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THE REGULATION OF CELLULAR VOLUME IN LIVER SLICES

By A. D. C. MACKNIGHT, JENNIFER P. PILGRIM AND BRIDGET A. ROBINSON

From the Department of Physiology, University of Otago Medical School, Dunedin, New Zealand

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SUMMARY

1. Rat, rabbit and guinea-pig liver slices were incubated in media at 37° C to study effects of ouabain and of metabolic inhibition on tissue composition.

2. Slices initially incubated in oxygenated balanced medium did not swell when exposed to either balanced media or potassium-free media containing ouabain in concentrations ranging from 0·1 to 10 mM though they lost potassium and gained a comparable amount of sodium.

3. Without prior incubation in oxygenated medium slices initially swelled when incubated with ouabain but then maintained their new volume.

4. Slices whose metabolism was inhibited by incubation with nitrogen and 1 or 10 mM iodoacetamide lost as much potassium as slices exposed to 10 mM ouabain but became swollen with the uptake of sodium, chloride and water.

5. The results show that liver cells, like renal cortical cells, possess a metabolically dependent, ouabain-insensitive, potassium-independent mechanism concerned in the regulation of cellular volume.

INTRODUCTION

The volume of mammalian cells is often thought to be determined by a balance between the influx of sodium to the cells down its electrochemical gradient and the energy-dependent extrusion of sodium from the cells (Woodbury, 1965). This transport of sodium from the cells has usually been attributed to the cardiac glycoside-sensitive sodium pump responsible for the maintenance of intracellular potassium. This pump prevents the continual entry of extracellular fluid to the cells which would otherwise occur as a result of the colloid osmotic force generated

by intracellular macromolecules to which the cellular membrane is impermeable (Leaf, 1956).

It would be predicted from this hypothesis that cells should swell in the presence of cardiac glycosides such as ouabain or conversely that swollen cells should fail to regain their volume when exposed to otherwise favourable conditions in the presence of ouabain (Tosteson, 1964).

Recently the validity of this simple model has been questioned. Several workers (Macknight, 1968*a*; Maude, 1969; Whittembury, 1968) have extended the work of Kleinzeller & Knotkova (1964*a*) and confirmed that renal cortical tissue seems to possess an ouabain-insensitive, potassium-independent mechanism which, when metabolism is intact, maintains cellular volume. Such a mechanism may also exist in both diaphragm (Kleinzeller & Knotkova, 1964*b*) and uterine smooth muscle (Daniel & Robinson, 1971). However, the nature of this mechanism and its relationship to transepithelial transport in renal cortical tissue remain controversial.

In contrast to the now quite extensive work with renal cortical tissue, relatively little attention has been paid to the mechanism of volume regulation by liver cells. Kleinzeller & Knotkova (1964*a*) were unable to demonstrate ouabain-insensitive volume regulation by slices of rabbit liver. However, Elsehove & van Rossum (1963) reported that rat liver slices leached at 0° C and subsequently reincubated lost sodium even in the presence of the cardiac glycoside strophanthin K. Unfortunately they provided no information about associated water movements under these conditions. Recently, McLaughlin (1972) obtained some results with slices of rat liver incubated with ouabain that led us to re-examine the relationship in liver slices between cellular volume regulation and ouabain-sensitive sodium transport. Our results establish that liver cells, like renal cortical tissue, diaphragm and uterine smooth muscle, can maintain their volume in spite of complete inhibition of ouabain-sensitive ion transport. A preliminary account of some of this work has been presented elsewhere (Pilgrim, Robinson & Macknight, 1973).

METHODS

Media

The basic medium used for the incubation of slices was similar in composition to synthetic interstitial medium described by Bretag (1969), and is subsequently referred to as SIF. It contained (in mM) Na^+, 145; K^+, 3·5; Ca^{2+}, 1·5; Mg^{2+}, 0·7; Cl^-, 117; HCO_3^-, 21·6; PO_4^{2-}, 1·7; SO_4^{2-}, 0·7; gluconate$^-$, 9·6; glucose, 5·6; sucrose, 7·6.

$Na^+ = K^+$ medium contained (in mM) Na^+, 75; K^+, 75; but was otherwise identical in composition to SIF.

In K^+-free medium, Na^+, 3·5 mM replaced K^+.

Media containing ouabain octahydrate (Sigma Chemical Co., U.S.A.) and media containing iodoacetamide (Koch-Light Laboratories Ltd. U.K.) were prepared by dissolving the appropriate masses of these chemicals in the media immediately before use.

Media were gassed with either O_2:CO_2, 95:5 or N_2:CO_2, 95:5 bubbled directly through the medium at a rate sufficient to provide continuous stirring of the slices throughout incubation. Unless otherwise indicated all incubations were performed at 37° C. All media were at pH 7·4.

