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ACTION OF POTASSIUM IONS ON BRAIN METABOLISM

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Potassium ions cause pronounced effects on the metabolism of glucose by slices of cerebral cortex, increasing aerobic glycolysis and respiration and inhibiting anaerobic glycolysis (Ashford & Dixon, 1935; Dickens & Greville, 1935). Gerard (1938) suggested that potassium may leak out of neurones during asphyxia and stated that he had some evidence in support of this contention (see also Cowan, 1934). Gerard further supposed that potassium released in asphyxia might then cause increase in respiration and stimulation of nervous activity. Dixon (1940) considered that liberation of potassium ions with attendant inhibition of anaerobic glycolysis might be responsible for the final paralytic symptoms which follow cerebral ischaemia. Recently it has been found (Dixon, 1949) that K leaks out of brain when it is deprived of both glucose and oxygen. Leakage of K from brain also occurs during convulsions (Colfer & Essex, 1947). Liberation of K from brain cells may thus take place in various forms of cerebral disease and it seems possible that such liberated K ions might then produce secondary toxic effects on brain metabolism. It is therefore of interest to know more precisely the conditions necessary to produce these metabolic effects and the rapidity with which they are exerted.

In the experiments of Ashford & Dixon with slices of rabbit cerebral cortex potassium chloride was added to the Ringer before the start of the experiments. Dickens & Greville, however, in some cases added solid KCl during the course of their experiments to the Ringer solution in which slices of rat cerebral cortex were suspended. Respiration (in phosphate-Ringer) was increased after 10 min., and anaerobic glycolysis (in bicarbonate-Ringer) was diminished during a 30 min. period following the addition. Owing to pressure changes produced by the addition of solid KCl a lapse of 10 min. occurred before metabolic observations could commence. To eliminate this difficulty the rapidity of onset of the K effect has now been studied by adding KCl already in solution. The present communication records continuous manometric observations on the glycolysis of slices of rabbit cerebral cortex before and after the addition of small volumes of KCl in aqueous solution to the bicarbonate saline in which the slices were

immersed. In the experiments of Ashford & Dixon (1935) and Dixon (1940) effects were observed with concentrations of added K as low as 0·02 M, but 0·1 M was the usual concentration employed. An attempt was therefore made to define more precisely the concentrations of added potassium necessary to produce the main alterations in metabolism. Some experiments have also been made to decide whether the K effect can occur under isotonic conditions when the saline solution used is diluted by an amount equivalent to the K added. Substantially the same results were obtained. It is thus apparent that the effects of K on metabolism may well take place under conditions realizable *in vivo* in living tissues.

METHODS

Slices of rabbit cerebral cortex were used throughout. The glycolysis was measured with the tissue immersed in glucose-bicarbonate-Ringer of the same composition as that described by Dickens & Greville (1935). This solution contains in g./100 ml. NaCl 0·7, KCl 0·018, $CaCl_2$ 0·019, $MgCl_2$ 0·0076, $NaHCO_3$ 0·21, and glucose 0·2; it was made up daily from stock solutions of the following composition in g./100 ml.:

Stock solution *A*	Stock solution *B*	Stock solution *C*
NaCl 7 KCl 0·18 $CaCl_2$ 0·19 $MgCl_2$ 0·076	$NaHCO_3$ 2·1 saturated with CO_2	Glucose 2 made up daily

