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Electrical Pulses and the Potassium and Other Ions of Isolated Cerebral Tissues

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The differential distribution of sodium and potassium salts which is found in most animal tissues is of special significance in neural systems, for here the substances take part in the rapid ion movements associated with the nerve impulse. Loss of K^+ ions and gain in Na^+ ions on electrical stimulation of peripheral nerve is well documented (Keynes, 1951; Hodgkin, 1958), and it is a general impression that a major part of energy utilization by cerebral tissues is connected with the work performed in maintaining their characteristic ion distribution. When cerebral tissues are removed from an animal, promptly sliced and placed in media similar in content of Na^+ and K^+ ions to the extracellular fluids of the body they lose K^+ ions and gain Na^+ ions and water. Respiration in glucose is, however, associated with restoration of a large part of the K^+ ions and with extrusion of Na^+ ions (Terner, Eggleston & Krebs, 1950). Studies with isotopically-labelled potassium (^{42}K) salts showed that this was accompanied by a considerable turnover of the K^+ ions of the slices, of approximately 3·5–4% of the tissue content/min. (Krebs, Eggleston & Terner, 1951).

The metabolism of isolated cerebral tissues in media similar to those employed in studying potassium movement, is affected by applied electrical pulses; energy-rich phosphates of the tissue are diminished and energy-yielding reactions accelerated (McIlwain, 1951, 1959). The content of Na^+ and K^+ ions and the exchange of K^+ ions have now been examined in electrically stimulated tissues. The tissues' respiratory and glycolytic responses to pulses have already been shown to be dependent on the addition of Na^+ ions to the medium in which they are incubated (Gore & McIlwain, 1952), and a similar dependence on K^+ ions is reported below.

EXPERIMENTAL

Ion movements

Tissues and electrodes. The procedures described by McIlwain (1960, 1961) were followed. Guinea pigs were used throughout the studies; they were stunned by a blow on the neck, killed by bleeding and the brain was removed in less than 3 min. The cerebral cortex was sliced to an average thickness of 0·35 mm. with a blade and recessed glass guide, weighed after draining on glass and placed in vessels which were incubated at 37·5° within 30 min. of the animal's death. Electrical pulses were supplied from the generator of Ayres & McIlwain (1953) to silver-grid electrodes at 100/sec., and were condenser pulses of peak potential 10v and time constant 0·4 msec.; for choice of these conditions see McIlwain (1954).

Ion movement and tissue composition. These studies were carried out in the rapid-transfer apparatus of Heald & McIlwain (1956) with electrodes with nylon gauze (McIlwain, 1960) and tissue slices of 80–100 mg. fresh wt. in 5 ml. of bicarbonate medium of composition (mM): NaCl, 120; KCl, 4·8; KH_2PO_4, 1·2; $MgSO_4$, 1·3; $CaCl_2$, 2·8; glucose, 10; $NaHCO_3$, 26. The initial content of Na^+ ions in the medium was thus 146 mM; its K^+ ion content was 6·0 mM and Cl^- ion content 130·4 mM. The medium was equilibrated with O_2+CO_2 (95:5, v/v), which was maintained in the vessels during incubation (Fig. 1).

In typical experiments, six weighed tissues were mounted in electrodes, incubated for 30–60 min., pulses were applied to certain of the tissues for periods of 10 sec.–30 min. and the electrode carrying its tissue was rapidly transferred to a second vessel for rinsing or extraction. Additional procedures adopted with ^{42}K are described below.

Rinsing and extraction. During the rapid transference of tissues for analysis, adhering fluid is inevitably also transferred; the effect of this on apparent tissue composition has previously been computed by calculation or minimized by a quick rinse in the cold fixing agent to be used in a subsequent extraction (Kurokawa, 1960). Because of the preponderance of Na^+ ions in the incubating fluids, rinsing is especially important in the present experiments, and has been appraised as shown in Fig. 2. Cold 0·3M-sucrose was chosen as an innocuous sodium-free medium and slices were released from the holder into an adjacent dish containing 100 ml. of the sucrose; after defined times they were picked from the dish with a mounted bent platinum wire and placed in test tubes containing 1 ml. of conc. HNO_3 or trichloroacetic acid for analysis. The minimum time occupied by the transference was 3 sec., and in this time a sudden loss occurred in sodium and chloride, without appreciable change in potassium. After 3 sec. all three substances changed only slowly, and thus ample latitude was given for satisfactory transfer. Apart from the experiments of Fig. 2, all transferences were made in 3–4 sec.

Our interpretation of the change during 3 sec. in sucrose is that it represents loss of adhering medium (note that the apparent sodium and chloride concentrations at zero time, when referred to unit weight of the pre-weighed tissue, are greater than those of the medium) and that loss of the substances determined, even from the extracellular spaces of the tissue, is relatively slow.

Experiments with ^{42}K. $^{42}K_2CO_3$ was received from the Atomic Energy Research Establishment, Harwell; usually

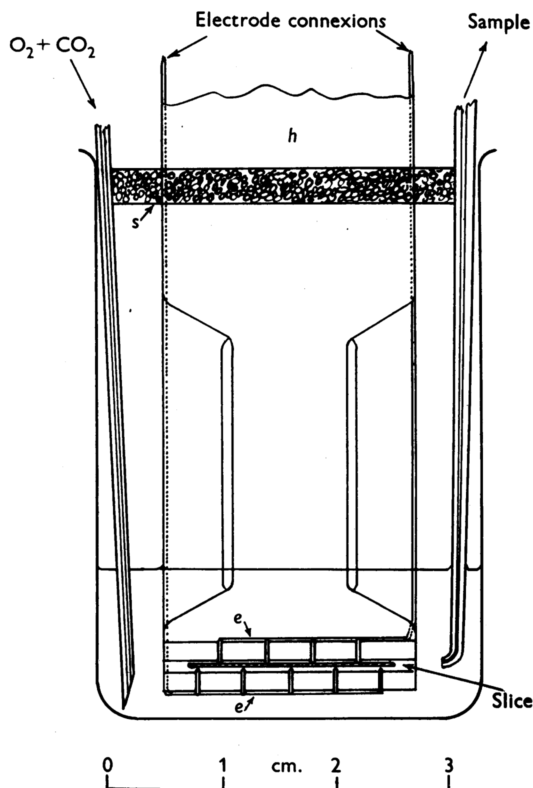

Fig. 1. Incubation and sampling in the quick-transfer apparatus. The slice is within the electrodes *e*, which form the lower parts of the holder *h*. The holder carries the sponge-rubber seal *s*, which enables the chosen gas atmosphere to be maintained in a 20 ml. beaker. For sampling, the capillary pipette is inserted at the position shown; for full details of the holder, its electrodes and thermostat, see Heald & McIlwain (1956) and McIlwain (1960).