Preparation of slices

Adult male black and white hooded rats of an inbred strain developed by the Animal Breeding Department, University of Otago Medical School, adult male N.Z. white rabbits or adult male guinea-pigs were used in these experiments. Slices from all species were prepared and handled identically. The animals were rapidly killed without prior anaesthesia by stunning and bleeding from the carotid arteries. The livers were then rapidly removed and slices of thickness 0·2–0·3 mm and of average dry weight 6 mg were prepared using a mechanical tissue chopper (McIlwain & Buddle, 1953) manufactured by Mickle Laboratory Engineering Co. U.K. In any experiment no more than 10 min elapsed from the death of the first animal to the preparation of the last slices. Unless otherwise indicated the slices were immediately transferred to a large volume (500 ml.) of $Na^+ = K^+$ medium at 37° C gassed with O_2:CO_2 where they remained for at least 10 min. This initial incubation served to wash the slices free of blood and damaged tissue. The presence of the high concentration of potassium in the medium has been shown by McLaughlin (1972) to enhance the ability of the liver slices to maintain their intracellular potassium during subsequent incubation in more physiological solutions.

After this initial incubation slices were generally transferred to SIF at 37° C bubbled with O_2:CO_2 where they remained for 60 min. Some slices were then taken for analysis. The remainder were randomly distributed to the experimental media. Normally some slices were removed for analysis from all media after a further 30 and 60 min incubation. For conciseness, many tables show only the values after 60 min incubation but in all experiments the compositions of slices analysed after 30 min were completely consistent with those presented.

Analyses

Tissue water content was determined by loss of weight after drying for at least 2 hr in a hot air oven at 105° C (Little, 1964). Ions were extracted from the tissues overnight in 0·1 M-HNO_3 (Little, 1964). Sodium and potassium in these acid extracts were measured with an EEL flame photometer and chloride determined by the method of Cotlove, Trantham & Bowman (1958) using an Aminco Chloride titrator.

Tissue water contents are expressed in kg H_2O/kg tissue dry wt., and ion contents are presented in m-mole/kg tissue dry wt. This method of expression seems more useful than the alternative of m-mole/kg tissue water for it allows direct comparison of ion and water movements in the tissue and does not imply that all the ions in the tissue were dissolved uniformly in all the water.

All Tables show the means ± S.E. of mean of the number of observations stated. The statistical significances of differences between means have been determined using Student's *t* test.

RESULTS

Preliminary experiments

These were performed to determine the optimal conditions for subsequent work. It was found that the incubation of liver slices in medium of more complex composition (Dulbecco's modified Eagle medium with 20% foetal calf serum used in tissue culture work which contains in addition to the ions, amino acids, proteins and vitamins) did not appreciably affect tissue composition. We therefore used the simpler SIF medium for all subsequent experiments.

It has been shown (Burg & Orloff, 1966) that it is difficult to achieve adequate oxygenation of renal tissue *in vitro* at 37° C. We found, however, that provided the rate of bubbling of gas through the medium was sufficient to maintain continual movement of the slices in the medium, tissue composition was satisfactorily maintained.

We also examined the effect of temperature of incubation on tissue composition. Slices of renal cortex are often incubated at 25° C rather than 37° C following the work of Mudge (1951) who found that slices at this temperature contained slightly more potassium than did slices incubated at 38° C. This seemed not to be true for slices of rat liver (Table 1). Though the compositions of the slices were similar after 10 min in $Na^+ = K^+$ medium in the early minutes in SIF medium slices at 25° C lost considerably more potassium and gained more sodium than did slices at 37° C. They also gained water initially before restoring their volume and reaching a water content similar to that of slices incubated at 37° C. However, even after 150 min incubation, though tissue water contents were virtually identical, tissue potassium contents at 25° C were 100 m mole/kg dry wt. lower than those at 37° C. Definitive experiments were therefore carried out at the higher temperature.

Effects of ouabain and of metabolic inhibition on liver slices

Since rat tissues are relatively insensitive to ouabain, slices were exposed to five concentrations over a hundred fold range from 0·1 to 10 mM (Table 2). As expected the greater the concentration of ouabain the greater the inhibition of sodium-potassium exchange, tissue potassium falling progressively while tissue sodium rose. Although at all ouabain concentrations sodium-potassium exchange was clearly inhibited, at no concentration did the presence of ouabain appreciably affect tissue water. This conclusion is confirmed by the relative constancy of tissue chloride contents and the sum of sodium and potassium contents. Ouabain, under these conditions, caused a one-for-one exchange of potassium for sodium without any cellular swelling. Unless ouabain at each concentration

blocked both passive sodium influx and active sodium extrusion equally, such behaviour would not be consistent with the activity of a single ouabain-sensitive sodium pump regulating both intracellular potassium and cellular volume.

