yield. A 2,4-*trans*-configuration was also assigned by Mitsui & Kasahara (1960) to the flavan-4-ol obtained by catalytic hydrogenation of flavanone in neutral solution.

The flavan-4-ols formed from (−)-butin (II, R = H) and (−)-robtin (II, R = OH) are therefore tentatively assigned the absolute configurations (2*S*,4*R*)-3′,4′,7-trihydroxyflavan-4-ol (III, R = H) and (2*S*,4*R*)-3′,4′,5′,7-tetrahydroxyflavan-4-ol (III, R = OH) respectively.

SUMMARY

1. The analogues robtein, (±)-robtin and (±)-3′,4′,5′,7-tetrahydroxyflavan-4-ol have been synthesized.

2. The sequence of conversions (+)-dihydrorobinetin → (−)-robtin → (+)-3′,4′,5′,7-tetrahydroxyflavan-4-ol and (+)-fustin → (−)-butin → (+)-3′,4′,7-trihydroxyflavan-4-ol has been effected in each instance by means of a Clemmensen-type reduction followed by catalytic hydrogenation.

3. These interconversions establish the absolute configurations of flavanones as (2*S*), and those of the flavan-4-ols tentatively as (2*S*, 4*R*).

4. The biochemical significance of the ready conversion of flavanonol into flavanone is discussed.

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REFERENCES

Arakawa, H. & Nakazaki, M. (1960*a*). *Liebigs Ann.* **663**, 111.
Arakawa, H. & Nakazaki, M. (1960*b*). *Chem. & Ind.* p. 73.
Bokadia, M. M., Brown, B. R., Kolker, P. L., Love, C. W., Newbould, J., Somerfield, G. A. & Wood, P. M. (1961). *J. chem. Soc.* p. 4663.
Clark-Lewis, J. W. & Roux, D. G. (1959). *J. chem. Soc.* p. 1402.
Freudenberg, K. & Hartmann, L. (1954). *Liebigs Ann.* **587**, 207.
Freudenberg, K. & Hübner, H. H. (1952). *Chem. Ber.* **85**, 1181.
Ivnäs, L. & Lindberg, B. (1961). *Acta chem. scand.* **15**, 1081.
Mitsui, S. & Kasahara, A. (1960). *J. chem. Soc. Japan*, **81**, 1583.
Pew, J. C. (1948). *J. Amer. chem. Soc.* **70**, 3031.
Roberts, E. A. H. & Wood, J. D. (1953). *Biochem. J.* **53**, 332.
Rosenmund, K. W. & Pfannkuch, E. (1922). *Ber. dtsch. chem. Ges.* **55**, 2357.
Rosenmund, K. W. & Zetzsche, F. (1918). *Ber. dtsch. chem. Ges.* **51**, 594.
Roux, D. G. & Maihs, A. E. (1960*a*). *Biochem. J.* **74**, 44.
Roux, D. G. & Maihs, A. E. (1960*b*). *J. Chromat.* **4**, 65.
Roux, D. G., Maihs, A. E. & Paulus, E. (1961*a*). *J. Chromat.* **5**, 9.
Roux, D. G., Maihs, A. E. & Paulus, E. (1961*b*). *Biochem. J.* **78**, 834.
Roux, D. G. & Paulus, E. (1960). *Biochem. J.* **77**, 315.
Roux, D. G. & Paulus, E. (1961*a*). *Biochem. J.* **78**, 120.
Roux, D. G. & Paulus, E. (1961*b*). *Biochem. J.* **80**, 62.
Roux, D. G. & Paulus, E. (1961*c*). *Biochem. J.* **80**, 476.
Roux, D. G. & Paulus, E. (1962). *Biochem. J.* **82**, 324.
Schmidt, E. (1919). *Ber. dtsch. chem. Ges.* **52**, 409.
Weinges, K. (1958). *Liebigs Ann.* **615**, 203.

Biochem. J. (1962) **84**, 421

The Influence of Potassium on Respiration and Glycolysis by Brain Slices

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Ashford & Dixon (1935) and Dickens & Greville (1935) observed that the respiratory and aerobic glycolytic activities of brain-cortex slices in a 'physiological saline' medium were greatly stimulated, and the anaerobic glycolytic activity inhibited, on addition of a high concentration (100 mM) of potassium chloride to the medium. These observations have been confirmed by various workers though the magnitude of the effects observed has varied [see, for example, Webb & Elliott (1951)]. Except for recent studies by Cummins & McIlwain (1961), mentioned below, little information is available with regard to the influence of the low concentrations of potassium normally present in extracellular fluids or of moderate changes in these concentrations. In their detailed study on the effects of various cations on the metabolism of brain slices Dickens & Greville (1935) reported no results for the condition in which potassium alone was omitted from the medium. Gore & McIlwain (1952) reported that absence of potassium salts from the medium had no

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marked effects on the rate of respiration and glycolysis of guinea-pig brain tissue.

