CLXXVII. THE METABOLISM OF NORMAL AND TUMOUR TISSUE.

XIII. NEUTRAL SALT EFFECTS.

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The study of the metabolism of surviving tissue in the form of slices, that is, in the form in which least damage has occurred, is being pursued by many workers as a method of elucidating the metabolism of the living body cells. This method, whilst having the great advantage of using intact cells, is limited in comparison with the analytical method in which tissue enzymes, carriers *etc.* are extracted and studied; nevertheless, much has been, and is being, learnt by making changes in the medium in which the slices are suspended, particularly by the addition of substances foreign to the body. A better understanding of the nature of the effects produced, and a correlation of the action of different substances, is however needed before theories of cell metabolism, based on these actions, can usefully be evolved.

It has recently been found that when 0·1 *M* KCl is added to the Ringer solution in which slices of rabbit brain cortex are respiring, both the respiration and aerobic glucolysis assume values much larger than the values in pure Ringer solution [Ashford and Dixon, 1935]. This profound alteration of the metabolism is of great interest to the writers, since they have observed similar actions on various kinds of tissue by certain organic coloured substances in very low concentrations. Thus, thionine increases the respiration of tumours without changing the aerobic glucolysis [Dickens, 1934], whilst phenosafranine increases the aerobic glucolysis of brain and tumours without affecting the respiration [Dickens, 1935]. Further, dinitro-*o*-cresol simultaneously increases the respiration and aerobic glucolysis, not only in tumours [Dodds and Greville, 1934], but also in brain tissue [Greville, unpublished observations], thus producing in the latter exactly the same effect as potassium chloride. We were led, therefore, to study the potassium effect more closely in order to seek its cause; to observe the result of other alterations of the cations in the medium; and to enquire whether the potassium effect, like the action of the coloured substances, occurs with tissues other than the brain cortex.

Experimental methods and results.

The measurements of respiration in phosphate medium and of anaerobic glucolysis in bicarbonate medium were made in the usual way [Warburg, 1926]. Simultaneous measurements of respiration and aerobic glucolysis were made sometimes by the two-vessel method [Warburg, 1924] and sometimes by the method of Dickens and Šimer [1930, 1] for the determination of R.Q. The latter,

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as ordinarily used, gives low values for the aerobic glucolysis when this is high compared with the respiration. If however the amount of tissue and the duration of the experiment be reduced so that the change in bound CO_2 does not exceed say 250 μl., then, although the R.Q. cannot be measured accurately, good values are obtained for the respiration and aerobic glucolysis, and the advantage of the method is retained, the actual measurement being made on one tissue slice.

Media.

Unless otherwise stated, the suspension media were the following:

	Bicarbonate medium		Phosphate medium	
	%	*M*	%	*M*
NaCl	0·70	0·120		
KCl	0·018	0·0024		
$CaCl_2$	0·019	0·0017	As in bicarbonate medium	
$MgCl_2$	0·0076	0·0008		
Glucose	0·20	0·011		
$NaHCO_3$	0·21	0·025	—	—
NaH_2PO_4	—	—	0·040	0·0033
Na_2HPO_4	—	—	0·21	0·015

The salt concentrations are those used by Warburg [1923], except that a small quantity of $MgCl_2$ is introduced (*cf.* Tyrode's solution).

Effect of high concentrations of neutral salts on the metabolism of brain tissue.

1. *Rat brain.* Ashford and Dixon used rabbit brain. We have found that addition of 0·1 *M* KCl increases the respiration and aerobic glucolysis in rat brain also; and rat tissues only have been used by us.

In the experiments in phosphate medium, solid KCl was weighed into the side-bulb of the manometric vessels, and after a period of 30–40 mins., during which the oxygen uptake was noted, the KCl was washed into the medium by tipping backwards and forwards. The readings for the next 10 mins. were neglected, owing to a pressure increase on addition of the solid to the solution; thereafter a greatly increased respiration was observed. This was not however steadily maintained; often it fell off severely with time, so that 90 mins. after the addition the respiration might be little higher than that in the control where no KCl was added. For this reason most of the experiments were of short duration.

In the experiments in bicarbonate medium the solid KCl was dissolved in the medium before it was measured into the vessels. The large increase in respiration (Q_{O_2}) and aerobic glucolysis ($Q_G^{O_2}$) which occurs under these conditions is seen in Exp. 4 of Table I.

2. *Effect of substrate.* 0·1 *M* KCl still produces a large increase in respiration when the glucose in the medium is replaced by an equal concentration of lactate, pyruvate or fructose (Exps. 1, 2, 3, Table I). It follows that the increased respiration in glucose is not a result of increased lactic acid production.

There is no increase in the absence of added substrate (Exp. 5); and when the tissue is removed from a glucose-containing solution in which an increased metabolism has been produced by 0·1 *M* KCl, the solution itself has a negligible oxygen uptake. Hence 0·1 *M* KCl produces a general stimulation of carbohydrate respiration, and the increased oxygen uptake is apparently not due to oxidation of cell material, either inside or outside the tissue. R.Q. measurements in phosphate medium by the method of Dickens and Šimer [1930, 1] showed

that the increased oxidation occurring in presence of excess KCl proceeds, like the normal glucose oxidation of brain tissue, at R.Q. unity:

Exp. 0.	*Medium.* Ordinary phosphate-Ringer solution		The same containing *M*/10 KCl	
	Q_{O_2}	R.Q.	Q_{O_2}	R.Q.
Duration, 80 mins.	−11·2	1·02	−18·5	1·01
	−10·0	1·00	−15·3	0·99

Table I.