Sodium-potassium transport by cells requires the presence of potassium in the medium, and as potassium concentration in the medium is decreased the effectiveness of any concentration of ouabain in inhibiting sodium-potassium transport may be enhanced. We therefore incubated tissues in potassium-free media with or without ouabain (Table 3). Though the media were initially free of potassium this ion was lost from the tissues throughout incubation. It proved impossible using the flame photometer to measure accurately the final medium potassium concentration because of interference from the great excess of sodium in the medium. However, knowing the amount of potassium lost from the tissue and the weight of tissue incubated it can be calculated that the medium never contained more than 0·6 mM potassium after incubation for 60 min. Since potassium is continually diffusing across the membranes from the cytoplasm its concentration in the region of membranes must be considerably higher than its over-all concentration in the medium, and this concentration should determine cellular potassium uptake. Hence, even in a nominally potassium-free medium, cellular potassium should remain higher than in the same medium containing ouabain. Such a result is shown in Table 3, and is similar to results reported for kidney slices incubated in potassium-free media (Macknight, 1968*a*).

Table 3 reveals that even under conditions of low medium potassium concentration with ouabain, tissue water remained unchanged after 30 and 60 min incubation in spite of the profound alterations in tissue potassium and sodium levels.

Taken together, Tables 2 and 3 therefore suggest that liver cells, like renal cortical cells, can maintain cellular volume independently of ouabain-sensitive cation transport. The mechanism depends upon metabolism (Table 3), for tissue incubated in potassium-free medium with 1 mM iodoacetamide and N_2:CO_2 as the gas phase not only lost potassium but also became considerably swollen. Table 4 extends this observation. When both aerobic and anaerobic metabolism were inhibited (by N_2 and iodoacetamide respectively) tissue lost potassium and became swollen with the uptake of sodium, chloride and water. Analysis of these results reveals that slices gained 1·37 kg water/kg dry wt. and 237 m-mole/kg chloride. They gained 388 m-mole/kg dry wt. of sodium but lost 168 m-mole/kg dry wt. of potassium so that their net cation gain was 221 m-mole/kg dry wt. Hence, within the limits of the method tissue swelling seemed to be associated with the uptake of an isotonic extracellular fluid as would

be predicted by the hypothesis that when metabolism is inhibited cells swell as a result of the now unopposed colloid osmotic force exerted by intracellular macromolecules. The results shown in Table 4, together with those in Table 3, exclude the possibility that the failure of cells to swell when exposed to ouabain could be simply the result of loss of cellular

TABLE 1. Kinetic study of rat liver slices incubated at 25 or 37° C

Slices were initially incubated in $Na^+ = K^+$ medium for 10 min. Six slices were then analysed, the remainder were transferred to SIF at the same temperature and analysed at the times shown (each value represents mean ± S.E. of mean of six observations)

	H_2O content (kg/kg dry wt.)		Na^+ (m-mole/kg dry wt.)		K^+ (m-mole/kg dry wt.)	
	37° C	25° C	37° C	25° C	37° C	25° C
Na = K	2·90 ± 0·05	3·01 ± 0·06	205 ± 5	242 ± 8	349 ± 3	337 ± 3
SIF (min)						
5	2·73 ± 0·07	3·14 ± 0·05	317 ± 30	409 ± 10	179 ± 16	180 ± 16
15	2·62 ± 0·05	2·86 ± 0·06	281 ± 20	439 ± 23	179 ± 12	117 ± 10
30	2·52 ± 0·06	2·85 ± 0·09	263 ± 17	421 ± 28	184 ± 15	94 ± 6
45	2·49 ± 0·07	2·76 ± 0·13	222 ± 20	412 ± 21	214 ± 9	96 ± 4
60	2·43 ± 0·02	2·53 ± 0·07	208 ± 16	359 ± 16	243 ± 6	128 ± 6
75	2·48 ± 0·06	2·72 ± 0·08	233 ± 10	378 ± 14	249 ± 10	128 ± 8
90	2·41 ± 0·03	2·38 ± 0·09	203 ± 14	307 ± 18	265 ± 7	148 ± 6
150	2·29 ± 0·05	2·33 ± 0·13	189 ± 13	285 ± 34	277 ± 5	179 ± 8

TABLE 2. Effect of various concentrations of ouabain on rat liver slices. Slices were incubated at 37° C, initially in $Na^+ = K^+$, O_2 medium for 10 min, then in SIF, O_2 medium for 60 min, before transfer to the media shown where they remained a further 60 min. Means of ten observations ± S.E. of mean