Previously observation of the effects of low concentrations of potassium was difficult because fresh tissue slices contain a high concentration of potassium: when they are immersed in saline medium they rapidly lose a major fraction of their potassium content to the medium so that the concentration in the medium cannot be precisely controlled and is never zero. We have found, however, that when brain slices are immersed in potassium-free saline medium at 0° they lose most of their potassium with little change in metabolic activity. With brain slices thus pretreated to render them nearly potassium-free the respiratory and glycolytic activities, subsequently measured at 38°, are markedly affected by low concentrations of potassium in the medium. The effects of potassium, and of calcium, are more complex than was previously realized. Moderate concentrations of potassium exert effects on glycolysis which are the opposite of those usually stated to be the effects of potassium. These observations are reported below.

METHODS

Slices of rat cerebral cortex were prepared in a humid chamber without moistening and were weighed. In most experiments the tissue was placed in 3 ml. of phosphate-buffered potassium-free medium in the presence of 100% oxygen and shaken at 0° for 1 hr. ('cold pretreatment'). The slices (referred to below as 'potassium-depleted slices') were drained on a perforated disk with suction and transferred to 3 ml. of medium in a Warburg flask for the determination of rates of oxygen uptake and glycolysis at 38°.

All media contained glucose (final concentration 10 mM) unless otherwise stated. Variations in the concentration of potassium in the suspending media were balanced by reciprocal equivalent variations in sodium concentration. The phosphate-buffered media iso-osmotic with plasma contained (final concentrations) NaCl + KCl (161 mM), $MgCl_2$ (1·2 mM) and either sodium phosphate, pH 7·8 (17·9 mM), and no $CaCl_2$, or sodium phosphate (11 mM) and $CaCl_2$ (1·7 mM). (The $CaCl_2$, in solution, was added just before starting the experiment.) The iso-osmotic bicarbonate-buffered media contained NaCl + KCl (150 mM), $MgCl_2$ (1·2 mM) and $NaHCO_3$ (24·5 mM) either with no $CaCl_2$ or with $CaCl_2$ (1·7 mM). In specified experiments the concentration of NaCl was increased by 100 mM.

For determinations under aerobic conditions phosphate-buffered medium was used and the flasks were filled with oxygen. Oxygen uptake was measured manometrically; the CO_2-absorbing papers in the centre wells were saturated with 20% (w/v) NaOH instead of KOH to avoid possible contamination of slices with potassium when potassium determinations were to be made. Aerobic glycolysis was measured by determining the total amount of lactic acid in the tissue and the medium by the method of Barker & Summerson (1941); at the end of the experimental period 3 ml. of 20% (w/v) trichloroacetic acid was added, the slices and fluid were homogenized and centrifuged, and the supernatant was analysed. The average total lactic acid, 6 μmoles/g., found in the tissue after cold pretreatment, but before incubation at 38°, was deducted from that found after the incubation at 38°. For anaerobic experiments bicarbonate-buffered medium was used, the gas phase was $N_2 + CO_2$ (95:5) and glycolysis was measured manometrically.

The potassium content of slices was estimated by flame photometry after digestion of the tissue with nitric acid according to the procedure of Pappius & Elliott (1956*b*). Results are given in μequiv./g. of fresh tissue. This is calculated from the initial weight of slices, their final weight after incubation and the potassium content of the incubated slices. It is assumed that an increase in weight of the slices during incubation, up to 40% of the initial weight, is due to uptake of medium with its potassium content unchanged and that this fluid is not truly part of the tissue. [A similar correction was applied by Terner, Eggleston & Krebs (1950) and its validity was examined by Pappius & Elliott (1956*a*).] Details of the method of calculation are given by Pappius & Elliott (1956*b*).

RESULTS

Cold pretreatment

Fig. 1 shows that when fresh brain-cortex slices are kept in cold potassium-free medium they lose potassium rapidly. The potassium content falls from an initial value of about 110 to about 10 μequiv./g. of tissue in 60 min. (Further loss, to about 3 μequiv./g., occurs at 38° in potassium-free medium during the few minutes required for equilibration.) The loss of potassium was matched by an equivalent uptake of sodium by the tissue from the medium. The course of the loss of potassium at 0° suggests that two time-constants apply, indicating that the potassium may be present in the tissue in two conditions.