2 ml. of *A*, 2 ml. of *B* and 2 ml. of *C* were added to a graduated glass-stoppered measuring cylinder and diluted to 18 ml. The mixture was then equilibrated with N_2 or O_2 containing 5% CO_2. Of this solution 1·8 ml. was placed in the main cup of each Warburg manometer and 0·2 ml. of water, KCl solution or NaCl solution was placed in the side bulb. The slices of rabbit brain were then immersed in the fluid in the main cups and each manometer was filled with N_2 or O_2 containing 5% CO_2. The manometers were then shaken in a bath at 38° and readings were commenced after 10 or 15 min. Thermobarometers containing the same fluid and gas as used in the cups containing the brain slices showed that equilibration had been reached by the time readings were commenced. No significant pressure changes followed the addition from the side bulbs of 0·2 ml. of water, KCl solution, or NaCl solution, to the 1·8 ml. of fluid in the main cups of the thermobarometers. When constant readings had been recorded over a few 10 or 15 min. intervals, the fluid contents of the side bulbs were tipped into the main cups. After the dilution of the 1·8 ml. of fluid in the main cups by the 0·2 ml. of water from the side bulbs the concentrations of salts and glucose in the final mixture were the same as described above in the glucose-bicarbonate-Ringer of Dickens & Greville. Before this addition the solutions were slightly stronger. No metabolic change was however detected following this dilution by 10% extra water, the glycolytic rates before and after being identical. In the parallel experiments in which KCl and NaCl solutions were added, these salts were dissolved in the 0·2 ml. fluid in the side bulbs in such amount as to give the varying required final concentrations of these added ions after dilution to 2 ml. by the 1·8 ml. of fluid in the main cup. This technique obviated the difficulty of adding solid KCl or NaCl.

The slices of rabbit cerebral cortex were washed with Ringer's solution before being placed in the cups. In the first experiments the washing fluid was made by diluting stock solution *A* 1:10 with water. This solution contained no bicarbonate and was thus slightly hypotonic. In later experiments either bicarbonate Ringer or the full glucose bicarbonate Ringer was used for washing the slices. Results were unaffected by using these salines of slightly different composition for the preliminary washing.

Anaerobic glycolysis was evaluated by manometric measurement of the CO_2 evolved. Aerobic glycolysis was estimated on the assumption that the fall in pressure due to respiratory absorption of oxygen cancels the pressure change due to the formation of respiratory CO_2. This is only approximately true with small volumes of fluid (2 ml. was used), and it is realized that the figures for aerobic glycolysis are probably on the low side. The comparative value of the figures for aerobic glycolysis is probably little diminished by this approximation. Furthermore, results obtained by this method were nearly the same as those obtained simultaneously with Warburg's two-vessel method (see Table 4).

RESULTS

Many of the results are expressed graphically (Figs. 1–6), but in some cases numerical values of anaerobic glycolysis ($Q_L^{N_2}$) and aerobic glycolysis ($Q_L^{O_2}$) are given (Tables 1–5).

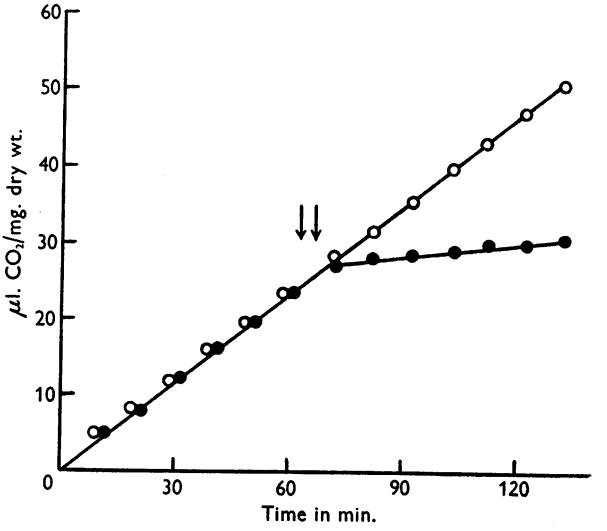

Fig. 1. Effect of addition of K on rate of anaerobic glycolysis. Both series of points record normal anaerobic glycolysis in two parallel experiments up to the period between the arrows. Here 0·2 ml. of water was added in curve ○—○—○, and the same volume of KCl solution of such strength as to give a final concentration of 50 m.equiv./l. of added K in the curve ●—●—●.

