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# CONTROL OF SODIUM, POTASSIUM AND WATER CONTENT AND UTILIZATION OF OXYGEN IN RAT LIVER SLICES, STUDIED BY AFFECTING CELL MEMBRANE PERMEABILITY WITH CALCIUM AND ACTIVE TRANSPORT WITH OUABAIN

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## SUMMARY

Slicing and incubating rat liver caused a rapid $Ca^{2+}$-independent exchange of $K^+$ for $Na^+$, followed by a $Ca^{2+}$-dependent recovery. Freshly cut slices washed for 10 min in a $Ca^{2+}$ medium containing equal concentrations of $Na^+$ and $K^+$ showed little replacement of $K^+$ by $Na^+$ during subsequent incubation in a normal medium. Changes in medium $Ca^{2+}$ caused immediate changes in slice $Na^+$ and $K^+$, before any substantial change in slice $Ca^{2+}$ and without altering gradients responsible for passive transfers of $Na^+$ and $K^+$. $Ca^{2+}$ did not influence an ouabain-sensitive $Na^+$ pump. It also appeared unlikely that $Ca^{2+}$ was required for an ouabain-insensitive $Na^+$ pump or for maintenance of intracellular structures concerned with $K^+$ sorption, even if these mechanisms existed in the slices. Instead $Ca^{2+}$ seemed to maintain the cell membrane relatively impermeable to $Na^+$ and $K^+$. An ouabain-sensitive $Na^+$ pump not normally dependent on oxygen supply to the cells appeared to alter its activity according to the work required of it. Control of slice water content could not be attributed to the activity of this pump.

## INTRODUCTION

Although $Ca^{2+}$ has been shown to alter the ability of incubated rat liver slices to maintain a low $Na^+$ content and high $K^+$ content[1] there is doubt about the mechanisms involved. It is generally assumed that $Ca^{2+}$ maintains a relatively low cell membrane permeability to $Na^+$ and $K^+$ although the evidence quoted for this is usually derived from other tissue preparations[2]. Loewenstein[3], studying the high permeability of cell–cell junctions in a number of tissues including liver, found this depended on low $Ca^{2+}$ in the (intracellular) region of the junctional membrane. Judah and Ahmed[4] investigated an apparent metabolic effect of $Ca^{2+}$, and suggested that $Ca^{2+}$ taken up by slices occupied and paralysed the active sites of the $Na^+$ transporting system. This effect was also studied by Van Rossum[2] who included some information of slice $Ca^{2+}$ contents. Others[5] have postulated that the $Na^+/K^+$ ratio of normal tissue is maintained by mechanisms other than the "pump and leak" or mem-

brane theory. This work presents further details of the behaviour of liver incubated with and without $Ca^{2+}$, using a novel incubation medium. Slice $Ca^{2+}$ concentrations were measured concurrently with $Na^+$ and $K^+$ concentrations and water contents, at all stages of incubation. Oxygen consumption was also measured in an attempt to follow metabolic integrity during incubation. The results indicated that calcium affected cell membrane permeability, and it was then possible to make a preliminary interpretation of oxygen utilization and control of water content in the slices.

## METHODS

### *Expression of results*

Water contents of slices are dimensionless numbers, being the ratio of the mass of water and the mass of solid material in the slice. Electrolyte concentrations are expressed as amounts of electrolyte in the water (mmole·$kg^{-1}$) contained in the slices. This is not intended to imply that all electrolyte was in solution, or that all slice water was solvent. Where appropriate, electrolyte contents (mmole·$kg^{-1}$ of dry wt) are also given.

Graphs show mean ± standard deviation of groups of relevant observations. Comparisons of samples for probability of equal means have been made using Student's *t* test.

### *Media*

The media were developed from the synthetic interstitial fluid of Bretag[6]. $Ca^{2+}$ was omitted from all the media except where specifically required, when it was added separately. Compositions were: Incubation medium (mM): $Na^+$, 145.0; $K^+$, 3.5; $Mg^{2+}$, 0.68; $Cl^-$, 115.6; $HCO_3^-$, 21.6; $H_2PO_4^-$, 1.7; $SO_4^{2-}$, 0.68; gluconate, 9.6; glucose, 5.55; sucrose, 7.6. pH 7.4 with $CO_2$–$O_2$ (5:95, v/v) in the gas phase, temperature 37 °C. $Na^+ = K^+$ medium (mM): $Na^+$, 75; $K^+$, 75; $Cl^-$, 117.1; otherwise identical to incubation medium. $Ca^{2+}$ (M $CaCl_2$) or EDTA was added to these media as required.

### *Animals*

Respiration studies were performed on slices from male rats of a closed colony albino strain from the Otago Medical School Animal Department. These rats were also used for the analyses of slice composition shown in Fig. 4. All other results were obtained with male black and white hooded rats of an inbred strain from the Otago Medical School Animal Department.

