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NET SODIUM AND POTASSIUM MOVEMENTS IN LIVER SLICES PREPARED FROM RATS OF DIFFERENT FOETAL AND POST-NATAL AGES

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SUMMARY

1. Net movements of sodium and potassium in liver slices prepared from rats at different stages of foetal and post-natal growth have been studied *in vitro*.

2. During incubation at 1° slices prepared from rats of most of the ages studied lost potassium and gained sodium and water. These changes could be accounted for by a 1:1 exchange of Na^+ from the medium for K^+ of the cells and by the further entry of sodium as a solution having the same composition as the medium.

3. In liver slices from the youngest rats studied (17–18 days gestation) the extent of the changes during incubation at 1° was much less than in slices prepared from older animals. There was no significant increase in the water content and the net gain of sodium was equivalent to the loss of potassium.

4. During subsequent incubation at 38° under aerobic conditions the slices from rats of all ages regained potassium and lost sodium.

5. The net uptake of potassium during the incubation at 38° of liver slices prepared from foetuses at 17–20 days gestation was completely inhibited by cyanide. At 21–22 days gestation about 40 % of the potassium uptake persisted in the presence of cyanide; after birth about 10 % of the potassium uptake was resistant to cyanide. The cyanide-resistant potassium uptake was inhibited by the further addition of iodoacetate to the incubation medium.

6. Liver slices prepared from foetuses at 17–20 days gestation showed a net loss of sodium during incubation at 38° in the presence of cyanide.

7. The relation of the sodium and potassium movements at 38° to the respiratory and anaerobic glycolytic activity of the rat liver during the growth of the animal is discussed.

INTRODUCTION

Cells of adult rat liver maintain a high potassium and low sodium content by means of metabolically linked transport mechanisms[1-3]. Liver cells of new-born and young

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rats also maintain high potassium and low sodium contents *in vivo*[4]. This situation is disturbed when the metabolic activity of the tissue is retarded[5], and it therefore seems likely that the cells of the young liver tissue also transport sodium and potassium by mechanisms which are linked to metabolism.

The results of FLINK *et al.*[1] suggest that the energy required for the transport of sodium and potassium by the adult rat liver is derived from oxidative metabolism. The transport of these cations by slices of the kidney cortex of adult rats and rabbits is also dependent upon respiration[6,7], but kidney-cortex slices of the new-born animals of these species are able to transport sodium and potassium when respiration is inhibited, provided that anaerobic glycolysis continues[8,9]. The liver of the rat foetus has a higher rate of anaerobic glycolysis than the adult tissue[10] and it therefore seems possible that the cells of the young rat liver are also able to obtain the energy for cation transport from anaerobic glycolysis.

Experiments have now been done to see if slices of young rat liver can transport sodium and potassium and to what extent the transport mechanisms are linked to respiration and anaerobic glycolysis. Simultaneous measurements of net sodium and potassium movements and of the respiratory and glycolytic activity of liver slices prepared from rats of different foetal and post-natal ages have been made. The results obtained on cation movements are given in this paper and the measurements of respiration and glycolysis are reported in the accompanying paper[11]. It has been found that liver slices prepared from rats of all the ages studied are able to transport sodium and potassium by mechanisms which are linked to metabolism. The extent to which the transport is dependent upon respiration or anaerobic glycolysis varies considerably during foetal and post-natal development.

A preliminary account of some of this work has already been published[12].

METHODS

Animals

Albino rats were used. The age of foetuses was determined from their crown-to-rump length[13]. Experiments were done with foetuses of the following age groups: 17–18 days, 19–20 days, 21 days and 22 days of gestation. Experiments were also done with rats of the following post-natal age groups: within 1 h of birth, 4–16 h after birth (*i.e.* rats born during the night preceding the experiment), and older than 3 months (referred to as "adults"). The adult group consisted only of male rats; at all other ages animals of both sexes were used at random.

Preparation of liver slices

The rats were killed by decapitation. Foetuses were rapidly removed from the uterus and foetal membranes. The livers were cut out and cooled on ice, after which they were cut freehand with a razor blade into slices, approx. 0.3 mm thick. A single lobe of the liver of an adult rat gave sufficient material for a single experiment, but at younger ages the whole livers of 3–12 animals (depending on the age) were required. A necessary consequence of this difference was that the time required for the preparation of all the slices was greater in experiments on foetal tissue (20–25 min from the death of the mother) than in experiments on adult tissue (5–7 min from the death of the animal).

Incubation procedure

In order to determine the ability of liver slices to transport sodium and potassium, the normal cation composition of the slices was first disturbed by incubation at a low temperature. The ability of the slices to regain their original composition could then be studied under different conditions. Essentially the same procedure has previously been used with liver slices by FLINK *et al.*[1], MCLEAN[2], and PARSONS AND VAN ROSSUM[3].