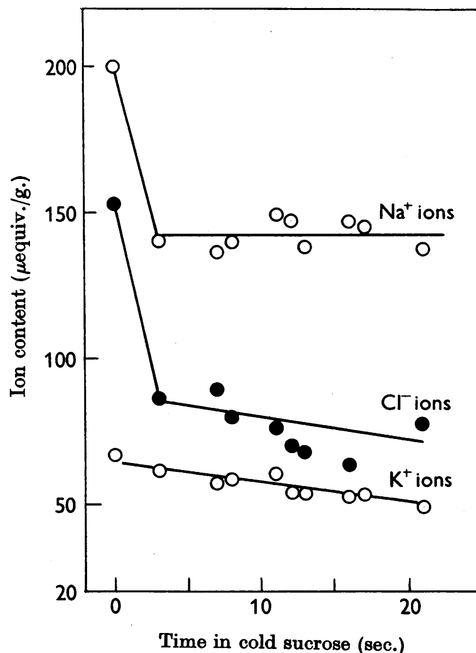

Fig. 2. Rinsing cerebral slices in cold sucrose. Tissue slices in quick-transfer electrodes were incubated in glucose–bicarbonate medium in quick-transfer electrodes for 30 min. and then were either released immediately into 3% (w/v) trichloroacetic acid (giving the values quoted at zero time) or released into 0·25M-sucrose at 0° and, after the periods shown, transferred to the trichloroacetic acid. Each vertical group of three points represents one slice.

two experiments were performed with one sample, the first as soon as practicable after its removal from the pile (about 12 hr.) and the second on the following day. The sample (100 mg.) was dissolved in 5 ml. of water and brought to pH 7·4 with 0·1 N-HCl (approx. 0·3 ml.). All the KCl of a batch of the bicarbonate medium described above was replaced by the ^{42}KCl to make a stock ^{42}K–bicarbonate medium. In the first day's experiments, the initial incubation medium was prepared from 1 ml. of the stock ^{42}K–bicarbonate medium plus 4 ml. of ordinary bicarbonate medium; on the second day the two solutions were used in the proportion of 2:3.

In each case, experiments comprised an initial incubation of 30 min. at 37·5°, after which the slice, still in its holder, was quickly rinsed in 5 ml. of non-radioactive glucose–bicarbonate medium also at 37·5°. With its holder it was then placed in a further 5 ml. of ordinary glucose–bicarbonate medium in a beaker (*A*) of the quick-transfer apparatus and with an $O_2 + CO_2$ atmosphere. Here incubation was continued and samples of 0·05 or 0·1 ml. of the medium were taken by capillary pipettes at the intervals indicated in the individual experiments. By inserting the capillary pipette through the sponge-rubber seal of the quick-transfer apparatus, as shown in Fig. 1, on the side opposite to that which receives the gas supply, it was possible to maintain the $O_2 + CO_2$ atmosphere during sampling.

During this period of incubation and sampling, electrical pulses were applied to some vessels. At its termination, the slices were successively released to cold sucrose and after 3 sec. were ground in 10 ml. of 3% trichloroacetic acid. Dilutions of the tissue suspension, and of the incubation medium, were placed in 10 ml. tube counters (M-6H of 20th Century Electronics Ltd.) and radioactivity was determined with an Ekco scaler model N530F.

The radioactivities of each sample (medium and tissue) from a given beaker at stage *A* of the experiment were counted in succession during a period of up to 30 min. These counts were corrected for radioactive decay during the counting period; corrections ranged from 0 to 3%. From these calculated values for radioactivity at the beginning of the counting period, addition in appropriate proportions gave a value for total radioactivity in each beaker. In Figs. 6 and 7, radioactivity in the fluid samples (corrected values) have been expressed as percentages of the total radioactivity in the appropriate beaker. This method of expression gave data with which one slice could be compared with another even though the amount of ^{42}K associated with the slices differed, which was the case when the slices differed in weight or were derived from different experiments.

In the experiments of Fig. 5, the course of ^{42}K movement during a period of 60 sec. was measured by transferring a slice from beaker *A* to one after another of a series of beakers each holding 5 ml. of bicarbonate medium at 37° and with an $O_2 + CO_2$ atmosphere. The time occupied by each transference was less than 0·5 sec. and five transferences of one slice were made in the course of 1 min.; the several slices of an experiment were handled in succession. Results were calculated and expressed as already described.

Influx of ^{42}K into the slices was also measured in the quick-transfer apparatus. After the preincubation of 30 min. at 37·5° in 5 ml. of glucose–bicarbonate medium, 0·1 ml. of ^{42}K–glucose–bicarbonate medium was added by capillary pipette. Electrical pulses were applied to some slices of an experiment and after 5 min. the slices were transferred through sucrose to trichloroacetic acid as described above. Determination of radioactivity in the media and in tissue extracts was carried out as described above.

Respiratory response to pulses

The tissues for these experiments were prepared in media lacking or diminished in potassium salts. The media were based on the glucose–glycylglycine medium of Kratzing (1953) and prepared by replacing its KCl and KH_2PO_4 by equimolar amounts of sodium salts; small additions of KCl were made in instances described in the individual experiments. Slices obtained as described above were floated from the cutting blade and guide into the potassium-deficient medium, trimmed to size (approx. 45 mg.), drained on a glass surface, weighed, returned to a second dish of potassium-deficient medium and mounted in grid electrodes *H* and, with the electrodes, placed in conical manometric vessels *E* (Ayres & McIlwain, 1953) containing 3·5 ml. of the medium. Respiratory rates were measured manometrically; for further details see Fig. 8.

Analytical methods

Sodium and potassium. Slices, after brief rinsing in cold sucrose, were placed in a test tube containing 2·5 ml. of conc. HNO_3 or were ground in 2·5 ml. of 10% (w/v) trichloroacetic acid. After 12–24 hr. at room temperature, samples, usually of 0·25 ml., were diluted to 10 ml. and the sodium and potassium determined with a flame photometer (Evans Electroselenium Ltd., model A) with the appropriate filters. Standard curves were constructed from readings taken in the presence of the concentration of HNO_3 or trichloroacetic acid in the diluted samples (Collins & Polkinhorne, 1952), and with Na^+ and K^+ ions present in a molar ratio of 2:1. This is the approximate ratio of Na^+ to K^+ ions in typical samples, and the procedure is similar to that of Pappius & Elliott (1956*a*, *b*).

Chloride. This was determined by the method of Lowry *et al.* (1954) and Ames & Nesbett (1958) carried out on a larger scale. Excess of mM-$AgNO_3$ was mixed with 0·1 ml. of a 0·1N-HNO_3 extract or of the trichloroacetic acid extract of the tissue (see above) in a 2 ml. centrifuge tube. After 30 min. at room temperature the tube was centrifuged and, to 0·1 ml. of the supernatant, 3 ml. of the rhodanine reagent was added and the determination completed according to Lowry *et al.* (1954).

