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Ion Transport in Rat-Liver Slices

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The relation between metabolism and ion transport is a subject of general interest. It has been studied in many tissues (Harris, 1960). One of the techniques for the study of ion transport has been to leach out part of the potassium from a tissue. When the tissue is then placed into a favourable environment, potassium reaccumulates. Leaf (1956) showed that cooling led to the entry of sodium and water into, as well as the loss of potassium from, cooled liver and kidney slices. Mudge (1951), using kidney slices, showed that potassium was lost on cooling, and that it reaccumulated when the slices were incubated in warm Ringer solution. Maizels (1951) and Maizels & Remington (1958) have used similar cooling and warming techniques for red cells, liver slices and kidney slices.

However, rat-liver slices, one of the most convenient laboratory preparations, have not given consistent results by this technique. The ion movements produced by cooling and rewarming were often small and slow (Maizels & Remington, 1958; D. Parsons, personal communication). The present paper describes a procedure that gives consistent and rapid ion transport in rat-liver slices. The results of using these methods to study the effects of drugs and nutritional state on ion transport are reported by Judah & McLean (1962) and by McLean (1963).

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MATERIALS AND METHODS

Preparation of liver slices. Albino rats of 100–200 g. weight were used. All rats were given 20 mg. of α -tocopherol acetate 1 day before they were killed. The cube diet used (MRC 41B cubes) was deficient in vitamin E, and liver slices from animals even mildly deficient in vitamin E show defects of ion transport (McLean, 1960*a, b*).

The rats were killed by a blow on the head and exsanguinated, and the livers were rapidly removed. The liver was cut into slices by hand with a long razor blade and a guide similar to that described by McIlwain (1961). Slices 0.3 mm. thick and weighing 100–200 mg. were easily produced in numbers up to 20 per liver.

The slices were put into ice-cold 'saline solution' [containing NaCl (150 mM) and KCl (5 mM)] as soon as they were cut. The slices and saline were kept in a squat beaker, surrounded by crushed ice and water to maintain a low temperature (about 2°). The slices were kept cold for 35 min., by which time 70% of their original potassium content had leaked out. The slices were then distributed to vessels for incubation at 38°.

When oxygen uptake was to be measured as well as slice composition, single slices were put into 3 ml. of Ringer solution in Warburg flasks.

To measure the time-sequence of ion movements, up to three slices were put into 10 ml. of Ringer solution in a 50 ml. conical flask. Slices were withdrawn at intervals from the flask. The flasks were corked and gassed through wide-bore hypodermic needles. A piece of polythene tubing led the gas to the surface of the incubation medium. Ringer phosphate solutions were gassed with oxygen, Ringer bicarbonate solutions with O₂ + CO₂ (95:5). Flasks were shaken on a Warburg bath at 80–100 strokes/min.

Incubation solutions. The Ringer phosphate solution with added calcium contained: NaCl (125 mM), KCl (6 mM),

MgSO₄ (1.2 mm), NaH₂PO₄ (1 mm), CaCl₂ (1.2 mm), glucose (200 mg./100 ml.) and NaH₂PO₄ buffer, pH 7.4 (15 mm). The Ringer bicarbonate solution contained: NaCl (125 mm), KCl (6 mm), MgSO₄ (1.2 mm), NaH₂PO₄ (1 mm), CaCl₂ (2 mm), glucose (200 mg./100 ml.) and NaHCO₃ (15 mm). The water used in these solutions was first distilled and then passed through a column of mixed-bed ion-exchange resin.

Determination of water, sodium and potassium contents of liver. The methods of Heckman & Parsons (1959) were used. The original composition of the liver was determined on a piece taken as soon as the rat was killed. The liver was washed free of blood and blotted on Whatman no. 54 filter paper, and a piece was cut off for analysis. When slices were taken out of cold saline or warm incubation solution, they were blotted. Each slice was first spread out on the filter paper, and then another paper was firmly pressed on top. The slice was picked off the filter paper into a tared weighing bottle (Quickfit WB 10 and 11). The thinnest slices sometimes fell apart during blotting, but could be scraped off the surface of the filter paper without difficulty; such slices had a composition similar to the slices that were picked up in one piece.

The slices were weighed, dried overnight at 105°, and weighed again after cooling, to give wet and dry weights. 0.15N-Nitric acid (5 ml.) was then run into each of the weighing bottles, which were then stoppered. After 24 hr. all the sodium and potassium was in the aqueous phase. The sodium and potassium contents of suitable dilutions of the extracts were determined by flame photometry.

When light ether anaesthesia was used in killing the rats, potassium uptake was not affected.

In preliminary experiments the ratio of fat and of DNA phosphorus to dry weight of slices did not vary significantly during incubation. The glycogen content also remained surprisingly constant. Hence it seemed reasonable to use dry weight as the basis of measurement.

RESULTS

Normal rat liver contains about 350 m-equiv. of potassium/kg. dry wt. When slices are incubated in cold saline the potassium content falls by about

70 % in 35 min. (Heckman & Parsons, 1959). On incubation in warm Ringer solution most of this potassium is regained. Table 1 shows the results of experiments on at least nine rats for each point. When slices from a single rat are used for multiple determinations the standard deviations are reduced to about half of those shown.