Fig. 1 and Table 1 show the effect of the addition of KCl on the anaerobic glycolysis of rabbit cerebral cortex. Using samples from the same brain, in seven parallel experiments, normal anaerobic glycolysis was observed during a preliminary control period. Then various amounts of KCl, dissolved in 0·2 ml. of water, were added to the 1·8 ml. of glucose-bicarbonate-Ringer containing each sample of the brain slices. In Fig. 1, for the sake of clarity, only the effects of adding 0·2 ml. of pure water and 0·2 ml. 0·5 N-KCl are recorded. In the former case the K concentration was not increased, and in the latter case the K concentration was increased by 50 m.equiv./l. The rate of anaerobic glycolysis from the start of the experiments up to the time of the addition was constant and was not affected to any detectable degree by the

addition of 0·2 ml. of water. Normal anaerobic glycolysis is in fact, under the conditions studied, a remarkably constant process during the whole period of incubation. The addition of 50 m.equiv. K/l. caused rapid inhibition of anaerobic glycolysis, which was well established (Fig. 1) within the first 10 min. period following the addition. Table 1 shows the changes produced in the quotients of anaerobic glycolysis by the varying amounts of KCl solution in this series of experiments.

TABLE 1. Anaerobic glycolysis before and after addition of varying amounts of KCl. in aqueous solution (seven parallel experiments with slices from same rabbit brain)

$Q_L^{N_2}$ before addition of KCl solution		$Q_L^{N_2}$ after addition of KCl solution		Final concentration of added KCl m.equiv./l.
1st ½ hr.	2nd ½ hr.	3rd ½ hr.	4th ½ hr.	
24	23	22	22	0
23	23	24	23	10
23	24	16	13	25
30	28	9	7	30
26	24	6	5	40
25	23	3	4	50
25	24	1	3	100

First ½ hr. period commenced 20 min. from start of incubation. KCl solutions (water in control) were added to each cup from side bulb between the end of the 2nd and beginning of 3rd ½ hr. period over a period of 4 min. (63–67 min. from the start of 1st ½ hr. period). The 3rd ½ hr. period began 5 min. later.

The symbol $Q_L^{N_2}$ represents anaerobic glycolysis measured in μl. CO_2/mg. dry wt. of tissue/hr. in N_2. The lactic acid formed by glycolysis liberates an equivalent amount of CO_2 from the bicarbonate-Ringer.

Fig. 2 shows graphically (in another series of parallel experiments) the effects of adding various amounts of KCl in solution so as to raise the final concentrations of K by 10–100 m.equiv./l. The level for a marked effect lies between 20 and 30 m.equiv./l. (see below). The inhibition was again rapid even at the lower effective concentrations, and could be measured during the first 10 min. interval after that in which K was added. More rapid inhibition could scarcely be detected by the manometric technique.

Fig. 3 and Table 2 refer to a similar series of parallel experiments in which isoequivalent quantities of NaCl were added in the control experiments instead of pure water. In each case 40 m.equiv./l. of total alkali metal ions were added. Na alone produced no effect, but profound and rapid inhibition was observed after the addition of 30 m.equiv. of K/l. With 20 m.equiv./l. of added K some inhibition was seen in this case. In a more recent series, after additions of 50 m.equiv./l. of Na, the glycolysis remained at 94% of the original in each of two parallel experiments. However, after the addition of 50 m.equiv./l. of K only 12 and 14% of the initial glycolysis remained in a pair of simultaneous duplicate experiments with samples of this same tissue. Here NaCl produced

no effect, but KCl rapidly inhibited glycolysis within 6–9 min. In this series other additions were not made.