Inorganic phosphate and phosphocreatine. Slices from the quick-transfer apparatus, with or without the rinse in sucrose, were placed in 2·5 ml. of ice-cold trichloroacetic acid in a test tube homogenizer with a loosely fitting pestle, ground and the phosphates determined as described by R. J. Woodman & H. McIlwain (in preparation); methods were based on those of LePage (1949) and Heald (1954).

Tissue content of lactic acid. Slices were quickly rinsed in sucrose and extracted with trichloroacetic acid as for sodium and potassium determination, and samples of extract were transferred to $CuSO_4$, lime was added and the determination completed according to Barker & Summerson (1941).

RESULTS

Tissue composition and its change with pulses

Phosphocreatine, inorganic phosphate and lactic acid. In the Experimental section are described the incubation and transfer procedures adopted during the present experiments. In order to appraise the metabolic status of the tissue after these procedures, its phosphocreatine and inorganic phosphate contents were determined on several occasions during the course of the studies and a few measurements were made of tissue lactate. These constituents are sensitive to inadequacies in the supply of O_2 or glucose to the tissue, or to other abnormalities. The values obtained (Table 1) indicate satisfactory metabolic conditions (see McIlwain, Buchel & Cheshire, 1951; McIlwain, 1959). Moreover, the application of electrical pulses caused the loss of phosphocreatine and increase in inorganic phosphate typical of the normal tissue (Anguiano & McIlwain, 1951; McIlwain & Gore, 1951). Phosphocreatine has also been determined in tissues incubated in media containing ^{42}KCl prepared in the laboratory from $^{42}K_2CO_3$, and also found to be normal and to undergo a normal fall with pulses. The tissue lactate and its change with pulses are in accordance with the observations of McIlwain & Tresize (1956).

Sodium, potassium and chloride. (*a*) Normal content. The present method of handling cerebral tissue has been found to give a potassium content of 60–65 μmoles/g. wet wt., or 5–25 μmoles/g. greater than those quoted by Terner *et al.* (1950) and Pappius & Elliott (1956*a*, *b*) after incubation in comparable media. This may be due to small differences in the preparation of the tissue, to the mechanical support given to it during incubation or to the subsequent transfer procedures. The majority of the experiments of Terner *et al.* (1950) and of Krebs *et al.* (1951) were carried out with the further addition of glutamate, which affords still higher values for the potassium associated with a given weight of tissue. It has, however, been observed that this is accompanied by intracellular uptake of fluid (Pappius & Elliott, 1956*a*, *b*) and that the cellular concentration of potassium salts falls. Glutamate also lowers the phosphocreatine of cerebral tissues (McIlwain, 1952), renders their respiration and glycolysis largely unresponsive to electrical pulses and diminishes the resting potentials observable in polarized elements of the slices (Hillman & McIlwain, 1961). For these reasons glutamate has generally not been added in the present experiments, but its effects were studied in a few instances (see below).

The tissue absorbed much aqueous fluid on preparation and incubation, as has also been found by

Table 1. *Tissue composition and response to pulses*

Slices of guinea-pig cerebral cortex or liver weighing about 80 mg. were incubated in 5 ml. of glucose–bicarbonate medium in the electrodes designed for rapid transfer. After 30 min., condenser pulses were applied to some of the tissues. The slices were then transferred to 0·25 M-sucrose and plunged into cold trichloroacetic acid. They were extracted by grinding and the phosphates and ions were determined. (See the Experimental section for further details.) Values give the mean result ± S.D. of the number of experiments indicated in parentheses. Pulses of time constant 0·4 msec., peak potential 10 V, were applied at 100/sec. for 10 min. except in the instance marked with an asterisk, when application was for 1 min.

Tissue	Substance determined	Tissue content (μmoles/g. fresh wt.) No pulses	With pulses
Cerebral cortex	Phosphocreatine	1·5 ± 0·14 (8)	0·45 ± 0·15 (6)
	Inorganic phosphate	3·1 ± 0·3 (8)	4·0 ± 0·6 (6)
	Lactic acid	2·1 ± 0·5 (4)	8·1 ± 0·7 (4)
			4·0 ± 0·9 (4)*
	K	63 ± 5 (8)	47 ± 2·7 (6)
	Na	146 ± 11 (8)	152 ± 13 (6)
Liver	K	36·2 ± 6·4 (4)	35·5 ± 5·0 (4)

Table 2. *Tissue water, potassium, sodium and chloride*

Guinea-pig cerebral cortex was taken for analysis: (*a*) immediately after excision; (*b*) after cutting in glucose–bicarbonate medium; (*c*) and (*d*) after incubation as described in Table 1. Dry wt. was obtained after 2 hr. at 105° and reweighing at 3 hr.

Determination	Condition of tissue: (*a*) Fresh	(*b*) Cut	(*c*) Incubated	(*d*) Incubated and pulses applied
Dry wt. (%)	20·3 ± 0·8 (4)	13·8 ± 1·1 (4)	11·9 ± 1·8 (5)	10·4 ± 1·7 (5)
K (μequiv./g. dry wt.)	550 ± 11 (6)	384 ± 64 (6)	442 ± 43 (8)	348 ± 29 (8)
Na (μequiv./g. dry wt.)	256 ± 8 (6)	862 ± 28 (6)	1015 ± 40 (5)	1036 ± 93 (6)
Cl (μequiv./g. dry wt.)	143 ± 14 (4)	406 ± 57 (4)	616 ± 48 (5)	478 ± 72 (6)

Leaf (1956) and Pappius & Elliott (1956*a*, *b*). In Table 2 the tissue dry weight and ionic constituents are shown, derived from experiments which include those of Table 1. Expressing the ionic constituents in terms of tissue dry weight, in comparison with the values obtained by Leaf (1956) using rat cerebral cortex, again shows tissue potassium to be well maintained on incubation. The sodium and chloride in the incubated tissue (*c*, Table 2) are also high, indicating the fluid absorbed to be comparable in composition to the medium rather than water. Thus comparison of the dry weights (*a*) and (*c*), Table 2, shows the uptake of 3·48 g. of water/g. dry wt. of tissue; the increase in chloride of 473 μequiv./g. dry wt. would be contained in 3·62 ml. of medium. Taking into account observations of Leaf (1956), Pappius & Elliott (1956*a*, *b*), Thomas & McIlwain (1957) and Tower (1960), this fluid may be concluded to be extracellular. The 3·62 ml. of medium would carry 530 μequiv. of sodium/g. dry wt. of tissue; the tissue acquires more sodium than this, as has been noted also by Leaf (1956) in cerebral tissues of the rat. Interpretation of this change, and of that in chloride, would require allocation of sodium and chloride to the intracellular and extracellular compartments of the tissue, which is not examined in the present study.

