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NET MOVEMENTS OF MONOVALENT AND BIVALENT CATIONS, AND THEIR RELATION TO ENERGY METABOLISM, IN SLICES OF HEPATOMA 3924A AND OF A MAMMARY TUMOUR

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SUMMARY

1. During incubation at 1° in saline medium buffered either with phosphate or bicarbonate, slices of Morris hepatoma 3924A, and of a chemically induced tumour of rat mammary gland, lost K^+ and gained Na^+, Ca^{2+} and water.

2. Upon subsequent incubation at 38° in oxygenated medium, these changes were partially reversed. In the hepatoma, the reaccumulation of K^+ was equally efficient in phosphate or bicarbonate medium, and in the presence and absence of glucose. Ca^{2+} was extruded in bicarbonate, but not in phosphate medium, and its extrusion was reduced in the presence of glucose.

3. When respiration was inhibited in the presence of glucose, K^+ transport by the hepatoma continued to an extent which varied with the glycolytic activity of the slices, suggesting that the rate of ATP synthesis was a limiting factor under these conditions.

4. In the absence of glucose, the transport of Na^+ and K^+ was completely stopped by respiratory inhibition. However, more than 50 % of the O_2 uptake had to be inhibited before any effect on transport was observed, suggesting that the rate of synthesis of ATP from endogenous respiration is in excess of that required to maintain transport.

5. Inhibition of transport by ouabain was accompanied by a 30 % fall in the rate of endogenous respiration, and by a fall of 33 % in the rate of glycolysis in the presence of cyanide *plus* glucose.

6. Comparison of the minimum rates of respiration and of glycolysis (in the presence of glucose *plus* cyanide) required to maintain the maximal extent of K^+ transport in the hepatoma slices, suggests that ATP derived from oxidative phosphorylation or from anaerobic glycolysis is equally efficient as a source of energy for ion transport.

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INTRODUCTION

The energy-dependent transport of cations has proved to be a useful model for studying the relation between energy-conserving and energy-utilising aspects of cell metabolism[1]. In many types of cell, transport is dependent on oxidative phosphorylation, apparently because of the low glycolytic activity, but in others it can be adequately supported by high-energy compounds synthesised by either glycolysis or oxidative phosphorylation[2–8]. The rate of the energy-conserving pathways is related to the demands of the transport mechanism[9–16], but only in one case so far, the ascites tumour, has control of both anaerobic glycolysis and respiration been demonstrated in the same type of cell[17,18].

Anaerobic gylcolysis is a less efficient source of ATP, per mole of substrate, than oxidative phosphorylation and it can be calculated that in many anaerobic cells the Pasteur effect only partially compensates for the loss of ATP production by oxidative phosphorylation; yet in such cells the transporting activity is often close to the aerobic level[2]. Thus, either the ATP produced by glycolysis is used more effectively for the support of transport, or the rate of ATP production from oxidative phosphorylation is well in excess of that required for transport. It should be possible to distinguish between these two possibilities by comparing the glycolysis-dependent or respiration-dependent transporting activities in the presence of varying rates of the appropriate energy-conserving pathway. Rapidly growing hepatomas offer a suitable material for such studies, since they have a high rate of glycolysis[19], and are known to support at least one anabolic process (protein synthesis) with equally efficient utilisation of the ATP formed by oxidative phosphorylation or glycolysis[20].

In addition to the above problem of general importance, we have considered two questions which are more specifically of interest in tumour physiology. The first, is whether mitochondria within tumour cells exhibit coupled respiration[20]. This has been approached by studying the effects of ouabain, since inhibition of O_2 uptake by this specific inhibitor of ion transport seems to be due, to a considerable extent, to the controlling effects of ADP (released upon consumption of ATP by the transport mechanism) on coupled respiration[1,2]. Secondly, although the abnormal K^+ and Ca^{2+} content of tumour tissues (compared to the normal tissues from which they are derived) has long been of interest[21–23], studies of active ion movements in them have been largely confined to ascites tumour cells[5,17,24] and to slices of the rather slowly growing Morris hepatoma 5123tc (refs. 25,26). We have therefore attempted to see whether the differences in composition between hepatoma and liver can be qualitatively related to differences in transport activity.

We show below that slices of the rapidly growing, Morris hepatoma 3924A are able to effect metabolism-dependent movements of K^+, Na^+ and Ca^{2+}. The K^+ transport can derive energy with equal efficiency from either aerobic or anaerobic metabolism, but while the rate of respiration is well in excess of that required, the rate of anaerobic glycolysis is often a limiting factor in the K^+ transporting activity. We also describe experiments done with samples of a chemically induced tumour of rat mammary gland.

METHODS

Tumours. Female rats of the ACI/T strain were inoculated in both hind legs with the cells of hepatoma 3924A in the laboratory of H.P.M.[27], and were shipped to the Johnson Foundation. The mammary tumours were induced in 50-day-old, female, albino rats (approx. 150 g body weight) by a single administration, *via* stomach tube, of 20 mg of 7,12-dimethylbenzanthracene in 1 ml sesame oil; the tumour developed 7 weeks later and was used after a further 2 weeks. In order to obtain enough material for the experiments, all tumours were allowed to grow to a diameter of at least 1 cm before use.