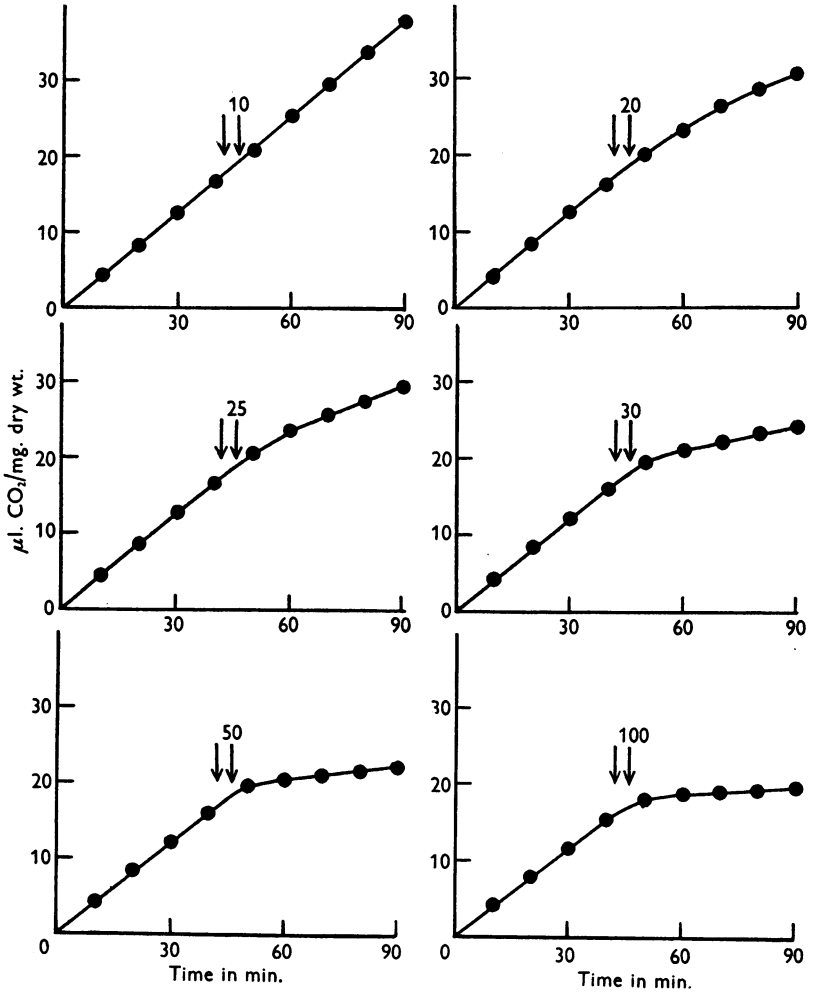

Fig. 2. Effects of varying additions of K on rate of anaerobic glycolysis. Each curve shows anaerobic glycolysis in parallel experiments up to period between arrows. At this point KCl solutions were added to give final concentrations of added K in m.equiv./l. indicated by number over right-hand arrow.

Fig. 4 summarizes the results of the above and other experiments. It shows anaerobic glycolysis after the addition of KCl expressed as percentage of the initial anaerobic glycolysis before addition, plotted against the concentration of added K in m.equiv./l. The results were obtained from six series of parallel experiments using in each series the brain of a different rabbit. In three cases

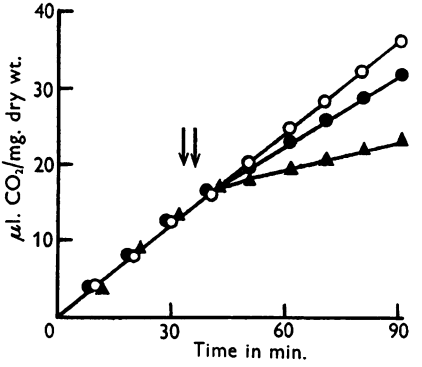

Fig. 3. Effect of addition of Na and K on rate of anaerobic glycolysis. Each series of points records normal anaerobic glycolysis up to period between arrows. Here NaCl and KCl were added in solution in such concentration as to give the following final strengths of each salt in m.equiv./l. ○—○—○, NaCl 40, KCl 0; ●—●—●, NaCl 20, KCl 20; ▲—▲—▲, NaCl 10, KCl 30.

TABLE 2. Anaerobic glycolysis before and after addition of varying amounts of KCl and NaCl in aqueous solution (five parallel experiments with slices from the same rabbit brain)

$Q_L^{N_2}$ before addition of salts in solution 1st ½ hr.	$Q_L^{N_2}$ after addition of salts in solution		Final concentration of added salts m.equiv./l.	
	2nd ½ hr.	3rd period (20 min.)	KCl	NaCl
25	25	24	0	40
27	29	24	10	30
25	19	18	20	20
26	8	7	30	10
29	8	4	40	0

First ½ hr. period commenced 15 min. from start of incubation. Salt solutions were added to each cup from side bulb between end of 1st and beginning of 2nd ½ hr. period over a period of 3 min. (33–36 min. from the start of 1st ½ hr. period). The 2nd ½ hr. period started 4½ min. later.