(*b*) Effect of pulses. Electrical pulses diminished the potassium content of cerebral tissues but not of similarly maintained slices of liver (Table 1). The change in guinea-pig cerebral cortex in 10 min. is seen to involve loss of 25 % of the tissue potassium, or a molar quantity very much greater than that in the other cerebral constituents which have been examined. Thus the change in inorganic phosphate or phosphocreatine was about 6 % of that in potassium. Expressed in terms of tissue dry wt., pulses are seen from Table 2 to cause the loss of an amount of potassium salts greater than the total quantity reassimilated on incubation *in vitro*. Certain constituents or metabolic activities in cerebral tissues have been found to differ in concentration or magnitude in slices cut from different depths in the cerebral cortex, and Table 3 shows an examination of potassium salts from this point of view. The samples represent two-thirds of the thickness of the cortex, and are seen to be uniform in potassium content; pulses in all cases caused loss of K^+ ions. Loss of K^+ ions on applying pulses is seen to be accompanied by a rather greater loss in Cl^- ions (Table 2); Na^+ ions changed little if at all (Tables 1 and 2). Appraising the changes in all three ions suggests that some sodium had entered the cells on application of pulses; specific examination of this is deferred.

Table 3. *Potassium content of slices from different depths in the cerebral cortex*

Slices designated 1 were 0·35 mm. thick and were cut from the outer convexity of the guinea-pig cerebral cortex; slices 2 were from the remaining block, and slices 3 were cut after slices 2. Incubation and other details were as described in Table 1, except that pulses were applied for 5 min. only.

Slice	Content of K after incubation (μequiv./g. fresh wt.) No pulses	With pulses
1	63±4·8 (8)	56·4±2·5 (5)
2	60±4·8 (8)	51·5±3·2 (4)
3	61±5·3 (8)	48·6±2 (5)

The effect of pulses on tissue potassium proved to be largely independent of added oxidizable substrates (Table 4). In the absence of such substrates, the potassium content of the cortical slices was lower than with glucose; glutamate afforded concentrations similar to those in the presence of glucose, and the greatest potassium content was found with both glucose and glutamate, in agreement with Terner *et al.* (1950). In each of these four cases, pulses diminished tissue potassium by amounts of 14–23 μmoles/g.

Course of change in tissue potassium

Net movement with pulses. Control observations during the preceding experiments showed the potassium content of cerebral slices to be constant between 30 and 60 min. of incubation in oxygenated glucose media. When observations were made at briefer intervals the stability was confirmed, but application of pulses brought about a discernible loss of potassium salts within 1 min. (Fig. 3). The

Table 4. *Effect of substrates and electrical pulses on tissue potassium*

Experiments were carried out and results are expressed as described in Table 1, except for changes in substrate.

Substrate (mM)	Tissue potassium (μequiv./g. fresh wt.) No pulses	With pulses	Change
None	48±2 (5)	25±2 (4)	−23
Glucose (10)	61±8 (35)	47±4 (8)	−14
L-Glutamate (20)	62±6 (3)	40±3 (3)	−22
L-Glutamate (20) and glucose (10)	87±8 (6)	71±10 (6)	−16

loss continued at a diminished rate until 10 min., after which little change occurred. These observations involved the analysis of many tissues, and the inherent variation in their potassium content precluded accurate estimation of the rate of potassium loss during the initial phase, though the loss in 1 min. was statistically significant, with $P < 0{\cdot}01$. The variation also made it unprofitable to attempt to examine shorter time intervals, or to analyse mathematically the course of change in potassium. However, the data of Fig. 3 imply an initial rate of loss of at least 300 μequiv. of potassium/g. fresh wt. of tissue/hr. during the first minute, and a continued loss of about 40 μequiv./g. fresh wt./hr. until, after 10 min., stability was approached.

Switching off pulses at this point was followed by a remarkably rapid reassimilation of potassium salts (Fig. 4). About 10 μmoles/g. of tissue were regained in 1 min., after which only a relatively small change occurred. Slices cut first from a hemisphere (outer slices) differed from those cut second or third in the concentration of potassium attained, and are indicated separately in Fig. 4 (see also Fig. 8). This gives an indication of the heterogeneity of cerebral tissues which is important in appraising the findings (see Discussion).

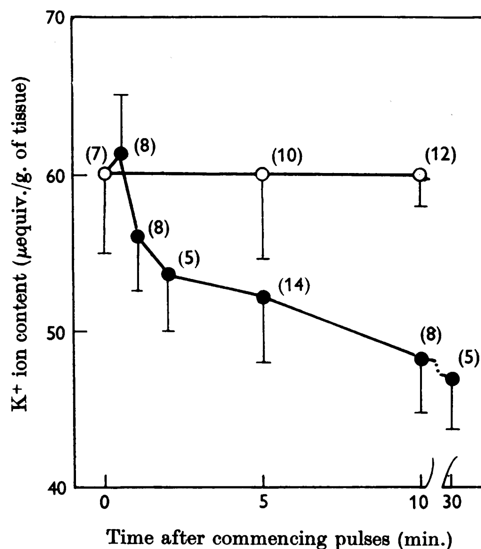

Fig. 3. Loss of potassium from guinea-pig cerebral cortex subjected to electrical pulses. The slices were in the quick-transfer apparatus and, after a preliminary incubation of 30 min., alternating condenser pulses of time constant 0·4 msec. and peak potential 10v were applied at 100/sec. Slices were then floated into 0·25M-sucrose and rapidly transferred into trichloroacetic acid. Potassium was determined by flame photometry; points give the contents from the number of slices indicated in parentheses; vertical lines indicate the S.D. of the means. Differences between the potassium content of slices at zero time and after 1 and 2 min. of pulses showed $P < 0{\cdot}01$; the tissue exposed to pulses for 5 and 10 min. also differed from their controls without pulses, with $P < 0{\cdot}01$. Outer slices: ○; inner slices, ● (see text).

Efflux of ^{42}K. Potassium movements during brief periods have been examined by using the isotope ^{42}K. In these experiments preincubation of the tissue took place in media containing ^{42}KCl, and after 30 min. the tissue was rinsed and incubated in a medium of the same composition except that it contained unlabelled potassium salts. Without application of pulses, ^{42}K rapidly appeared in the medium during incubation, indicating that tissue potassium was undergoing considerable exchange with that of the medium even while its total quantity was stable. The initial rate of efflux corresponded to 11% of the tissue ^{42}K/min., or 400 μequiv./g. of tissue/hr. (Fig. 5).

