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THE CONTROL OF ANAEROBIC GLYCOLYSIS BY GLUCOSE TRANSPORT AND OUABAIN IN SLICES OF HEPATOMA 3924A

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SUMMARY

1. The activities of glycolysis and K^+ transport have been studied in slices of Morris hepatoma 3924A incubated under anaerobic conditions in the presence of different concentrations of glucose (1–50 mM).

2. Ouabain-sensitive net transport of K^+ was observed at all glucose concentrations greater than 1 mM; ouabain reduced the rate of glycolysis by about 25% at all glucose concentrations able to support ion transport.

3. The net entry of glucose into the intracellular phase was studied at varying glucose concentrations. The rate of glucose entry was similar to the rate of glucose utilisation by anaerobic glycolysis at medium concentrations of 10 mM and less, but exceeded the rate of glycolysis at 20 mM and above.

4. The glucose entry was not Na^+-dependent and was not inhibited by ouabain.

5. The results suggest (a) that the reduction in glycolytic activity caused by ouabain is not due to an inhibition of glucose transport and (b) that the glucose transport system of this poorly differentiated hepatoma has properties similar to that of normal liver.

INTRODUCTION

We have reported that slices of the poorly differentiated, rapidly glycolysing Morris hepatoma 3924A are able to support the net transport of Na^+ and K^+ with energy derived from anaerobic glycolysis [1]. We also showed that inhibition of ion transport by ouabain was accompanied by a reduction in the rate of glycolysis, one interpretation for which is that the reduced utilisation of ATP by the ion-transporting mechanism renders the relative concentrations of the adenine nucleotides less favourable for glycolysis [2, 3]. Alternatively, the effect of ouabain on glycolysis could be explained if the entry of glucose into the cells is the rate-limiting step of

anaerobic glycolysis and if the glucose transport system of the hepatoma is of the Na^+-dependent, ouabain-sensitive type [4].

Studies on glucose transport in liver and hepatoma cells are relatively few in number. The sugar appears to enter liver cells very rapidly [5, 6] but, at low external concentrations, its entry into the cells of the rapidly glycolysing Novikoff hepatoma has been implicated as a rate-limiting step for glycolysis [7]. In both liver and Novikoff hepatoma, glucose transport proceeds by facilitated diffusion [6, 7]; it therefore seems unlikely to be a Na^+-dependent process, as such dependence appears to be confined to active transport mechanisms. However, a direct test for Na^+-dependence does not appear to have been made in cells of either liver or the hepatoma. There was apparently a marked point of difference between the normal and neoplastic tissue in that the apparent K_m for glucose transport was reported to be about 17 mM for liver [6] but only 1–2 mM in Novikoff hepatoma [7].

We report here experiments which studied the relationship of glucose entry to the rate of glycolysis in slices of hepatoma 3924A, as a step in the understanding of the effect of ouabain on glycolysis. The apparent K_m for transport in this tumour was similar to that reported for normal liver. The results suggest that glucose entry may have a rate-limiting role on glycolysis at low external concentrations, but provide no evidence for a Na^+ dependence or direct ouabain sensitivity of the transport mechanism. The effect of ouabain on glycolysis in this tissue is thus not mediated by a reduction in glucose entry.

METHODS

Cells of Morris hepatoma 3924A were transplanted subcutaneously into both thighs of female rats of the ACI/T strain and were taken for use after 20–25 days growth. The majority of the individual tumours used were grown in Rome, where hepatoma 3924A has been maintained for the last four years. The ACI/T rats used to start the colony, and the initial samples of 3924A, were obtained from the laboratory of H.P.M. in Washington. Other experiments were done in Philadelphia, using tumour-bearing rats inoculated in H.P.M.'s laboratory and shipped by air.

Preparation of the tumour slices has been described previously [1]. The slices were immediately placed into a phosphate-buffered Ringer medium (see below) at 1 °C and allowed to pre-incubate for 120 min, during which time they were transferred 3 times to fresh portions of medium. The pre-incubation allowed equilibration of the extracellular marker, inulin, with its available space in the tissue water [8], and also caused depletion of cellular K^+, thus making these experiments directly comparable with our earlier work [1]. The vessels containing the final portions of medium each held 100–200 mg wet wt. of tissue slices in 3 ml medium and were gassed with O_2 or N_2, as appropriate. The experimental periods were initiated by placing the stoppered incubation vessels in a shaking water-bath at 38 °C. Duplicate or triplicate flasks were incubated at each point in each experiment. At least three experiments, using different animals, were done for each of the series of experiments summarised in the tables and figures.