Exp. 1. P.* Substrate ...	Glucose	Pyruvate	Lactate	Fructose
Q_{O_2} before 0·1 *M* KCl addition (30 mins.)	−15·0	−15·0	−19·8	−13·8
Q_{O_2} after 0·1 *M* KCl addition (30 mins.)	−25·8	−21·4	−26·8	−23·6

Exp. 2. P. Substrate ...	Glucose		Lactate	
KCl addition ...	None	0·1 *M*	None	0·1 *M*
Q_{O_2} before KCl addition (30 mins.)	−11·4	−11·4	−15·0	−15·2
Q_{O_2} after KCl addition (first 30 mins.)	−13·0	−23·9	−14·5	−22·9
Q_{O_2} after KCl addition (further 60 mins.)	−14·2	−17·3	−15·0	−15·6

Exp. 3. P. Substrate ...	Glucose			Fructose		
KCl addition ...	None	0·1 *M*	0·05 *M*	None	0·1 *M*	0·05 *M*
Q_{O_2} before KCl addition (30 mins.)	−10·2	−10·2	−10·4	−10·0	−10·8	−12·2
Q_{O_2} after KCl addition (first 30 mins.)	−10·5	−19·3	−20·6	−10·1	−21·6	−24·7
Q_{O_2} after KCl addition (further 30 mins.)	−10·9	−14·4	−16·6	−10·4	−15·0	−16·6

Exp. 4. BW. Substrate: glucose.

	No KCl		0·1 *M* KCl	
	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$
First 30 mins.	−10·0	+4·2	−21·0	+18·3
Second 30 mins.	−10·0	+4·7	−17·6	+15·6

Exp. 5. BW. Substrate: none added.

	No KCl		0·1 *M* KCl	
	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$
First 30 mins.	−5·1	−1·0	−0·7	−0·2
Second 30 mins.	−2·1	−1·2	—	—

* Experiments done in phosphate medium denoted P. Experiments done by Warburg method (bicarbonate medium) denoted BW. Experiments done by Dickens-Šimer method (bicarbonate medium) denoted BD.

Table II.

Exp. 6. P. Substrate: glucose.

Cl addition (0·1 *M*) ...	None	Li	Na	K	Rb	Cs
Q_{O_2} before Cl addition (30 mins.)	−14·0	−12·8	−13·0	−11·4	−11·7	−11·5
Q_{O_2} after Cl addition (first 30 mins.)	−13·7	−15·9	−14·0	−23·8	−22·4	−21·6
Q_{O_2} after Cl addition (second 30 mins.)	−14·9	−12·6	−14·8	−19·5	−18·1	−17·7

(Repeated with similar result.)

Exp. 7. P. Substrate: glucose.

Cl addition (0·1 *M*) ...	K	Mg
Q_{O_2} before Cl addition (30 mins.)	−15·0	−16·5
Q_{O_2} after Cl addition (30 mins.)	−25·8	−14·0

Exp. 8. P. Substrate: glucose.

Salt added ...	None	0·07 *M* NaCl	0·09 *M* NaCl	0·07 *M* NaCNS	0·09 *M* NaCNS
Q_{O_2} before salt addition (40 mins.)	−11·5	−11·5	−11·7	−11·3	−12·4
Q_{O_2} after salt addition (40 mins.)	−11·0	−14·4	−13·3	−14·3	−15·2

3. *Effect of other cations.* Rubidium and caesium chlorides in 0·1 *M* concentration produce increases in respiration of the same order as KCl; 0·1 *M* NaCl and 0·1 *M* LiCl have small effects; 0·1 *M* $MgCl_2$ has none (Table II). Hence for the effect produced by the alkali cations, Li, Na < K, Rb, Cs. It follows that the "potassium effect" is not an effect specific to the potassium ion, but rather that the increase of respiration in brain may be produced by the metals at a particular end of the lyotropic series of the cations.

Ashford and Dixon [1935] observed that 0·1 *M* KCl decreased the anaerobic glucolysis of rabbit brain: this was confirmed for rat brain; and it was found that the same concentration of NaCl had little effect:

Exp. 9. Anaerobic glucolysis.

(0·1 *M*)	Na	K
$Q_G^{N_2}$ before Cl addition (20 mins.)	+16·2	+18·0
$Q_G^{N_2}$ after Cl addition (30 mins.)	+13·6	+ 4·0

Influence of the constituents of the medium on the metabolism of brain.

The effects described above, increases of respiration and aerobic glucolysis, are produced by raising the proportion of one constituent of the suspension medium. Now this medium is a "balanced" salt solution, the cations being in proportions which have been found most suitable for the maintenance of physiological function. Decreasing the concentration of the other constituents might well have a similar effect to increasing the concentration of K. It has indeed been found that Ca, K and Mg, in the concentrations in which they are usually present in the suspension medium, have a profound effect on the respiration of brain.

Effect on respiration. A number of experiments in which Ca, K and Mg were left out in turn are summarised in Table III. The concentration of Na was kept

Table III.

Q_{O_2} in phosphate medium.

Cations present...	Na	Na+Ca	Na+K	Na+Ca+K	Na+Ca+K+Mg
Exp. 25	—	—	−18·7	—	−10·4
„ *46*	—	—	−18·1	−15·8	—
„ *47*	—	—	−18·9	−17·2	—
„ *10*	−17·8	−15·8	−18·0	−16·3	−13·2
„ *48*	−16·9	−14·1	−17·7	−17·2	− 9·0
„ *26*	−12·6	−13·2	−17·2	−13·1	—
„ *49*	—	—	—	−15·2	−12·4
„ *50*	—	−13·5	−17·0	−13·1	−11·6
„ *23*	−14·2	−10·8	−15·0	−11·2	—
„ *51*	−15·0	−14·1	−18·8	−17·2	—
„ *11*	—	—	−20·0	−17·2	−12·7
„ *52*	—	—	−19·3	−16·0	−11·5
„ *53*	—	—	−17·9	—	−12·6
„ *54*	—	—	−15·8	—	−10·6

constant; the table shows the effect of adding the chlorides of calcium, potassium and magnesium singly, in pairs, or altogether, in the concentrations in which they are normally present in the suspension medium (*i.e.* 0·0024 *M* K, 0·0017 *M* Ca and 0·0008 *M* Mg), to the sodium chloride-sodium phosphate mixture. Glucose was present in 0·2 % concentration, and the Q_{O_2} values, most of which represent the mean values of several simultaneous measurements, refer to the first 30 or 40 mins. of the experiment.