In the case of the hepatomas, the diameters varied from 1.0 to 2.5 cm and the age from inoculation ranged from 27 to 41 days. Such tumours always contained a necrotic centre; their peripheral areas were white and free of obvious necrosis, although histological examination of these areas (for which we are indebted to Dr. P. Gambetti of the Department of Neurology, University of Pennsylvania Hospital) indicated the presence of microscopic foci of necrosis which could account for up to 10 % of the total tissue volume.

Experimental procedure. Rats were killed by decapitation, the tumours rapidly removed and cooled in ice-cold Ringer solution. Further dissection was done on a Petri dish cooled with ice. The capsules and adhering foreign tissue were cut away. Several blocks were cut of the uniformly white, peripheral areas, free of obviously necrotic and haemorrhagic tissue, and these were then cut with a razor blade into slices that were 0.15–0.3 mm thick. The slices cut from the two tumours of a single animal were placed in fresh, ice-cold medium, and mixed randomly. The time between death of the animal and completion of slicing was approximately 15 min.

The slices were first incubated at 1°, and subsequently at 38° in the Warburg manometric apparatus gassed with O_2 or CO_2–O_2 (5:95, v/v) (as appropriate to the medium), according to the procedures published previously[11,28]. When respiration was to be measured, 20 % NaOH was placed in the centre well of the manometric flasks. In the titration experiments with cyanide (Fig. 4), the centre wells contained mixtures of KCN and KOH as described by ROBBIE[29]. Initial cyanide concentrations of 0.5 mM or more cannot be accurately maintained in this way[29]; however, this does not affect the results, since at each initial concentration of cyanide the rate of respiration was constant throughout the experiment, and the plot of Fig. 4 is concerned with the rate of respiration rather than with the concentration of cyanide.

After the appropriate incubation periods, the flasks were detached from the manometers; when lactate was measured, an aliquot of the medium was removed by pipette and transferred to a centrifuge tube containing ice-cold, 10 % $HClO_4$. The slices were separated from the remaining medium on a sintered glass filter, gently blotted with filter paper (Whatman No. 54), and placed into a tared, hard-glass weighing bottle. The tissue was dried at 105° and the fats and ions extracted as described previously[11,28]. The ions were estimated by atomic-absorption spectrophotometry. The samples of lactate in the $HClO_4$ were neutralised with K_2CO_3–triethanolamine[30] and analysed enzymically[31].

RESULTS

Incubation at 1°. Unincubated tissues of the mammary tumour and hepatoma 3924A were very similar with respect to their water and cation composition, except that the latter contained rather more Ca^{2+} and less Mg^{2+} (Tables I and II). Compared to normal liver[28], the hepatoma contained 50 % more K^+ and Mg^{2+}, 100 % more

TABLE I

CHANGES IN COMPOSITION, AND LACTATE PRODUCTION, OF SLICES OF HEPATOMA 3924A INCUBATED IN BICARBONATE MEDIUM

Slices were incubated for 120 min at 1°, followed by 70 min at 38°. The medium contained (mM): Na^+ 161.5, K^+ 5.0, Ca^{2+} 1.3, Mg^{2+} 1.0, Cl^- 147.5, HCO_3^- 25.0, SO_4^{2-} 1.0, phosphate 2.0, and was gassed with O_2–CO_2 (95:5, v/v), giving a pH of 7.4; other additions were made after 60 min at 1°. Water and cation values in lines 1 and 2 are the total slice contents; those given in lines 3–6 represent the differences in composition from line 2. Line 2 contains pooled values from slices incubated with and without glucose, cyanide and ouabain, since none of these additions affected the cold slices. Lactate was measured in the medium only. Values are means ± S.E.

Incubation	*(n)*	*kg water/kg fat-free dry wt.*	*mmoles/kg fat-free dry wt.*				
			K^+	*Na^+*	*Ca^{2+}*	*Mg^{2+}*	*Lactate in medium*
1. None (fresh tissue)	(21)	4.86 ± 0.08	447 ± 15	512 ± 22	23.3 ± 2.1	51.9 ± 1.2	—
2. 120 min at 1°	(20)	6.20 ± 0.12	205 ± 9	1242 ± 33	58.0 ± 5.7	48.7 ± 2.0	8.3 ± 1.5
120 min at 1° followed by 70 min at 38° with:							
3. Endogenous substrate	(10)	−1.02 ± 0.13	122 ± 13	−324 ± 32	−28.2 ± 7.0	7.1 ± 6.6	4.9 ± 2.3
4. Glucose (20 mM)	(16)	−1.20 ± 0.06	145 ± 12	−428 ± 39	−14.5 ± 6.9	8.8 ± 5.3	324 ± 35
5. Glucose (20 mM) + CN^- (1 mM)	(17)	−0.77 ± 0.08	120 ± 13	−270 ± 36	−17.5 ± 6.5	−11.2 ± 1.9	771 ± 50
6. Glucose (20 mM) + ouabain (1 mM)	(10)	−0.78 ± 0.16	−41 ± 11	45 ± 37	−18.3 ± 8.5	3.4 ± 1.7	277 ± 39