The phosphate-buffered Ringer medium contained: Na^+ 155.0 mM, K^+ 5.0 mM, Mg^{2+} 1.0 mM, Ca^{2+} 1.25 mM, Cl^- 147.5 mM, SO_4^{2-} 1.0 mM, phosphate 10.0 mM (pH 7.4). Inulin, when used, was at 0.5 % w/v. For anaerobic experiments,

the medium contained 2 mM KCN and was gassed with N_2: for aerobic experiments, the medium was gassed with O_2. Glucose and other additions were made to the final concentrations indicated in the text.

After completion of the appropriate incubation period, a sample of medium was withdrawn from the incubation vessels and deproteinised with 1 % w/v $HClO_4$. The remaining flask contents were rapidly tipped onto a circle of hardened filter paper supported on a sintered-glass filter, and the slices separated from medium by suction followed by gentle blotting. Samples of the slices were transferred to a tared, hard-glass weighing bottle for drying, extraction with 0.1 M HNO_3 and analysis of water, Na^+, K^+ and dry weight. Other samples were deproteinised in 8 % w/v $HClO_4$ (at 1 °C), homogenized and centrifuged. The pellets were assayed for total protein by the biuret reaction. Perchloric acid extracts of tissue and medium were neutralised with a mixture of K_2CO_3 and triethanolamine [9], and assayed for lactate, glucose and glucose 6-phosphate, as appropriate. Lactate was assayed by use of lactate dehydrogenase (EC 1.1.1.27), glucose 6-phosphate with glucose-6-phosphate dehydrogenase (EC1.1.1.49) and glucose with hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase [9,10]. Further details of the procedures and assay methods are given by van Rossum [11].

Changes in slice composition or lactate production during the experimental period were determined by comparing samples of tissue and medium taken at the end of the pre-incubation period with samples taken after varying periods at 38 °C. Intracellular contents have been calculated by assuming that the inulin-containing compartment of the slice water contained all diffusible substances at their concentrations in the medium. Results are expressed in terms of the tissue protein content. In cases where tissue dry weight, rather than protein, was directly assayed, the conversion to units per weight of protein was made on the basis of estimates that the protein content was 0.592 ± 0.015 (26) kg protein/kg dry weight. The results are expressed as mean $\pm$ standard error of the mean. The number of observations is noted in parentheses, where a single observation is the mean of duplicate analyses carried out on the contents of a single reaction vessel.

RESULTS

Glycolysis

The time-course of anaerobic glycolysis was determined in slices that were incubated in the presence of 1 and 5 mM glucose; it should be noted that (unless otherwise stated) the pre-incubation (1 °C) of each set of slices was conducted in medium containing the same concentration of glucose as that used for the experimental incubation (38 °C). Control slices were incubated in glucose-free medium throughout, in order to estimate the contribution of endogenous substrates to the lactate formed. Fig. 1 shows that the extra lactate production in the presence of 1 mM glucose was linear for 45 min at 38 °C but fell off somewhat at longer times. Analysis of the medium showed that the glucose concentration had fallen to 0.65 ± 0.06 (11) mM by 45 min, a concentration which was below the apparent K_m for glycolysis (see below) and which would thus account for the declining rate of glycolysis. Slices incubated with 5 mM glucose showed a linear production of lactate during the whole period at 38 °C, and after 70 min the medium glucose concentration was 3.53 ± 0.24 (7) mM.

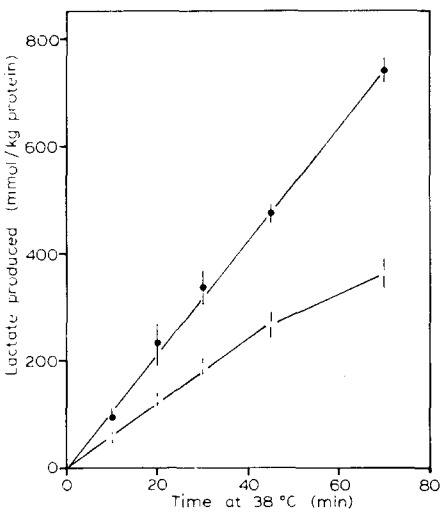

Fig. 1. Time-course of anaerobic lactate production by slices of hepatoma incubated in 1 mM or 5 mM glucose. Slices were incubated for 120 min at 1 °C followed by incubation at 38 °C in the presence of KCN (2 mM) and a N_2 atmosphere for the time indicated; glucose was present in the media throughout. ●, 5 mM glucose; ○, 1 mM glucose.

In the medium initially containing 1 mM glucose the ratio, mol total glucose utilised/mol total lactate produced, varied from 0.56±0.08 (11) after 10 min at 38 °C, to 0.38 ± 0.09 (11) after 70 min, and so did not differ significantly from the value of 0.5 expected for glycolysis. Similar results were obtained in the presence of 5 mM glucose, the ratio being 0.65±0.07 after 10 min and 0.54±0.03 after 30 min. The tissue content of free glucose decreased during incubation at 38 °C, the initial level of 25.7±1.5 mmol/kg protein after pre-incubation in 5 mM glucose decreasing by 5.6 ±1.0 mmol/kg after 10 min at 38 °C and by 11.3 ±1.5 mmol/kg after 30 min. By 30 min, the tissue glucose 6-phosphate showed only a very small increase of 0.48±0.20 mmol/kg protein. It is concluded that, in these anaerobic cells, all the glucose utilised was accounted for by the increased lactate production observed in the presence of the exogenous substrate.