### *Procedure*

Rats were anaesthetised with diethyl ether and bled from the carotid artery. The liver was removed and slices cut on a mechanical chopping device[7] (Mickle Laboratory Engineering Co.), then transferred to the desired medium without prior leaching at low temperature, or equilibration. This procedure could be accomplished in 4 min and enabled the initial behaviour of incubated rat liver slices to be followed. Slices were incubated in open beakers in controlled-temperature water-baths, stirred by the bubbling gas phase.

*Analytical methods*

Slices required for analysis were gently blotted and their water content determined by weighing after at least 2.5 h at 105 °C (ref. 8). The dried slices were extracted overnight with 2.7 ml of 0.1 M $HNO_3$ and the extracts analysed for $Na^+$ and $K^+$ by flame photometry and for $Ca^{2+}$ by atomic absorption spectrophotometry.

For measurement of $O_2$ consumption with an oxygen electrode, slices were incubated in a 134-ml water-jacketed glass vessel with no gas phase. The rate of $O_2$ consumption by slices was constant over the range of $O_2$ concentrations 0.95–0.35 of saturation. The medium was recharged with $CO_2$–$O_2$ (5:95, v/v) when the $O_2$ content fell to 0.4–0.5 of saturation. Rates of $O_2$ consumption are expressed in $cm^3 \cdot s^{-1} \cdot (kg \text{ initial wet wt})^{-1}$. The oxygen electrode provided greater accuracy and sensitivity to rapid changes in $O_2$ consumption than a manometric technique would have done, and also enabled a $HCO_3^-$-buffered medium to be used for studies of both electrolytes and $O_2$ consumption. In these and other cases, sets of related observations together with their individual time coordinates were subjected to polynomial regression analysis to obtain rate equations. On graphs these took the form of two lines representing fitted dependent values ± S.E. of fit.

## RESULTS

*Effect of $Ca^{2+}$ on incubated liver slices*

Freshly cut slices sustained a rapid loss of $K^+$ and gain of $Na^+$ when suspended in incubation medium both with and without $Ca^{2+}$ (Fig. 1). These electrolyte changes,

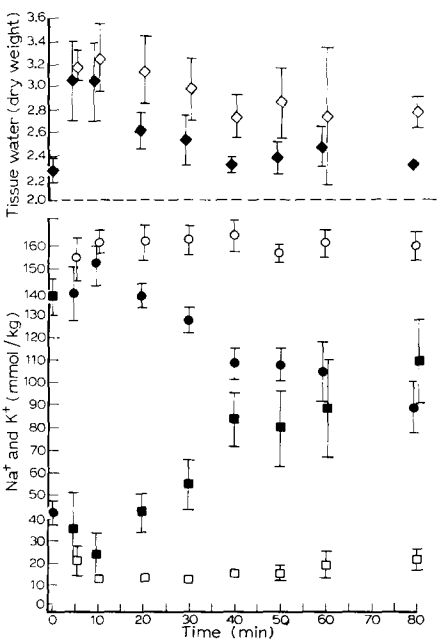


Fig. 1. Amounts of $Na^+$, $K^+$ and water in slices maintained in incubation medium and in incubation medium containing 1.5 mM $Ca^{2+}$. Each point represents mean ± S.D. of 6–8 separate observations. Composition of unincubated liver plotted at 0 min. Incubation medium: ○, $Na^+$; □, $K^+$; ◇, $H_2O$. Incubation medium + $Ca^{2+}$: ●, $Na^+$; ■, $K^+$; ◆, $H_2O$.

which lasted for 10 min, were accompanied by considerable swelling of the slices, also shown in Fig. 1. The $Ca^{2+}$ concentration of slices meanwhile increased rapidly from 1.45 to 6.49 mmole·$kg^{-1}$ in the presence of $Ca^{2+}$. In the absence of medium $Ca^{2+}$ there was a small drop in slice $Ca^{2+}$ during the first 10 min.

The changes during the first 10 min of incubation were not subsequently maintained. In the absence of $Ca^{2+}$, $Na^+$ and $K^+$ concentrations remained at approximately the levels previously achieved. But in the presence of $Ca^{2+}$ there was a steady recovery of slice $K^+$ (86.0 mmole·$kg^{-1}$) and a parallel extrusion of $Na^+$ (62.2 mmole·$kg^{-1}$) during 70 min of further incubation. Slice $Ca^{2+}$ did not alter much in the presence of $Ca^{2+}$, but there was a steady decrease of 0.95 mmole·$kg^{-1}$ over 80 min in the absence of medium $Ca^{2+}$. The water contents of slices decreased after the first 10 min of incubation, and in the presence of $Ca^{2+}$ they returned to the level in fresh liver tissue. They remained significantly higher than this in the absence of $Ca^{2+}$, although some recovery was seen.