	H_2O content (kg/kg dry wt.)	Na^+ content (m-mole/kg dry wt.)	K^+ content (m-mole/kg dry wt.)	Cl^- content (m-mole/kg dry wt.)
SIF, O_2 60 min	2·55 ± 0·06	271 ± 14	237 ± 10	251 ± 15
60 min				
SIF, O_2	2·45 ± 0·05	305 ± 13	210 ± 8	247 ± 12
SIF, O_2, 0·1 mM ouabain	2·41 ± 0·08	326 ± 14	168 ± 6	240 ± 10
SIF, O_2, 0·5 mM ouabain	2·39 ± 0·07	387 ± 12	116 ± 6	251 ± 5
SIF, O_2, 1 mM ouabain	2·56 ± 0·06	423 ± 13	98 ± 6	269 ± 10
SIF, O_2, 10 mm ouabain	2·67 ± 0·05	486 ± 11	40 ± 4	288 ± 7

potassium, for cells lost as much potassium when exposed to iodoacetamide as when incubated with ouabain, yet they became markedly swollen. Indeed, passive loss of cellular potassium could not alone maintain cellular volume indefinitely though it might theoretically allow transient

restoration of volume, as suggested by Kregenow (1971) to explain the behaviour of duck erythrocytes exposed to hypotonic solutions. The conclusion that lack of swelling of liver slices exposed to ouabain is not due simply to potassium loss is consistent with the demonstration in kidney slices that potassium loss plays no part in the ouabain-insensitive restoration of cellular volume in previously leached tissue (Macknight, 1968*a*).

TABLE 3. Effect of ouabain in K^+-free medium on rat liver slices. Slices were incubated at 37° C, in $Na^+ = K^+$, O_2 for 10 min and then in SIF, O_2 for 60 min. Slices were then transferred to media shown. Means of nine to ten observations ± S.E. of mean

	H_2O content (kg/kg dry wt.)	Na^+ content (m-mole/kg dry wt.)	K^+ content (m-mole/kg dry wt.)	Cl^- content (m-mole/kg dry wt.)
SIF, O_2 60 min	2·46 ± 0·05	222 ± 16	266 ± 10	228 ± 9
30 min				
SIF, O_2	2·47 ± 0·12	254 ± 17	220 ± 13	236 ± 16
K-free O_2	2·23 ± 0·05	291 ± 11	172 ± 10	204 ± 6
K-free, O_2, 1 mM ouabain	2·55 ± 0·08	364 ± 25	111 ± 11	240 ± 12
K-free, O_2, 10 mM ouabain	2·30 ± 0·03	381 ± 11	70 ± 6	223 ± 8
K-free, N_2, 1 mM iodoacetamide	3·13 ± 0·10	472 ± 21	111 ± 9	327 ± 16
60 min				
SIF, O_2	2·43 ± 0·11	278 ± 26	223 ± 11	227 ± 16
K-free, O_2	2·40 ± 0·08	376 ± 13	114 ± 7	236 ± 13
K-free, O_2, 1 mM ouabain	2·48 ± 0·06	404 ± 12	58 ± 10	250 ± 7
K-free, O_2, 10 mM ouabain	2·51 ± 0·08	449 ± 11	26 ± 3	263 ± 7
K-free, N_2, 1 mM iodoacetamide	4·22 ± 0·10	718 ± 19	32 ± 3	504 ± 10

Although rat tissues are relatively insensitive to cardiac glycosides, the observation that tissues exposed to 10 mM ouabain lost as much potassium as tissues whose metabolism was inhibited shows that ouabain at this concentration must virtually completely block potassium accumulation in liver slices as it does in rat renal cortical tissue (Macknight, 1968*a*).

Table 4 also shows results of experiments in which slices were incubated in medium with both 1 mM iodoacetamide and 10 mM ouabain. The presence of ouabain here had no effect on tissue ion contents, and did not prevent the cellular swelling associated with inhibition of metabolism. Ouabain did not therefore inhibit sodium influx into non-metabolizing cells. This observation, together with the profound alterations in tissue sodium which accompanied the loss of intracellular potassium from

metabolizing tissue exposed to ouabain excludes the possibility that failure of cellular swelling in metabolizing slices exposed to ouabain reflects inhibition by ouabain of sodium entry to the cells.

TABLE 4. Effect of anoxia and iodoacetamide on rat liver slices. Slices incubated at 37° C, in $Na^+ = K^+$, O_2 medium for 10 min, and then in SIF, O_2 medium for 60 min. Slices then transferred to media shown. Means of eight to ten observations ± S.E. of mean

	H_2O content (kg/kg dry wt.)	Na^+ content (m-mole/kg dry wt.)	K^+ content (m-mole/kg dry wt.)	Cl^- content (m-mole/kg dry wt.)
SIF, O_2 60 min	2·33 ± 0·05	241 ± 9	221 ± 7	226 ± 9
60 min				
SIF, O_2	2·36 ± 0·05	243 ± 9	240 ± 13	197 ± 5
SIF, N_2	2·71 ± 0·08	374 ± 21	165 ± 12	264 ± 15
SIF, N_2, 1 mM iodoacetamide	3·73 ± 0·14	631 ± 37	73 ± 14	434 ± 25
SIF, N_2, 1 mM iodoacetamide 10 mM ouabain	3·78 ± 0·18	615 ± 33	69 ± 6	414 ± 25