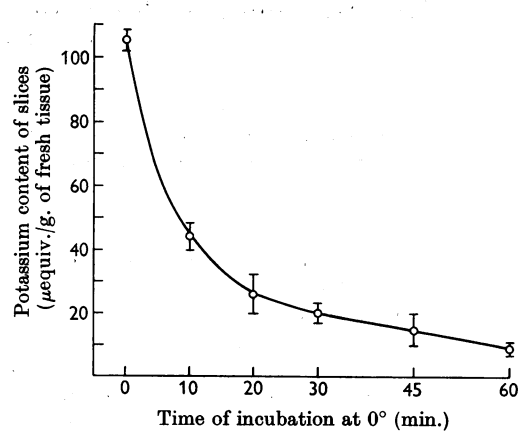

Fig. 1. Changes in potassium content of rat-cerebral-cortex slices during incubation in cold potassium-free medium. Experimental details are given in the text. Points represent the means of 7–16 determinations on different slices; vertical lines indicate S.D.

Fig. 2 gives results of a typical experiment which shows that the above treatment does not seriously affect the respiratory activity of the tissue. When potassium-depleted slices were placed in potassium-containing medium at 38° they respired nearly as rapidly as slices that had not been pretreated or as slices that had been kept at 0° and kept humid but not immersed in medium so that loss of potassium was prevented.

Oxygen uptake

Figs. 2, 3A and 3B show that the rate of oxygen uptake by potassium-depleted slices is definitely lower in potassium-free medium than in medium containing 3·6 m-equiv. of potassium/l. The latter is about the concentration in cerebrospinal fluid. The rate of oxygen uptake remains constant in the presence of potassium but falls with time at 38° in the absence of potassium. Other experiments have shown that the rate can be largely restored, and rendered constant, by adding potassium even after deprivation for 60 min. at 38°.

Figs. 3A and 3B show the effects of various concentrations of potassium on the oxygen-uptake rate of potassium-depleted slices in the absence or presence of calcium. The curves show the effects obtained with the media which were iso-osmotic with plasma and with media in which the concentration of sodium chloride had been increased by 100 mM. In the latter case, even when 160 m-equiv. of potassium/l. was used, there was at least 100 m-equiv. of sodium/l. present but the medium was somewhat hyperosmotic. The standard method used by Dickens & Greville (1935) and later workers (e.g. Ghosh & Quastel, 1954; Kini & Quastel, 1959; Gore & McIlwain, 1952) consisted of comparison of the rate of oxygen uptake in an iso-osmotic medium containing 2·5 m-equiv. or more of potassium/l. with that in the same medium in which the concentration of potassium chloride had been in-

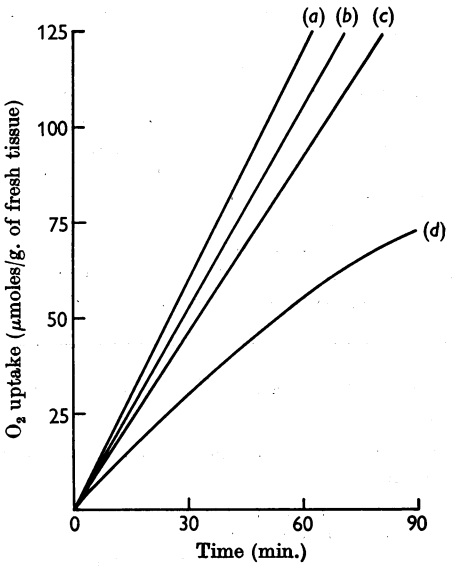

Fig. 2. Effect of cold pretreatment and potassium in the medium on the oxygen uptake of brain slices at 38°. Experimental details are given in the text. (*a*) Slices subjected to no pretreatment; (*b*) slices kept for 1 hr. at 0° at 100% humidity but not suspended in medium; (*c*) slices shaken for 1 hr. at 0° in potassium-free medium. During the measurement of oxygen uptake the slices were incubated in phosphate-buffered calcium-free medium. For (*a*), (*b*) and (*c*) this medium contained 3·6 m-equiv. of potassium/l.; for (*d*) the medium contained no potassium.