NaCl was added simultaneously with the KCl so as to produce an equimolar rise in total salt concentration in parallel experiments. In the other cases KCl only was added. The glycolysis was measured over a 20 or 30 min. period before the addition of KCl, and again over a 30 min. period after the addition neglecting the glycolysis during the 10 min. period in which the addition was made. The main inhibition of glycolysis was established between 20 and 40 m.equiv./l. of added K. Further increases produced little additional effect.

The rapidity of the KCl effect on aerobic glycolysis has also been studied. Here the aerobic glycolysis has been evaluated approximately (see above) from the pressure changes produced in manometers filled with O_2 95%, CO_2 5% and containing 2 ml. of fluid. Fig. 5 records some of the results from a series

of five parallel experiments in which Na and K salts in solution were added in such amounts as to produce in each case an increase by 40 m.equiv./l. in the concentration of total alkali metal ions. No effect was observed with NaCl alone nor with 10 nor 20 m.equiv./l. of K. With the addition of 30 m.equiv./l. of K, a definite increase in aerobic glycolysis was produced, and with 40 m.equiv./l. of K intense aerobic glycolysis developed with such rapidity that the full rate was reached within 10 min. of adding the KCl solution. For the sake of clarity only three of these parallel experiments are recorded in Fig. 5. Other similar series of experiments have given the same results.

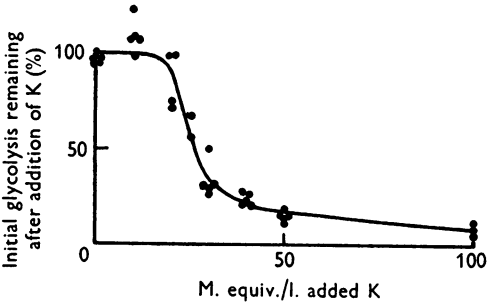

Fig. 4. Relation of inhibition of anaerobic glycolysis to concentration of added K.

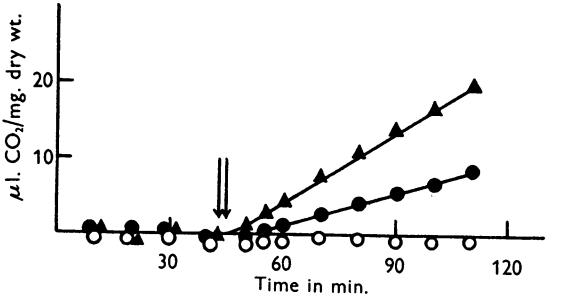

Fig. 5. Effect of addition of Na and K on the rate of aerobic glycolysis. Each series of points records normal aerobic glycolysis up to the period between the arrows. At this point NaCl and KCl were added in solution in such concentration as to give the following final strengths of each salt in m.equiv./l.: ○—○—○, NaCl 40, KCl 0; ●—●—●, NaCl 10, KCl 30; ▲—▲—▲, NaCl 0, KCl 40.

Fig. 6 and Table 3 show the effect of adding varying amounts of KCl solution alone on the aerobic glycolysis in another series of five parallel experiments. A definite increase in aerobic glycolysis first became evident with 30 m.equiv./l. of added K. With 50 m.equiv./l. of added K the aerobic glycolysis had reached the normal high anaerobic rate (Table 3). The full rate of enhanced glycolysis was attained in each case within 6–8 min. of the addition of KCl. Owing to the method used, however, small increases of aerobic glycolysis at lower concentrations may have escaped detection.

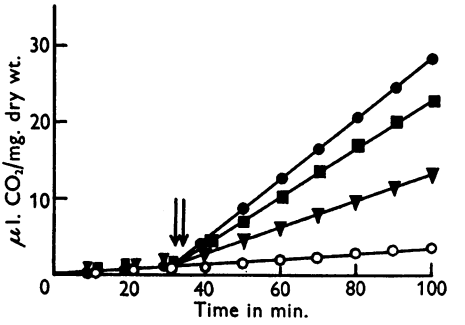

Fig. 6. Effect of addition of K on rate of aerobic glycolysis. Each series of points records normal aerobic glycolysis up to the period between the arrows. At this point KCl was added in solution in such concentration as to give the following final strengths of K in m.equiv./l. ○—○—○, KCl 20; ▼—▼—▼, KCl 30; ■—■—■, KCl 40; ●—●—●, KCl 50.