Groups of liver slices, each group having a total wet weight of 30–100 mg, were placed in 3 ml of incubation medium contained in a Warburg manometric apparatus. The slices prepared from a single litter of young animals, or from one adult animal, were distributed over 4–8 manometric flasks. Unless otherwise stated, the slices were incubated for 90 min in a water bath maintained at 1.0 ± 0.5°; during the last 15 min of the cold incubation the apparatus was gassed with oxygen. At the end of the cold incubation the contents of one flask were removed for analysis and the remaining flasks were placed for varying periods in a water bath maintained at 38 ± 0.2°. After incubation the slices were poured out of the flasks and separated from the medium by filtration and blotting[14]. The centre wells of the flasks contained concentrated alkali solution (see below) and this was removed with absorbant paper before the flask contents were poured out.

The incubation medium had the following composition (mM): Na^+, 146.7; K^+, 6.4; Ca^{2+}, 1.2; Mg^{2+}, 1.0; Cl^-, 137.0; SO_4^{2-}, 1.0; phosphate, 10.0; D-glucose, 10.0. The initial pH was 7.4 and this fell to 7.2–7.0 during the incubation.

Stock solutions of the inhibitors, KCN and iodoacetic acid, were made up in portions of the incubation medium. The appropriate inhibitor solution was placed in the side-arm of the manometer flask and was added to the contents of the main flask during the transfer from the cold to the warm bath. The final concentration of each inhibitor in the medium was then 1 mM. The centre wells of the flasks without cyanide contained 0.2 ml of 10 % NaOH and those of the flasks with cyanide contained an appropriate mixture of KOH and KCN[15].

Analyses

The water, fat-free dry solid, sodium and potassium contents of the blotted liver slices were determined by methods described elsewhere[4,14] except that the EEL flame photometer was used for the cation analyses. For the determination of the haemoglobin content, liver slices were finely chopped with a razor blade and extracted with distilled water at 1° for 12–20 h. The haemoglobin content of the extract was determined after conversion to cyanohaemoglobin[16].

RESULTS

Net cation movements during incubation at 1°

During incubation at 1° for 90 min the liver slices prepared from rats of all age groups lost potassium and gained sodium, and there was usually also an increase in the water content of the slices (Table I). The amount of potassium lost per unit tissue solids increased with increasing age of the animals from which the slices were prepared, and the loss represented an increasing fraction of the initial potassium content of the

slices as the age increased. The gain of sodium and water by the slices also increased with age. Slices of the youngest age group (17–18 days gestation) showed only a small, statistically insignificant increase in their water content. The calculations summarized in Table II show that at all ages the increase in the sodium content of the tissue during incubation could be largely accounted for if some of the Na^+ entered the slices in a 1:1 exchange with the K^+ that was lost, and most of the remainder entered as a

TABLE I

EFFECT OF INCUBATION FOR 90 MIN AT 1° ON THE COMPOSITION OF SLICES OF RAT LIVER

For a description of the incubation conditions, see text. The values given are the mean ± standard error of mean.

Animal age (days)	Number of observations	Potassium		Sodium		Water	
		Loss during incubation	Content after incubation	Gain during incubation	Content after incubation	Gain during incubation	Content after incubation
		(mmoles/kg fat-free dry wt.)				(kg/kg fat-free dry wt.)	
Foetal							
17–18	7	137 ± 34	493 ± 22	131 ± 56	413 ± 46	0.45 ± 0.32	4.68 ± 0.17
19–20	13	157 ± 21	466 ± 12	293 ± 58	511 ± 39	0.84 ± 0.29	5.12 ± 0.19
21	7	203 ± 33	221 ± 20	437 ± 80	600 ± 50	1.18 ± 0.39	4.60 ± 0.25
22	15	192 ± 12	172 ± 7	376 ± 27	562 ± 19	1.16 ± 0.17	4.22 ± 0.11
Post-natal							
1 h	4	246 ± 13	184 ± 13	423 ± 39	648 ± 39	1.16 ± 0.15	4.82 ± 0.15
4–16 h	8	261 ± 12	204 ± 8	508 ± 38	727 ± 31	1.84 ± 0.23	5.62 ± 0.18
Adult	10	206 ± 6	97 ± 3	418 ± 23	652 ± 19	1.88 ± 0.12	4.31 ± 0.10

TABLE II

FRACTIONS OF THE SODIUM ENTERING RAT-LIVER SLICES DURING INCUBATION AT 1°

The values given represent the increase in the sodium content of liver slices during incubation for 90 min at 1°, and are calculated from the data of Table I. For the calculation of the amount of sodium entering the tissue in exchange for potassium, it was assumed that 1 Na^+ ion entered in exchange for each K^+ ion lost from the tissue. The fraction of sodium entering the slices with water was calculated by assuming that the water entering the tissue during incubation was accompanied by the same amount of sodium as contained in an equivalent volume of the incubation medium. The values given are the mean ± standard error of mean.