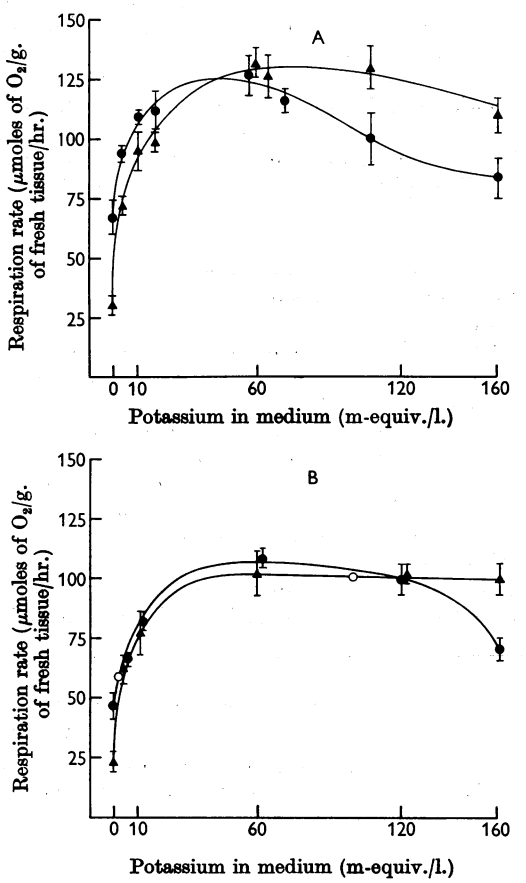

Fig. 3. Effect of the potassium concentration in the medium on the rate of oxygen uptake by brain slices. Experimental details are given in the text. A, Calcium absent; B, calcium (1·7 m-equiv./l.) present. ●, Iso-osmotic media; ▲, same media with the concentration of sodium chloride increased by 100 mM. The points represent the means of five or more determinations on different slices and the vertical lines show the S.D. Circles (○) in Fig. 3B indicate the results (interpolated) under the conditions of experiments of Dickens & Greville (1935).

creased by 100 mM without reducing the concentration of sodium chloride. These conditions are indicated by the circles in Fig. 3B (and also in Figs. 6B and 7B). It is evident that the classical increase in oxygen uptake rate can be obtained with cold-pretreated slices.

Figs. 3A and 3B show that the maximum increase in respiration occurs with about 60 m-equiv. of potassium/l. and the relative increase with this concentration is greater in the presence than in the absence of calcium though the highest actual rates occur in the absence of calcium. The variety of effects at very high potassium concentrations (above 60 m-equiv./l.) under various conditions is complex. The differences between results obtained in iso-osmotic media and those obtained in the presence of extra salt (100 mM extra sodium chloride added) are apparently due to maintenance of sodium in the medium and not to the hyper-osmotic condition of the medium. The addition of 200 mM-sucrose to the medium in place of the 100 mM extra sodium chloride did not reproduce the effects given by the extra sodium chloride.

Substrates other than glucose

The respiration of untreated brain slices in the absence of glucose falls off with time as endogenous lactate is consumed, pyruvate can be utilized as well as glucose, and glutamate also can serve as substrate (see, for example, Elliott, 1955). Results summarized in Table 1 show that, with potassium-depleted slices, the respiration in the absence of glucose is greatly diminished and is not increased by the presence of potassium in the medium. Respiration in the presence of pyruvate is stimulated by 60 m-equiv. of potassium/l. (see also Dickens & Greville, 1935). Respiration with glutamate is appreciably stimulated by 3·6 or 60 m-equiv. of potassium/l. The tricarboxylic acid-cycle intermediates, α-oxoglutarate and malate, cause small increases in oxygen uptake in the absence of glucose but these are not stimulated by potassium. The small effect of γ-aminobutyrate on oxygen uptake is somewhat stimulated by potassium. The effects of γ-aminobutyrate on the respiration of untreated brain slices observed in this Laboratory (N. van Gelder, unpublished work; and Table 1) are much smaller than those observed by others (Tsukada, Nagata & Takagaki, 1957; Tower, 1958) in the absence of glucose; we observed almost no effect in the presence of glucose (see also Kurokawa, 1960).

Potassium uptake

As Fig. 4 shows, potassium-depleted slices re-accumulate potassium rapidly initially when they are placed in a medium containing potassium and glucose in the presence of oxygen at 38°. From the curves it can be estimated that in these experiments the rates of potassium uptake during the first 3 min. in media containing 3·6 and 18 m-equiv. of potassium/l. were respectively about 5 and 10 μequiv./g. of tissue/min. The rate in the medium containing 3·6 m-equiv./l. was considerably higher than that obtained by Pappius & Elliott (1956*b*) with slices which had been depleted of potassium by anaerobic incubation at 38°.

Fig. 5 shows that the maximum concentration of potassium reached in the slices increased with increasing potassium concentration in the medium. When the concentration of potassium in the medium was more than 18 m-equiv./l., however, the difference between the concentrations in the slices and the medium, i.e. the amount accumulated against the concentration gradient, progressively decreased. In no case did the difference in concentration between that in the slice and that in the medium reach that which obtains in fresh tissue.

Table 1. *Effects of potassium on oxygen-uptake rates of potassium-depleted slices in the presence of various substrates*

Iso-osmotic phosphate-buffered calcium-free medium was used. Experimental details are given in the text. The results are given as means ± S.D. where four or more determinations were made.