TABLE 5. Effect on rat liver slices of immediate anoxia compared with anoxia after incubation in SIF, O_2. Slices incubated at 37° C for 30 min in SIF, O_2, and then transferred to media shown. Means of ten observations ± S.E. of mean

	H_2O content (kg/kg dry wt.)	Na^+ content (m-mole/kg dry wt.)	K^+ content (m-mole/ kg dry wt.)	Cl^- content (m-mole/kg dry wt.)
SIF, O_2 30 min	2·58 ± 0·08	376 ± 13	108 ± 5	229 ± 6
60 min				
SIF, O_2	2·50 ± 0·09	315 ± 21	175 ± 9	225 ± 10
SIF, N_2	2·73 ± 0·12	404 ± 23	120 ± 7	267 ± 15
SIF, N_2, 1 mM iodoacetamide	4·26 ± 0·09	718 ± 12	37 ± 2	471 ± 7
Slices incubated in SIF, N_2 medium, at 37° C for 60 min without previous incubation in SIF, O_2	3·49 ± 0·08	544 ± 25	89 ± 7	375 ± 13

Finally, Table 4 contains results obtained in slices in which only aerobic metabolism was inhibited. These slices became less swollen and retained more potassium than slices in which anaerobic glycolysis was also blocked. Anaerobic metabolism seems to provide some energy for volume restoration in leached rat renal cortical slices (Macknight, 1968*b*). However it has been claimed (Seidman & Cascarano, 1966) that anaerobic metabolism alone is insufficient to allow ion transport by rat liver slices. We therefore examined the effect of inhibition of aerobic metabolism alone (Table 5). We initially incubated tissue from the same group of rats in either oxygenated SIF or SIF gassed with N_2:CO_2. After 30 min

the slices in the oxygenated medium were transferred either to fresh oxygenated medium or medium with or without iodoacetamide and gassed with N_2, where they remained for a further 60 min. In contrast, slices initially exposed to anaerobic conditions simply remained under these conditions for 60 min before analysis. The results showed that initial exposure to oxygenated medium allowed slices subsequently incubated anaerobically to maintain a more nearly normal composition than slices incubated anaerobically throughout. However, even these slices were less swollen and contained more potassium than slices whose anaerobic metabolism was also inhibited by iodoacetamide. It is concluded that in rat liver slices energy derived from anaerobic metabolism can contribute significantly to the maintenance of both cellular volume and cellular potassium.

TABLE 6. Effect of ouabain on rabbit and guinea-pig liver slices. Slices incubated at 37° C in $Na^+ = K^+$, O_2 for 10 min, then SIF, O_2 for 60 min. Slices were then transferred to the media shown. Means of eight to ten observations ± S.E. of mean

	H_2O content (kg/kg dry wt.)	Na^+ content (m-mole/kg dry wt.)	K^+ content (m-mole/ kg dry wt.)	Cl^- content (m-mole/kg dry wt.)
	Rabbit			
SIF, O_2 for 60 min	2·07 ± 0·07	216 ± 10	188 ± 8	202 ± 10
60 min				
SIF, O_2	2·26 ± 0·05	256 ± 10	178 ± 6	201 ± 5
SIF, O_2, 0·1 mM ouabain	2·55 ± 0·03	431 ± 6	40 ± 2	280 ± 7
SIF, O_2, 1 mM ouabain	2·64 ± 0·04	449 ± 10	36 ± 2	380 ± 8
SIF, O_2, 10 mM ouabain	2·40 ± 0·09	431 ± 15	36 ± 2	278 ± 13
	Guinea-pig			
SIF, O_2 for 60 min	2·35 ± 0·07	309 ± 17	126 ± 8	222 ± 13
60 min				
SIF, O_2	2·58 ± 0·05	355 ± 12	114 ± 4	255 ± 10
SIF, O_2, 0·1 mM ouabain	2·89 ± 0·04	499 ± 6	32 ± 1	312 ± 8
SIF, O_2, 1 mM ouabain	2·81 ± 0·05	505 ± 12	29 ± 1	303 ± 16
SIF, O_2, 10 mM ouabain	2·70 ± 0·04	477 ± 10	33 ± 2	280 ± 9

The experiments so far described were all performed using slices of rat liver. We therefore extended our observations to liver slices from two other species, rabbit and guinea-pig, whose tissues are more sensitive to cardiac glycosides. Renal cortical tissue from both rabbit (Kleinzeller & Knotkova, 1964*a*) and guinea-pig (Whittembury, 1968) possesses an ouabain-insensitive mechanism regulating cellular volume. Table 6 shows the effects of ouabain on liver slices from both species. The much greater sensitivity to ouabain is demonstrated by the fact that 0·1 mM ouabain

caused as much loss of potassium as did 10 mM ouabain. As in rat liver slices, though ouabain produced profound inhibition of sodium-potassium transport, it did not cause cellular swelling.