Animal age (days)	Calculated fractions of sodium entering tissue			Observed total entry of sodium (mmoles/kg fat-free dry wt.)
	In exchange for potassium (1)	Entering with water (2)	Sum of fractions (1) + (2)	
	(mmoles/kg fat-free dry wt.)			
Foetal				
17–18	137 ± 34	66 ± 47	203	131 ± 56
19–20	157 ± 21	124 ± 43	281	293 ± 58
21	203 ± 33	174 ± 57	377	437 ± 80
22	192 ± 12	170 ± 25	362	376 ± 27
Post-natal				
1 h	246 ± 13	170 ± 22	316	423 ± 39
4–16 h	261 ± 12	270 ± 34	531	508 ± 38
Adult	206 ± 6	276 ± 18	482	418 ± 23

solution having the same composition as the medium (*cf.* LEAF[17]). In agreement with the observation that the slices of the youngest age group did not show a significant increase in their water content, the gain of sodium by these slices could be entirely accounted for by exchange with the potassium lost.

A study of the time course of the changes in the foetal tissue during incubation at 1° showed that liver slices from foetuses at 21–22 days gestation rapidly lost potassium and gained sodium and water during the first 30-min incubation (Fig. 1a). There followed a slower increase in the sodium and water content with no further change in the potassium content. These changes were similar to those previously observed in liver slices from rats of various post-natal ages[5,14]. Liver slices prepared from foetuses 19–20 days old also showed the initial rapid changes in composition, but differed from the slices of older age groups in that they did not undergo the further, slow increase in their water and sodium contents after the loss of potassium had ceased (Fig. 1b).

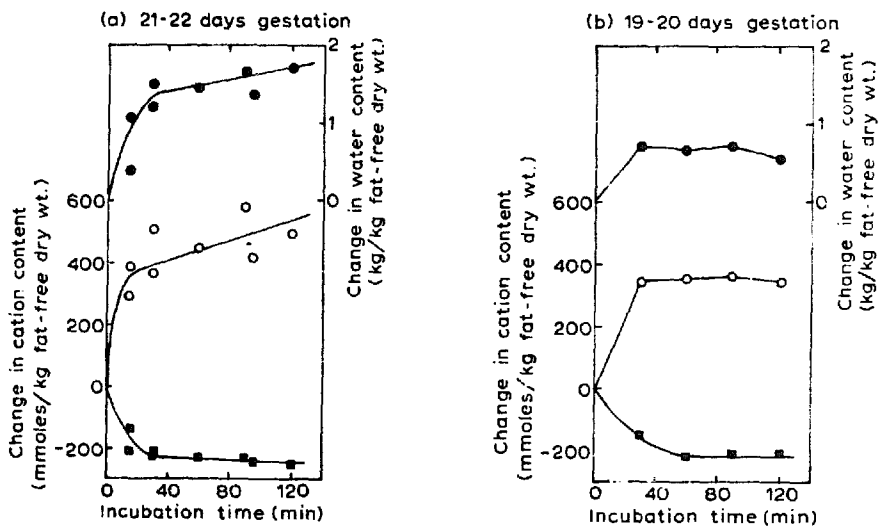

Fig. 1. Change in composition of rat-liver slices during incubation at 1°. Each point represents a single observation of the difference between the composition of unincubated liver slices and the composition of liver slices incubated at 1° for the length of time indicated. a, Slices prepared at 21–22 days gestation; b, slices prepared at 19–20 days gestation. ●—●, water; O—O, sodium; ■—■, potassium.

Since liver slices prepared from foetal rats at 17–20 days gestation only underwent relatively small changes in their water and cation contents during the cold incubation, and in particular retained some 75 % of their potassium content, it seemed possible that they retained some metabolic activity under these conditions. However, the addition of cyanide to the incubation medium, either alone or with iodoacetate, did not affect the changes in composition at 1° (Table III), although these substances greatly inhibited the ion transport (see below) and the respiration and glycolysis of slices incubated at 38° (see ref. 11). Another possible explanation for the resistance of the liver slices from young foetuses to changes in composition during the cold incubation is suggested by the fact that the foetal liver is a haemopoietic organ and so contains a relatively high proportion of red blood cells. It has been shown by PONDER[18] that red blood cells only lose potassium very slowly during incubation at low temperatures. The haemoglobin content of liver tissue from rats of various ages was therefore determined. Fresh (unincubated) liver tissue was used. It can be seen from Table IV

that haemoglobin accounted for about 10 % of the total fat-free solids of the liver of rats at 17–18 days gestation. If it is assumed that in the rat, as in certain other species[19], the amount of potassium per kilogram haemoglobin in foetal red cells is little different from that of the adult cells, then using the potassium content of the adult rat erythrocyte as given by LEVITT *et al.*[20], it can be calculated that red cells contained within

TABLE III

THE COMPOSITION OF LIVER SLICES, PREPARED FROM RATS AT 17–18 DAYS GESTATION, AFTER INCUBATION FOR 90 MIN AT 1° IN THE PRESENCE OF METABOLIC INHIBITORS

For a description of the incubation conditions, see text. The figures on each line represent the analysis of the liver slices prepared from a single foetus; all the foetuses were taken from the same litter.