The dependence of glycolysis on exogenous glucose was further studied over a range of concentrations from 1 to 50 mM. Incubation at 38 °C was continued for a standard time of 30 min, this being on the linear portion of all the time-course curves (Fig. 1). The endogenous lactate production (mean value 70±5 mmol/kg protein per 30 min) was determined as above, and was subtracted from the experimental values in order to estimate the rate of glycolysis from exogenous glucose only.

The experiment was done with tumours from each of the two sources (see Methods), and Fig. 2 illustrates that there was no more than 10 % difference between them at any glucose concentration. In each case, the maximal rate of glycolysis occurred at 20 mM glucose. Since glycolysis is the sole source of ATP in these anaerobic slices, the ion-transporting activity of the cells may also be expected to depend upon the concentration of glucose. Fig. 3 shows the net reaccumulation at 38 °C of the K^+ lost during pre-incubation at 1 °C. The rate of glycolysis induced by 1 mM medium glucose was insufficient to support a significant net uptake of K^+, but higher glucose concentrations permitted an accumulation which was closely

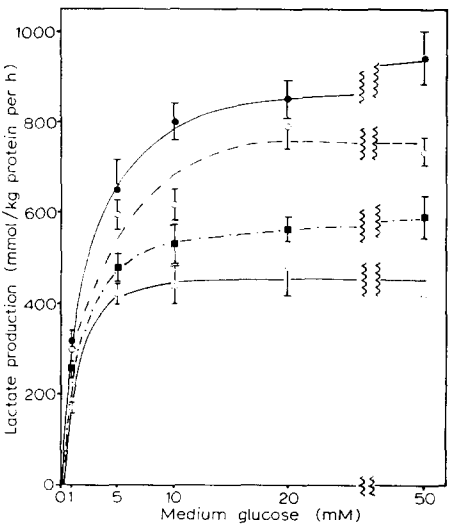

Fig. 2. Effect of medium glucose concentration and ouabain on the rate of lactate production by tumour slices from different sources and under varying conditions of incubation. Slices were incubated for 120 min at 1 °C and 30 min at 38 °C; glucose and inhibitors were present throughout. ●, Hepatomata inoculated in the laboratory of H.P.M.; incubation in the presence of KCN (2 mM) and N_2 atmosphere ($n = 6$). ○, Hepatomata inoculated in the laboratory of T.G.; incubation in the presence of KCN (2 mM) and N_2 atmosphere ($n = 10$). ■, Hepatomata from T.G., incubated in the presence of ouabain (1 mM), KCN (2 mM) and N_2 atmosphere ($n = 10$). △, Hepatomata from H.P.M.; incubation in O_2 atmosphere ($n = 6$).

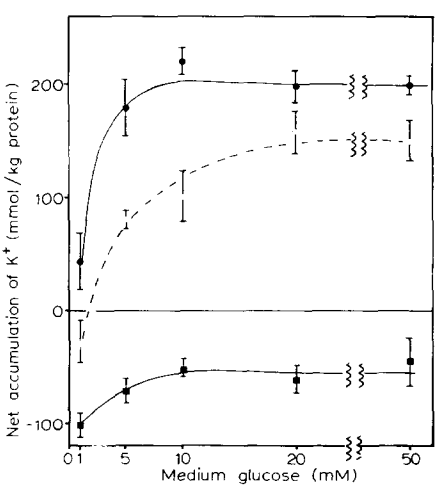

Fig. 3. Effect of medium glucose concentration on the net accumulation of K^+ by anaerobic slices of hepatoma. The analyses were done on the slices whose lactate production is shown in Fig. 2. ●, Hepatomata from H.P.M.; incubation in the presence of KCN (2 mM) and N_2 atmosphere ($n = 6$). ○, Hepatomata from T.G.; incubation in presence of KCN (2 mM) and N_2 atmosphere ($n = 10$). ■, Hepatomata from T.G.; incubation in presence of ouabain (1 mM), KCN (2 mM) and N_2 atmosphere ($n = 10$).