TABLE 7. Effect of ouabain and iodoacetamide on rabbit liver slices. Slices were either (*A*) incubated at 37° C for up to 120 min in SIF, O_2, SIF, O_2 with 10 mM ouabain, or SIF, N_2 with 1 mM iodoacetamide; or (*B*) incubated at 37° C in SIF, O_2 for 60 min, then transferred to SIF, O_2, SIF, O_2 with 10 mM ouabain, or SIF, N_2 with 1 or 10 mM iodoacetamide for a further 60 or 120 min. Means of ten observations ± S.E. of mean

	H_2O content (kg/kg dry wt.)	Na^+ content (m-mole/kg dry wt.)	K^+ content (m-mole/ kg dry wt.)	Cl^- content (m-mole/kg dry wt.)
A 60 min				
SIF, O_2*	2·73 ± 0·13	478 ± 27	53 ± 6	277 ± 19
SIF, O_2, 10 mM ouabain	3·40 ± 0·05	560 ± 22	31 ± 1	309 ± 4
SIF, N_2, 1 mM iodoacetamide	3·79 ± 0·07	662 ± 20	45 ± 2	410 ± 11
120 min				
SIF, O_2*	2·45 ± 0·04	405 ± 18	83 ± 4	235 ± 6
SIF, O_2, 10 mM ouabain	3·43 ± 0·06	573 ± 11	27 ± 1	302 ± 7
SIF, N_2, 1 mM iodoacetamide	5·25 ± 0·18	980 ± 30	41 ± 4	635 ± 24
B SIF, O_2, 60 min*	2·73 ± 0·13	478 ± 27	53 ± 6	277 ± 9
60 min				
SIF, O_2*	2·45 ± 0·04	405 ± 18	83 ± 4	235 ± 6
SIF, O_2, 10 mM ouabain	2·87 ± 0·04	551 ± 16	20 ± 1	344 ± 6
SIF, N_2, 1 mM iodoacetamide	3·42 ± 0·14	612 ± 23	21 ± 1	371 ± 19
SIF, N_2, 10 mM iodoacetamide	3·68 ± 0·07	679 ± 17	20 ± 1	441 ± 9
120 min				
SIF, O_2	2·43 ± 0·03	363 ± 15	132 ± 6	218 ± 5
SIF, O_2, 10 mM ouabain	2·91 ± 0·02	566 ± 10	19 ± 1	306 ± 3
SIF, N_2, 1 mM iodoacetamide	5·30 ± 0·19	969 ± 36	23 ± 1	657 ± 23
SIF, N_2, 10 mM iodoacetamide	4·83 ± 0·12	904 ± 19	22 ± 1	630 ± 16

* For ease of comparison the values in SIF, O_2 medium marked with an asterisk in this, and in Tables 8 and 9 are shown in two places.

Our results in rabbit liver slices appeared to conflict with those reported by Kleinzeller & Knotkova (1964*a*) who had immediately incubated freshly cut rabbit liver slices in oxygenated medium containing ouabain for 60 min. We therefore decided to follow both their protocol and our own to investigate this discrepancy. The results are shown in Table 7 together with an outline of the experimental protocol. To enable direct comparison with the procedure of Kleinzeller & Knotkova (1964*a*) the initial incubation in oxygenated $Na^+ = K^+$ medium was omitted. This resulted in tissue potassium contents considerably lower than those shown for rabbit slices in Table 6. Our results for freshly cut slices (Table

7*A*) exposed immediately to oxygenated medium containing ouabain were very similar to those reported by Kleinzeller & Knotkova (1964*a*) with swelling within the first 60 min of incubation. They thought this showed that rabbit liver slices possessed no ouabain-insensitive mechanism regulating cellular volume, but the present results do not support this conclusion. Though slices did swell in the first 60 min their water content then remained constant over a further 60 min. Slices incubated with iodoacetamide and N_2 were more swollen at the end of 60 min and indeed swelled continuously throughout incubation. Slices therefore appeared still to regulate their volume in ouabain-containing medium but the water content was maintained constant at a greater than normal level.

In contrast, slices incubated initially for 60 min in oxygenated medium (Table 7*B*), though they lost potassium and gained sodium did not subsequently change in volume when transferred to medium with ouabain and incubated a further 120 min. Slices which remained in oxygenated medium without ouabain continued to accumulate cellular potassium and showed a small but significant loss of tissue water. Slices exposed to N_2 and 1 or 10 mM iodoacetamide swelled throughout incubation and their potassium fell to levels similar to those found in slices with ouabain. The similarity of the results with 1 and 10 mM iodoacetamide confirms that a concentration of 1 mM virtually abolished anaerobic glycolysis.