Inhibitor	*Water content (kg/kg fat-free dry wt.)*	*Sodium content*	*Potassium content*
		(mmoles/kg fat-free dry wt.)	
None	4.84	605	398
	4.74	488	421
KCN (1 mM)	4.88	516	425
	4.64	455	425
KCN (1 mM) + iodoacetate (1 mM)	4.84	450	423
	5.08	456	430

TABLE IV

HAEMOGLOBIN CONTENT OF UNINCUBATED RAT-LIVER TISSUE

The values for the amount of potassium associated with the haemoglobin were calculated by assuming that red blood cells contained approx. 400 mmoles potassium/kg haemoglobin. This approximate value was deduced from the data of LEVITT *et al.*[20] and MCCANCE AND WIDDOWSON[19].

The values represent the mean ± standard error of mean (number of observations).

Animal age (days)	*Haemoglobin content of liver (g/kg fat-free dry wt.)*	*Estimated potassium content of red blood cells present in rat liver (mmoles/kg fat-free dry wt. of liver)*
Foetal		
17–18	105 ± 8 (3)	42
19–20	80 (2)	32
21	77 ± 6 (5)	31
22	32 (1)	13
Post-natal		
4–16 h	53 ± 10 (3)	21
24 h	39 ± 5 (3)	16

the liver can account for about 40 mmoles potassium/kg fat-free solids of the whole liver at 17–18 days gestation. This represents only a small fraction of the potassium retained within the liver slices of this age group after incubation at 1° for 90 min (see Table I). In liver tissue prepared 4–16 h after birth, the potassium content of the red cells can account for about 20 mmoles potassium/kg whole liver fat-free solids.

Electrolyte movements during incubation at 38°

When liver slices which had been pre-incubated at 1° for 90 min were subsequently incubated at 38° in an oxygenated medium, they took up potassium and lost sodium and water. The time course of the recovery process was studied in liver slices of three age groups, namely 21 days gestation, 4–16 h after birth and adults (Fig. 2). At these ages the net changes in composition were largely completed within the first 60-min incubation at 38°. In all subsequent experiments the slices were incubated at 38° for a standard time of 60 min.

A comparison of the results of Table I with those given in the first column of Table V shows that the percentage of the lost potassium which was re-accumulated

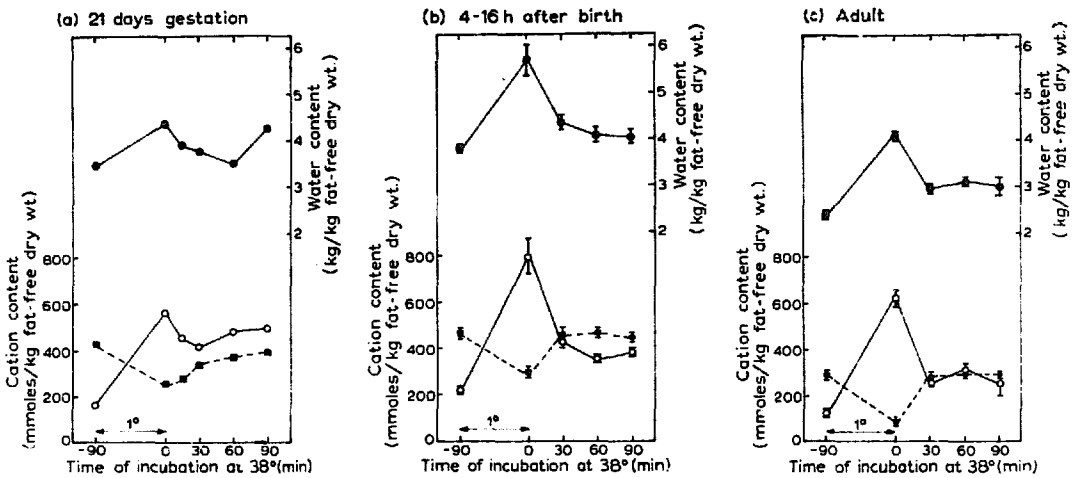

Fig. 2. Changes in the composition of rat-liver slices during incubation at 1° for 90 min followed by incubation at 38°. a, Slices prepared at 21 days gestation. Each point represents the mean of 2 observations; b, slices prepared 4–16 h after birth. Each point represents the mean ± standard error of mean of 6 observations; c, slices prepared from adult rats. Each point represents the mean ± standard error of mean of 3 observations. ●—●, water; O—O, sodium; ■—■, potassium.

TABLE V

CHANGES IN THE POTASSIUM CONTENT OF RAT-LIVER SLICES AFTER INCUBATION AT 38°

Liver slices were incubated for 90 min at 1° followed by 60 min at 38°. For a further description of the incubation conditions, see text The values given are the mean ± standard error of mean (number of observations) of the difference between the potassium content of the slices after incubation at 1° and the potassium content after subsequent incubation at 38°.