When condenser pulses were applied to tissue preincubated with ^{42}K, efflux of the isotope increased by 60–80% of its control value (Fig. 5). This increased rate corresponded to 600–750 μ-equiv. of potassium/g. of tissue/hr. and was established within the first 10 sec. application of pulses, despite its involving diffusion of ^{42}K from the tissue to the medium. In the experiment of

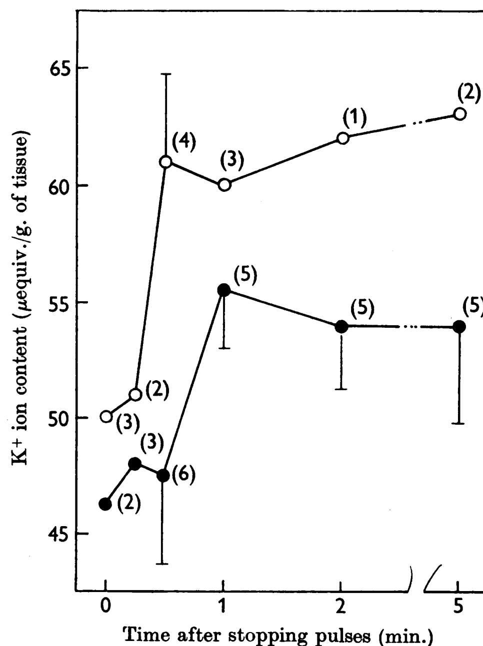

Fig. 4. Reassimilation of K^{+} ions after stimulation for 10 min. Condenser pulses were applied for 10 min. and, at appropriate intervals after stopping the pulses, slices were floated into 0·25M-sucrose; other details were as given in Fig. 3.

Fig. 5 (*a*), pulses were applied for 10 sec. only. This brief application of pulses doubled the rate of efflux both during and subsequently to their application, indicating an increased efflux of the isotope even during the period of recovery of total tissue potassium.

The efflux of ^{42}K induced by pulses was found to be relatively independent of the metabolic status of the tissue. In the absence of glucose (Fig. 6) the unstimulated efflux was greater than in the normal medium, and was further increased by applied pulses. Expressing the efflux with pulses as a percentage of that without pulses gives, however, a greater proportional change in the presence of glucose; tissue content of total potassium was greater with glucose (Table 4), as in the experiments of Terner *et al.* (1950). Glutamate added to glucose-containing media also caused increased efflux of ^{42}K, and this again was further augmented by application of pulses (Fig. 7).

Influx of ^{42}K. Labelled potassium salts added to media in which cerebral tissues are incubated were shown by Krebs *et al.* (1951) to enter the tissue rapidly; with similar experimental arrangements and calculations with the present glucose–bicarbonate medium, the values of Table 5 were obtained. The rate of entry in normal tissues corresponded to 5·4 % of the tissue potassium/min. over the interval

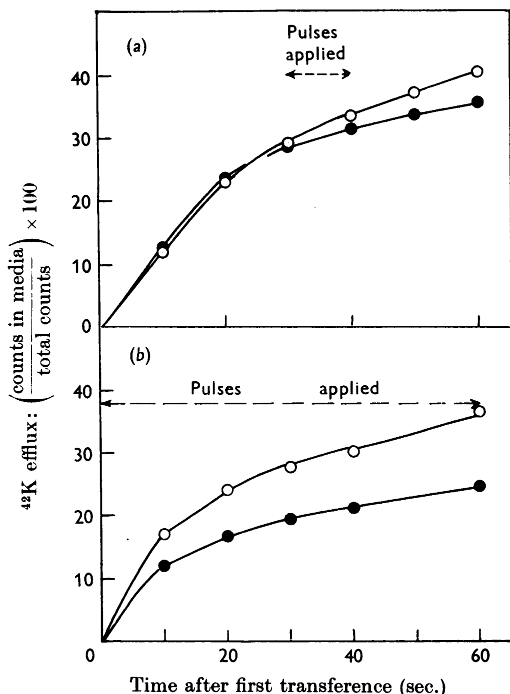

Fig. 5. Radioactive potassium efflux during brief periods. The slices, held in rapid-transfer electrodes, were incubated for 30 min. in 5 ml. of ^{42}K–glucose–bicarbonate medium (see the Experimental section). Tissue was then rinsed quickly and transferred at 10-sec. intervals from one to another of a series of beakers, each containing 5 ml. of fresh medium. The tissue was then floated into 0·25 M-sucrose and rapidly transferred into 10 ml. of trichloroacetic acid. Alternating condenser pulses (of time constant 0·4 msec., peak potential 10v at 100/sec.) were applied: (*a*) to slices (○) between 30 and 40 sec.; (*b*) to slices (○) between 0 and 60 sec. The filled-in points (●) give their respective controls without pulses. The radioactivity of each beaker and slice was counted and the efflux calculated as the sum of the counts in the media at different stages, as a percentage of the total counts from the slice and all media through which it had passed. Corrections for radioactive decay were made.

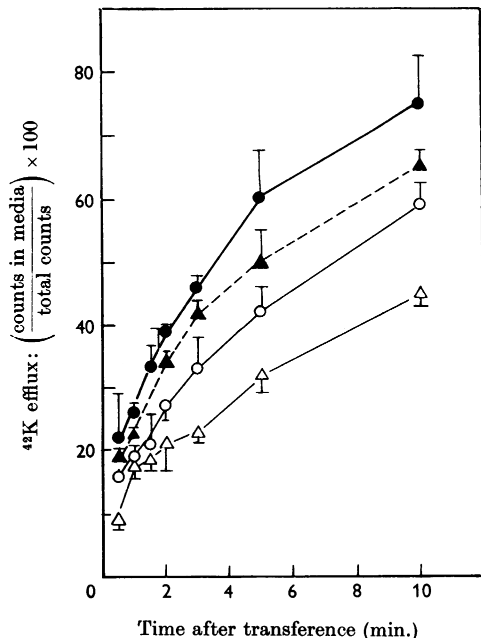

Fig. 6. Movements of ^{42}K from cerebral slices with and without glucose. The slices were preincubated at 37° in ^{42}K medium, rinsed and transferred quickly into 5 ml. of medium: ○, containing no glucose; ●, containing no glucose and with application of pulses; △, with glucose; ▲, with glucose and applied pulses. Samples (0·1 ml. of medium) were taken at appropriate time intervals. After 10 min. the tissue was floated into cold 0·25 M-sucrose and ground in trichloroacetic acid. Condenser pulses of the characteristics described in Fig. 5 were applied to the two sets of tissues indicated, for the whole 10 min. shown. Radioactivity of the samples was counted in a 10 ml. liquid tube counter, and expressed as total counts in the medium at that time. Corrections for radioactive decay and change in volume were made. Points give the amount of ^{42}K appearing in the media as the percentage of total ^{42}K added; vertical lines indicate S.D. of mean; each point corresponds to four determinations on different slices.