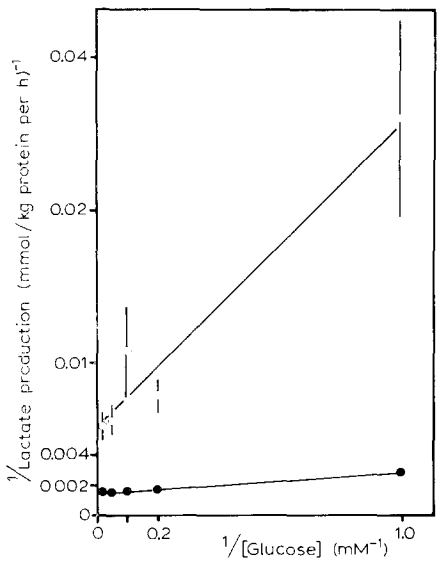

Fig. 4. Double reciprocal plot of the effect of glucose on anaerobic lactate production. Results are replotted from Fig. 2. Tumours from T. G. were incubated with KCN in a N_2 atmosphere, with or without ouabain (1 mM). ●, Lactate production in presence of ouabain; ○, total lactate production minus lactate production in presence of ouabain.

related to the rate of anaerobic glycolysis. This may be compared with our earlier finding that the inherent variation between animals in the rate of anaerobic glycolysis, at a constant glucose concentration, was also linearly related to the K^+ uptake [1]. Comparison of the curves obtained with slices from the two sources showed that there was a tendency for those obtained directly from H.P.M.'s laboratory to accumulate more K^+ at lower rates of glycolysis, but that there was no significant difference between the maximal amounts of accumulation achieved at 20 and 50 mM glucose.

The presence of 1 mM ouabain completely inhibited K^+ reaccumulation and, indeed, permitted some further loss of K^+ beyond that occurring during the pre-incubation period (Fig. 3). It also reduced the anaerobic lactate production by 20–30 % at all those concentrations of glucose which supported a significant, net active transport of K^+, but not at 1 mM glucose (Fig. 2). By subtraction, the anaerobic glycolytic activity may be separated into ouabain-sensitive and ouabain-insensitive fractions. From double reciprocal plots (Fig. 4) the apparent K_m values for glucose of the two fractions were found to be somewhat different: the values were 0.9 mM for ouabain-insensitive glycolysis and 3.4 mM for the ouabain-sensitive activity.

For comparison, Fig. 2 includes results on the glucose-dependence of aerobic glycolysis in the slices of 3924A. The form of the curve is generally similar to that shown by the ouabain-resistant anaerobic lactate production.

Net entry of glucose

We next studied the rate at which glucose enters the cells, in order to compare it with the rate of utilisation of the sugar by glycolysis. The slices were pre-incubated

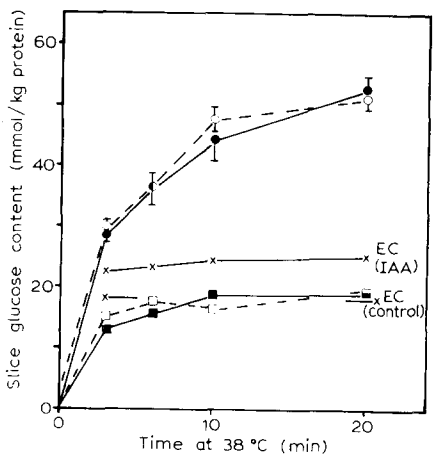

Fig. 5. Effect of iodoacetate (2 mM) and medium Na^+ on the entry of glucose into hepatoma slices incubated in presence of KCN (2 mM) and a N_2 atmosphere. Medium glucose concentration was 5 mM. For experimental procedure, see text. ●, ○, 2 mM iodoacetate present; ■, □, no iodoacetate (control). ●, ■, Incubated at 38 °C in Ringer with normal Na^+ concentration. ○, □, Incubated at 38 °C in Ringer containing Li^+ instead of Na^+. ×—×, E.C. (IAA) or E.C. (control) represent the equilibrium glucose contents of the extracellular (inulin-containing) tissue compartments incubated with and without iodoacetate.

in glucose-free medium containing the necessary inhibitors and inulin. Entry of glucose was initiated by transferring the slices to medium at 38 °C which contained glucose at the appropriate concentration. Samples of the slices were then taken for analysis at intervals.

The study of the entry of glucose into an actively glycolysing tissue is complicated by the rapid phosphorylation and metabolism of the sugar. The effect of metabolic activity in removing free glucose as soon as it enters the cells is illustrated in Fig. 5, where it can be seen that the presence of 2 mM iodoacetate (to inhibit glycolysis) greatly increased the amount of free glucose detectable in the slices that were incubated anaerobically in 5 mM glucose. In the slices incubated without iodoacetate, the glucose space was not significantly different from the inulin space, suggesting immediate phosphorylation of intracellular glucose. In the presence of iodoacetate and cyanide all reactions synthesising ATP are inhibited and it is reasonable to conclude that glucose entering the cells remains unaltered. Therefore, the majority of the remaining experiments on glucose entry were carried out in the presence of iodoacetate. This device has been used previously in work on sugar transport by brain slices [12, 13].