Some typical experiments are reported in Table IV and from these and Figs. 1 and 2 it will be seen that the respiration falls off rapidly in the absence of Ca, K and Mg. Addition of K makes the respiration steadier, but the respiration is most nearly constant when Ca, K and Mg are all present. In every experiment the respiration in the first half hour is highest when the cations present

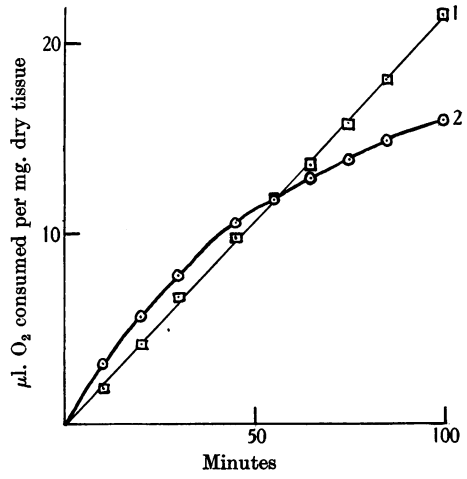

Fig. 1.

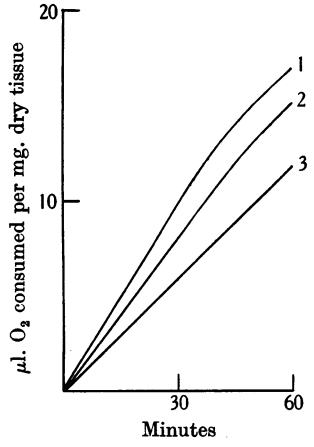

Fig. 2.

Fig. 1. Rat brain cortex. Respiration in glucose-phosphate.
1. Na+Ca+K. 2. Na.

Fig. 2. Rat brain cortex. Respiration in glucose-phosphate.
1. Na+K. 2. Na+K+Ca. 3. Na+K+Ca+Mg.

Table IV.

Exp. 10. P.	Na	Na+Ca	Na+K	Na+Ca+K	Na+Ca+K+Mg
Q_{O_2} (first 30 mins.)	−17·8	−15·8	−18·0	−16·3	−13·2
Q_{O_2} (second 30 mins.)	−11·6	−10·0	−15·4	−13·6	−12·5
Exp. 11. P.	Na+K	Na+K+Ca	Na+K+Ca+Mg	Na+K+Mg	Na+K+Mg*
Q_{O_2} (first 30 mins.)	−20·0	−17·2	−12·7	−16·2	−14·5
Q_{O_2} (second 30 mins.)	−18·0	−16·2	−12·3	−15·8	−14·3

(Experiment repeated with similar result.)

* Mg present as 0·0008 *M* $MgSO_4$.

are Na and K. Addition of Ca to this mixture always lowers the respiration, and the addition of Mg to the mixture of Na, K and Ca lowers the respiration still further. This is seen best from the mean values given below.

Simultaneous measurements in all the media to be compared were not made in all the experiments of Table III. It is therefore necessary to take the media in pairs, and in reckoning the mean values for each pair, only to consider those

experiments in which measurements were made simultaneously in both media. We then obtain the following mean values of Q_{O_2}:

Na + K	−18·0	Na + K	−18·0
Na + Ca + K	−15·4	Na + Ca + K + Mg	−11·5
(10 experiments)		(8 experiments)	
Na + Ca + K	−15·8	Na + K	−17·3
Na + Ca + K + Mg	−11·8	Na + Ca	−13·6
(6 experiments)		(6 experiments)	

Not much attention should be paid to the respiration figures in the medium containing Na as the only cation, owing to the rapid fall of Q_{O_2} with time (Fig. 1), but it is probable that the initial rate is very high.

The effect of Mg in lowering the respiration not only in the Na + K medium (Exp. 11), but also in the medium containing Na, K and Ca, is very striking, the concentration of Mg achieving this effect being only $8 \times 10^{-4} M$. As certain balanced solutions contain magnesium sulphate (*e.g.* artificial sea-water and the suspension medium used by Krebs and Henseleit [1932]), the effect of this salt was tried (Exp. 11). It lowers the respiration, and apparently slightly more so than an equivalent concentration of magnesium chloride.

After the completion of this work, it was reported by Chang *et al.* [1935] that the equivalence of Mg and Ca in lowering the respiration of nerve "does not extend to the grey matter of the brain, magnesium ion appearing indifferent to the respiration of this tissue despite its well-known 'anaesthetic' action". Our own experiments are so clear-cut that we can only ascribe these contradictory findings to the very different experimental conditions employed.

Effect on aerobic glucolysis. It will be remembered that 0·1 *M* KCl increases not only the respiration but also the aerobic glucolysis of brain. It might therefore be that removal of Ca from the suspension medium would also lead to an increased aerobic glucolysis, as well as an increased respiration. This is indeed so (Table V). In a solution containing Na as the only cation, the aerobic

Table V.