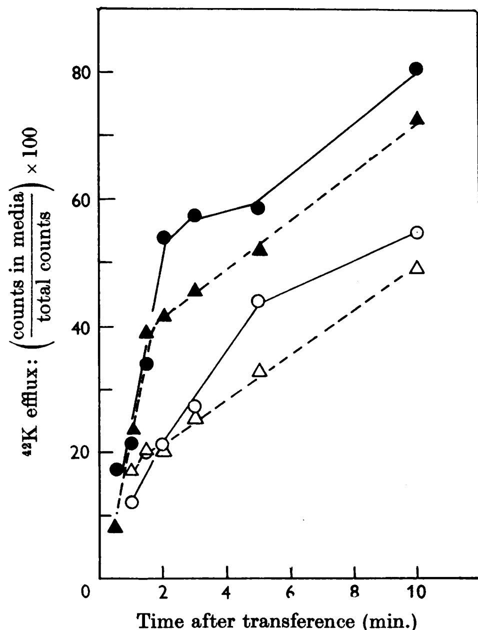

Fig. 7. Glutamate and the efflux of radioactive potassium from cerebral slices. Experimental conditions were as given in Fig. 6, except that glutamate (to 10 mM) was added to some vessels; △, glutamate alone; ▲, glutamate with pulses; ○, with glutamate and glucose; ●, with glucose, glutamate and pulses. Points give the average of two determinations of the percentage of the total ^{42}K appearing in the medium.

of 5 min. When pulses were applied, influx of ^{42}K calculated with this formula on the basis of the final K^+ ion content of the tissue rose to an average value of 12·6 %. This took place despite a net movement of potassium in the opposite sense (Fig. 3). The net movement of potassium averages 2·5 % of the potassium content/min. during the first 5 min. of pulses (Fig. 3). Although this makes the formula employed in calculation not strictly correct, increase in ^{42}K influx with pulses is a direct experimental observation (Table 5).

Dependence of metabolic response to pulses on potassium salts

Preliminary experiments showed that to lower effectively the concentration of potassium salts in slices of guinea-pig cerebral cortex it was necessary to handle the tissues throughout in media without added potassium salts. Even so, the media in which the tissues were first placed could accumulate appreciable quantities of K^+ ions. Thus in a typical experiment slices (0·7 g.) were cut and floated, from the blade and guide used in cutting, into a dish of 15 ml. of medium; here they were cut to size before weighing and placing in manometric vessels. In experiments in which the medium of the dish was initially potassium-free, it was found after receiving the slices to have become 2·6–2·8 mM in potassium salts; thus the concentration in this medium had risen to about half that of an ordinary Krebs–Ringer fluid (6 mM-K^+ ions). This implies a loss of 55–60 μmoles/g. of tissue from an

Table 5. *Electrical stimulation of the entry of* ^{42}K *into slices of guinea-pig cerebral cortex*

All slices were held in the quick-transfer apparatus and, after 30–40 min. preincubation, 0·1 ml. of stock ^{42}K medium was added. Condenser pulses of the characteristics given in Table 1 were applied to one of duplicate pairs of vessels, immediately on addition of the ^{42}K. After 5 min. further incubation, slices were released to sucrose and extracted with trichloroacetic acid as described in Table 1; radioactivity in tissues and incubation fluids was counted, and total potassium determined by flame photometer.

Wt. of slice (mg.)	Total potassium (μequiv.)		Radioactivity (counts/min.)			Calculated* rate of entry into slice	
	Slice	Medium	Initially (medium)	Finally (medium)	Finally (tissue)	(μequiv. of K/min.)	(% of K/min.)
			Slices without applied pulses				
88	5·1	40·6	11 710	11 292	418	0·349	6·8
79	5·3	40·8	10 734	10 408	326	0·285	5·4
129	7·1	40·6	8 628	8 391	237	0·247	3·5
91	5·2	46·2	37 707	36 760	947	0·280	5·4
66	3·6	47·0	36 371	35 466	907	0·206	5·7
Mean ± S.D.	—	—	—	—	—	—	5·4 ± 0·8
			Slices with 5 min. application of pulses				
77	4·6	47·8	38 116	36 752	1 364	0·416	9·1
68	3·6	46·5	37 534	36 369	1 165	0·378	10·5
76	3·4	46·0	36 765	35 229	1 536	0·590	17·4
79	3·6	46·4	37 405	36 034	1 371	0·475	13·2
Mean ± S.D.	—	—	—	—	—	—	12·6 ± 3

* Calculated according to Krebs, Eggleston & Terner (1951).

initial value of 100–110 μmoles/g., which is a loss comparable with that observed by Terner *et al.* (1950) to occur from cerebral tissues to media of normal potassium content.

Potassium content of incubated tissue

Tissue that had been prepared in the fashion described, and which had already lost potassium salts to potassium-free media, was found to lose further K^+ ions on incubation in a fresh batch of potassium-free medium. After incubation for 1–2 hr. slices (about 45 mg.) placed in 3·5 ml. of medium contained only 4–12 μmoles of potassium/g.

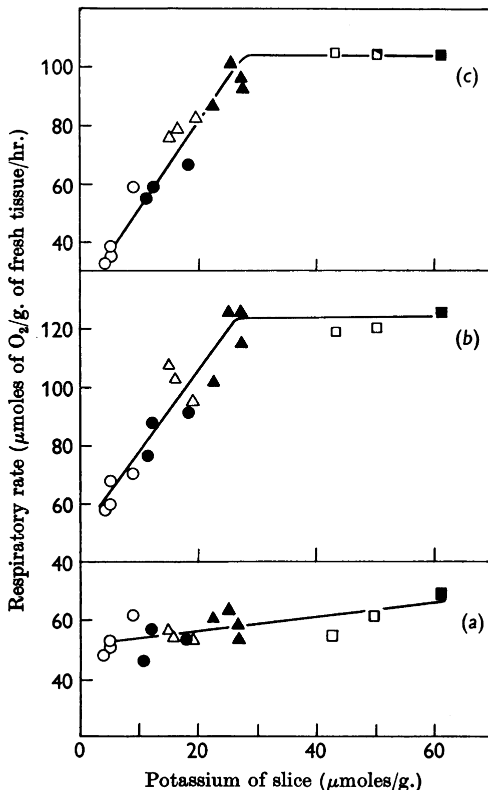

Fig. 8. Dependence of respiratory response to pulses, in slices of guinea-pig cerebral cortex, on potassium salts of medium and tissue. Slices were prepared and incubated in media which contained no added K^+ ions (○ and ●), or 1·1 mM-K^+ ions (△ and ▲) or 11 mM-K^+ ions (□ and ■). Slices ●, ▲ and ■ were from the outer convexity of the cortex and slices ○, △ and □ were inner slices. Respiration was measured in three successive periods: (*a*) up to 40 min. of incubation, without applied pulses; (*b*) from 40 to 70 or 80 min., when pulses of peak potential 10v, 0·4 msec. time constant, were applied at 100/sec.; (*c*) from 70 or 80 to 100 or 120 min., with continued application of pulses of the same characteristics. At the end of the experiments the slices were drained free of medium and their K^+ ion content was determined.

