testing the significance of a difference between two means has been used, although the same results can of course be obtained from the direct application of the probable error concept. The analysis shows that the difference between the R.Q. of avitaminous cerebral tissue with and without added vitamin is undoubtedly significant; the difference between the R.Q. of normal brain and normal cerebrum is probably significant; between the R.Q. of cerebral tissue of rice-fed birds, with and without added vitamin, there is probably no significant difference; and the addition of vitamin to normal cerebral tissue does not significantly change the R.Q.

DISCUSSION.

Many authors have claimed that vitamin B₁ is not specifically related to tissue oxidation processes, but that the results obtained have been due to the loss in weight or general inanition of the animals studied [*e.g.* Drummond and Marrian, 1926; Galvao and Cordoso, 1932; Abderhalden and Vlassopoulos, 1931]. Meiklejohn *et al.* [1932] proved that vitamin B₁ deficiency and inanition were independent, but Westenbrink [1932] has revived the controversy. The results presented here show that the minced avitaminous tissue has a decreased R.Q. as well as a lowered respiration, and that the addition of minute amounts of vitamin B₁ *in vitro* raises both nearly to the normal values. Further, the vitamin has no effect upon the normal tissue and probably no significant effect upon the tissue of birds fed with polished rice but not showing the symptoms of polyneuritis. The results obtained therefore lend strong support to the conclusions of Peters and his colleagues.

These results can be interpreted in many ways. Taking the simplest explanation and assuming that no protein is being oxidised¹, the R.Q. of 0.9 for the

¹ The aerobic production of ammonia by brain is well established [Loebel, 1925; Schwarz and Dibold, 1931; Riebeling, 1931]. Any such production will affect the values neither of the R.Q. nor of the oxygen uptake.

normal tissue may be taken to represent a respiration of which 66 % is carbohydrate and 34 % is lipid. It is interesting to recall that Peters and Sinclair [1933] found that rather more than 60 % of the respiration was sensitive to cyanide or fluoride. The avitaminous tissue has an R.Q. of about 0.68, but it is unlikely that this represents an oxidation of fat alone since lactate markedly stimulates the respiration of this tissue (although not to the same extent as with the normal) and since Meiklejohn [1933] found that the avitaminous tissue readily removed lactate. It is more probable that an incomplete oxidation is occurring in the avitaminous tissue whereby some substance is produced which is only oxidised in presence of the vitamin, thus producing the apparent R.Q. of 1.5 of the extra respiration induced by the vitamin.

The fate of the lactic acid is indicated by the experiments in bicarbonate. The aerobic glycolysis can be measured in these.

The figures given for the $Q_M^{O_2}$ in presence of phosphate do not represent true values and so have been placed in brackets (see Tables IV, V and VI). The apparatus does not give a direct measure of the $Q_M^{O_2}$ if phosphate is present, since buffering by the phosphate minimises any changes in the acid content of the medium. Therefore the true value for the disappearance of acid (negative $Q_M^{O_2}$) in presence of phosphate should be very much larger than the figure given in the Tables.

The removal of acid in the avitaminous brain in absence of phosphate is found to be very small ($Q_M^{O_2} = -0.45$), but upon adding vitamin B₁ there is a large increase ($Q_M^{O_2} = -0.75$) approaching the value for the normal tissue ($Q_M^{O_2} = -1.1$). There is a corresponding rise in the respiration and in the R.Q. Yet the latter is only 0.81 (for the normal tissue, and avitaminous + added vitamin), and the R.Q. of the extra respiration produced by the vitamin is about 1.4. Therefore it seems improbable that the increased disappearance of acid merely indicates an oxidation of lactic acid.

Adding phosphate hardly affects the oxygen uptake either of normal or of avitaminous brain with added vitamin, but raises the R.Q. from 0.81 to 0.91 (representing an increase of 35 to 69 % in the carbohydrate part of the respiration). Also, the disappearance of acid is greatly increased¹. But since this large disappearance can hardly all be accounted for by the increase in the carbohydrate part of the respiration, it is simplest to regard it as a synthetic removal of lactic acid. Speculation, however, would not be justifiable.

SUMMARY.

1. The respiration of brain tissue from normal, rice-fed and polyneuritic pigeons has been studied in presence of lactate buffered with either phosphate or bicarbonate.

2. The respiratory quotient of the tissue from polyneuritic pigeons is low and is raised nearly to the normal value by the addition *in vitro* of minute amounts of crystalline vitamin B₁.

3. The low value is independent of inanition, since the addition of vitamin does not influence the R.Q. of tissue from normal pigeons and hardly influences the quotient of tissue from pigeons fed on polished rice but not showing symptoms.

4. The specific effect of vitamin B₁ in raising the respiration of avitaminous brain tissue is found in lactate buffered with bicarbonate, and there is also a

¹ It is improbable that the negative aerobic glycolysis is due in any large degree to production of ammonia or breakdown of creatinephosphate, since it is unlikely that either would be greatly increased by the addition of phosphate.

rise in the respiratory quotient. These results are independent of the addition of inorganic phosphate to the medium.

5. The aerobic glycolysis of the avitaminous tissue is slightly negative and becomes more so when vitamin B₁ is added or with normal tissue. The addition of phosphate markedly increases this negative value.

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