	O_2 uptake (μmoles/g. of fresh tissue/hr.)		
Concn. of K (m-equiv./l.)	0	3·6	60
Substrate (10 mM)			
Glucose	67±7	91±6	126±9
No substrate	29±6	32±8	31±5
Pyruvate	83±11	92±6	140±7
Glutamate	46±9	66±6	87±3
Glutamate + glucose	66±6	103±7	134±3
γ-Aminobutyrate	32±6	44±8	54±3
γ-Aminobutyrate + glucose	77±6	102±9	128±9
α-Oxoglutarate	38±7	39±8	41±4
Malate	50	53	47±3

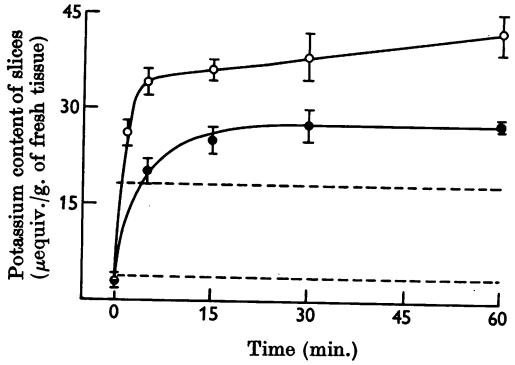

Fig. 4. Uptake of potassium by potassium-depleted slices during aerobic incubation at 38°. Experimental details are given in the text. Slices were kept at 0° for 60 min. in potassium-free medium and then transferred to medium (phosphate-buffered, calcium-free) containing 3·6 (●) or 18 (○) m-equiv. of potassium/l., and incubated aerobically at 38° for various periods. The slices were then reweighed and their potassium contents were determined. The potassium contents/g. of fresh tissue were calculated from the determinations as noted in the Methods section. The points represent the means of five or more determinations and the vertical lines show the S.D. The two broken lines represent the potassium concentrations in the media (3·6 and 18 m-equiv./l.).

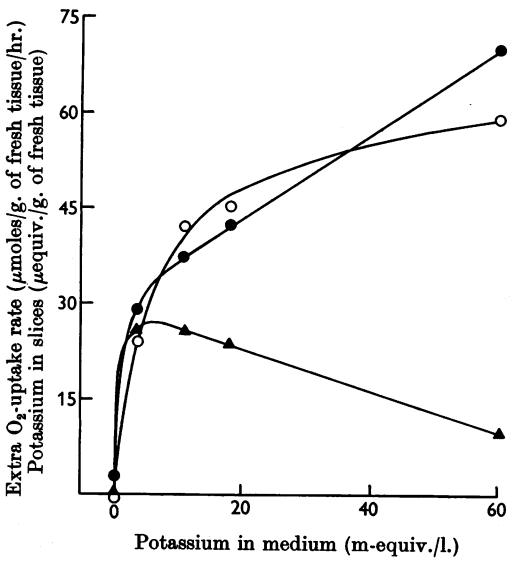

Fig. 5. Relation of potassium concentration in tissue to that in the medium after incubation of slices at 38° for 60 min. The procedure and calculation were as for Fig. 4. ●, Average potassium concentration in the slice; ▲, average difference in potassium concentration between slice and medium; ○, average increase in oxygen uptake rate over that in medium containing no potassium.

Fresh untreated brain slices lose about 60 % of their potassium content on immersion and then regain some, but by no means all, during aerobic incubation (Terner *et al.* 1950; Pappius & Elliott, 1956*b*).

Fig. 5 shows also that the increase in oxygen uptake that occurs with increase in concentration of potassium in the medium is approximately paralleled by the increase in tissue potassium up to about 45 μequiv./g. (20 m-equiv./l. in the medium). As the potassium content of the medium is further increased the increase in the oxygen-uptake rate is less than proportional to the increase in tissue potassium. With guinea-pig-cortex slices preincubated for 1–2 hr. in warm (38°) potassium-free media and then placed in potassium-containing media, Cummins & McIlwain (1961) observed little variation in respiration rate with the potassium content of the tissue unless electrical pulses were applied. In the latter case the respiration rate increased in proportion to the potassium content of the tissue up to 30 equiv. of potassium/g. of tissue.

Aerobic glycolysis

Figs. 6A and 6B show the influence of potassium on the aerobic glycolysis of brain slices. As the open circles indicate, the stimulation of aerobic glycolysis in the presence of a high potassium concentration, which is the commonly stated effect of potassium, can be observed. However, with potassium-depleted slices, in iso-osmotic medium, aerobic glycolysis is active in the absence of potassium and is inhibited in the presence of moderate potassium concentrations, the maximum inhibition occurring with about 10 m-equiv. of potassium/l. Further increase in potassium concentration causes the glycolysis to rise again but, at concentrations above about 60 m-equiv./l., the glycolysis tends to fall again especially if the medium lacks sodium.