The rate of entry of glucose into the slices was determined in media containing glucose at concentrations from 0.5 to 50 mM, and the extracellular glucose was estimated from simultaneous measurements of the inulin distribution in the same samples of slices. The tissue contents were corrected for the small amount of endogenous glucose (mean value 0.54 ± 0.06 (32) mmol/kg protein) found in control slices incubated in glucose-free medium. Table I shows that the presence of iodoacetate prevented net production of lactate during incubation at 38 °C at any concentration of glucose, the lactate detected being equal to that present after glucose-free pre-incubation at

TABLE I

Products of glucose metabolism in hepatoma slices incubated in the presence of 2 mM CN^-, 2 mM iodoacetate and different concentrations of glucose. Results from experiments shown in Fig. 6. After preincubation for 120 min at 1 °C in the presence of the inhibitors, slices were transferred to media at 38 °C which contained the inhibitors and varying concentrations of glucose. Glucose 6-phosphate was analyzed in the tissue and lactate in media and tissue. Results obtained in media containing 0.5, 1.0, 2.0 and 5.0 mM glucose have been pooled in one group, and those in 10, 20 and 50 mM glucose in another.

Medium glucose	(mM)	Min at 38 °C			
		0	1	6	20
Glucose 6-phosphate	0	0.4±0.1	–	–	3.9±0.2
(mmol/kg protein)	0.5–5.0	–	1.0±0.1	2.7±0.3	4.0±0.6
	10–50	–	2.2±0.6	2.7±0.2	3.9±0.6
Lactate	0	50±13	–	–	–
(mmol/kg protein)	0.5–5.0	–	56±16	47±5	55±8
	10–50	–	32±8	43±13	51±11

1 °C. There was a small increase of tissue glucose 6-phosphate during the course of incubation, but since this was not affected by the external glucose concentration it must have arisen from endogenous sources (possibly glycogen) and does not indicate phosphorylation of transported sugar (see also above). During the first minute of incubation at 38 °C in all glucose- containing media there was a rapid entry of the sugar into the extracellular water. At 1 min, the glucose space was not significantly different from the inulin space in slices incubated in media containing 0.5–5.0 mM glucose, while at higher medium concentrations the apparent volume occupied by

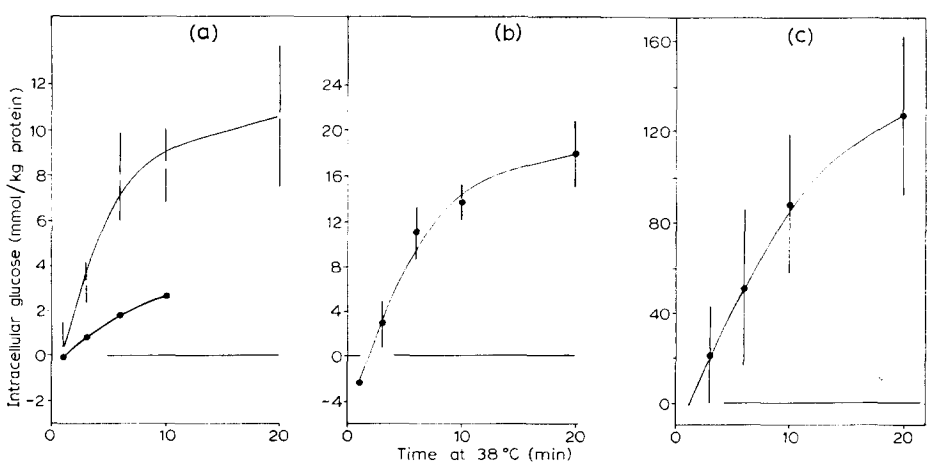

Fig. 6. Entry of glucose into intracellular compartment of hepatoma slices incubated in the presence of varying glucose concentrations. For experimental procedure, see text. The ordinate represents the tissue glucose content in excess of the equilibrium glucose content of the inulin-containing compartment. All media contained KCN (2 mM) and iodoacetate (2 mM). Glucose concentrations in media were (a) 0.5 mM (●) and 2 mM (○); (b) 5 mM; (c) 50 mM.

glucose came to exceed that occupied by inulin at about 2 min (as indicated by interpolation between the observations at 1 and 3 min). Fig. 6 illustrates the experiments at four glucose concentrations, the values plotted being the glucose content in excess of that calculated to be in the inulin-containing compartment of the tissue water. The rate at which glucose was transported into the cellular compartment was estimated from the initial slopes of the curves of the type illustrated in Fig. 6. In Fig. 7a the rate of entry has been plotted against the medium glucose concentration. The relationship shows a tendency to saturation of the transport system and a double-reciprocal plot allows calculation of an apparent K_m for transport of 13.5 mM glucose (Fig. 7b). However, the further increase in the rate of glucose uptake at even the highest glucose concentrations studied suggests that there is a component of glucose entry which occurs by simple diffusion, in addition to the saturable component. The calculated value of the apparent K_m may thus be an overestimate (cf. refs 7 and 14).