*Preincubation of slices in $Na^+ = K^+$ medium in presence and absence of $Ca^+$*

A method of incubation was sought that would provide a short wash, and protect slices from the drastic changes in composition that occurred during the first 10 min in incubation media. Washing in media in which the concentration of $Na^+$ and $K^+$ were respectively equal to those of $K^+$ and $Na^+$ in incubation media gave slices which contained little $Na^+$ and were rich in $K^+$, but this composition was not maintained on subsequent incubation in incubation media.

Slices washed for 10 min in $Na^+ = K^+$ media with or without $Ca^{2+}$, then transferred to incubation media and incubated for up to 6 h were more satisfactory (Fig. 2). Concentration of the medium by evaporation was not studied, but there was no obvious depletion of medium volume.

During the 10-min washing, $Na^+$ concentration in these slices had increased either in the presence or absence of $Ca^{2+}$. The $K^+$ concentration was reduced in the absence of $Ca^{2+}$, and had not altered significantly in the presence of $Ca^{2+}$, but since this medium would have increased extracellular $K^+$, intracellular $K^+$ was probably lower than that of cells *in vivo*. The water content was slightly elevated after 10 min washing.

On transfer of the washed slices to incubation media there was a rapid increase in $Na^+$ and decrease in $K^+$ concentrations in the absence of $Ca^{2+}$; these changes continuing for about 15 min, after which relatively stable levels were reached and maintained over 4 h. In the presence of $Ca^{2+}$, the initial increase in slice $Na^+$ and decrease in $K^+$ on transfer to incubation medium were smaller. On continuing incubation, these slices lost small amounts of $Na^+$ and gained a little $K^+$ over 6 h, finally showing a $Na^+$ concentration somewhat greater than fresh liver and a $K^+$ concentration almost indistinguishable from that of fresh liver tissue ($P > 0.05$).

In the absence of medium $Ca^{2+}$, slice $Ca^{2+}$ declined throughout the 6 h of incubation, finally reaching almost zero values. In the presence of $Ca^{2+}$, slice $Ca^{2+}$ increased by 2.35 mmole·$kg^{-1}$ during the incubation in $Na^+ = K^+$ medium then remained at an approximately constant level for 6 h.

The water content, which increased slightly during washing in $Na^+ = K^+$ media, declined during a further 30 min of incubation to about the level in unsliced liver tissue. This value was then maintained either with or without $Ca^{2+}$ for 4 h. But while

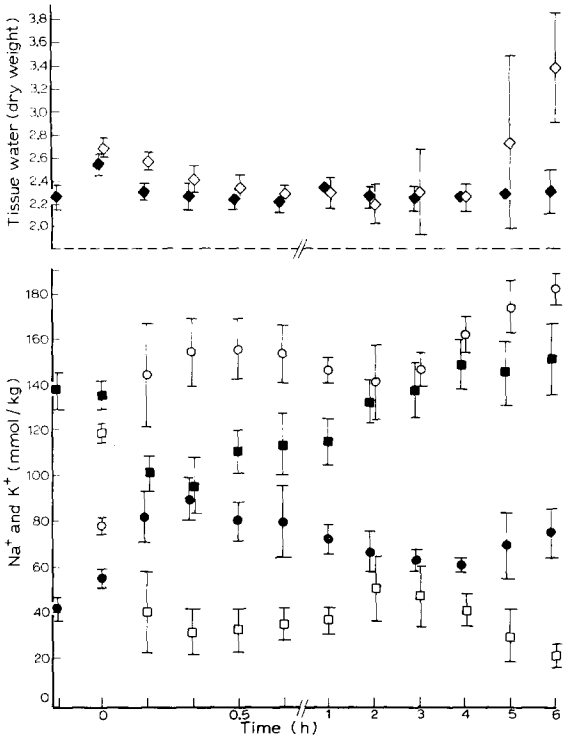


Fig. 2. Amounts of $Na^+$, $K^+$ and water in slices washed for 10 min in $Na^+ = K^+$ medium and in $Na^+ = K^+$ medium + 1.5 mM $Ca^{2+}$ then incubated in incubation medium and in incubation medium + 1.5 mM $Ca^{2+}$. Each point represents mean ± S.D. of 6–8 separate observations. Slices transferred from $Na^+ = K^+$ media to incubation media at 0 h. Composition of unincubated liver plotted at −10 min copied from previous experiment. $Ca^{2+}$-free media: ○, $Na^+$; □, $K^+$, ◇, $H_2O$. Media + $Ca^{2+}$: ●, $Na^+$; ■, $K^+$ ◆, $H_2O$.

in the presence of $Ca^{2+}$, a further 2 h of incubation revealed no change in water content, in the absence of $Ca^{2+}$ a considerable swelling had occurred. This was associated with uptake of $Na^+$ and loss of $K^+$ from the tissue.