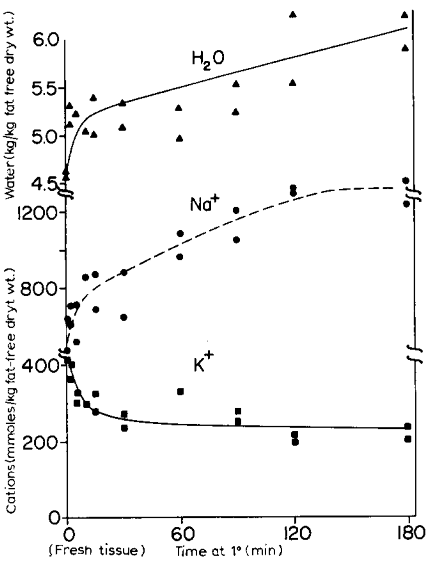
TABLE II

CHANGES IN COMPOSITION AND RESPIRATORY RATE OF SLICES OF RAT MAMMARY TUMOUR INCUBATED IN PHOSPHATE MEDIUM

The medium contained (mM): Na^+ 161.5, K^+ 5.0, Ca^{2+} 1.3, Mg^{2+} 1.0, Cl^- 151.5, SO_4^{2-} 1.0, phosphate (pH 7.4) 10.0; vessels were gassed with O_2 during the last 10 min of incubation at 1°. Other details as for Table I.

Incubation	*(n)*	*kg water/kg fat-free dry wt.*	*mmoles/kg fat-free dry wt.*				*Q_{O_2} (μl/mg fat-free dry wt. per h)*
			K^+	*Na^+*	*Ca^{2+}*	*Mg^{2+}*	
1. None (fresh tissue)	(4)	4.97 ± 0.15	446 ± 13	520 ± 58	35.6 ± 1.8	41.7 ± 0.4	—
2. 90 min at 1°	(4)	6.77 ± 0.33	143 ± 14	1250 ± 109	41.3 ± 4.8	40.5 ± 1.9	—
90 min at 1° followed by 70 min at 38° with:							
3. Endogenous substrate	(2)	−1.13	294	−311	—	—	10.1
4. Glucose (20 mM)	(6)	−0.92 ± 0.14	212 ± 14	−349 ± 29	2.3 ± 6.9	3.0 ± 4.6	8.0 ± 0.5
5. Glucose (20 mM) + CN^- (2 mM)	(6)	−0.98 ± 0.46	54 ± 11	−321 ± 66	7.6 ± 4.7	−4.5 ± 2.7	—
6. Glucose (20 mM) + ouabain (1 mM)	(6)	0.09 ± 0.19	−29 ± 18	92 ± 61	−0.9 ± 3.0	1.1 ± 2.4	4.8 ± 0.5

Na^+ and water, and 500 % more Ca^{2+} per unit fat-free dry wt. These and other aspects of the fresh tissue composition will be described in more detail elsewhere[32]. When metabolism was slowed by incubation at 1° in the phosphate medium, slices of both types of tumour lost K^+ and gained Na^+ and water (Figs. 1 and 2); identical effects were seen with the hepatoma in the bicarbonate medium (Table I). Similar changes occur in most tissues, including normal rat liver[33,34], but whereas the liver finally retains only 25 % of its initial K^+ content, hepatoma 3924A retained almost 50 %. Using the reasoning of HECKMANN AND PARSONS[35], it can be calculated that most of the residual K^+ is 'bound' in some non-diffusible form within the slices*. The average amount of 'bound' K^+ thus calculated to be present in cold-incubated hepatoma slices was 164 ± 6 (33) mmoles/kg fat-free dry wt., whereas liver slices contain approximately 50 mmoles/kg[36]. Like normal liver slices[28], the hepatoma gained Ca^{2+} during incubation at 1° in either medium (Table I), but this was not true of the mammary tumour (Table II). In neither tumour was the Mg^{2+} content affected by cold incubation.



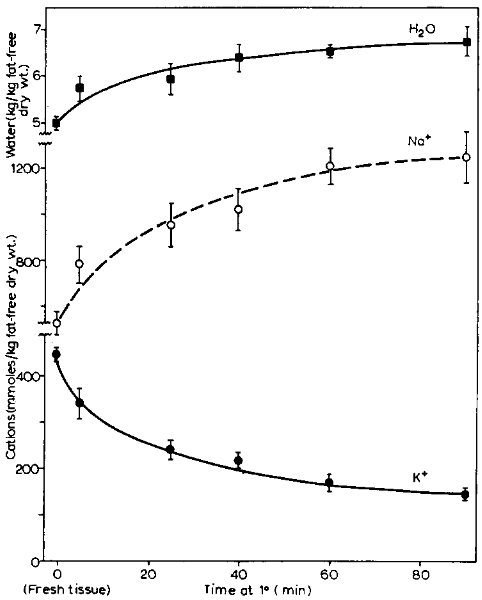


Fig. 1. Changes in water and cation content of slices of hepatoma 3924A during incubation at 1° in phosphate medium. Each point represents a single observation. For composition of medium, see Table II. ▲, H_2O; ●, Na^+; ■, K^+.

Fig. 2. Changes in water and cation content of slices of mammary tumour during incubation at 1° in phosphate medium. Each point is the mean ± S.E. of 4 observations. For composition of medium, see Table II. ■, H_2O; ○, Na^+; ●, K^+.

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* These arguments are based on the findings that the distribution of Na^+ and Cl^- (as the main, diffusible ions) between the multi-compartment tissue water of liver slices, and the medium, is determined by a modified Donnan equilibrium, in which the distribution ratio

$$\frac{[Na^+]_t \cdot [Cl^-]_t}{[Na^+]_m \cdot [Cl^-]_m}$$

is slightly greater than unity (ref. 35; the subscripts 't' and 'm' refer to concentrations in the total tissue water and the medium). In other experiments with slices of hepatoma 3924A incubated at 1° for 120–180 min, the distribution ratio was found to have the value, 1.12 ± 0.08 (18) (ref. 26), which is similar to values obtained with liver slices[37].