Exp. 12. BW.	Na		Na + Ca		Na + K		Na + Ca + K	
	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$
First 30 mins.	−19·6	+28·2	−16·6	+8·1	−14·3	+8·5	−10·8	0·0
Second 30 mins.	−16·5	+17·3	−14·5	+6·2	−13·2	+4·5	−10·9	0·0
Further 20 mins.	− 9·6	+16·7	−16·1	+9·5	−10·7	+5·2	—	—

Exp. 13. BD.	Na				Na + Ca + K + Mg	
	Q_{O_2}		$Q_G^{O_2}$		Q_{O_2}	$Q_G^{O_2}$
90 mins.	−15·6	−13·6	+18·9	+18·3	−14·3	+4·2

glucolysis is very high. This high acid production does not result from damage to the respiration without accompanying damage to the glucolysis (as with cyanide addition or as a result of physical damage to the tissue [Warburg, 1929]), since it appears while the respiration is higher than in the medium containing bivalent cations. Its appearance has been confirmed by the method of Dickens and Šimer (Exp. 13). Lactic acid determinations by the method of Friedemann and Graeser [1933] have shown that the acid produced is in the highest probability lactic acid.

Exp. 14. Duration 90 mins.

Medium ...	Na+Ca+K+Mg	Na	
Lactic acid at beginning (mg.)	0·12	0·13	0·11
Lactic acid at end (mg.)	0·16	0·91	0·77
Lactic acid formation expressed as $Q_G^{O_2}$	+1·0	+29·9	+20·2

Whilst, as we have seen, the respiration is highest in the absence of bivalent cations and the presence of K, this is not true of the aerobic glucolysis. The presence of either K or Ca in the NaCl medium lowers the aerobic glucolysis, though perhaps not so efficiently as the presence of these two ions together:

Duration 60 mins.

Medium ...	Na+Ca		Na+K		Na+Ca+K		Na only	
	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$
Exp. 15	−13·0	+3·8	−17·0	+2·4	−13·2	+0·9	—	—
Exp. 16	−15·3	+7·8	−13·2	+4·5	−10·9	+0·0	−13·1	+17·0

Reversal of the NaCl effect. To test whether the NaCl action could be reversed by the addition of Ca and K ions, the brain slices were suspended in NaCl-$NaHCO_3$-glucose solution in Warburg box-shaped vessels (two-vessel method) with side-bulbs. The latter contained amounts of isotonic solutions of KCl and $CaCl_2$ which on addition to the medium would give the usual concentrations of those salts. It was first found that the separate addition of either $CaCl_2$ or KCl to the NaCl medium caused the aerobic glucolysis immediately to fall from its high level. At the same time, however, the respiration decreased rapidly. On addition of Ca and K together the glucolysis fell to a still lower value, but the respiration was now maintained at a level only slightly below that of the control in which all three cations had been present throughout (Table VI). Hence the action of Na ion on brain is reversed by addition of the other cations of Ringer solution. The exposure of the tissue to NaCl before the addition should not exceed about 40 mins.

Table VI. *Reversal of "Na" effect.*

	Medium ...	Na		Na		Na (control)		Na+Ca+K (control)	
		Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$
Exp. 17	0–20 mins.	−24·7	+16·8	−16·6	+12·6	−14·2	+16·6	−9·1	+2·1
		Add $CaCl_2$		Add KCl		↓	↓	↓	↓
	After addition	−4·0	+3·6	−1·9	+7·5	−10·4	+16·6	−11·1	+3·5

		Na		Na		Na+Ca+K	
		Q_{O_2}	$Q_G^{O_2}$				
Exp. 18	0–30 mins.	−17·9	+13·9	−13·2	+9·4	−8·0	+0·2
		Add $CaCl_2$+KCl				↓	↓
	After addition	−7·9	+0·3	—	—	−8·3	+0·2

		Na		Na	
Exp. 19	0–20 mins.	−17·0	+12·1	−16·6	+12·7
		Add $CaCl_2$+KCl		↓	↓
	After addition	−7·0	+0·4	−10·4	+12·7

Effect on anaerobic glucolysis. We have seen that removing all the cations except Na from the suspension medium has the same effect on the aerobic glucolysis as adding 0·1 *M* KCl to the medium. Is the effect on the anaerobic

glucolysis the same? Addition of 0·1 *M* KCl to the suspension medium decreases the anaerobic glucolysis of brain [Ashford and Dixon, 1935]: we find that removal of all the cations except Na lowers the anaerobic acid production in just the same way (Table VII).

Table VII.

	Medium ...	Na + Ca + K + Mg	Na
Exp. 20	$Q_G^{N_2}$ (first 30 mins.)	21·2, 21·4	9·8
	$Q_G^{N_2}$ (second 30 mins.)	19·6, 19·0	8·2
Exp. 21	$Q_G^{N_2}$ (first 30 mins.)	35·8, 22·0	13·8, 14·0
	$Q_G^{N_2}$ (second 30 mins.)	26·2, 15·8	8·0, 8·4

Influence of the constituents of the medium on the effect of high concentrations of potassium chloride.

In all experiments so far described in which an increase of brain metabolism is evoked by the addition of 0·1 *M* KCl to the medium, the tissue has been under conditions of considerable hypertonicity. We have also studied the effect of 0·1 *M* KCl in an isotonic medium, by replacing part of the NaCl with isotonic KCl to give a final K concentration of *M*/10 (Table VIII). The respiration was

Table VIII.