Anaerobic glycolysis

Ashford & Dixon (1935) and Dickens & Greville (1935) observed that addition of potassium salt to an already iso-osmotic saline medium strongly inhibited anaerobic glycolysis by brain slices. Pappius & Elliott (1958) on the other hand found that, in a medium in which sodium was completely replaced by potassium, anaerobic glycolysis was stimulated. In both cases the comparison was with medium containing 2·5–5 m-equiv. of potassium/l. Figs. 7A and 7B show that both these observations can be made with cold-pretreated slices. The relation of glycolytic activity to the potassium content of the medium is more complex than the above-mentioned workers appreciated. The rate of anaerobic glycolysis is low in the complete absence of potassium and it rises as the potassium content

of the medium is increased, reaching a maximum at a concentration of about 10 m-equiv./l. Further increase in potassium concentration causes, under most conditions, an inhibition of glycolysis, but again the relation between glycolytic activity and potassium concentration varies in a complex way with other conditions. The strong inhibition of anaerobic glycolysis by high potassium concentrations described by the above-mentioned authors and others depends on (*a*) a high concentration of potassium, (*b*) the presence of sodium and (*c*) the presence of calcium.

Anaerobic glycolysis is stimulated by the presence of pyruvate (see Elliott, 1955) and inhibited by glutamate (Weil-Malherbe, 1938) in the medium. As Table 2 indicates, pyruvate-stimulated glycolysis, like glycolysis in the absence of pyruvate, is stimulated by 3·6 m-equiv. of potassium/l. and inhibited by 60 m-equiv. of potassium/l. in iso-osmotic medium. The inhibitory effect of glutamate also is apparent in the presence or absence of potassium. When glutamate and pyruvate are present together their effects balance each other so that the addition of 3·6 m-equiv. of potassium/l. no longer stimulates glycolysis.

Liver and kidney tissues

Dickens & Greville (1935) showed that the effects of potassium occur only with brain tissue. The oxygen-uptake rates of slices of liver and kidney-cortex tissue, pretreated in the same way as brain-cortex slices, did not vary significantly in media containing 0, 3·6 and 60 m-equiv. of potassium/l. The respiration of these tissues, like that of brain, was inhibited by complete replacement of sodium in iso-osmotic medium by potassium.

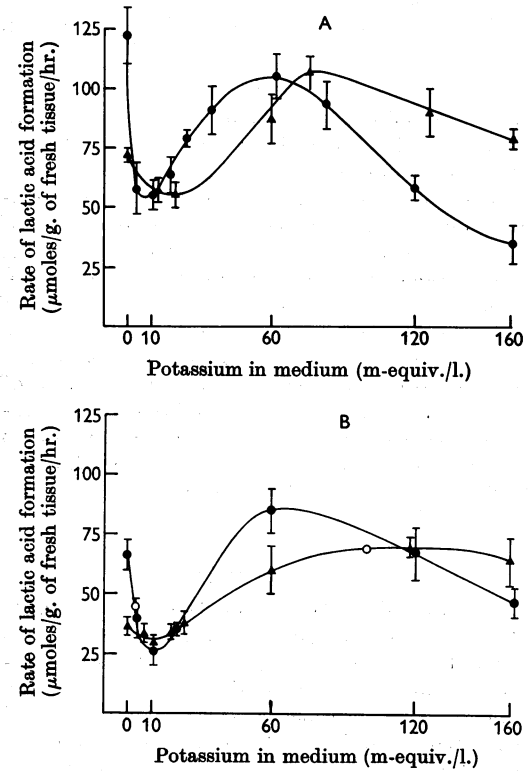

Fig. 6. Effect of potassium concentration in the medium on the aerobic glycolytic activity of brain slices. Experimental details are given in the text. A, Calcium absent; B, calcium (1·7 m-equiv./l.) present. ●, Iso-osmotic media; ▲, same media with the concentration of sodium chloride increased by 100 mM. The points represent the means of five or more determinations on different slices and the vertical lines show the S.D. Circles (○) in Fig. 6B indicate the results (interpolated) under the conditions of experiments of Dickens & Greville (1935).

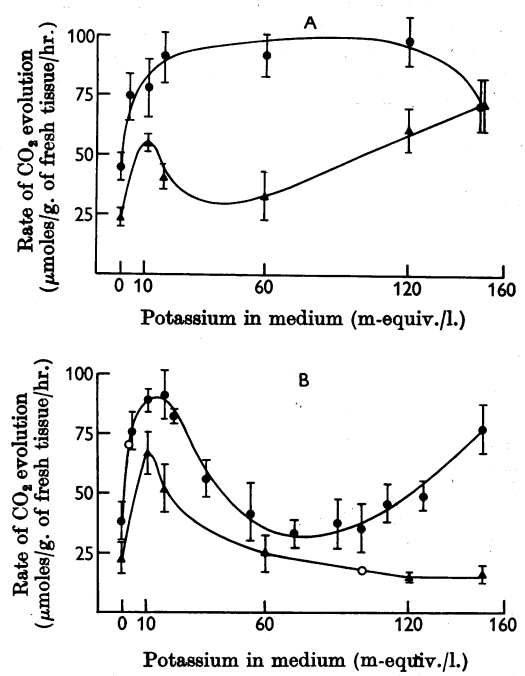

Fig. 7. Effect of potassium concentration in the medium on the anaerobic glycolytic activity of brain slices. Experimental details are given in the text. A, Calcium absent; B, calcium (1·7 m-equiv./l.) present. ●, Iso-osmotic media; ▲, same media with the concentration of sodium chloride increased by 100 mM. The points represent the means of five or more determinations on different slices and the vertical lines show the S.D. Circles (○) in Fig. 7B indicate the results (interpolated) under the conditions of experiments of Dickens & Greville (1935).