These results prompted an examination of the behaviour of both rat and guinea-pig liver slices under these same experimental conditions. Table 8 shows results obtained with rat tissues. Again, tissue potassium contents were somewhat lower than those observed after initial exposure to $Na^+ = K^+$ medium. The results are exactly comparable to the findings in rabbit liver slices. Table 9 shows the results when slices of guinea-pig liver were similarly incubated. Without initial exposure to $Na^+ = K^+$ medium, tissue potassium contents were very low and even slices in oxygenated medium, showed virtually no potassium accumulation over the first 120 min incubation. (Guinea-pig liver slices even when initially incubated in $Na^+ = K^+$ medium (Table 6) had considerably lower potassium contents than did either rabbit or rat slices. The reasons for those species differences remain to be investigated.) In contrast to both rabbit and rat liver slices, the water contents of freshly cut guinea-pig slices (Table 9*A*) after 60 min incubation were nearly the same in oxygenated medium and in medium containing ouabain. However, both values were abnormally high. While slices in oxygenated medium lost water during the next 60 min, the water content of the slices incubated with ouabain remained constant. Once again the tissue exposed to ouabain maintained its cellular volume though at a greater than normal level,

TABLE 8. Effect of ouabain and iodoacetamide on rat liver slices. Protocol as for rabbit slices (Table 7). Means of ten observations ± S.E. of mean

	H_2O content (kg/kg dry wt.)	Na^+ content (m-mole/kg dry wt.)	K^+ content (m-mole/kg dry wt.)	Cl^- content (m-mole/kg dry wt.)
A 60 min				
SIF, O_2*	2·63 ± 0·04	324 ± 7	157 ± 7	224 ± 7
SIF, O_2, 10 mM ouabain	3·13 ± 0·05	570 ± 21	31 ± 1	331 ± 6
SIF, N_2, 1 mM iodoacetamide	3·82 ± 0·12	620 ± 29	67 ± 5	393 ± 18
120 min				
SIF, O_2*	2·49 ± 0·07	283 ± 14	216 ± 10	206 ± 7
SIF, O_2, 10 mM ouabain	3·12 ± 0·06	587 ± 26	27 ± 1	335 ± 11
SIF, N_2, 1 mM iodoacetamide	4·57 ± 0·12	892 ± 56	34 ± 2	538 ± 23
B SIF, O_2, 60 min*	2·63 ± 0·04	324 ± 7	157 ± 7	224 ± 7
60 min				
SIF, O_2*	2·49 ± 0·07	283 ± 14	216 ± 10	206 ± 7
SIF, O_2, 10 mM ouabain	2·55 ± 0·04	465 ± 10	38 ± 5	241 ± 6
SIF, N_2, 1 mM iodoacetamide	3·90 ± 0·16	756 ± 39	62 ± 4	425 ± 17
SIF, N_2, 10 mM iodoacetamide	4·04 ± 0·09	725 ± 31	45 ± 5	457 ± 14
120 min				
SIF, O_2	2·35 ± 0·08	283 ± 12	203 ± 4	199 ± 8
SIF, O_2, 10 mM ouabain	2·63 ± 0·04	510 ± 5	21 ± 1	290 ± 6
SIF, N_2, 1 mM iodoacetamide	4·61 ± 0·16	831 ± 40	27 ± 4	581 ± 52
SIF, N_2, 10 mM iodoacetamide	4·84 ± 0·11	903 ± 31	22 ± 2	613 ± 22

* For explanation, see Table 7.

whereas tissue whose metabolism was inhibited by iodoacetamide and N_2 swelled progressively.

Table 9*B* shows the behaviour of slices first incubated in SIF for 60 min before transfer to the media shown. Guinea-pig slices initially incubated in oxygenated medium for 60 min were markedly swollen. When transferred to ouabain-containing medium, they lost some potassium but their tissue water content did not change over the next 120 min. Since these slices were as swollen initially as were those fresh slices immediately incubated with ouabain for 60 min (Table 9*A*), the water contents of all guinea-pig slices incubated with ouabain were virtually identical. In contrast, slices in oxygenated medium slowly accumulated potassium and lost sodium and water. By the end of a total incubation of 180 min their water content was approaching a more normal value (compare the water contents of Table 9 with those for guinea-pig in Table 6) but their potassium content was still considerably lower than that usually found in metabolizing tissue. Once again inhibition of metabolism resulted in progressive swelling and loss of potassium.