*Effect of transferring slices from medium containing $Ca^{2+}$ to $Ca^{2+}$-free medium*

An attempt was made to separate effects of lack of $Ca^{2+}$ on incubated slices from changes caused by cutting and incubating slices. Slices were washed for 10 min in $Na^+ = K^+$ medium containing 1.5 mM $Ca^{2+}$, then maintained in incubation medium containing 1.5 mM $Ca^{2+}$ for 60 min. The slices were then placed in incubation medium devoid of $Ca^{2+}$ and analysed for their electrolyte and water contents during incubation over 60 min. The results are shown in Fig. 3.

Introduction of the slices into $Ca^{2+}$-free medium was followed immediately by a slowly declining increase in $Na^+$ concentration and a corresponding immediate decrease in $K^+$ concentration; $Na^+$ after $t$ min $= (79.2 + 3.00\ t - 0.0312\ t^2)$ mmole · $kg^{-1}$; $K^+$ after $t$ min $= (110.0 - 3.03\ t + 0.0326\ t^2)$ mmole · $kg^{-1}$.

Both these changes appeared to commence without any time lag. They con-

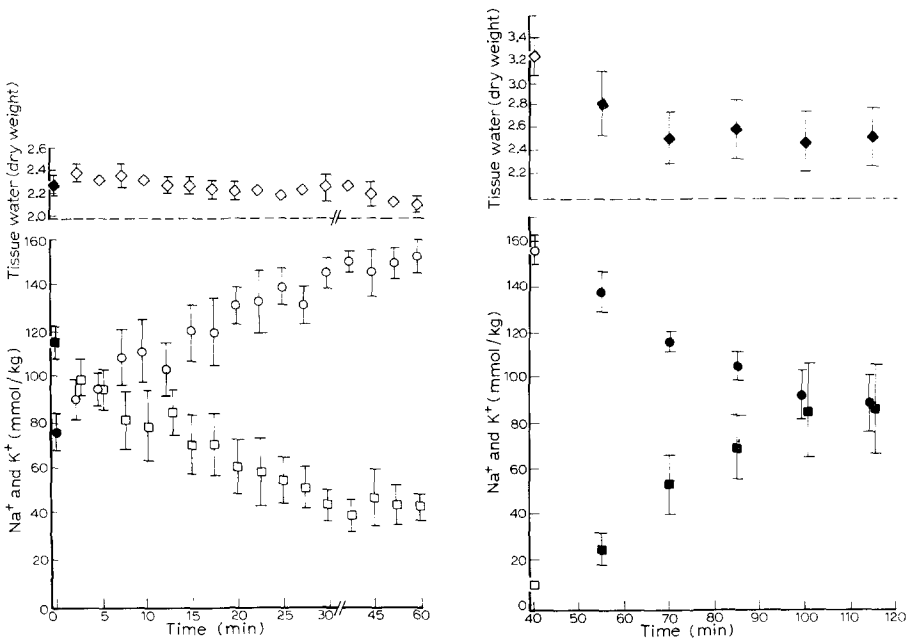


Fig. 3. Amounts of $Na^+$, $K^+$ and water in slices maintained in incubation medium after preliminary incubation for 1 h in medium + 1.5 mM $Ca^{2+}$. Each point represents mean ± S.D. of 6–10 separate observations. Composition of slices after incubation for 1 h in medium + $Ca^{2+}$ plotted at 0 min. Incubation medium: ○, Na; □, $K^+$; ◇, $H_2O$. Incubation medium + $Ca^{2+}$: ●, $Na^+$; ■, $K^+$; ◆, $H_2O$.

Fig. 4. Amounts of $Na^+$, $K^+$ and water in slices after addition of 1.5 mM $Ca^{2+}$ to incubation medium after incubation for 40 min in medium devoid of $Ca^{2+}$. Each point represents mean ± S.D. of 8 separate observations. Composition of slices after 40 min in absence of $Ca^{2+}$ plotted at 40 min. Incubation medium: ○, $Na^+$; □, $K^+$; ◇, $H_2O$. Medium + $Ca^{2+}$: ●, $Na^+$; ■, $K^+$; ◆, $H_2O$.

tinued for about 30 min, after which approximately stable levels of both $Na^+$ and $K^+$ were maintained.

The $Ca^{2+}$ concentration decreased from 4.25 to 3.05 mmole·$kg^{-1}$ ($P<0.001$) within 2.5 min of introduction to $Ca^{2+}$-free medium. This was followed by a slower steady decline for the remainder of the incubation. In contrast to the very large changes in ions, the water content remained quite constant ($P>0.5$) in the absence of $Ca^{2+}$.

To check that these changes were not merely due to the transfer process, slices were incubated under identical conditions to those described above but were transferred from incubation medium containing $Ca^{2+}$ to identical fresh medium. In this case there was no noticeable alteration in any of the measured parameters.