Exp. 22. P.	Control		0·1 *M* KCl (isotonic solution)	
	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$
60 mins.	− 9·9	—	−8·8	—
BW.				
First 15 mins.	−11·1	+4·1	−8·5	+5·9
Next 30 mins.	−10·5	+2·5	−6·8	+5·3
Exp. 23. BW.				
First 30 mins.	−12·7, −10·7	+2·0, +2·5	−14·8, −11·3	+10·0, +5·5

actually decreased in three experiments out of four, but there was a slight increase in the aerobic glucolysis (Table VIII). These results, which are in striking contrast to the effects in hypertonic solution, we ascribe to the lowering of the Na ion concentration. This view has received a certain amount of confirmation. In Exp. 24 the NaCl in the medium (which contained the usual concentrations of $CaCl_2$ and KCl but no $MgCl_2$) was replaced by glucose to give a final glucose concentration of 4·5 %. It was considered from the experiments of Loeb [1913] on the change in weight of muscle in glucose solutions that this solution should be roughly "isotonic". The addition of 0·1 *M* KCl (as solid) to this medium caused no marked increase in respiration.

Exp. 24. P.	Usual medium		Medium containing 4·5 % glucose	
KCl addition ...	None	0·1 *M*	None	0·1 *M*
Q_{O_2} before KCl addition (40 mins.)	−11·7	−12·6	−14·0	−14·1
Q_{O_2} after KCl addition (40 mins.)	−13·9	−28·8	−10·1	−14·7

It thus seems to be necessary to have a fairly high concentration of Na ions present before the addition of 0·1 *M* KCl can increase the respiration. In the experiments of Table IX we added 0·1 *M* KCl to brain tissue respiring in media

from which the other cations were omitted in turn. From these experiments we draw the following conclusions:

(1) In the absence of all cations but Na, addition of 0·1 *M* KCl provokes no increase in the respiration.

(2) When in addition to Na the usual concentrations of K, or K+Ca or K+Ca+Mg are present, addition of 0·1 *M* KCl increases the respiration. In all three media the respiration is raised to about the same level, but the relative increase is least in Na+K, since the initial respiration in this medium is highest.

(3) When Ca alone is present in addition to the Na, addition of 0·1 *M* KCl causes no increase in the respiration.

(4) It thus seems that the tissue undergoes some change in a medium free from K, as a result of which the respiration can no longer be increased by the addition of 0·1 *M* KCl; and that low concentrations of K, but not of Ca, can prevent this change.

Table IX.

Exp. 25. P.	Na+K		Na+K+Ca+Mg		Na+Ca+K+Mg (1 % glucose)	
KCl addition ...	None	0·1 *M*	None	0·1 *M*	None	0·1 *M*
Q_{O_2} before KCl addition (30 mins.)	−19·6	−17·8	−10·9	− 9·8	−10·5	−10·5
Q_{O_2} after KCl addition (30 mins.)	−17·7	−22·8	−12·4	−21·9	−12·1	−21·6

Exp. 26. P.	Na		Na+K		Na+Ca	Na+Ca+K	
KCl addition ...	None	0·1 *M*	None	0·1 *M*	0·1 *M*	None	0·1 *M*
Q_{O_2} before KCl addition (45 mins.)	−14·3	−10·9	−16·0	−18·4	−13·2	−13·1	−13·1
Q_{O_2} after KCl addition (45 mins.)	− 5·5	− 7·4	−13·5	−23·4	− 8·9	−12·7	−21·8

Exp. 27. B.*	Na		Na+K		Na+Ca		Na+Ca+K
KCl addition ...	None	0·1 *M*	None	0·1 *M*	None	0·1 *M*	None
Q_{O_2} before KCl addition (first 20 mins.)	−19·1	−15·8	−19·1	−19·4	−12·2	−10·1	−13·3
Q_{O_2} before KCl addition (second 20 mins.)	−13·8	−11·7	−18·6	−18·8	−11·9	− 8·9	−14·4
Q_{O_2} after KCl addition (40 mins.)	− 7·4	− 9·7	−15·8	−22·4	− 9·2	− 8·4	−14·0

* This experiment was carried out by the original Warburg [1923] technique (low bicarbonate concentration: CO_2 absorption by potash).

Effect of neutral salts on the metabolism of tissues other than brain cortex.

It is important to discover whether the neutral salt effects, which have been observed with brain, can be shown in other surviving tissues; especially since the coloured substances, which produce changes in brain metabolism similar to those produced by neutral salts, also cause these effects in kidney, tumour and other surviving tissues.

In the experiments of Table X, KCl, in 0·1 *M* and lower concentrations, was added to the usual medium, in which slices of various kinds of rat tissue were respiring. The substrate, unless otherwise stated, was glucose. Not once did the addition of KCl provoke an increase of respiration: usually there was a

Table X.

Exp. 28. P. Kidney cortex.

Substrate	Glucose		Lactate		
KCl addition ...	None	0·1 *M*	None	0·05 *M*	0·1 *M*
Q_{O_2} before KCl addition (40 mins.)	−20·0	−18·0	−40·9	−39·3	−40·5
Q_{O_2} after KCl addition (40 mins.)	−17·1	− 9·5	−41·1	−33·0	−31·4

Exp. 29. P. Testis.

KCl addition ...	None	0·05 *M*	0·1 *M*
Q_{O_2} before KCl addition (30 mins.)	− 9·9	−10·0	− 8·4
Q_{O_2} after KCl addition (30 mins.)	−10·0	− 9·2	− 7·8

Exp. 30. P. Liver.

KCl addition ...	None	0·05 *M*	0·1 *M*
Q_{O_2} before KCl addition (30 mins.)	−10·3	−11·3	−11·0
Q_{O_2} after KCl addition (30 mins.)	−11·3	−11·4	− 9·9

Exp. 31. P. Rat yolk-sac (20 days).

KCl addition ...	None	0·02 *M*	0·05 *M*	0·1 *M*
Q_{O_2} before KCl addition (30 mins.)	−12·9	−12·6	−13·0	−13·3
Q_{O_2} after KCl addition (40 mins.)	−11·6	−10·9	− 9·8	− 8·6

Exp. 32. P. Retina.