Animal age (days)	*Change in potassium content after incubation at 38° (mmoles potassium/kg fat-free dry wt.)*		
	No inhibitor	*Cyanide (1 mM)*	*Cyanide (1 mM) + iodoacetate (1 mM)*
Foetal			
17–18	110 ± 15 (8)	−50 ± 29 (6)	−252 ± 28 (6)
19–20	111 ± 10 (14)	−24 ± 11 (19)	−226 ± 16 (11)
21	98 ± 11 (6)	42 ± 15 (6)	−54 ± 34 (3)
22	104 ± 6 (20)	42 ± 3 (20)	−2 ± 11 (5)
Post-natal			
1 h	64 ± 9 (4)	28 ± 8 (4)	−19 (2)
4–16 h	161 ± 6 (8)	19 ± 6 (10)	−21 ± 8 (8)
Adult	177 ± 11 (9)	17 ± 6 (10)	−3 ± 4 (9)

during the 60 min at 38° fell from 80 to 50 % as the foetal age increased. However, the actual amount of potassium taken up per unit of tissue remained constant at about 100 mmoles/kg fat-free dry wt. Slices prepared from animals taken immediately after birth only took up some 60 mmoles potassium/kg fat-free dry wt., or about 25 % of the potassium which they had lost during the cold incubation, but within the first day of post-natal life the ability of the liver slices to re-accumulate potassium approached the adult level. Accompanying the increase in potassium content per unit solids during incubation at 38° there was also an increase in the potassium concentration in the tissue water of the slices of all age groups (Table VI). The final concen-

TABLE VI

CONCENTRATION OF POTASSIUM IN THE WATER OF RAT-LIVER SLICES INCUBATED *in vitro*

The values represent the mean ± standard error of mean (number of observations).

Animal age (days)	*Concentration of potassium in tissue water (mmoles/kg water)*		
	After incubation for 90 min at 1°	*After incubation for 90 min at 1° followed by 60 min at 38° in the presence of:*	
		No inhibitor	*Cyanide (1 mM)*
Foetal			
17–18	108 ± 4 (7)	164 ± 5 (8)	110 ± 4 (6)
19–20	95 ± 3 (13)	148 ± 3 (14)	100 ± 2 (19)
21	48 ± 4 (7)	87 ± 7 (6)	66 ± 4 (6)
22	41 ± 2 (15)	75 ± 3 (20)	52 ± 2 (20)
Post-natal			
1 h	39 ± 3 (4)	59 ± 5 (4)	45 ± 2 (4)
4–16 h	37 ± 1 (8)	77 ± 2 (9)	37 ± 1 (12)
Adult	23 ± 1 (10)	86 ± 3 (11)	21 ± 1 (13)

TABLE VII

CHANGES IN THE SODIUM CONTENT OF RAT-LIVER SLICES AFTER INCUBATION AT 38°

Experimental procedure as in Table V. The values given are the mean ± standard error of mean (number of observations) of the difference between the sodium content of the tissue after incubation at 1° and the sodium content after subsequent incubation at 38°.

Animal age (days)	*Change in sodium content after incubation at 38° (mmoles sodium/kg fat-free dry wt.)*		
	No inhibitor	*Cyanide (1 mM)*	*Cyanide (1 mM) + iodoacetate (1 mM)*
Foetal			
17–18	−191 ± 32 (7)	−90 ± 40 (6)	151 ± 33 (6)
19–20	−175 ± 22 (13)	−104 ± 16 (16)	187 ± 23 (8)
21	−219 ± 71 (5)	−162 ± 70 (6)	101 ± 83 (3)
22	−105 ± 25 (15)	9 ± 16 (11)	142 ± 73 (4)
Post-natal			
1 h	−89 ± 64 (4)	−13 ± 15 (4)	152 (2)
4–16 h	−274 ± 27 (8)	70 ± 47 (10)	192 ± 55 (8)
Adult	−348 ± 23 (9)	133 ± 39 (10)	268 ± 44 (9)

tration of potassium in the tissue water was 10–25 times the medium potassium concentration, depending on the age group.

In liver slices of all age groups there was a loss of sodium relative to the fat-free solids during incubation at 38° (Table VII) and a fall in the sodium concentration in the tissue water to well below its concentration in the medium (Table VIII). The net

TABLE VIII

CONCENTRATION OF SODIUM IN THE WATER OF RAT-LIVER SLICES INCUBATED *in vitro*

The values represent the mean ± standard error of mean (number of observations).

Animal age (days)	*Concentration of sodium in tissue water (mmoles/kg water)*		
	After incubation for 90 min at 1°	*After incubation for 90 min at 1° followed by 60 min at 38° in the presence of:*	
		No inhibitor	*Cyanide (1 mM)*
Foetal			
17–18	90 ± 9 (6)	63 ± 7 (7)	86 ± 4 (6)
19–20	103 ± 6 (10)	75 ± 5 (11)	95 ± 5 (15)
21	130 ± 7 (7)	116 ± 11 (5)	120 ± 11 (6)
22	136 ± 3 (10)	119 ± 4 (15)	140 ± 6 (11)
Post-natal			
1 h	134 ± 6 (4)	131 ± 10 (4)	134 ± 1 (4)
4–6 h	132 ± 3 (8)	97 ± 2 (9)	132 ± 7 (12)
Adult	151 ± 2 (10)	103 ± 6 (11)	143 ± 3 (13)

TABLE IX

CHANGES IN THE WATER CONTENT OF RAT-LIVER SLICES AFTER INCUBATION AT 38°

Experimental procedure as in Table V. The values represent the mean ± standard error of mean (number of observations) of the difference between the water content of the tissue after incubation at 1° and the water content after subsequent incubation at 38°.