*Effects of adding $Ca^{2+}$ to $Ca^{2+}$-free medium during incubation of slices*

Slices were washed for 10 min in incubation medium, then transferred to fresh medium and incubated a further 30 min, after which 1.5 mM $Ca^{2+}$ was added and the incubation continued. Water content and electrolyte concentrations of slices were measured after addition of $Ca^{2+}$ (Fig. 4).

Following addition of $Ca^{2+}$, $Na^+$ concentration decreased at a declining rate:

and $K^+$ increased concurrently: $Na^+$ after $t$ min $= (234.0 - 2.26\ t + 0.00870\ t^2)$ mmole·kg$^{-1}$; $K^+$ after $t$ min $= (-80.5 + 2.53\ t - 0.00909\ t^2)$ mmole·kg$^{-1}$. Both these equations were calculated on the basis that $Ca^{2+}$ was added at 40 min. Although slices were not analysed until 15 min after addition of $Ca^{2+}$, it appeared by interpolation that the changes in $Na^+$ and $K^+$ commenced without any time lag.

As in previous cases the $Ca^{2+}$ concentration increased rapidly on addition of $Ca^{2+}$ to the medium, reaching 5.7 mmole·kg$^{-1}$.

The water content, which was 3.24 before the addition of $Ca^{2+}$, decreased after the addition by 0.72 within 30 min and then remained constant.

*Effect of ouabain during incubation of slices in presence and absence of $Ca^{2+}$*

Ouabain was used to inhibit active ion transport in slices incubated in the presence and absence of $Ca^{2+}$. Slices were washed in $Na^+ = K^+$ media for 10 min, transferred to incubation medium, and maintained for 60 min. Ouabain at 3 mg·ml$^{-1}$ (4.12 mM) was then added and slices analysed during the subsequent 40 min (Fig. 5). Half of the slices so treated were incubated in 1.5 mM $Ca^{2+}$ throughout. Fig. 6 shows the $O_2$ consumption of slices incubated in the presence of 4.12 mM ouabain added after 30 min of incubation. Preliminary experiments with lower concentrations of ouabain had demonstrated a less complete inhibition than that described here.

On addition of ouabain, slices incubated in the presence of $Ca^{2+}$ gained $Na^+$ at an initial rate of 1.97 mmole·kg$^{-1}$·min$^{-1}$ and lost $K^+$ at a rate of 1.80

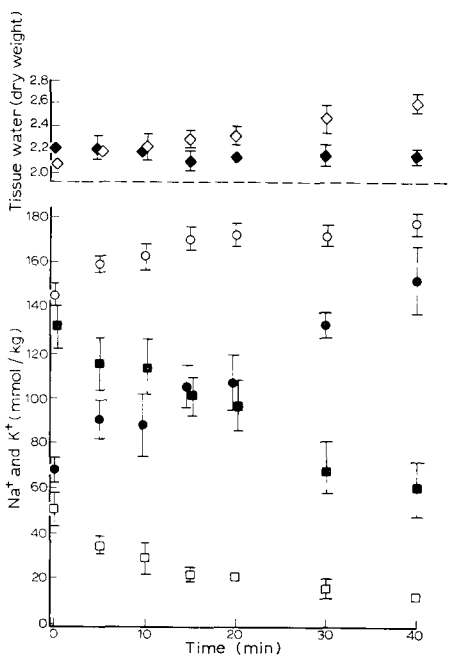


Fig. 5. Amounts of $Na^+$, $K^+$ and water in slices incubated in media containing 4.12 mM ouabain. Each point represents mean ± S.D. of 6 separate observations. Composition of slices before addition of ouabain is plotted at 0 min. Incubation medium: ○, $Na^+$; □, $K^+$; ◇, $H_2O$. Medium + $Ca^{2+}$: ●, $Na^+$; ■, $K^+$; ◆, $H_2O$.