Fig. 7a also compares the rate of glucose entry with the rate of glucose consumption by anaerobic glucolysis, calculated as half the lactate production shown in Fig. 2. At concentrations less than 10–15 mM, the rate of glucose entry did not exceed the rate of glucose utilisation and could thus represent a rate-limiting step for glycolysis. At 20 mM glucose and above, the rate of glucose transport exceeded that of its utilisation by anaerobic glycolysis, and is thus not a rate-limiting factor in the latter. This agrees well with our observations that glycolysing slices (i.e. without iodoacetate) did not show free, intracellular glucose when incubated in the presence of 5 mM glucose (Fig. 5), but did contain it when incubated in 50 mM glucose (see below).

The possibility that the effect of ouabain on glycolysis might be due to a direct effect on glucose transport, independently of its effect on ion transport, was tested in experiments of the type just described. In the presence of cyanide and iodoacetate all active ion transport would be impossible because of the complete absence of ATP

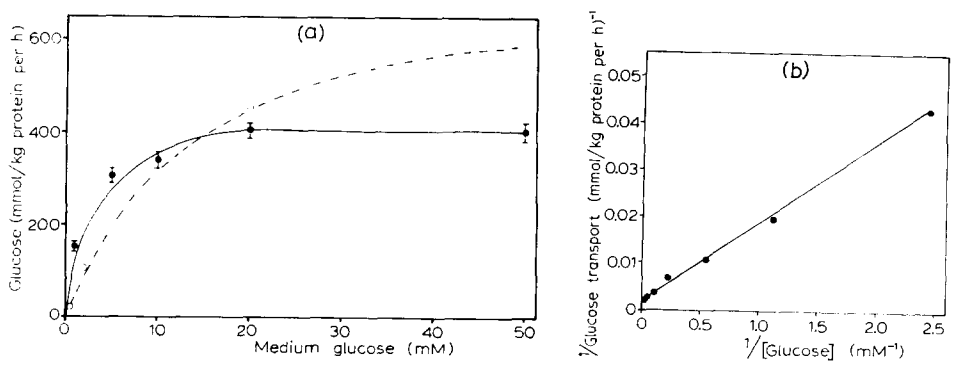

Fig. 7. (a) A comparison of the rates of glucose transport and glucose utilisation by glycolysis in hepatoma slices incubated in the presence of different concentrations of glucose. ○, Rates of glucose transport are calculated from results of experiments of the type illustrated in Fig. 6, using the maximal rate of net glucose penetration beyond the inulin space (see text for further details). ●, Rates of glucose utilisation in glycolysis have been taken to be half the rate of lactate production under anaerobic conditions (in the absence of ouabain) shown in Fig. 2. Results from the two sources of hepatomata have been pooled, since they show no significant difference in glycolytic rate. (b) Double reciprocal plot of the effect of glucose concentration on rate of glucose transport. Results are taken from (a).

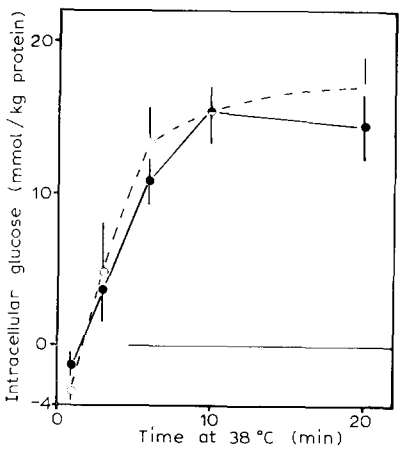

Fig. 8. Effect of ouabain on the time-course of glucose entry in the presence of KCN (2 mM) and iodoacetate (2 mM); medium glucose concentration was 5 mM. Procedure as for Fig. 6. ●, Control; ○, ouabain (2 mM).

synthesis, and any direct effect of ouabain on the glucose transport system should be apparent. Fig. 8 shows that the entry of glucose into slices incubated in the presence of 5 mM glucose was unaffected by 2 mM ouabain.