The net changes in composition were largely completed by 90 min and 120 min in the mammary tumour and hepatoma, respectively, and in subsequent experiments these were taken as the standard times for preincubation at 1°.

Recovery during incubation at 38°

Na$^+$ and K$^+$. When tumour slices which had been preincubated in the cold were restored to metabolically favourable conditions (*i.e.* an oxygenated medium at 38°), the changes in composition were reversed, to a considerable extent, by metabolism-dependent transport processes. Fig. 3 shows the time-course of the recovery for slices of hepatoma 3924A which were incubated in the phosphate medium containing glucose. The net changes were completed by 120 min, at which time the slices had regained 70 % of the lost K$^+$ and had extruded 60 % of the extra Na$^+$ and water. The rate of respiration was constant throughout incubation at 38° (the mean rate was 5.6 ± 0.3 (7) μl O$_2$/mg fat-free dry wt. per h); the aerobic lactate production is included in Fig. 3. Inhibition of the respiration with 1 mM cyanide stimulated lactate production, especially during the first 30–60 min, but had no effect on the rate or extent of the net transport of Na$^+$ or K$^+$. Thus, in this series of experiments anaerobic glycolysis was an adequate source of high-energy compounds for the support of active transport. However, the degree of recovery in the presence of cyanide varied somewhat in different series of experiments (see below).

Table I shows that the active movement of water, Na$^+$ and K$^+$ by slices of hepatoma 3924A was also observed in the bicarbonate medium. Despite the low glycogen content of this tumour[38], endogenous substrates alone were adequate to support the transport when respiration was active. Addition of glucose gave a slight, but statistically insignificant increase of the net transport. In these experiments cyanide, in

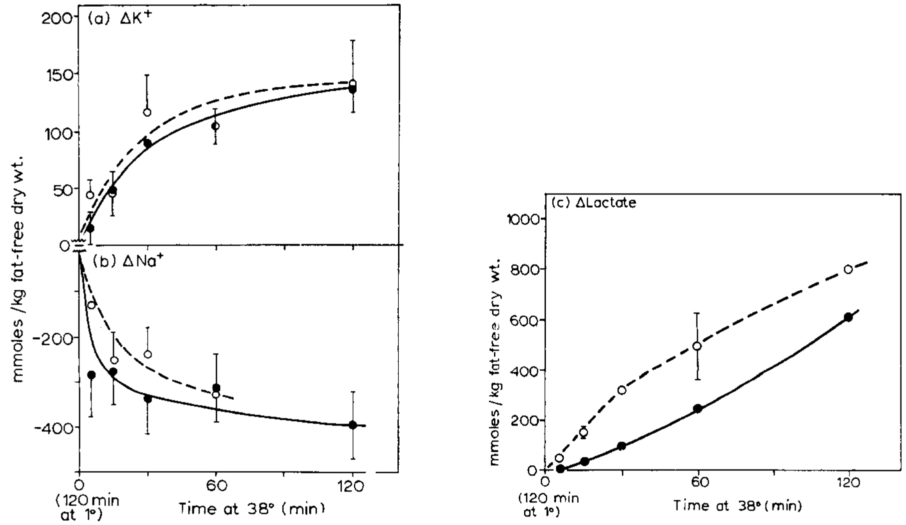

Fig. 3. Effect of cyanide on the movements of K$^+$ (a) and Na$^+$ (b), and production of lactate (c), by slices of hepatoma 3924A. The slices were incubated at 1° for 120 min, and then at 38°, in phosphate medium containing 20 mM glucose without (●) or with (○) 1 mM cyanide. The points represent the net change of tissue cation content, or medium lactate; during the incubation at 38°. Each point is the mean of 2–4 observations.

the presence of glucose, inhibited the loss of water and Na^+ by about 30 %. Ouabain completely inhibited Na^+ and K^+ transport, but reduced water extrusion by only 30 %. A similar dissociation of cation and water movements has been observed in slices of kidney cortex[39].

Slices of the mammary tumour were also able to extrude Na^+ and accumulate K^+ aerobically, whether or not glucose was present (Table II). Unexpectedly, however, these cation movements were differently affected by respiratory inhibition, the K^+ transport being considerably inhibited by cyanide in the presence of glucose, while the movements of Na^+ and water were unaffected. Ouabain completely inhibited active movements of K^+, Na^+ and water.

Ca^{2+} and Mg^{2+}. Upon incubation at 38° in the bicarbonate medium, the hepatoma slices extruded almost all of the Ca^{2+} taken up at 1° (Table I). The presence of glucose appeared to reduce the extrusion, but a significant loss of Ca^{2+} still took place, and neither cyanide nor ouabain had any further effect. No extrusion of Ca^{2+} by slices of either tumour was observed in phosphate medium (Table II; *cf.* liver slices in ref. 28); the mammary tumour slices were not studied in bicarbonate medium. The Mg^{2+} content of both tumours was much more stable than that of the other three ions. The only significant effect observed was a small loss of Mg^{2+} from hepatoma slices incubated in the presence of cyanide (Table I; *cf.* rat liver slices, ref. 28).