TABLE 9. Effect of ouabain and iodoacetamide on guinea-pig liver slices. The protocol as for rabbit slices (Table 7). Means of ten observations ± S.E. of means

	H_2O content (kg/kg dry wt.)	Na^+ content (m-mole/kg dry wt.)	K^+ content (m-mole/ kg dry wt.)	Cl^- content (m-mole/kg dry wt.)
A 60 min				
SIF, O_2*	4·00 ± 0·07	663 ± 11	41 ± 1	413 ± 8
SIF, O_2, 10 mM ouabain	3·84 ± 0·05	653 ± 8	35 ± 2	397 ± 9
SIF, N_2, 1 mM iodoacetamide	4·68 ± 0·07	763 ± 12	43 ± 3	526 ± 31
120 min				
SIF, O_2*	3·34 ± 0·08	560 ± 15	47 ± 2	377 ± 23
SIF, O_2, 10 mM ouabain	3·90 ± 0·05	672 ± 14	34 ± 4	410 ± 5
SIF, N_2, 1 mM iodoacetamide	5·13 ± 0·08	886 ± 9	35 ± 1	576 ± 15
B SIF, O_2 60 min*	4·00 ± 0·07	663 ± 11	41 ± 1	413 ± 8
60 min				
SIF, O_2*	3·34 ± 0·08	560 ± 15	47 ± 2	377 ± 23
SIF, O_2, 10 mM ouabain	3·98 ± 0·07	694 ± 12	27 ± 1	438 ± 12
SIF, N_2, 1 mM iodoacetamide	4·04 ± 0·09	697 ± 13	26 ± 1	416 ± 13
SIF, N_2, 10 mM iodoacetamide	4·39 ± 0·13	742 ± 16	23 ± 1	476 ± 14
120 min				
SIF, O_2	2·93 ± 0·06	462 ± 8	80 ± 2	276 ± 7
SIF, O_2, 10 mM ouabain	3·93 ± 0·10	706 ± 16	24 ± 1	430 ± 13
SIF, N_2, 1 mM iodoacetamide	4·82 ± 0·06	831 ± 11	24 ± 1	547 ± 8
SIF, N_2, 10 mM iodoacetamide	4·82 ± 0·04	842 ± 7	22 ± 1	577 ± 12

* For explanation, see Table 7.

DISCUSSION

These experiments were performed to determine whether or not liver cells possess a metabolically dependent, ouabain-insensitive, potassium-independent mechanism concerned in the regulation of cellular volume. The results clearly demonstrate that they do. Liver slices from rat and rabbit both maintained a relatively normal volume when incubated with ouabain, provided that they had first been exposed to oxygenated medium. Without such prior incubation they initially became somewhat swollen but then maintained a new though greater water content. Guinea-pig liver slices required, in addition, an initial period in a high potassium medium if they were to maintain a relatively normal water content either in oxygenated medium alone or in oxygenated medium with ouabain. Without this treatment they swelled initially but like rat and rabbit slices the presence of ouabain did not prevent their maintaining this increased volume during subsequent incubation. This dependence of volume regulation on an initial period in oxygenated medium without

ouabain suggests important relationships between cellular volume, metabolism, ouabain and intracellular potassium which are being examined in detail.

From a consideration of the experimental results, alternative explanations for the maintenance of cellular volume in the presence of ouabain, namely passive loss of cellular potassium balancing passive sodium entry from the medium, or inhibition by ouabain of passive sodium influx to the cells are rendered unlikely.

The present results establish the existence in liver cells of ouabain-insensitive volume regulation but do not themselves favour any particular hypothesis for the mechanism of this regulation. Since liver cells, unlike renal cortical cells, are not highly specialized for transepithelial sodium transport the results offer no support to the suggestion that the ouabain-insensitive mechanism is a second sodium pump primarily involved in transepithelial sodium transport (Giebisch, Boulpaep & Whittembury, 1971). An alternative hypothesis that such regulation involves a contractile mechanism situated in the cellular membrane (Kleinzeller, 1965) seems to demand an impossibly high tensile strength in the membrane. A third possibility, that volume regulation involves metabolically dependent interrelationships between intracellular macromolecules (Wiggins, 1964) remains to be excluded.

Though the precise nature of the mechanism involved in ouabain-insensitive volume regulation remains to be established, its demonstration in both skeletal muscle (rat diaphragm, Kleinzeller & Knotkova, 1964*b*) and smooth muscle (Daniel & Robinson, 1971), kidney (Kleinzeller & Knotkova, 1964*a*; Macknight, 1968*b*; Maude, 1969; Whittembury, 1968) and now in liver raises the possibility that this process is a general property of mammalian cells. It has also been shown that isolated toad bladder epithelial cells maintain a normal volume with ouabain in the medium (Macknight, DiBona, Leaf & Civan, 1971). It is therefore of interest that work with both duck (Kregenow, 1971) and mammalian erythrocytes (Poznansky & Solomon, 1972) has suggested that variations in their volume in response to hypotonic media may involve mechanisms other than the conventional sodium-potassium linked, ouabain-sensitive pump. More recently, Parker (1973) has made a similar suggestion from experiments with dog erythrocytes.

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