KCl addition ...	None	0·05 *M*	0·1 *M*
Q_{O_2} before KCl addition (30 mins.)	−20·2	−19·0	−19·8
Q_{O_2} after KCl addition (30 mins.)	−19·6	−17·9	−17·4

Exp. 33. P. Jensen rat sarcoma.

KCl addition ...	None	0·05 *M*	0·1 *M*
Q_{O_2} before KCl addition (30 mins.)	−8·2	−8·8	−8·6
Q_{O_2} after KCl addition (30 mins.)	−8·5	−6·6	−5·4

BD. J.R.S.	Usual medium			Usual medium + 0·1 *M* KCl		
	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$
Exp. 34. 120 mins.	−12·1	+15·8	—	−6·2	+7·6	—
Exp. 35. 100 mins.	−12·3	+18·7	+32·4	−6·8	+8·0	+19·9
	−10·5	+18·2	—	−7·2	+8·5	+19·0

Exp. 36. P. Walker rat carcinoma 256.

	Usual medium	Usual medium + 0·1 *M* KCl
40 mins.	−8·7	−6·1

Exp. 37. BD. J.R.S. In "0·1 *M* KCl medium" part of the sodium chloride was replaced by isotonic KCl to give isotonic medium containing 0·1 *M* KCl.

	Usual medium		"0·1 *M* KCl medium"	
	Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$
100 mins.	−9·2	+23·8	−9·2	+15·7
			−7·8	+15·5
Exp. 38. BW. Walker 256. Media as in Exp. 49.				
First 30 mins.	−8·8	+19·6	−7·0	+ 9·8
Second 30 mins.	−8·8	+23·8	−7·2	+10·0

Exp. 39. B. J.R.S. Anaerobic glucolysis.

KCl addition ...	None	0·1 *M*
$Q_G^{N_2}$ before KCl addition (40 mins.)	+31·4	+32·2
$Q_G^{N_2}$ after KCl addition (30 mins.)	+29·4	+22·4

slight fall, the more marked the higher the KCl concentration. We would lay stress on the following:

(1) Testis resembles brain cortex in having a slight aerobic glucolysis.

(2) Rat yolk-sac resembles brain cortex in having a high anaerobic glucolysis and a R.Q. of unity [Dickens and Šimer, 1930, 2].

(3) Retina resembles brain cortex in having a high anaerobic glucolysis and a R.Q. of unity [Dickens and Šimer, 1930, 2], a respiration dependent on the presence of carbohydrate, and a capability of oxidising fructose directly [Dickens and Greville, 1933].

But as none of these tissues suffers an increase of respiration on the addition of 0·1 *M* KCl we may conclude that the effect on brain is not associated with any particular metabolic characteristic of that tissue.

(4) Tumour tissues show a marked decrease in aerobic glucolysis, both when 0·1 *M* KCl is added to the usual medium and also when part of the NaCl of the medium is replaced by isotonic KCl to give an isotonic medium containing 0·1 *M* KCl (Exps. 34–38 inclusive). This effect is in marked contrast to the effect of KCl on brain and to the effect of dinitro-*o*-cresol on both brain and tumour tissue.

The anaerobic glucolysis of tumours is also decreased by the addition of 0·1 *M* KCl to the usual bicarbonate medium (Exp. 39).

We have seen that the respiration of brain is very considerably raised when the calcium and magnesium are left out of the usual medium. With the other tissues tested (kidney, testis, tumour) the respiration is unaffected, or insignificantly compared with that of brain, under these conditions (Table XI).

Table XI.

	Na + Ca + K + Mg	Na + K
Exp. 40. P. Kidney.		
Q_{O_2} (first 30 mins.)	−20·8, −21·2	−19·6, −19·0
Q_{O_2} (second 30 mins.)	−21·4, −20·6	−16·8, −15·6
	(Repeated with similar result.)	
Exp. 41. P. Testis.		
Q_{O_2} (first 30 mins.)	−10·1, −10·2	−11·8, −10·4
Q_{O_2} (second 30 mins.)	−10·3, −10·1	−11·6, −10·6
	(Repeated with similar result.)	
Exp. 42. P. J.R.S.		
Q_{O_2} (first 30 mins.)	−7·6, −7·2	−7·0, −7·3
Q_{O_2} (second 30 mins.)	−7·6, −7·3	−7·4, −7·1
	(Repeated with similar result.)	

Table XII.

	Na + Ca + K			Na		
	Q_{O_2}	$Q_G^{O_2}$		Q_{O_2}	$Q_G^{O_2}$	
Exp. 43. BW. Testis.						
60 mins.	−9·5	+3·7		−8·4	+3·6	
				−10·5	+4·3	
	Na + Ca + K			Na		
	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$
Exp. 44. BW. Walker 256.						
First 30 mins.	−18·5	+24·0	+46·0	−17·2	+17·4	+24·4
Second 30 mins.	−21·6	+24·8	+42·4	−16·1	+18·0	+19·6
	(Repeated with similar result.)					
Exp. 45. BW. Mouse yolk-sac.						
	−12·5	+8·7	—	−13·9	+8·1	—

We have seen that the aerobic glucolysis of brain is enormously increased when the K, Ca and Mg are left out of the usual medium. The aerobic glucolysis of testis and yolk-sac is unaffected, and that of tumours is slightly decreased, under these conditions (Table XII).

We may therefore say that there is no evidence that any of the effects of neutral salts on the aerobic metabolism of brain tissue, which are described in this paper and that of Ashford and Dixon, are to be observed with slices of any surviving tissue other than brain.

Discussion.