Effect of varying rates of energy metabolism

Respiratory inhibition. In the absence of glucose, slices of hepatoma 3924A were completely unable to support the active movements of cations at cyanide concentrations of 1 mM or greater; thus, under these conditions the transport processes derived their energy exclusively from respiratory metabolism. In order to determine the form of the relationship between respiratory and transport activities, the rate of respiration of slices oxidising only endogenous substrates was varied by using different concentrations of cyanide (in the range, $1 \cdot 10^{-5}$ to $5 \cdot 10^{-3}$ M). When the net, active movement of water or cation is plotted against the rate of respiration, it can be seen that the Q_{O_2} had to be reduced below 2 μl/mg fat-free dry wt. per h before any inhibition of the net movements of Na^+ or water occurred (Fig. 4). In the case of K^+, the variation in amount of K^+ accumulated was rather large at intermediate values of Q_{O_2}, but here also a significant inhibition was not seen until the rate of respiration was less than 2–3 μl/mg per h. A similar discontinuous relationship between net ion movement and the rate of respiration has recently been reported for slices of normal liver[2]. Clearly, the normal O_2 consumption of slices of these tissues is considerably in excess of that required to maintain the maximal active transport attained *in vitro*.

Variations of glycolysis. As a counterpart to the last experiments, the relation of glycolysis-dependent transport to the rate of lactate production was studied in hepatoma slices which were incubated in the presence of glucose *plus* cyanide. Slices with differing rates of glycolysis were obtained in two ways: (i) There was found to be a large 'spontaneous' difference in the rate of lactate production between hepatomas from different individual rats. The K^+ accumulation in each sample (expressed as a percentage of the respiration-dependent K^+ transport observed in control slices in the same experiments) varied directly with the rate of glycolysis until it attained a value not significantly different from the aerobic level (100 %). This point was reached at a lactate production of about 800 mmoles/kg fat-free dry wt. per h (Fig. 5). However,

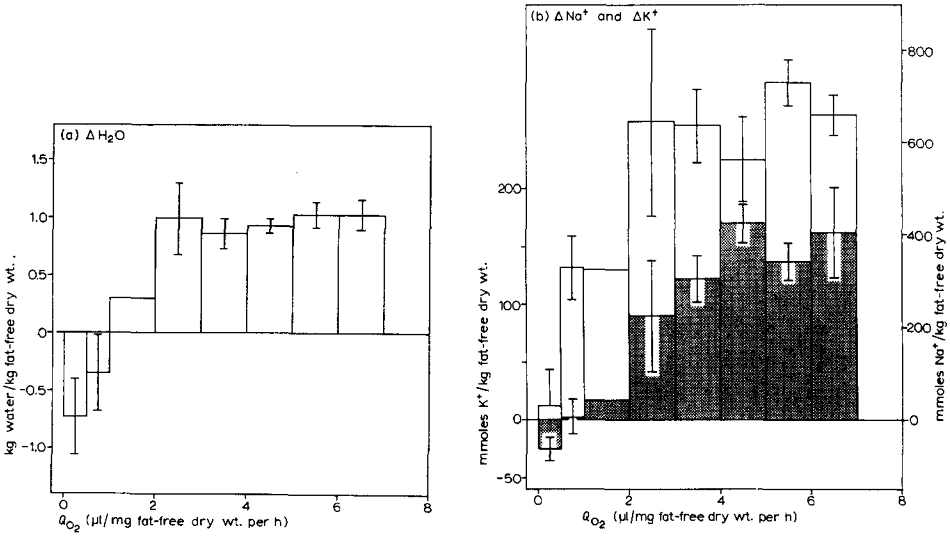

Fig. 4. Relation between net movements of cations and water and the rate of respiration of slices of hepatoma 3924A, in the presence of varying concentrations of cyanide. Slices were incubated for 120 min at 1° and 70 min at 38° in substrate-free, phosphate medium containing cyanide in concentrations of 0, $1 \cdot 10^{-5}$, $5 \cdot 10^{-5}$, $1 \cdot 10^{-4}$, $5 \cdot 10^{-4}$, $1 \cdot 10^{-3}$ and $5 \cdot 10^{-3}$ M. The net movements occurring during the incubation at 38° are grouped according to the rate of respiration, irrespective of cyanide concentration. In diagram (b), the entire bars represent Na^+ movements, hatched portions represent K^+ movements.