Table 2. *Effect of potassium on anaerobic glycolysis of potassium-depleted brain slices in the presence of pyruvate and glutamate*

Glucose (10 mM) was present in all cases. Iso-osmotic bicarbonate-buffered medium (calcium present) was used. Experimental details are given in the text. Results are given as means ± S.D. where four or more determinations were made.

	CO_2 evolved (μmoles/g. of fresh tissue/hr.)		
Concn. of K (m-equiv./l.)	0	3·6	60
Added substance			
None	54±2	74±2	23±9
Pyruvate (2 mM)	76±8	122±12	43±6
L-Glutamate (10 mM)	28±6	43±10	18
Pyruvate (2 mM) + glutamate (10 mM)	80±4	80±17	48±11

DISCUSSION

It has been shown, by using potassium-depleted slices, that low concentrations of potassium in the medium exert a stimulatory effect on the respiration of brain tissue. Previous workers (e.g. Ashford & Dixon, 1935; Dickens & Greville, 1935) had found that a very high concentration of potassium (100 m-equiv./l.) was needed to obtain strong stimulation. The observations of these workers are explained by the fact that in their controls the medium always contained some potassium so that the respiration already was stimulated and therefore further addition of a relatively large concentration of potassium was necessary to produce obvious further stimulation. Also, since the respiratory activity tends to be decreased when potassium is substituted for most of the sodium in the medium, the above-mentioned workers had to add potassium salt to the medium without decreasing the sodium salt. The inhibitory effect of potassium in low concentration on aerobic glycolysis and the stimulatory effect on anaerobic glycolysis were not well known because the controls in most studies had been made in 'normal' media, which contained about 2 m-equiv. or more of potassium/l., in which the effects of potassium approach maximum (see, for example, Dixon, 1949). Dickens & Greville (1935), however, did note that the rate of aerobic glycolysis was high in a medium containing sodium as the only cation and that the addition of small amounts of potassium lowered this activity. They also observed that the rate of anaerobic glycolysis was lower in a medium containing only sodium than in this medium with the normal low potassium concentration added.

The rate of uptake of potassium by brain slices (Fig. 4) is too rapid to permit us to determine whether the rate of metabolism changes as the potassium content of the slice increases or is constant at a rate determined by the potassium concentration in the medium. The results shown in Fig. 5, however, suggest that at moderate potassium concentrations it is the potassium content of the slice, and not that in the medium, that determines the rate of metabolism. Further evidence for this is the fact that in the absence of glucose, when slices do not accumulate potassium, no stimulation of respiration occurs on the addition of potassium to the medium. Studies on the interference with potassium uptake in brain slices by protoveratrine and certain other substances have indicated that this interference is correlated with lack of effect of potassium in the medium on oxygen uptake (F. Bilodeau & K. A. C. Elliott, unpublished work).

Quastel & Quastel (1961) suggested that the potassium:calcium ratio in the medium determines the stimulation of respiration. The results given in Fig. 3, however, do not agree with this. The absolute increases in oxygen uptake caused by addition of potassium to the medium are about the same in the presence and absence of calcium; the relative increases are actually greater in the presence of calcium. The effects of potassium and calcium thus seem to be independent of each other.

McIlwain (1952) suggested that the effect on respiration of high potassium in the medium is the result of increased expenditure of energy in maintaining the intracellular/extracellular potassium concentration gradient. The results as illustrated in Fig. 5 are not in obvious agreement with this postulate since the respiration rate continued to increase while the intracellular/extracellular potassium gradient decreased.

It is probable that potassium exerts effects of more than one kind; effects on permeability of membranes and on the activity of individual enzymes are presumably involved. Kini & Quastel (1959) suggested that potassium stimulates glucose oxidation through acceleration of the aerobic conversion of pyruvate into acetyl-coenzyme A, the rate of which conversion limits the whole process of glucose utilization. As Fig. 6 shows, aerobic lactate production is high in iso-osmotic medium in the absence of potassium, presumably owing to a relatively low rate of oxidation of pyruvate.