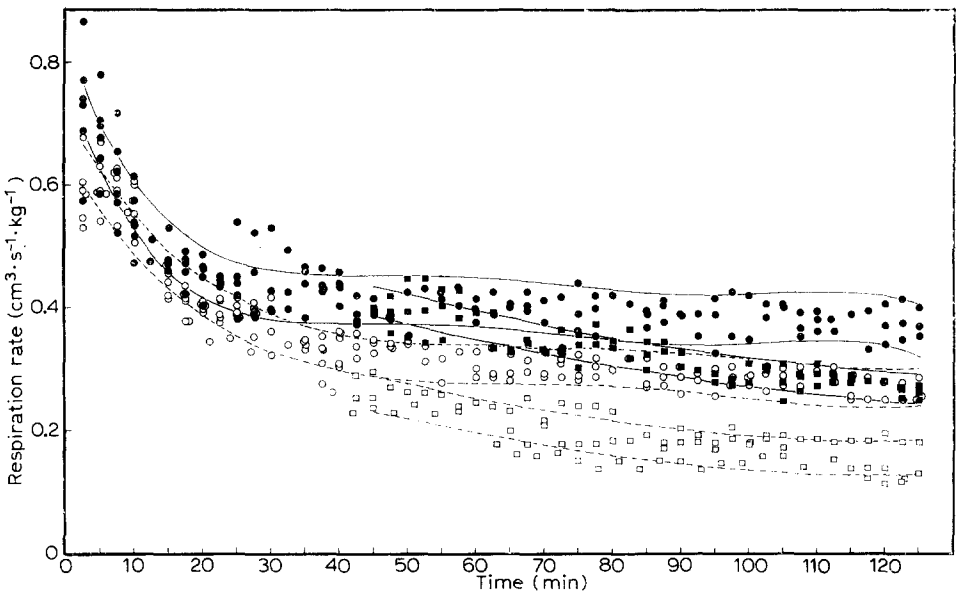


Fig. 6. Respiration rates of slices before and after addition of 4.12 mM ouabain to incubation medium. Lines indicate ± S.E. of equation fitted by polynomial regression analysis of relevant points. Incubation medium: ○- - -○, rate without ouabain; □- - -□, rate with ouabain. Incubation medium + 1.5 mM $Ca^{2+}$: ●—●, rate without ouabain; ■—■, rate with ouabain.

mmole·$kg^{-1}$·$min^{-1}$. The accumulation of $Na^+$ and loss of $K^+$ continued throughout 40 min of incubation and the slice concentrations appeared to be approaching those of slices without $Ca^{2+}$. In the absence of $Ca^{2+}$, accumulation of $Na^+$ and loss of $K^+$ were also observed following addition of ouabain. These changes were faster initially than in the presence of $Ca^{2+}$, although the gradients driving passive movements of $Na^+$ and $K^+$ were smaller.

There were no obvious changes in $Ca^{2+}$ concentration after addition of ouabain. during incubation either in the presence or absence of $Ca^{2+}$. Slices incubated in the presence of $Ca^{2+}$ showed no increase in water content 40 min after addition of ouabain but those incubated in the absence of $Ca^{2+}$ had gained water ($P < 0.001$).

The addition of ouabain caused a marked and quite similar decline in respiratory rate, both in the presence and absence of $Ca^{2+}$, of the order of 0.004 $cm^3 \cdot s^{-1} \cdot kg^{-1} \cdot min^{-1}$ during the first few minutes after the addition. While respiratory rates of slices not subjected to the influence of ouabain remained constant or declined only slightly, the decline continued during 80 min of incubation in the presence of ouabain but at a decreasing rate.

In the absence of $Ca^{2+}$, the addition of ouabain not only initiated a continuing decline in respiration rate but also resulted in an immediate fall which could not be detected in the presence of $Ca^{2+}$.

## DISCUSSION

The concentration of $Ca^{2+}$ inside the cells of liver and many other tissues is too small to be in equilibrium with external $Ca^{2+}$ at measured membrane potentials, and

active outward transport must be assumed. However, the membrane potential of incubated rat liver slices has been reported as 0.028 V, inside negative, which is considerably lower than the value for intact liver, but even slices with this low potential, incubated in 1.5 mM $Ca^{2+}$, should have an intracellular equilibrium concentration of $Ca^{2+}$ of 12.2 mM. In these experiments, as in other work[10–12], incubation of slices or prolonged perfusion of liver resulted in accumulation of $Ca^{2+}$. In the case of slices this was complete in 10 min and no more $Ca^{2+}$ was accumulated on continuing incubation. Van Rossum[12] reported that $Ca^{2+}$ accumulated at 1 °C was lost on increasing the temperature to 38 °C, but some of this $Ca^{2+}$ may have been associated with water lost at about the same time[13]. At all events, the concentration of $Ca^{2+}$ in normal incubated slices never approached the equilibrium concentration (12.2 mM) and since other work in this laboratory indicated that only 0.17 of the $Ca^{2+}$ in incubated slices could be found in a supernatant devoid of mitochondria, the concentration of free $Ca^{2+}$ in cytoplasmic solution was likely to be so low that active outward transport of $Ca^{2+}$ would need to be postulated in slices.

Medium $Ca^{2+}$ was not only required for incubated rat liver slices to maintain a normal steady state composition with low $Na^+$ and high $K^+$ (Fig. 2); there were also immediate changes in composition when $Ca^{2+}$ alone was altered in the medium (Figs 3 and 4), without altering the gradients of $Na^+$ and $K^+$. The addition of ouabain also caused changes in $Na^+$ and $K^+$ concentrations, and these were additional to the effects arising from absence of $Ca^{2+}$ (Fig. 5).