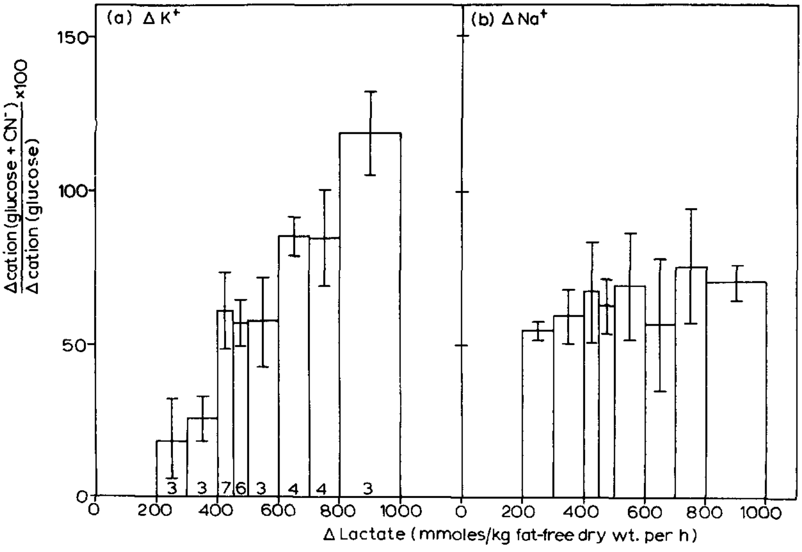

Fig. 5. Relation between net movements of cations and the rate of glycolysis in the presence of cyanide, in slices of hepatoma 3924A. Slices were incubated for 120 min at 1° and 70 min at 38°, in either phosphate or bicarbonate medium containing 20 mM glucose and 1 mM cyanide. There was no consistent difference between results in the phosphate and bicarbonate media. The net movements of K^+ (a) and Na^+ (b) during the incubation at 38° with cyanide are expressed as a percentage of the net movements occurring in control slices incubated without cyanide in the same experiments. The net movements of ions are grouped according to the rate of glycolysis. Numbers of observations are shown at the base of the bars in (a).

the Na^+ extrusion was much less sensitive to variations of glycolytic rate over the range occurring 'spontaneously'. (ii) Variations in the rate of anaerobic glycolysis were also induced with varying concentrations of iodoacetate (in the range, $1 \cdot 10^{-5}$ to $2 \cdot 10^{-3}$ M); at a concentration of 1 mM, this inhibitor reduced lactate production by 90 % and completely abolished the active movements of Na^+ and K^+. In this series of experiments, the maximal rate of anaerobic glycolysis (*i.e.* the rate with no iodoacetate present) was approximately 500 mmoles lactate/kg per h and so, by comparison with the results of Fig. 5, any further reduction of glycolysis, by iodoacetate, would be expected to lead to a concomittent decline of K^+ uptake. This was found to be the case (Fig. 6). The results with Na^+ extrusion in the same experiments were too scattered to draw definite conclusions, but there is some indication that, in agreement with the findings of Fig. 5, Na^+ extrusion was not inhibited until the rate of lactate production fell below 200–300 mmoles/kg fat-free dry wt. per h.

Control of respiration and glycolysis by energy requirements of ion transport

In a variety of tissues, the specific inhibition of ion transport (*e.g.* by ouabain) is accompanied by a fall in the activity of respiration or glycolysis, depending upon which pathway provides the energy for the transport[9–18]. In Table III both of these effects are demonstrated for the hepatoma slices incubated in phosphate medium. Under aerobic conditions, with endogenous substrate only, the Q_{O_2} was inhibited by 29.8 ± 5.8 (11) % when ouabain caused complete inhibition of the net, active movements of K^+ and water. In the presence of glucose *plus* cyanide, the inhibition of transport was accompanied by a reduction of lactate production amounting to 33.3 ± 4.7 (11) %. In contrast, the results of Table I, lines 4 and 6, show that aerobic glycolysis proceeding in the presence of glucose was not significantly affected by ouabain.

In Table II, lines 4 and 6, the inhibition of transport in the mammary-tumour slices was also accompanied by a 40 % decline of the respiratory rate.

TABLE III

EFFECTS OF OUABAIN ON THE RESPIRATION AND ANAEROBIC GLYCOLYSIS OF SLICES OF HEPATOMA 3924A, IN PHOSPHATE MEDIUM

Slices were incubated for 120 min at 1°, followed by 70 min at 38°. Values for tissue water, K^+, and for medium lactate, represent the differences between slices incubated at 38° and control slices incubated only at 1°.

Substrates and inhibitors	(*n*)	*kg water/kg fat-free dry wt.*	*mmoles K^+/kg fat-free dry wt.*	Q_{O_2} (*µl/mg fat-free dry wt. per h*)	*mmoles lactate in medium/kg fat-free dry wt.*
1. Endogenous substrate	(8)	−0.77 ± 0.13	154 ± 24	5.5 ± 0.3	−3.0 ± 3.9
2. Endogenous + ouabain (1 mM)	(10)	+0.59 ± 0.16	−53 ± 14	4.0 ± 0.3*	6.8 ± 5.5
3. Glucose (20 mM) + CN^- (2 mM)	(12)	−0.52 ± 0.15	86 ± 18	—	470 ± 29
4. Glucose + CN^- + ouabain (1 mM)	(11)	+0.31 ± 0.18	−61 ± 8	—	304 ± 25**

* Significantly different from value without ouabain, by Student's *t* test, $P < 0.01$.

** Significantly different from value without ouabain, by Student's *t* test, $P < 0.001$.

DISCUSSION

Our results show that after a period of lowered metabolic activity, cells of hepatoma 3924A are able to bring about energy-dependent, net movements of Na^+, K^+, water and (in bicarbonate medium) Ca^{2+}, so as to restore their composition towards normal; the same is true of the mammary tumour studied, with respect to Na^+, K^+ and water. In the hepatoma, at least, either oxidative phosphorylation or anaerobic glycolysis can provide the necessary high-energy compounds, and both of these energy-conserving pathways are partially inhibited by ouabain. The following discussion will consider the various questions raised in the INTRODUCTION.