Animal age (days)	*Change in tissue water content after incubation at 38° (kg/kg fat-free dry wt.)*		
	No inhibitor	*Cyanide (1 mM)*	*Cyanide (1 mM) + iodoacetate (1 mM)*
Foetal			
17–18	−1.00 ± 0.15 (9)	−0.62 ± 0.22 (8)	−0.38 ± 0.26 (7)
19–20	−1.00 ± 0.14 (16)	−0.67 ± 0.13 (19)	0.15 ± 0.15 (11)
21	−1.12 ± 0.27 (6)	−0.91 ± 0.19 (6)	0.44 ± 0.46 (3)
22	−0.44 ± 0.16 (20)	−0.08 ± 0.08 (20)	0.79 ± 0.29 (5)
Post-natal			
1 h	−0.57 ± 0.31 (4)	−0.07 ± 0.14 (4)	1.03 (2)
4–16 h	−0.94 ± 0.19 (8)	0.53 ± 0.15 (10)	1.38 ± 0.26 (8)
Adult	−1.26 ± 0.13 (9)	1.19 ± 0.22 (10)	2.11 ± 0.24 (9)

loss of sodium from the slices exceeded the potassium uptake, on a molar basis, and from earlier results[3] it seems probable that most of the excess positive charge of the Na^+ leaving the tissue was balanced by a simultaneous loss of Cl^-. The extrusion of this sodium and chloride was accompanied by a loss of water from the slices (Table IX), as has already been shown with slices of adult rat liver by PARSONS AND VAN ROSSUM[3].

Effect of metabolic inhibitors on electrolyte movements at 38°

The net uptake of potassium by liver slices from foetuses at 17–18 and 19–20 days gestation was completely inhibited in the presence of 1 mM potassium cyanide, and the slices lost some of the potassium that was retained after the incubation at 1° (Table V). At 21 and 22 days gestation, and during the first hour after birth, about 40 % of the potassium re-accumulation persisted in the presence of cyanide. The cyanide-resistant potassium uptake led to an increase in the potassium concentration in the tissue water (Table VI). In liver slices prepared 4–16 h after the birth of the rats the amount of potassium taken up in the presence of cyanide had fallen to 10 % of the amount taken up in the absence of the inhibitor, and this value was maintained in the adult liver slices. The potassium uptake in the presence of cyanide was completely inhibited by the further addition of 1 mM iodoacetic acid to the incubation medium.

The effect of cyanide on the loss of sodium from liver slices of different age groups during incubation at 38° differed somewhat from its effect on potassium uptake. Thus, in slices from rats at 17–18, 19–20 and 21 days gestation the loss of sodium was only partially inhibited by cyanide, while the loss of sodium from the slices of all other age groups was completely inhibited (Table VII). The further addition of iodoacetic acid to the medium completely inhibited the cyanide-resistant loss of sodium from liver slices prepared at 17–21 days gestation.

Incubation of the liver slices at 38° in the presence of cyanide and iodoacetic acid together, thus effectively inhibiting both respiration and anaerobic glycolysis[11], not only led to an inhibition of the active cation movements but in most cases led to further changes in composition in the same direction as those occurring during incubation at 1°. Thus, slices of all age groups older than 21 days gestation showed a further increase in their sodium and water content, but only an insignificant loss of potassium. This result is in accordance with the observation that the increase in sodium and water content may continue for several hours[14] when metabolism is inhibited by cooling. Slices prepared from foetuses at 17–20 days gestation differed from those of older age groups in that they lost much of the potassium retained after incubation at 1° and gained some sodium. The gain of sodium did not exceed the loss of potassium and there was no further increase in the water content.