$Ca^{2+}$ might have acted by several possible mechanisms. It could have reduced leakage of $Na^+$ and $K^+$ in a pump-leak system. Alternatively it could have enhanced active ion transport by being necessary either for metabolism or for an ion pump, which would have to be other than the ouabain-inhibited $Na^+$–$K^+$ exchange pump, for which there is no evidence of $Ca^{2+}$ dependence[14]. It is also possible that $Ca^{2+}$ was required for accumulation of $K^+$ by sorption on some cellular component. If the structure of such components was energy dependent, this could also be "active transport".

Because of the difficulties of making reliable determinations[15,16] the extracellular space in the slices was not measured. The amount of extracellular $Ca^{2+}$ was therefore not known, but this fraction could be expected to change more rapidly in response to alterations in the concentration of $Ca^{2+}$ in the medium. Hence although no compartment could be considered $Ca^{2+}$ free and unable to contribute an effect, the behaviour of slices transferred to $Ca^{2+}$ free medium (Fig. 3) is evidence against an intracellular action, as, for example, on metabolism or on $K^+$ sorption. The $Ca^{2+}$ which was quickly lost on transfer to $Ca^{2+}$-free medium was probably extracellular. If the maintenance of normal concentrations of $Na^+$ and $K^+$ had depended upon intracellular $Ca^{2+}$, then the continuing $Ca^{2+}$ loss should have been accompanied by increasing rates of $Na^+$ and $K^+$ flux, not decreasing rates as observed. Moreover if intracellular $Ca^{2+}$ was required for metabolism[17,18], the slower depletion of this fraction, together with reserves of metabolites and energy-rich compounds[19] should have introduced a time lag between changes in medium $Ca^{2+}$ and changes in $Na^+$ and $K^+$, but this was not observed (Figs 3 and 4).

The possibility that $Ca^{2+}$ enhanced active ion transport in any of the ways outlined above is not supported by the effects of ouabain on slices (Fig. 5). Table I shows some of the values from Fig. 5 converted to electrolyte contents of slices, *i.e.*

TABLE I

EFFECT OF $Ca^{2+}$ BEFORE AND AFTER OUABAIN TREATMENT

The $Na^+$ and $K^+$ concentrations shown at 0 and 40 min in Fig. 5 are expressed on a dry-weight basis.

| | *$Na^+$ content ($mmole \cdot kg^{-1}$ dry wt)* | | *$K^+$ content ($mmole \cdot kg^{-1}$ dry wt)* | |
|---|---|---|---|---|
| | *Before ouabain* | *After ouabain* | *Before ouabain* | *After ouabain* |
| $Ca^{2+}$-free (a) | 302.6 | 457.7 | 104.7 | 30.9 |
| $Ca^{2+}$ present (b) | 150.6 | 321.4 | 289.8 | 125.4 |
| Effect of $Ca^{2+}$ (b − a) | −152.0 | −136.3 | 185.1 | 94.5 |

$mmole \cdot kg^{-1}$ of dry wt. It is apparent from the first line of Table I that $Ca^{2+}$ was not required for a ouabain-sensitive system that was able to maintain at least part of the normal slice composition. Slices with $Ca^{2+}$ had less $Na^+$ and more $K^+$ than slices without (third line of Table I). If $Ca^{2+}$ had been necessary for an active transport mechanism then as ouabain reduced the $Ca^{2+}$-independent contribution (first line of Table I), the contributions due to $Ca^{2+}$ should have increased, as the $Ca^{2+}$-dependent system would then be working against smaller gradients. In fact, the third line of Table I shows that for both $Na^+$ and $K^+$, the contribution due to $Ca^{2+}$ was reduced after treatment with ouabain, Fig. 5 indicating that the effect of $Ca^{2+}$ would have become minimal if incubation had been continued longer. This could be expected if the hypothetical $Ca^{2+}$-dependent active ion transport was ouabain-sensitive. However, if both $Ca^{2+}$-dependent and $Ca^{2+}$-independent transport were blocked simultaneously, and if membrane permeability was not affected by $Ca^{2+}$, the proportion of $K^+$ lost from any initial content in a given time should also be independent of $Ca^{2+}$. In fact, in the first 5 min after addition of ouabain, 0.3 of the $K^+$ in $Ca^{2+}$-free slices had been lost compared with only 0.13 of the $K^+$ in slices with $Ca^{2+}$.

The results could be explained if $Ca^{2+}$ reduced $Na^+$–$K^+$ leakage in a pump-leak system. The immediate onset of effects following changes in medium $Ca^{2+}$ (Figs 3 and 4) favours an action of $Ca^{2+}$ at the surface of the cells, probably to reduce membrane permeability. The fact that in slices inhibited with ouabain (Fig. 5), $Na^+$ and $K^+$ changed more slowly in presence of $Ca^{2+}$ than in its absence, although they eventually approached the same values, suggests that $Ca^{2+}$ reduced membrane permeability. Since almost the entire $K^+$ content of the slices seemed to be attributable to the combined effects of a ouabain-sensitive pump and a $Ca^{2+}$-sensitive leak, it is doubtful if any other mechanisms for accumulating $K^+$ were important, whether $Ca^{2+}$ dependent or not.