The possibility of a sodium-dependent component of the glucose transport mechanism in hepatoma 3924A was next examined. Current ideas on the Na^+-dependent sugar-transport by other types of cells indicate that the essential factor is the existence of a concentration gradient of the ion in the direction of transport [4, 15]. In our experiments, we studied the effect of such a gradient on sugar transport in the presence of cyanide and iodoacetate by the following procedure. The slices were pre-incubated at 1 °C in a Na^+-free medium (Na^+ of the Ringer being replaced by Li^+). The slices were transferred to fresh portions of this medium four times during the 120 min period of pre-incubation so that their Na^+ content fell to an apparent concentration of 14.7 ± 2.5 mmol/kg tissue water (Table II). The study of glucose uptake was then initiated by transferring the slices to glucose-containing medium at 38 °C. Half of the slices were placed into such a medium containing the normal Na^+ concentration (155 mM) so that a 10-fold concentration gradient, outside greater than inside, was immediately established: after the full incubation period of 20 min at 38 °C, the apparent tissue concentration of Na^+ was 88 ± 3 mmol/kg water so that a concentration gradient (approximately two-fold) for Na^+ still existed in the same sense (Table II). The remaining slices were used as controls, being placed into glucose-containing Na^+-free (Li^+) medium. In this case, the tissue Na^+ remained roughly constant, so that a small Na^+ gradient in the adverse direction for sugar uptake was maintained throughout the incubation at 38 °C. From Fig. 5 it can be seen that the different initial Na^+ gradients produced no change in the rate of entry of glucose into slices from medium containing 5 mM glucose.

This experiment was repeated under conditions in which iodoacetate was absent, so that the cells were able to synthesise ATP by anaerobic glycolysis to support any possible active transport mechanism for glucose. As noted earlier, the rate of glu-

TABLE II

Effect of incubation in normal Na^+ or Na^+-free medium on tissue Na^+ concentration. The results are of analyses done on the slices used for studies of the Na^+ dependence of glucose transport. KCN (2 mM) was present in all media. Glucose was added at the onset of incubation at 38 °C. In the Na^+-free medium, Li^+ was substituted for Na^+. Line (1). Control experiment; slices incubated in Na^+ Ringer for 120 min at 1 °C followed by 20 min at 38 °C. The fall of Na^+ concentration is due to active transport deriving energy from glycolysis. Line (2). Slices incubated for 120 min at 1 °C in Li^+ Ringer, followed by transfer to Li^+ or to Na^+ Ringer for 20 min at 38 °C. Line (3). Similar to line (2), except that 2 mM iodoacetate was present.

Medium glucose during incubation at 38 °C	Slice [Na^+] (mmol/kg tissue H_2O)			
	Li^+ Ringer (Na^+-free)		Na^+ Ringer (155 mM Na^+)	
	1 °C	38 °C	1 °C	38 °C
(1) 50 mM	—	—	147.0±1.9	113.3±8.8
(2) 50 mM	20.0	22.8	—	97.3
(3) 5 mM (with iodoacetate, 2 mM)	14.7±2.5	12.0±3.0	—	88.3±2.6

cose entry from a 5 mM glucose medium was insufficient to permit the presence of free glucose in the actively-metabolising cells. However, 50 mM glucose is sufficiently in excess of the apparent K_m for glycolysis to permit such accumulation, and to give an indication, at short incubation times, of the rate of glucose entry into the tumour cells. Under these conditions also, the presence of external Na^+ and of a supposedly favourable Na^+ gradient (Table II) had no influence on the initial rate at which glucose entered the tissue (not illustrated).

After 20 min at 38 °C in the presence of 50 mM glucose, the slices incubated without iodoacetate in the Na^+ medium show an intracellular concentration of 28.4 mmol glucose/kg intracellular water, while those in the Li^+ medium contained 30.9 mmol/kg. Thus, there was no indication of an accumulation of glucose against a concentration gradient either in the presence or absence of medium Na^+. The concentration of glucose in the intracellular water of slices incubated for the same time (20 min) in the medium containing 50 mM glucose plus 2 mM iodoacetate was 21.0±5.6 (5) mmol/kg water. This value is not significantly different from those obtained in glycolysing slices, suggesting that the failure of the glucose to attain equilibrium with the 50 mM glucose medium in the absence of iodoacetate was not due to its rapid utilisation by the glycolytic activity. These results therefore provide no evidence for an active transport mechanism for glucose in this hepatoma, although the possibility is not rigorously excluded.

DISCUSSION

Glucose entry

The determination of the rate at which glucose enters the cells of tissue slices is made difficult, and the accuracy of the estimates impaired, by the rapid, metabolic utilisation of the sugar, the presence of endogenous glucose and the correction needed for the presence of glucose in the extracellular compartment. The first two of these factors may be avoided by studying a non-metabolisable derivative of the sugar.

However, there are indications in the literature that, in other cells, the maximal rate of transport of glucose may differ by 50–100 % from that of its commonly used derivatives [7, 14]. We therefore chose to use glucose itself, particularly because of the comparison we wished to make between the rate of glucose entry and its rate of glycolysis (Fig. 7a). The metabolism of glucose was prevented by the presence of iodoacetate in addition to cyanide (discussed below). Endogenous glucose levels were determined and subtracted: the maximal relative correction for this factor had to be applied to the slices incubated for 1 min in medium with 0.5 mM glucose, where it amounted to approximately 20 % of the total glucose content. The greatest source of inaccuracy was, however, that due to the correction for the glucose content of the extracellular compartment. This is a source of error inherent in the use of multicellular preparations of tissues, irrespective of the nature of the transported substance.