This is shown in Fig. 8 (reading the points only in relation to the abscissa), which indicates also that it is the outer slices which retain the greater quantities of potassium salts (see also Fig. 4). Tissues prepared in media without added potassium salts retained more K^+ ions if incubated as just described, but in media with added K^+ ions. Thus after 2 hr. in media 1·1 mM in K^+ ions their K^+ ion content was 15–30 μmoles/g. and in 11 mM-K^+ ions it was 40–50 μmoles/g., the outer slices in each case contributing the higher values.

Response to electrical pulses

Previous investigation had shown that tissue prepared in media of normal potassium content, incubated in media initially lacking potassium salts, was normal in its initial respiratory rate. Respiration increased in rate on applying electrical pulses to an extent similar to that observed in media of normal potassium content (Gore & McIlwain, 1952). Tissue prepared in media lacking potassium has, however, been found more susceptible. Before application of pulses, respiration tends to be low (Fig. 8*a*); when tissues were prepared in media without K^+ ions, values averaged 54 μmoles/g./hr. in place of 61 for tissues prepared in 1·1–11 mM-K^+ ions; this difference is just significant at $P < 0.05$.

The difference on application of electrical pulses is, however, much more striking (Fig. 8*b* and *c*). Tissues lowest in potassium salts (5 μequiv./g.) increased in respiration by only 10 μmoles of O_2/g./hr., whereas those with more than 25 μequiv. of K^+ ions/g. increased in respiratory rate by 60 μmoles of O_2/g./hr. Between the values of 5 and 25 μequiv. of K^+ ions/g. of tissue, the rate with pulses doubles, showing an approximately linear increase with increase in K^+ ion content. If the lines of Fig. 8 (*a*) and (*b*) are extrapolated to zero potassium content, they each cut the ordinate at 50 μmoles of O_2/g./hr., implying that no respiratory response would be expected in a tissue without potassium.

It is evident from Fig. 8 that tissues treated similarly with respect to the K^+ ions of the media in which they are handled can nevertheless differ in potassium content, either because of the depth in the cortex from which the slice was cut or for undetermined reasons. The relationship between potassium content and response to pulses is not disturbed by these variations; it holds for most of the subgroups shown by different points in the diagram: for example, among inner slices handled in media lacking K^+ ions (○) and among outer slices in media with 1·1 mM-K^+ ions (▲).

After pulses have been applied for 30–40 min., the respiratory rate of most tissues falls by 15–20 μmoles of O_2/g./hr. (Fig. 8*c*). Although in

Table 6. *Rates of change in tissue potassium*

Measurements followed 30–40 min. preincubation of guinea-pig cerebral cortex in glucose media; for other details see the sources quoted.

Movement	Source of data	Period measured	Rate (μequiv./g./hr.) No pulses	With pulses
Net loss from tissue	Fig. 3	0–60 sec.	0	240
Net gain after pulses	Fig. 4	0–60 sec.	550–600	—
Efflux measured by ^{42}K	Fig. 5	20–60 sec.	400	600–750
Influx measured by ^{42}K	Table 5	0–5 min.	330	645

tissues under optimum conditions this implies a fall of only 10–15 % of their stimulated rates, in tissues from media without K^+ ions the fall can mean that pulses cause a decrease, rather than an increase, in respiratory rate. This is seen to occur when the tissue K^+ ion content is about 5 μmoles/g.

The lactic acid which accumulated in the media during the experiments of Fig. 8 was found to average $70 \pm 12(19)$ μmoles/g. of tissue, and not to show a marked dependence on the potassium content of the medium or of the tissue.

DISCUSSION

These experiments have given an opportunity of observing the course of the several distinct aspects of potassium movement which are listed in Table 6. These show major changes to be caused by the application of electrical pulses. The numerical values quoted in Table 6 refer in most cases to an arbitrarily chosen period in the first minute after applying or ceasing to apply pulses, or after adding the potassium isotope whose change is measured. Fuller information of the course of change is given in Figs. 3–5, most of which include periods in which the rates concerned are faster or slower than during the periods selected. Certain of the data give approximately linear relationships between the potassium content of tissues, at different times after initiating ion movement, and the square root of the time interval, a relationship described by Harris (1960), as shown by processes in which diffusion is a limiting factor. Diffusion is clearly involved in the passage of ions from the cells of the slice to intercellular fluids, and from these fluids to the external solution. Logarithms of potassium fluxes did not show a linear relationship to time. Closer analysis of the course of potassium movement has not been made, in view of the variety of potassium-containing structures, at both cellular and subcellular levels, which are contained in the tissue. This heterogeneity is discussed in more detail by Hillman & McIlwain (1961) in relation to the polarized elements observed (by micropipette electrodes) to be present in cerebral slices comparable with those used in the present study.

Ion movements and tissue metabolism

The movements of potassium now observed in cerebral tissues in response to pulses appear in several respects to be analogous to those occurring in peripheral nerve during excitation and recovery (see Hodgkin, 1958). Here the following points are relevant.

(i) No corresponding changes in ion movement were induced in liver slices by electrical pulses, although liver slices maintain intracellular concentrations of potassium salts several times those of the medium and display resting membrane potentials when examined with micropipette electrodes (Li & McIlwain, 1957). Cerebral but not liver tissues have previously been shown to respond to electrical pulses by increases in respiration and glucose utilization (McIlwain, 1951; Kratzing, 1951).

(ii) Nevertheless, the potassium efflux induced in cerebral tissues by stimulation is not secondary to these changes in respiration or in glucose utilization, for the potassium efflux occurs in media without glucose, and applied pulses do not increase respiration under these conditions (McIlwain, 1953). Loss of phosphocreatine from cerebral tissues also normally accompanies their electrical stimulation, but is not necessary to the efflux of potassium in response to pulses, for this efflux takes place under conditions in which the tissue has little or no phosphocreatine: in the absence of glucose or in the presence of both glucose and glutamate. These conditions, it should be noted, still allow the tissue to maintain a concentration of potassium much above that of the surrounding medium, and to exhibit intracellular potentials (Hillman & McIlwain, 1961; H. H. Hillman, unpublished work).