Sodium and water move into the slices during cooling, and are rapidly extruded in the warm. In the first 10 min. of incubation little accumulation of potassium takes place. In the next 25 min. potassium is accumulated at a rate of about 5 m-equiv./kg. dry wt./min. As the original potassium content of the liver is approached, so the rate of potassium uptake slows. In most experiments the final potassium content did not rise above 90 % of the original content. Potassium could be maintained at this level for 2 hr. or more.

When the oxygen consumption of slices was measured at the same time as the ion changes, Q_{O_2} values of about 6 were obtained. The oxygen uptake did not fall off when the potassium content had reached plateau values. No difference was found between the oxygen uptake of slices incubated immediately after cutting and those loaded with sodium by cooling in saline.

Incubation of a single 100 mg. slice, instead of three slices, in 10 ml. of Ringer solution did not affect the potassium uptake. When more than three slices were incubated in one flask a marked fall in the rate of potassium uptake was found; the slices tended to stick together as they were shaken.

Slices incubated in Ringer bicarbonate solution took up potassium at a rate similar to that of slices in Ringer phosphate. If the calcium concentration was lowered to 0.1 mm or less, the rate of potassium uptake fell, but even in saline solution [sodium chloride (150 mm) plus potassium chloride (5 mm)] some potassium uptake took place at 38°

Table 1. *Sodium, potassium and water contents of liver and liver slices before and after incubation*

Each value given is the mean $\pm$ S.D. of the results of experiments with at least nine rats. The conditions of incubation are described in the text.

	Water content (kg./kg. dry wt. of liver)	Sodium content (m-equiv./ kg. dry wt. of liver)	Potassium content (m-equiv./ kg. dry wt. of liver)	Total sodium plus potassium content (m-equiv./ kg. dry wt. of liver)	Sodium content (m-equiv./ l. of liver- slice water)	Potassium content (m-equiv./ l. of liver- slice water)	Total sodium plus potassium content (m-equiv./ l. of liver- slice water)
Original liver	2.50 $\pm$ 0.20	99 $\pm$ 12	348 $\pm$ 28	447	40	140	180
Slices cooled in saline	3.45 $\pm$ 0.31	568 $\pm$ 62	117 $\pm$ 29	685	160	33	193
Slices incubated in Ringer phosphate with oxygen at 38° after cooling							
For 10 min.	2.89 $\pm$ 0.26	429 $\pm$ 76	119 $\pm$ 31	548	148	41	189
For 35 min.	2.68 $\pm$ 0.13	298 $\pm$ 30	236 $\pm$ 22	534	111	88	199
For 60 min.	2.65 $\pm$ 0.13	251 $\pm$ 31	305 $\pm$ 24	556	95	115	210

with oxygenation. The rate of potassium uptake was about 1 m-equiv./kg. dry wt./min. and was not sustained for more than about 20 min. The addition of the disodium salt of EDTA (1 mM) completely prevented potassium uptake in these circumstances. The addition of the calcium salt of EDTA did not affect potassium uptake.

The addition of pyruvate as substrate for slices made no difference to the rate of potassium accumulation.

DISCUSSION

The water and sodium contents of liver slices depend almost entirely on how hard the slices are blotted. The same factor makes the potassium concentration in liver-slice water an unreliable basis. The potassium content on a dry-weight basis is hardly altered by adherent extracellular fluid, and this is therefore the best measurement to use. The maximal rate of potassium accumulation is found in the interval 10–35 min. after the start of incubation. At this time the rate of accumulation is about 5 m-equiv. of potassium/kg. dry wt./min.

Leaf (1956) pointed out that tissue slices gain sodium chloride and water, as well as exchanging potassium for sodium, when kept in cold saline solutions. These two processes take place simultaneously during cooling. But when slices are warmed it seems that the processes are dissociated. In the first 10 min. of incubation the slices lose sodium at a rate of about 14 m-equiv./kg. dry wt./min. whereas the potassium content does not alter. Most of the fall in water content also takes place in the first 10 min., and presumably chloride is also lost at this time. The same initial rapid loss of sodium and of water is found in vitamin E-deficient slices which are incapable of accumulating potassium (McLean, 1963). The amount of sodium removed during blotting presumably depends in part on mechanical factors such as the elasticity of collagen fibres, and it may be that the initial rapid loss of sodium and of water is independent of cell metabolism.

After the initial 10 min., sodium-potassium exchange begins and is maintained in a 1:1 ratio. The total sodium plus potassium, in m-equiv./kg. dry wt., hardly alters. When the concentrations of sodium and potassium are considered it is seen that the ion conditions existing in the whole liver are to a large extent re-established after incubation for

60 min. There is a rise in the concentration of sodium plus potassium similar to that found by Aebi (1953).

In conclusion, this method for measuring ion transport in liver slices is easily carried out and is a convenient method for assessing the interaction of nutritional state, or the action of drugs, on active transport of ions.

SUMMARY

1. Rat-liver slices lose potassium when leached in cold saline solution, and reaccumulate potassium when incubated in warm oxygenated Ringer solution. The maximum rate of accumulation is about 5 m-equiv. of potassium/kg. dry wt./min.

2. After the initial 10 min. of incubation sodium loss and potassium uptake are in a 1:1 ratio.

3. In the first 10 min. of incubation no potassium accumulates, but much sodium and water are lost.

4. Conditions necessary for a sustained uptake of potassium to take place are described; α -tocopherol must be given to rats 24 hr. before they are killed.

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