Since the smaller $O_2$ consumption in absence of $Ca^{2+}$ (Fig. 6) could not readily be attributed to a direct effect of $Ca^{2+}$ on energy supplies or ion pumps, another interpretation would be that high $Na^+$ and low $K^+$ in the absence of $Ca^{2+}$ were detrimental to the normal function of the cells, resulting in impaired respiration. A number of cell functions are dependent on or activated by $K^+$ or are inhibited by $Na^+$ (refs 20–24) and in addition the basis of the $K^+$ sorption theories is that intracellular structure is maintained by $K^+$ but not by $Na^+$.

The initial rates of $O_2$ consumption of slices were similar to those for perfused liver[25,26], but with slices these rates were not maintained. The initial, $Ca^{2+}$-insensitive, decline in respiration rate in Fig. 6 might have been related to the rapid changes in composition of incubated fresh slices (Fig. 1) and both phenomena presumably reflected a deterioration of freshly-cut tissue. However, the two phenomena were probably not directly related, for the slices in Fig. 6 had been prewashed in $Na^+ = K^+$ medium, and in the presence of $Ca^{2+}$ they did not experience large upsets in composition. After 30 min the slices incubated in $Ca^{2+}$ medium had progressed substantially towards a normal $Na^+$–$K^+$ ratio and their respiration rates remained relatively static. But slices incubated in the absence of $Ca^{2+}$ did not recover a normal $Na^+$–$K^+$ ratio and their respiration rate continued to decline, though more slowly than initially.

Ouabain has been found to decrease $O_2$ consumption concurrently with inhibition of ion transport in gall bladder[27], kidney[28–30] and liver[31] and an inhibitory effect on glycolysis in tumor cells and erythrocytes has been noted[32]. Since in the present experiments addition of ouabain had no immediate effect on the $O_2$ consumption of slices incubated in presence of $Ca^{2+}$ (Fig. 6), but was followed by alterations in ion content with no lag phase, active ion transport did not appear to need a direct $O_2$ supply under these conditions[33]. Other workers[31] have recorded higher respiration rates and have also obtained evidence of ion transport coupled to $O_2$ consumption. Possibly the extent of such coupling may depend upon experimental conditions, and upon how these influence the load on the pumping system. The absence of $Ca^{2+}$ leading to high $Na^+$–low $K^+$ might have been such a factor, placing a greater load on the pumping system, so that the system would respond with increased activity, depending on coupled $O_2$ consumption to do so. The small immediate fall of 0.2 in respiration rate on addition of ouabain (Fig. 6, absence of $Ca^{2+}$) could be a measure of $O_2$ required for ion transport, which was not required when ouabain inhibited that transport.

Finally, the control of water content is of interest. Like ouabain in $Ca^{2+}$ medium (Fig. 5), lack of $Ca^{2+}$ resulted in a large deviation of $Na^+$ and $K^+$ from their "normal" values, but no prolonged change in water content was detected if slices were preincubated in $Na^+ = K^+$ medium (Figs 2 and 3). This behaviour would not be predicted if volume were regulated by a potassium-dependent, ouabain-sensitive sodium pump, but is like behaviour observed in kidney slices[34–36] and suggests that liver slices may also regulate their water content by an ouabain-insensitive (and $Ca^{2+}$-independent) mechanism[37].

Some swelling occurred in the absence of $Ca^{2+}$ if slices were not preincubated in $Na^+ = K^+$ medium, (Figs 1 and 4) or if ouabain was added in the absence of $Ca^{2+}$ (Fig. 5), but this was under conditions in which slice $K^+$ had reached very low levels and was presumably secondary to movements of ions. With similar low levels in rat kidney slices there is some swelling[35].

It is concluded that cells in liver slices maintain their composition by an ouabain-sensitive system which pumps out $Na^+$ in exchange for $K^+$. This active system works against the continual loss of $K^+$ and gain of $Na^+$ through the cell membrane. The permeability of the membrane to $Na^+$ and $K^+$ is dependent on the presence of extracellular $Ca^{2+}$. In absence of $Ca^{2+}$ permeability is increased so that $K^+$ leaks out and $Na^+$ leaks in at such rates that the active pumping system cannot maintain normal intracellular composition, even though pumping activity may be increased under such conditions. Thus in the presence of $Ca^{2+}$ an active ion pumping system with a rela-

tively ion-impermeable membrane leads to a steady-state intracellular composition of high $K^+$ and low $Na^+$ while in the absence of $Ca^{2+}$ the more permeable membrane allows loss of $K^+$ and gain of $Na^+$ leading to a steady state intracellular composition of low $K^+$ and high $Na^+$.

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