Table 1 shows that, when pyruvate itself was added, the respiratory activity was rapid and a low concentration of potassium stimulated respiration very little, presumably because a high concentration of pyruvate can compensate for a deficiency of potassium. The stimulation by potassium of the respiratory activity in the presence of glutamate and γ-aminobutyrate may be due to an effect of potassium on the pyridoxal phosphate-dependent enzymes (see Happold, 1955) involved in the metabolism of these amino acids. Only by close analysis of such primary effects could the complex overall effects of varying potassium concentrations be explained.

It is well known that displacements of potassium are a concomitant of physiological activity in nervous structures and it is now apparent that such displacements, even though they involve quite moderate changes in extracellular potassium concentrations, would be co-ordinated with marked changes in intracellular potassium and in energy metabolism. The production of acid (lactic acid) would also be definitely affected by potassium shifts *in vivo* and this would undoubtedly affect the dilatation of blood vessels and, consequently, the local circulation.

SUMMARY

1. When brain slices are kept in a potassium-free medium at 0° they rapidly lose most of their potassium without serious change in their oxygen uptake as subsequently measured at 38°.

2. The rate of oxygen uptake by potassium-depleted slices in the absence of potassium falls with time. It is rendered constant, and increased by 40–150 %, depending on the conditions, by the presence of 3·6 m-equiv. of potassium/l. in the medium, and reaches a maximum with about 60 m-equiv. of potassium/l.

3. The respiratory activity with or without potassium is higher in the absence than in the presence of calcium but the effect of potassium is more marked in the presence of calcium.

4. Stimulation of respiration by potassium in the medium does not occur in the absence of added substrate; it occurs appreciably with pyruvate, with glutamate and slightly with γ-aminobutyrate as added substrates.

5. Under aerobic conditions in the presence of glucose, cold-pretreated slices accumulate potassium rapidly during the first few minutes at 38°. The maximum difference reached between the concentration of potassium in the slices and that in the medium is only about one-third of that found *in vivo*. This difference becomes smaller in media containing high concentrations of potassium.

6. When the medium contains between 0 and 18 m-equiv. of potassium/l. the stimulation of respiration is approximately proportional to the concentration of potassium in the slice.

7. The aerobic glycolytic activity of potassium-depleted slices is appreciable in the absence of potassium. It is inhibited by concentrations of potassium in the medium of up to 10 m-equiv./l. but rises again in the presence of higher potassium concentrations.

8. The anaerobic glycolytic activity of potassium-depleted slices is low in the absence of potassium. It is stimulated by low concentrations of potassium and may be decreased again in the presence of higher concentrations.

9. Respiration and glycolysis are affected by sodium in the medium and complete replacement of sodium by potassium gives effects which cannot be attributed to potassium alone.

REFERENCES

Ashford, C. A. & Dixon, K. C. (1935). *Biochem. J.* **29**, 157.
Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
Cummins, J. T. & McIlwain, H. (1961). *Biochem. J.* **79**, 330.
Dickens, F. & Greville, G. D. (1935). *Biochem. J.* **29**, 1468.
Dixon, K. C. (1949). *J. Physiol.* **110**, 87.
Elliott, K. A. C. (1955). In *Neurochemistry*, p. 53. Ed. by Elliott, K. A. C., Page, I. H. & Quastel, J. H. Springfield, Ill.: Charles C. Thomas.
Ghosh, J. J. & Quastel, J. H. (1954). *Nature, Lond.*, **174**, 28.
Gore, M. B. R. & McIlwain, H. (1952). *J. Physiol.* **117**, 471.
Happold, F. C. (1955). *Abstr., 3rd int. Congr. Biochem., Brussels*, p. 32.
Kini, N. N. & Quastel, J. H. (1959). *Nature, Lond.*, **184**, 252.
Kurokawa, M. (1960). *J. Neurochem.* **5**, 283.
McIlwain, H. (1952). *Biochem. J.* **52**, 289.
Pappius, H. M. & Elliott, K. A. C. (1956*a*). *Canad. J. Biochem. Physiol.* **34**, 1007.
Pappius, H. M. & Elliott, K. A. C. (1956*b*). *Canad. J. Biochem. Physiol.* **34**, 1053.
Pappius, H. M. & Elliott, K. A. C. (1958). *Canad. J. Biochem. Physiol.* **36**, 217.
Quastel, J. H. & Quastel, D. M. J. (1961). *The Chemistry of Brain Metabolism in Health and Disease*, p. 11. Springfield, Ill.: Charles C. Thomas.
Terner, C., Eggleston, L. V. & Krebs, H. A. (1950) *Biochem. J.* **47**, 139.
Tower, D. B. (1958). *Proc. 4th int. Congr. Biochem., Vienna*, **3**, 213.
Tsukada, Y., Nagata, Y. & Takagaki, G. (1957). *Proc. imp. Acad. Japan*, **33**, 510.
Webb, J. L. & Elliott, K. A. C. (1951). *J. Pharmacol.* **103**, 24.
Weil-Malherbe, H. (1938). *Biochem. J.* **32**, 2257.