DISCUSSION

Passive ion movements

It has previously been observed that liver slices prepared from rats of various post-natal ages gain sodium and water and lose potassium during incubation at low temperatures[5,14]. According to the hypothesis proposed by WILSON[21] and by LEAF[17], these changes in composition arise as a result of the inhibition by cooling of the mechanism which extrudes sodium from the cells. Sodium, accompanied by anions of the medium (mainly chloride), is then free to enter the cells and becomes distributed between the tissue and the medium in accordance with the requirements of a Gibbs–Donnan system. The establishment of such a system results from the presence in the tissue of large anions which are unable to diffuse into the medium. The entry of sodium and chloride into the cells is secondarily accompanied by water. Further Na^+ enters the cells in exchange for K^+ which diffuses from the intracellular fluid to the incubation medium. The results described above suggest that the changes in the composition at

1° of liver slices from rats of most of the foetal and post-natal ages studied, are compatible with this hypothesis. Thus, the increase in the sodium content of the slices could be accounted for by assuming that part entered the slices in a 1:1 exchange for potassium that was lost and that the remainder entered as a solution having the same composition as the incubation medium. This observation agrees with that of LEAF[17] on slices of adult rat liver, but differs somewhat from the results of HECKMANN AND PARSONS[14] and PARSONS AND VAN ROSSUM[5] in that the latter authors found that the entry of sodium into liver slices considerably exceeded the amount that could be accounted for by exchange for potassium and entry as sodium chloride solution. They suggested that the extra sodium entered the slices in exchange for unknown cations which diffused out of the tissue during incubation at low temperatures. This difference in results seems to be correlated with the use of different volumes of medium for the incubation of similar amounts of tissue. Thus, PARSONS and co-workers used a volume of 20–50 ml renewed 2 or 3 times during the incubation, while in the experiments of LEAF and those in the present work the slices were incubated in only 3 ml medium. Possibly the loss of unknown cations from the cells is reduced when the slices are incubated in a small volume of medium.

Liver slices prepared from rat foetuses at 17–20 days gestation differed from those of older age groups in that they showed only a small increase in their water and sodium content during incubation at 1°. The results presented in Table III show that this resistance of the slices to changes of composition at low temperatures was not due to residual metabolic activity. According to the hypothesis of WILSON and LEAF, the absence of the entry of sodium accompanied by water into the tissue when metabolism is inhibited could be due to impermeability of the cell membranes to sodium and/or chloride, or to a rapid leakage of large intracellular anions from the cells in exchange for anions of the medium. The results of Table I and Fig. 1 show that there was a rapid exchange of external sodium for potassium of the slices at 17–20 days gestation. Thus, at least a considerable fraction of the cells was readily permeable to sodium. It therefore seems likely that the explanation for the lack of an increase in the water content of the liver slices from young foetuses may be found in one of the other two possibilities mentioned.

Liver slices prepared from rats at 17–20 days gestation also differed from those of older age groups in that they retained a much larger fraction of their initial potassium content during incubation at 1°. The results of Fig. 1 appear to exclude impermeability of the liver cells to potassium as an explanation for the potassium retention, while potassium retained within erythrocytes can only account for a small part of the total retention. HECKMANN AND PARSONS[22] have presented evidence for the existence of a "bound" fraction of the potassium of liver slices prepared from adult rats. It is possible that in the foetal liver this "bound" potassium represents a greater fraction of the total potassium content than it does in the adult tissue, and thus accounts, at least in part, for the retention of potassium in the liver slices prepared from foetuses at 17–20 days of gestation.

Active ion movements

Estimates of the size of the extracellular and intracellular water compartments of the liver slices were not made in this work and in the absence of such measurements the demonstration of sodium and potassium transport by the cells depends on the

extent to which changes in the potassium and sodium content of the whole slices are indicative of changes in the cell contents. The potassium concentration of the medium and so, presumably, of the extracellular fluid is much lower than that of the whole slice water. Changes in the potassium content of the whole slices will therefore be almost entirely due to changes in the potassium of the cells. On the other hand, the sodium concentration of the medium and extracellular fluid is high and small changes in the quantity of extracellular fluid can therefore lead to relatively large changes in the sodium content of the whole slices. It has previously been shown[3] that during experiments of the type described here, the extracellular compartment of adult liver slices tends to increase in size at the expense of the intracellular compartment during the incubation at 38°. This leads to an increase in the sodium content of the whole slices which may partly or completely mask the active extrusion of sodium from the liver cells. It would therefore seem that in these experiments the observation of a fall in the sodium content relative to the tissue solids is indicative of an extrusion of sodium from the cells, particularly if accompanied by a fall in the concentration of sodium in the whole slice water, but the absence of a fall in the sodium content of the slices does not necessarily imply that there was no extrusion of sodium from the cells.

Liver slices prepared from rats of all the age groups studied showed an increase in their potassium content and a decrease in their sodium content when incubated at 38° in an oxygenated medium after pre-incubation at 1°. There were simultaneous changes in the concentrations of sodium and potassium in the tissue water. In view of the above considerations it may be concluded that these changes in the composition of the whole slices were a reflection of changes in the cation content of the cells and represented net movements of potassium and sodium by the cells against the concentration gradients. The contents of adult liver cells incubated *in vitro* are electrically negative with respect to the incubation medium[23]. If it can be assumed that this is also true for liver cells of all the age groups examined here, then the movement of sodium fulfills a definition of active transport[24].