(iii) On the other hand, the tissues' respiratory response to applied pulses has now been shown to be dependent on the presence of an appreciable concentration of potassium. Similar dependence on sodium salts was demonstrated previously (Gore & McIlwain, 1952). A primary action of the pulses thus appears to be on ion movement rather than on the other metabolic changes mentioned

(these being associated, rather, with processes of recovery). Present investigations have not given information on whether such recovery processes operate primarily on sodium or potassium salts, or on both.

(iv) Within the earliest periods observed in the present study, measurement of ^{42}K showed not only an increased efflux of potassium in response to pulses but also an augmented influx. These findings indicate an initial effect of the pulses to be one of causing not so much a particular movement of potassium as of causing an increase in permeability to potassium. This is the situation also in peripheral nerve.

Speed of potassium movement

(i) Table 6 indicates that the normal stability of tissue potassium involves approximately equal rates of influx and efflux of some 400 μequiv./g./hr.; pulses increased the net loss (and also the fluxes measured by ^{42}K) by some 240 μequiv./g./hr. This increase, caused by application of 100 pulses/sec., amounts to 0·67 μm-equiv./g. of tissue/applied pulse. The total efflux under these conditions is about three times this amount. These values may be compared with that of Hodgkin & Huxley (1947, Table 2) for the isolated axon of *Carcinus maenas*, from which efflux of 2·1 μm-moles of potassium/g. of tissue/impulse can be calculated. The present investigation appears likely to give a minimum value only, for stable rather than maximal values have been used in interpreting Figs. 3–5, and no examination has been made of the effect of pulse frequency or duration on potassium movement. The pulse characteristics adopted represent values a little above the minima found necessary for maximal increase in tissue respiration in an earlier study (McIlwain, 1954).

(ii) On cessation of application of pulses, the tissue content of potassium increased at the considerable rate of 550–600 μequiv./g./hr., returning to a stable value within a minute. Increased respiration and glycolysis have been observed to persist after cessation of pulses (McIlwain, 1954; McIlwain & Tresize, 1956); the conditions under which these observations were made were not entirely comparable with those of the present experiments, and suggested persistence of increased respiration and glycolysis for periods of 5–20 sec. rather than 1 min. Assuming, however, that the increase in rate of respiration of 60 μmoles of oxygen/g./hr. and in glycolysis of 100 μmoles of lactate/g./hr. represent the source of energy for processes leading to reassimilation of potassium, provision of $(60 \times 6) + 100$ μmoles of energy-rich phosphate, or 460 μmoles of $\sim$P/g. of tissue/hr., is implied. This gives a ratio of K^+ ions/$\sim$P of 575/460, or 1·25. This is the order of magnitude suggested for comparable processes in other tissues (where the work to be performed is similar, differential ion concentrations being comparable). Hodgkin & Keynes (1954; see also Davies, 1954) suggested 4 sodium atoms to be transported/mole of oxygen consumed, both in *Sepia* axon and in frog muscle, implying a sodium/$\sim$P ratio of 0·67. Similar ratios were found by Caldwell, Hodgkin, Keynes & Shaw (1960). In the erythrocyte, Glynn (1958) considered the probable ratio of sodium and potassium exchanged/$\sim$P to be greater than 1.

The rate of energy consumption in reassimilating potassium to the tissue in the present conditions may be calculated by using the formula

$$-\Delta F = \boldsymbol{R}T \log_n C_1/C_2,$$

as did Krebs *et al.* (1951; though this does not take into account the electrical potential across the membrane) with the values $\boldsymbol{R}$ 1·987 cal./degree and T 310°; but with C_2, the external concentration of potassium salts, as 6 mM, and C_1, the internal concentration, as 100 mM (making allowance for the tissue extracellular space: see above and Pappius & Elliott, 1956*a*, *b*). This gives a value of 1740 cal. required/g. equiv. of potassium assimilated. Assimilation at the rate of 575 g.-equiv./g. of tissue/hr. therefore requires 1 kcal./g./hr. Taking the free energy (F) available from a mole of adenosine triphosphate as 12 kcal., the provision of 460 μmoles of $\sim$P/g. of tissue/hr. is equivalent to 5·5 cal./g./hr. The augmented rate of potassium reassimilation after stimulation thus appears to use at least 18% of the additional energy supply. Scope nevertheless remains for its utilizing the higher proportion suggested above, and also for the concomitant resynthesis of phosphocreatine or performance of other energy-consuming processes.

SUMMARY

1. Tissue from the cerebral cortex of the guinea pig, incubated in glucose media in apparatus permitting the application of electrical pulses and subsequent rapid transference of tissue, resynthesized phosphocreatine and reassimilated potassium salts to a concentration of 63 μequiv./g. wet. wt.

2. Electrical pulses diminished the potassium content at an initial rate of 240 μequiv./g./hr., until after 10 min. a new steady state was reached with about 80% of the original potassium content. No comparable phenomenon occurred with slices of liver. Stopping pulses after 10 min. was followed in cerebral tissues by rapid reassimilation of potassium, the net gain being at about 600 μequiv./g./hr.

3. During preincubation in media containing ^{42}K, the tissue assimilated the isotope, which was

subsequently released to fresh media at rates implying an initial loss of potassium of 400 μequiv./g./hr., increased to 600–750 μequiv./g./hr. by electrical pulses.

4. The rate of entry of ^{42}K to the tissue was also increased by electrical pulses, implying a change of potassium movement from an unstimulated rate of some 330 to one of 645 μequiv./g./hr.

5. When incubation media contained no added oxidizable substrate, less potassium was assimilated to the tissue but it underwent a similar fall with applied pulses. ^{42}K was again taken up by the tissue, but on transference to fresh media loss of the isotope was more rapid than when glucose was present. Pulses increased loss of ^{42}K as with added glucose.

6. In media with glutamate as only oxidizable substrate, slices were normal in potassium content and again lost potassium with pulses. In media with glucose and glutamate, more potassium was associated with a given initial weight of tissue and again pulses diminished potassium content.

7. Slices prepared and incubated in glucose media which lacked potassium salts contained 5–20 μequiv. of potassium/g. of tissue, according to their former position in the brain and their exposure to potassium-free media. In media 1·1 mM in potassium salts, slices contained 15–30 μequiv. of potassium/g.

8. In slices with potassium contents of 5–30 μequiv./g. of tissue, an approximately linear relationship was found between content of potassium and the increase in respiration brought about in the tissue by applied electrical pulses.

9. It is concluded that an early effect of pulses is to increase the permeability of the tissue to potassium salts, permitting the increase in both influx and, preponderantly, efflux of K^+ ions; the increased efflux corresponded to a minimum loss of 0·67 μm-equiv. of potassium/g. of tissue/applied pulse.

10. Computations suggest that some 1·25 μ-equiv. of potassium is reassimilated/equiv. of energy-rich phosphate made available by the concomitant increase in respiration and glycolysis; the reassimilation may take 18 % of this additional energy supply.

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