Transport activity and fresh-tissue composition

The energy-dependent movements of ions in hepatoma 3924A were qualitatively similar to those seen previously in normal, rat liver[11, 40, 41]; however, the K^+ transport shows certain quantitative differences from that of liver slices:

(i) Slices of hepatoma and liver differ in the amount of K^+ recovered during incubation at 38°. In a large number of experiments, rat-liver slices have been found to re-accumulate 205–255 mmoles/kg fat-free dry wt., or 81–97 % of the K^+ originally lost during cold incubation (G. D. V. VAN ROSSUM, unpublished observation). In contrast, slices of the hepatoma incubated aerobically, either with or without glucose, re-accumulated 126–172 mmoles/kg fat-free dry wt., or 43–66 % of the lost K^+.

(ii) From the results of Fig. 3, the maximal rate of K^+ uptake was 192 mmoles/kg fat-free dry wt. per h, which is considerably less than the rate in liver slices (300–492 mmoles/kg per h; G. D. V. VAN ROSSUM, unpublished observation).

Neither of these differences in K^+- transporting activity of liver and the hepatoma is in the direction required to account for the greater initial K^+ content of the latter. On the other hand, our results provide evidence that the difference in K^+ content is at least partly due to differences in the amount of 'bound' K^+ which is not free to diffuse out of the tissues. Thus, the fresh hepatomas we studied contained an average total K^+ content of 498 ± 18 mmoles/kg fat-free dry wt., which is about 180 mmoles/kg more than the total K^+ of normal liver. The 'bound' K^+ content of the hepatoma, as estimated after cold incubation, was greater than that of liver by some 110 mmoles/kg, and thus accounts for a considerable part of the difference in total K^+ contents.

A difference in the transporting activity does not appear to underlie the wide differences in Ca^{2+} content of hepatoma 3924A and liver either, since under appropriate conditions the hepatoma slices extrude Ca^{2+} more completely than liver slices do[28]. More detailed investigations of the content and transport of Ca^{2+} by hepatoma 3924A are in progress[26, 32].

Coupling of metabolism to Na^+ and K^+ transport

When the transport of Na^+ and K^+ by hepatoma 3924A or the mammary tumour was inhibited by ouabain, the O_2 uptake was reduced. Following the suggestion of WHITTAM[4], this is probably due to a coupling of respiration to the transport mechanism by way of ADP released by the latter, and hence provides evidence that well-coupled, phosphorylating respiration occurs in the mitochondria within cells of both of these tumours. However, recent results[12] suggest that in liver slices part of the lower respiratory activity in the presence of ouabain arises from the persistence of an intracellular environment with low K^+ and high Na^+ concentrations. Such an environment appears to be detrimental to the oxidative metabolism. Our present results do not allow us to estimate what proportion of the ouabain-sensitive respiration of the tumour slices was due to this latter effect.

In the case of the fall in the rate of anaerobic glycolysis induced by ouabain, the abnormal intracellular environment cannot have been a factor causing inhibition, since glycolysis is known to be somewhat more, not less, active in the presence of low concentrations of K^+ and high concentrations of Na^+ (ref. 42). Another possible explanation for the effect of ouabain is that the transport of glucose into the cells may be coupled to Na^+ transport (*cf.* glucose transport by the small intestine, ref. 43) and may be the rate-limiting step in anaerobic glycolysis. Na^+ dependence is not a universal property of glucose transport systems[41,45], and two findings of the present work may indicate that the entry of glucose into the cells of hepatoma 3924A is also independent of Na^+. (i) Aerobic glycolysis was not significantly affected by ouabain (Table I). (ii) The most rapid rate of glycolysis occurred during the first 10 min at 38°, at which time relatively little Na^+ had been extruded in the presence of cyanide (Fig. 3). The Na^+ dependence of glucose transport in other types of cells appears to be related to the existence of a gradient of Na^+ across the plasma membrane[43], but measurements of intracellular water content suggest that no such gradient exists in cells of hepatoma 3924A during the first 10 min, because of the simultaneous extrusion of water[26]. Thus, although neither of these observations rigorously excludes the possibility of an effect of ouabain on glucose entry, we incline to the view that the effect of ouabain was probably entirely attributable to a control of the rate of glycolysis by the rate at which high-energy compounds are utilised by the active transport of Na^+ and K^+—an effect which has been clearly demonstrated in erythrocytes[13,16]. If this is accepted, and if it is further assumed that none of the ATP supporting ion transport is diverted to other reactions when transport is inhibited, then the ouabain-sensitive glycolytic activity provides an estimate of the energy requirement of the transport mechanism (see further below).

Relative efficiency of ATP from glycolysis and oxidative phosphorylation as source of energy for K^+ transport

The results of Figs. 4 and 5 allow estimates to be made of the rates of ATP production required to maintain the maximal level of K^+ accumulation (defined as the K^+ uptake in aerobic conditions, in the absence of inhibitor) when oxidative phosphorylation and anaerobic glycolysis, respectively, are the sources of ATP. From the results of Fig. 4, a Q_{O_2} of 3.5 μl/mg fat-free dry wt. per h may be taken as the average, minimum requirement for the maintenance of the maximal, respiration-dependent K^+ transport. Assuming a P/O ratio of 3 for the oxidative phosphorylation of the tumour mitochondria[46], the rate of ATP production corresponding to this Q_{O_2} would be 850 mmoles/kg fat-free dry wt. per h. This is very similar to the rate of anaerobic lactate production (which, with glucose as substrate, is numerically equal to the rate of net ATP synthesis) that was found (Fig. 5) to be required for the maintenance of net K^+ uptake at the aerobic level. This suggests that for ion transport, as for protein synthesis[20], rapidly growing hepatomas can utilise the ATP produced by anaerobic glycolysis or by oxidative phosphorylation equally effectively.