Liver slices prepared at 21 and 22 days of gestation also showed a considerable increase in their potassium content when incubated at 38° in the presence of cyanide. At 21 days gestation, but not at 22 days, a fall in the sodium content of the slices was also observed in the presence of cyanide. In adult-liver slices there appears to be a tight coupling between the active movements of sodium and potassium[25]. The reciprocal movements of these two cations during the incubation at 38° under aerobic conditions of slices prepared from foetuses suggest that here, too, there may normally be a coupling between the transport of sodium and potassium. The failure to observe a net loss of sodium from the slices at 22 days gestation was therefore probably due to changes in the size of the extracellular water compartment masking the extrusion of sodium from the cells (as discussed above) rather than to the absence of sodium transport. If this is indeed the case, then the movements of these cations by the slices prepared at 21–22 days gestation, in the presence of cyanide, are essentially similar to the active ion movements under aerobic conditions.

In contrast to the slices prepared from the later foetuses, those prepared at 17–18 and 19–20 days gestation lost a small amount of potassium and a considerable amount of sodium during incubation at 38° in the presence of cyanide. The loss of potassium may indicate that the inward transport of this cation was completely inhibited by cyanide. However, it will be noted that there was a much greater loss of potassium

(amounting to about half of the potassium retained after the cold incubation) when iodoacetate was present in addition to cyanide, so that energy derived from anaerobic glycolysis was apparently concerned in maintaining the potassium content of the slices in the presence of cyanide. Whether the energy was required for the operation of an inward potassium flux or for the maintenance of structures which "bind" potassium cannot be decided from these experiments. On the other hand, sodium was clearly actively transported out of the cells in the presence of cyanide. If it should prove that potassium transport was completely inhibited by cyanide, then the transport of sodium was uncoupled from potassium transport under these conditions. Such an uncoupling of sodium and potassium movements has been observed in squid-giant axons incubated in the presence of cyanide[26,27] and in uterine muscle incubated with 2,4-dinitrophenol[28]. The positive charge of the sodium lost from the liver slices in the absence of potassium uptake may have been partly accompanied by a loss of chloride ions[3] and partly by lactate ions formed during glycolysis, since the latter largely diffused into the incubation medium[11].

Dependence of potassium transport on metabolism

In view of the uncertainty attending the interpretation of the net sodium movements in the presence of inhibitors, the discussion of the relation between the energy-providing metabolism of the tissue and the transport of cations, which follows, will be confined to a consideration of the transport of potassium.

The effects of cyanide and iodoacetate, which are specific inhibitors of respiration and glycolysis respectively, show that the uptake of potassium by liver slices was dependent upon the energy obtained from the respiratory and glycolytic activity of the cells and that the extent to which the transport depended on each of these activities varied during growth. At all ages studied the greatest part of the energy needed for potassium uptake was obligatorily obtained from respiration, since the uptake was greatly reduced in the presence of cyanide. Thus, despite the uncertainty as to whether or not the liver slices prepared from foetuses at 17–20 days gestation transport potassium at all in the presence of cyanide, the absence of a net uptake of potassium under these conditions shows that the transport mechanism works more efficiently when respiration is not inhibited. In liver slices prepared during the last two days of the gestation period 60 % of the net potassium uptake was inhibited by cyanide, but within the first day after birth the percentage of the potassium uptake which was obligatorily dependent upon respiration increased to 90 %. It should be pointed out that the concentration of cyanide used in these experiments was sufficient to give maximal, but not complete inhibition of the respiration of the liver slices; some 10–20 % of the respiration persisted[11]. That the cyanide-resistant potassium uptake did not obtain its energy from this cyanide-resistant respiration is shown by the effect of iodoacetate. The addition of this inhibitor to the medium in the presence of 1 mM cyanide resulted in the complete inhibition of the cyanide-resistant potassium uptake although the cyanide-resistant respiration was not affected[11]. Iodoacetate did, however, greatly reduce the glycolytic activity of the slices in the presence of cyanide. It is therefore concluded that the energy for the potassium uptake which persisted in the presence of cyanide was derived from anaerobic glycolysis.

A comparison of these observations on potassium transport with those on the rate of anaerobic glycolysis of the same slices[11] shows that the period of growth at which

the ability of the slices to take up potassium anaerobically was greatest did not correspond to the period at which the glycolytic activity was greatest. The net uptake of potassium in the presence of cyanide was maximal at 21–22 days of gestation, while at this age the rate of anaerobic glycolysis of the slices *in vitro* was considerably lower than it was at 17–18 days gestation. The ability of foetal rat-liver slices to take up potassium anaerobically was therefore not simply a function of the amount of energy which was made available in the cell under anaerobic conditions. Towards the end of gestation, the liver cell *in vitro* appears to utilize the available anaerobic energy more efficiently for potassium transport than at earlier stages.

The ability of the rat liver to transport potassium anaerobically fell to the adult level within the first day after birth. This contrasts with the situation in the renal cortex of the rabbit where the anaerobic potassium transport declines gradually over a period of two to three weeks after birth[9]. This difference is apparently not due to the changes in the rate of anaerobic glycolysis of the two tissues, since the rate of glycolysis appears to decline gradually after birth in both tissues (*cf.* WHITTAM[9] and VAN ROSSUM[11]).

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