From our results we draw these conclusions. When brain grey matter is respiring in a salt solution, the respiration is steadiest when, in addition to Na, K and Ca are present. K tends to increase the respiration, the bivalent cations Ca and Mg to lower it. Thus, if we leave the Ca out of the Ringer solution, the respiration is higher in the solution containing Na and K only than in the full Ringer solution. The action of the Ca in keeping the respiration small is not overcome by the low concentration of K in the Ringer solution, but it can be overcome by higher concentrations, so that the respiration reaches high values when KCl is added in concentrations of the order of $0{\cdot}1\,M$ to the full Ringer solution. When bivalent ions are absent from the medium, the respiration is not a maximum with K present in the concentration in which it is put in Ringer solution; the respiration can be raised further by the addition of $0{\cdot}1\,M$ KCl. It may be that the effect of residual bivalent ions in the tissue has to be overcome.

The effect of neutral salts on aerobic glucolysis cannot be easily formulated. Aerobic glucolysis is high when all cations are absent except Na, or when excess K is added to a medium containing Na and Ca. Here the effect of the excess K cannot be regarded as overcoming that of the low concentration of Ca, since the aerobic glucolysis is not high when a low concentration of K is added to the calcium-free medium. The aerobic glucolysis, however, depends on several factors, including, possibly, the respiration. The nature of the Pasteur reaction in muscle, the tissue most studied, is a controversial matter. New ideas on its mechanism in brain have just appeared [Geiger, 1935; see also Dixon, 1935]. It would therefore be unwise to attempt to explain the effect of neutral salts on the aerobic glucolysis at the present time.

Lyotropic effects. We have seen that the acceleration of brain respiration by a high concentration of KCl is associated with no particular substrate and no particular metabolic characteristic of the tissue. We may consider that it is the result of some physical change in the tissue. This is left beyond doubt by our observation that an increase is produced by other cations, the order of activity being:

$$\mathrm{Mg} < \mathrm{Li},\ \mathrm{Na} < \mathrm{K},\ \mathrm{Rb},\ \mathrm{Cs}.$$

This is clearly a lyotropic series. A vast amount of work on many different types of living cell [*v.* Höber, 1926; Gellhorn, 1929] has shown that K has a much greater effect than Li and Na in promoting the swelling of tissues, in increasing the permeability of cell membranes to salts and to cell constituents, in breaking down the normal impermeability of membranes or even in visibly damaging them [*e.g.* Spek, 1921]. Of particular interest is the following. Haldi *et al.* [1927] found that solutions of the alkali chlorides permitted water imbibition by the cerebral hemispheres of the rabbit brain in the order Li, Na < K, Rb, Cs. Thus the swelling of brain tissue and its respiration are increased by alkali cations

in the same order. There can be no doubt that the action of these cations on brain respiration results from an alteration by them of the colloidal state of the tissue protoplasm.

Ion antagonism. It is well known that a large number of organisms of most varied type cannot survive in pure solutions of alkali metal salts, but that the addition of a small quantity of the salt of a bivalent metal such as calcium "detoxicates" the solution and permits survival and even growth [for literature *v.* Beutner, 1933; Höber, 1926; Lillie, 1932; Stephenson, 1930]. The apparent "antagonism" of sodium and calcium may be regarded as an expression of their distance apart in the lyotropic series. Roughly we may write:

$$\xrightarrow[\text{Permeability}]{\qquad\qquad}$$
$$\text{Ca} < \text{Ba} < \text{Mg} < \text{Li} < \text{Na} < \text{Cs} < \text{Rb} < \text{K}$$
$$\xrightarrow[\text{Swelling}]{\qquad\qquad}$$

and say that Ca diffuses slowly, lowers permeability and favours contraction, whilst K diffuses rapidly, increases permeability and favours imbibition. If we start with the tissue in the condition corresponding to one cation, then addition of a cation to the right of the first in the series will cause a swelling and increased permeability, a cation to the left, a contraction and decreased permeability. Thus it is that an "antagonism", somewhat similar to that found between Na and Ca, is shown by Na and K, as in the growth of wheat roots [Osterhout, 1908], hypotonic haemolysis of red cells [Neuschloss, 1920] and the motility of spermatozoa [Gellhorn, 1922].

Neutral salt effects on brain respiration fall in well with these ideas. Ca lowers the respiration, K raises it. In the presence of Na alone, the respiration falls rapidly: the fall is considerably checked by the addition of small quantities of K. As a further expression of the disturbed condition when Na is the only cation, a large aerobic glucolysis appears which is to a great extent removed if K be present. The tissue is in its least deranged state when K and Ca are both present. This state is characterised by a low, steady respiration. Alteration of the colloidal state by removal of Ca or by addition of excess K results in a raised, rather unsteady, respiration, to be regarded as accompanying a deranged condition of the cell.

Na and Ca form a less satisfactory medium than Na and K, since on addition of 0·1*M* KCl to the former medium the respiration is not increased. This we cannot explain: the reverse might be expected here, if the controlling factor were simply the salt balance in the solution.

Nature of neutral salt effects. Whilst we have no doubt that the effects of Ca and K on brain respiration are connected with a change in the colloidal state of the protoplasm, the nature of this change remains undecided. It may be a change in the cell membrane, so that the permeability towards reactants or resultants in the respiratory reactions is altered (see for example Warburg's interpretation of his finding that the poisonous action of pure NaCl solution on the ova of *Strongylocentrotus lividus* is exerted through a great increase of the respiration of the eggs [Warburg, 1910]); or it may be a change in the protoplasm affecting the activity of the enzymes, possibly by altering the extent of their active surfaces. As examples of the latter, we may cite the demonstration of ion antagonisms in the effect of neutral salts on the activity of impure invertase solutions [Neuschloss, 1920], and the demonstration by Raab [1927] of a lyotropic series in the inhibitory effect of cations on the oxygen uptake of lysed avian erythrocytes [*cf.* Warburg, 1911].