Two qualifications must be made to this conclusion. First, the argument assumes that the effects of inhibitors on the net ion movements during 70 min at 38° are an indication of their effects on the rate of the transport process. This assumption is justified by a study of the response of the rate of transport in rat-liver slices to various concentrations of respiratory inhibitor[47]. The second qualification is that, because of the apparent differences in response of Na^+ transport and K^+ transport to variations in the rate of anaerobic glycolysis, the argument is only strictly valid for the transport of K^+. However, in the absence of measurements of changes in the extracellular water content, it is not certain that changes in total Na^+ content are always an accurate reflection of intracellular Na^+. The apparent differences between anaerobic K^+ and Na^+ movements thus require further study.

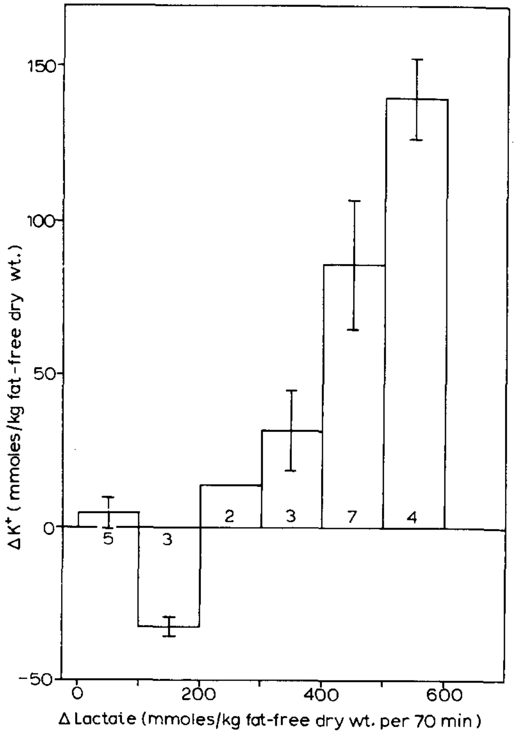

Fig. 6. Relation between the net movement of K^+ and the rate of glycolysis of slices of hepatoma 3924A incubated in phosphate medium in the presence of glucose (20 mM), cyanide (1 mM) and varying concentrations of iodoacetate (0, $1 \cdot 10^{-5}$, $5 \cdot 10^{-5}$, $1 \cdot 10^{-4}$, $5 \cdot 10^{-4}$, $1 \cdot 10^{-3}$, $2 \cdot 10^{-3}$ M). Values of K^+ accumulation are grouped according to the rate of glycolysis, irrespective of the concentration of iodoacetate. Numbers of observations are indicated on the bars.

Energy requirements for K^+ transport

It has been indicated above that a rate of ATP synthesis of approximately 800 mmoles/kg fat-free dry wt. per h was needed to maintain the optimal extent of K^+ accumulation in the hepatoma slices. However, this is a maximal estimate of the ATP requirement of the transport mechanism *per se*, because it is likely that other processes can compete with K^+ transport for the available ATP. As noted earlier, the ouabain-sensitive lactate production may offer an estimate of the requirement of ATP for the support of the transport mechanism itself. From Table III, this was seen to amount to an average of 470 *minus* 304 = 176 mmoles lactate/kg per h for a series of experiments in which the glycolysis-dependent K^+ transport was 86/154 = 0.56 of the aerobic level. Thus, the maximal level of transport activity would require a glycolytic rate of 314 mmoles/kg per h in order to support the transport mechanism itself. This is substantially less than the 800 mmoles/kg per h actually found to be needed for the maintenance of transport, and a large difference remains even when the former value is increased by 30 % in order to allow for the stimulation of glycolysis by the high Na^+ content of the slices incubated with ouabain[42]. This difference would appear to indicate the extent to which reactions other than transport utilise ATP under anaerobic conditions. We cannot, at present, make the corresponding comparison of the minimal O_2 uptake required to support transport upon limitation of respiration, with the extent of O_2 uptake coupled to transport inhibition, since the results do not permit an allowance for the effects of altered cellular ionic composition[12] to be applied to the latter.

The question of the significance of that part of the respiration which can be inhibited without affecting the transport, requires further investigation. In liver slices, at least, this fraction is coupled to ATP synthesis[2]. If the same is true for hepatoma 3924A, then it seems likely that this respiration supports energy-requiring processes which show a smaller affinity for ATP than the ion transport mechanism does. Such reactions would become preferentially inhibited when the ATP supply is limited.

Energy coupling of Ca^{2+} transport

The fact that ouabain did not reduce the Ca^{2+} extrusion observed in the presence of glucose, suggests that in hepatoma 3924A, as in liver[28] and erythrocytes[48], the transport of this cation is independent of the mechanism transporting Na^+ and K^+. The failure of cyanide to inhibit shows that glycolysis can provide the necessary source of energy, as well as respiration. Glucose reduced the Ca^{2+} extrusion below that obtained with endogenous substrate, aerobically, and this may indicate a competition for ATP between the Ca^{2+} transport system and hexokinase. In contrast, the Na^+ and K^+ transport is unaffected by the addition of glucose under aerobic conditions so that, if this explanation for glucose inhibition of Ca^{2+} transport is correct, the Ca^{2+} transport mechanism must have a lower affinity for ATP than the Na^+ 'pump'.

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