TABLE 3. Approximate values of aerobic glycolysis before and after the addition of varying amounts of KCl in aqueous solution (five parallel experiments with slices from the same rabbit brain)

$Q_L^{O_2}$ before addition of KCl solution 1st ½ hr.	$Q_L^{O_2}$ after addition of KCl solution		Final concentration of added KCl m.equiv./l.
	2nd ½ hr.	3rd ½ hr.	
2	3	3	20
3	10	11	30
3	19	19	40
3	25	24	50
3	21	18	100

First ½ hr. commenced 12 min. from start of incubation. KCl solutions were added to each cup from side bulb between the end of the 1st and beginning of the 2nd ½ hr. period over a period of 2 min. (32–34 min. from the start of the 1st ½ hr. period). The 2nd ½ hr. period started 6 min. later. The symbol $Q_L^{O_2}$ represents aerobic glycolysis measured in μl. CO_2/mg. dry wt. of tissue/hr. in O_2. The lactic acid formed by glycolysis liberates an equivalent amount of CO_2 from the bicarbonate-Ringer. It is assumed that the pressure change due to the formation of CO_2 in respiration is cancelled by the respiratory absorption of O_2. This approximation gives useful comparative values which appear to be only slightly smaller than those obtained by Warburg's two vessel method (see Table 4).

In the experiments so far described KCl was added in addition to the normal constituents of the Ringer solution. It might be argued that these ionic effects on metabolism thus depend on the final solution being hypertonic. This point was raised by Dickens & Greville (1935) who found that with rat brain the effect of addition of 0·1 M-KCl was largely eliminated when an equivalent amount of NaCl was simultaneously subtracted from the Ringer solution. To test this question further some experiments have now been made in which the Ringer solution was diluted by an amount equivalent to the added K. Since an addition of 0·04 N-KCl produces practically the full effect it was decided to add this amount of KCl with simultaneous dilution of the other metallic

chlorides of the Ringer. This would not cause such a profound modification of Na content as the addition of as much as 0·1N-KCl with corresponding subtraction of other salts. The Ringer solution normally used (see Dickens & Greville, 1935) is 0·1275N with respect to metallic chlorides. In these experiments 69% of the usual amount of stock concentrated Ringer solution was employed. Thus the normality of the final solution would be diminished by 0·04N. To offset this dilution one-tenth of the final volume of 0·4N-KCl was added, thereby raising the final concentration of KCl by 0·04N (actually 0·039N when correction is made for initial dilution of KCl originally present in the stock concentrated Ringer solution). At the same time the total salt concentration of the final solution is maintained at isotonic level. The ratio of bivalent to univalent ions in these solutions is so small that their osmotic pressure may be taken as proportional to their normality (within 1%). The dilution of Ca and Mg involved in this change was not responsible for metabolic effects, since controls in which 0·04N-NaCl instead of 0·04N-KCl was added gave substantially normal glycolytic quotients. The results obtained are recorded in Table 4.

TABLE 4. Anaerobic and aerobic glycolysis in the presence of added NaCl and KCl under isotonic conditions (glucose content 0·2% and bicarbonate 0·025M as previously)

Brain cortex slices from rabbit no.	Normal Ringer solution		Diluted Ringer solution + NaCl		Diluted Ringer solution + KCl			
	$Q_L^{N_2}$	$Q_L^{O_2}$	$Q_L^{N_2}$	$Q_L^{O_2}$	$Q_L^{N_2}$	$Q_L^{O_2}$	$Q_L^{O_2}W$	Q_{O_2}
1	23	2	—	—	5	22	—	—
2	26	1	—	—	—	26	29	−40
3	—	—	26	2	4	22	23	−21
4	—	—	28	3	4	24	26	−29

Normal Ringer: stock solution *A* (conc. Ringer solution) 2 ml.; 0·25M-$NaHCO_3$, 2 ml.; 2% glucose, 2 ml.; diluted to 20 ml. *Diluted Ringer* + NaCl: (The use of this control was kindly suggested to me by Dr G. D. Greville): stock solution *A*, 1·37 ml.; 0·25M-$NaHCO_3$, 2 ml.; 0·4M-NaCl, 2 ml.; 2% glucose, 2 ml.; diluted to 20 ml. *Diluted Ringer* + KCl: stock solution *A*, 1·37 ml.; 0·25M-$NaHCO_3$, 2 ml.; 0·4M-KCl, 2 ml.; 2% glucose, 2 ml.; diluted to 20 ml.