Anaerobic glucolysis. We have seen that the addition of 0·1 *M* KCl to the usual medium, or the removal of all the cations but Na, leads to a fall in the anaerobic glucolysis, the opposite of the effect on the aerobic glucolysis. This may be explained by an increased permeability in anaerobiosis; it has been shown that anaerobiosis renders muscle membranes more permeable to chloride [Winterstein and Hirschberg, 1927; see also Gerard, 1932, for nerve]. Then 0·1 *M* KCl, which in aerobiosis is favourable to metabolism, becomes on account of increased diffusibility toxic in anaerobiosis. Again, when the K and Ca are left out of the Ringer solution, the increased permeability which results is favourable to aerobic metabolism, but, since the permeability is already raised by anaerobiosis, unfavourable to anaerobic metabolism. Anaerobic glucolysis by tumour as well as brain decreases when Ca and K are removed from the medium [Lasnitzki, 1933; Lasnitzki and Rosenthal, 1933; see also Exp. 44].

Anions. In most neutral salt actions, the action of the cations is much modified by the nature of the anions [*e.g.* Pauli, 1903; Mathews, 1904; Overton, 1904; Kahho, 1921]. Thiocyanate and iodide are in general similar in their effects to K and promote its action. We have however found that 0·05–0·1 *M* KI and KCNS in their effect on brain respiration differ little from KCl of the same concentration, and that NaCNS has no greater action than NaCl. In this indifference to anions, brain resembles nerve but is in contrast to intact muscle [Chang *et al.*, 1935].

Other tissues. We have seen that the effect of addition of excess KCl on the respiration of brain is in great contrast to the effect on the respiration of all the other surviving tissues tested. The same is true of the effect of Ca removal on the respiration, and of Ca and K removal on the aerobic glucolysis. Our observations on kidney agree with those of Siebeck [1912], who found that the respiration of whole frog kidneys *in vitro* is slightly decreased by the addition of KCl.

Of all the tissues, intact muscle seems to be the only one which resembles brain in the way in which the respiration is affected by KCl. Claude Bernard observed that muscles become non-excitable in KCl. Frog muscle can become non-excitable when immersed in Ringer solution containing only three times the normal amount of K: lowering the Ca has the same effect [Sereni, 1925]. K also produces a contracture [Ringer, 1886]. The contracture and loss of irritability are accompanied by increased oxygen uptake, whilst with the higher concentrations of K glycolysis appears [Fenn, 1931; Hegnauer *et al.*, 1934]. It seems, however, that K effects on intact muscle are hardly to be compared with those on brain slices. According to Fenn [1931], there is "strong evidence in favour of the view that chemical stimuli act through the normal contractile mechanism"; and Chang *et al.* [1935] go so far as to say that the increase of muscle respiration caused by KCl "is most probably due to the contracture induced by this salt, and the fundamental effect may be a depression. This is at least the case for heart muscle, the respiration (and tone) of which is decreased by excess potassium, as in nerve".

One is tempted to suggest that the peculiar behaviour of brain tissue is at any rate in part due to its lipin nature: this is its chief difference from retina, for example. Höber [1926] suggested that a state of dispersion between lyophile and lyophobe is the most favourable for ion antagonisms; whilst Schürmeyer [1925] found that the activity of purified invertase was not ordinarily subject to salt effects, but became so when lecithin, but not gelatin or albumin, was added.

Whether this hypothesis be acceptable or no, we cannot escape the conclusion that the metabolism of brain tissue is subject to control by alterations

in the medium in a way that is not open to most other surviving tissues, and that this control bears a remarkable resemblance to the control of muscle contraction.

SUMMARY.

1. Following the observation of Ashford and Dixon that the respiration and aerobic glucolysis of rabbit brain slices are increased by the addition of 0·1 *M* KCl to the medium, a study has been made of the effect of neutral salts on the metabolism in salt solutions of brain and other surviving tissues.

2. The results of Ashford and Dixon have been confirmed with rat brain, and rat tissues have been used throughout.

3. 0·1 *M* KCl added to the medium increases the respiration of brain tissue whether glucose, lactate, pyruvate or fructose is the substrate. The increased glucose oxidation is complete.

4. All the alkali metal chlorides increase the respiration of brain tissue when added in 0·1 *M* concentration to the medium. The order of activity is

$$\text{Li, Na} < \text{Rb, Cs, K.}$$

5. The effect on brain metabolism of leaving the various cations in turn out of the medium has been tested:

(*a*) Ca and Mg lower the respiration, K raises it.

(*b*) In the presence of Ca, K and Na the respiration is low and steady.

(*c*) In the presence of K and Na the respiration is high and not very steady.

(*d*) When Na is the only cation the respiration is initially high but falls rapidly.

(*e*) When Na is the only cation there is a high aerobic glucolysis, which does not appear when Ca and K are added.

6. In agreement with Ashford and Dixon, it is found that 0·1 *M* KCl added to the medium decreases the anaerobic glucolysis. When Na is the only cation, increased aerobic glucolysis is again accompanied by decreased anaerobic glucolysis. It is suggested that these effects are due to increased cell permeability in anaerobiosis.

7. The conditions in the media which produce large changes in brain metabolism do not produce such effects on any other surviving normal or tumour tissue tested. In these:

(*a*) 0·1 *M* KCl added to the medium never causes a rise of respiration or aerobic glucolysis. Usually a fall is observed.

(*b*) The respiration is no higher in presence of K and Na than in presence of Na, Ca and K.

(*c*) When Na is the only cation there is no increased aerobic glucolysis.

8. The effects of neutral salts on brain metabolism are ascribed to changes in the colloidal state of the protoplasm. The similarity between the control of brain metabolism by changes in the medium and the control of muscle contraction is pointed out.

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