The entry of glucose into the cells of the rapidly glycolysing hepatoma 3924A appears to involve a process which is saturable as the external sugar concentration is raised. However, saturation does not appear to be completely attained at external concentrations as high as 50 mM and this strongly suggests that a simple, passive diffusion process was also contributing to the overall sugar entry. The apparent K_m for the total glucose entry (approximately 13 mM) was of the same order as that reported for perfused, normal liver (minimally 17 mM, see ref. 6) where there also appears to be a component of simple diffusion. Both of these values are substantially greater than the apparent K_m for glucose transport reported for cells of the rapidly glycolysing Novikoff hepatoma in tissue culture, which was 1–2 mM (ref. 7). This may represent a real difference between the two hepatomata, or it may be a reflection of some of the technical difficulties which, as mentioned above, attend the study of glucose transport in the slice preparation. However, an important contributory factor is likely to be the fact that the superimposition of a simple diffusion onto a carrier-mediated transport mechanism can lead to the estimate of the apparent K_m of the overall process being substantially greater than the K_m for the saturable mechanism [7, 14]. It thus seems likely that the K_m for the facilitated diffusion of glucose in hepatoma 3924A, and probably in liver also, is lower than the observed values of 13–17 mM. However, the values of Fig. 7a do not indicate a linear portion of the curve, even at the highest concentrations of glucose studied, and we cannot therefore reliably correct for the simple diffusion component of the total uptake. Nevertheless, the observation that the estimated apparent K_m values for total glucose transport in multicellular preparations of both normal liver and hepatoma 3924A are in good agreement, does suggest that there are no marked alterations in the nature of the glucose transport mechanism in the cells of this tumour.

The glucose-transporting systems of liver and Novikoff hepatoma were unable to produce intracellular concentrations greater than that of the medium. Our results with 3924A incubated in 50 mM glucose in the absence of iodoacetate (and therefore in the presence of active ATP synthesis by glycolysis) also failed to show an accumulation against the concentration gradient or even to show an intracellular concentration significantly higher than that attained with 50 mM glucose in the presence of iodoacetate. This seems to indicate that a mechanism of facilitated diffusion is involved rather than active transport, although we have not given a rigorous proof of this, e.g. the equality of concentration with and without iodoacetate could be a fortuitous result of the rate of glucose removal by glycolysis in the absence of the inhibitor, rather

than independence from ATP provision. Neither the glucose-transporting system of liver, nor that of Novikoff hepatoma, has been tested for sensitivity to Na^+, either by use of Na-free medium or by treatment with ouabain. Our results indicate, by both of these criteria, that glucose transport in hepatoma 3924A, whether in the presence or absence of iodoacetate, is independent of Na^+ coupling. This finding may in itself be taken as a further, indirect indication that a facilitated diffusion rather than active transport is involved, since active transport of sugars in mammalian cells has so far only been shown in Na-coupled systems [4].

Control of glycolysis

Comparison of the observed rates of anaerobic glycolysis and of glucose entry indicate that glucose consumption may be controlled by its rate of entry when the external substrate is present at less than 10–15 mM, but not at higher concentrations. The validity of this comparison depends upon the absence of an effect of iodoacetate on the carrier system since the rate of uptake was estimated in its presence. However, Joanny et al. [12] were unable to find evidence of inhibition of facilitated diffusion of at least one sugar (D-xylose) by this agent in brain slices (see also ref. 13). Our conclusion is supported by the finding that, in the absence of iodoacetate, free intracellular glucose can be detected when slices are incubated in 50 mM glucose, but not in 5 mM glucose medium.

The absence of Na or ouabain sensitivity of the glucose entry, as discussed above, indicates that the inhibitory effect of ouabain on glycolysis is not mediated by an effect on the glucose transport system. This is further shown by the finding that the inhibitory effect of ouabain on glycolysis is undiminished at concentrations of glucose at which the rate of sugar entry is clearly not rate-limiting for glycolysis. Thus, at 50 mM glucose, the maximal rate of glucose entry exceeded the rate of its utilisation by approximately 180 mmol/h per kg protein, while ouabain inhibited glucose conversion to lactate by less than this difference (i.e. by only 140 $mmol \cdot h^{-1} \cdot kg^{-1}$). An indication from the present work that the effect of ouabain is closely related to the utilisation of energy for cation transport is provided by the finding that ouabain only significantly reduced anaerobic glycolysis when the rate of this process was sufficient to support a net accumulation of K^+ (i.e. at glucose concentrations greater than 1 mM). Thus, we continue to favour the original suggestion of Whittam et al. [2], further supported by Eckel et al. [3], that the effect of ouabain is mediated via the reduced interconversion of ADP and ATP during K^+ transport and glycolysis.

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