The substitution of 1·37 ml. instead of the normal 2 ml. of stock solution *A* entails a dilution of the final solution by 0·04 equiv. of total salts per l. This subtraction is equivalent to the added Na or K.

The quotients $Q_L^{N_2}$ and $Q_L^{O_2}$ are used as defined in footnotes to Tables 1 and 3. Q_{O_2} and $Q_L^{O_2}W$ indicate respectively the quotients for oxygen uptake and lactic acid production observed in experiments using the two-vessel method of Warburg. The simplified method of calculating lactic acid production indicated by $Q_L^{O_2}$ (using one vessel) gives very similar values to $Q_L^{O_2}W$ found by the two-vessel method.

From Table 4 we see that the addition of 40 m.equiv./l. of KCl produced its full effect in inhibiting anaerobic glycolysis and stimulating aerobic glycolysis even when an equivalent subtraction was made from the other salts. Similar dilution followed by addition of 40 m.equiv./l. of NaCl produced no such effect. This addition of KCl in isotonic solution also gave very high values for

respiration. It is thus apparent that the effect of K ions on metabolism do not depend on the presence of a hypertonic saline environment and may thus take place under conditions likely to be realized *in vivo*.

Irritation followed by paralysis is a common sequence of events in cerebral disease. Stone (1938) suggested that convulsions (including those of cyanide poisoning) and increased activity might result in increased lactic acid production, whereas narcosis and diminished activity might inhibit glycolysis. Dixon (1939, 1940) supposed that increase in glycolysis might generate the convulsions and irritative phenomena which occur initially in cerebral anoxia, and that subsequent inhibition of glycolysis would account for the later paralytic symptoms which are the final outcome of interruption of blood supply. This final inhibition of glycolysis would result directly from defective supply of glucose to the ischaemic area, and also possibly indirectly from the liberation of K which takes place when brain is deprived of both glucose and oxygen. Such liberated K could cause rapid and profound inhibition of glycolysis in anaerobic regions still supplied with glucose. In regions with full aeration, on the other hand, a rapid increase in glycolysis above normal would be anticipated, which might lead to excessive activity at the periphery of the damaged area (see Gerard, 1938).

TABLE 5. Irreversibility of effect of K on anaerobic glycolysis

Concentration of added KCl (m.equiv./l.)	0	10	20	30	50
$Q_L^{N_2}$ in Ringer solution containing this added KCl	25	34	11	7	5
$Q_L^{N_2}$ of same slices of rabbit brain after transference to normal Ringer solution:					
1st 30 min.	21	21	11	10	—
2nd 30 min.	20	20	10	8	7

The anaerobic inhibition of glycolysis by K is probably irreversible. Ashford & Dixon (1935) showed that the effect of 0·1 M-KCl on anaerobic glycolysis was not removed by subsequent immersion of the brain slices in normal Ringer. Table 5 shows this irreversible effect of added KCl with concentrations down to 0·02 M. It is thus possible that even a transitory increase in K concentration under anaerobic conditions may be followed by lasting interference with the cellular glycolytic system and concomitant irreversible damage to the neurones.

SUMMARY

1. The rapidity of onset and the effective concentration limits of the action of potassium ions on brain glycolysis were studied.

2. Inhibition of anaerobic glycolysis became evident at about the level of 20 m.equiv./l. of added potassium. With 40 m.equiv./l. of potassium 75% of the anaerobic glycolysis was suppressed. Inhibition was established within less than 10 min.

3. Aerobic stimulation of glycolysis by the addition of potassium ions was equally rapid in onset. Its main development occurred following the addition of 30–50 m.equiv./l.

4. Diminution of the total salt concentration by an amount equivalent to the added potassium did not interfere with the effects of potassium ions on glycolysis.

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