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The pH-Dependence of Sugar Transport and of Glycolysis in Cultured Ehrlich Ascites-Tumour Cells

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1. pH-dependence of glycolysis has generally been ascribed to the effects of pH on the activities of glycolytic enzymes. The present study shows that sugar transport is pH-dependent in cultured Ehrlich ascites-tumour cells. 2. The rates of glucose consumption, of 3-*O*-methylglucose transport, and of 2-deoxyglucose transport and phosphorylation increased as linear functions of pH, as the pH of the cell culture medium was increased from 6.1 to 8.5. Transport of glucose, as measured in ATP-depleted cells, was pH-dependent to the same extent as transport of the non-metabolizable sugars. 3. Glucose consumption rates were about 8-fold higher at pH 8.5 than at pH 6.4. About 65–85% of glucose was converted into lactate. Sugar transport rates were 2.5-fold higher at pH 8.5 than at pH 6.3. 4. pH affected both simple diffusion and facilitated diffusion. pH effect was mainly on the $V_{max.}$ of 2-deoxyglucose uptake, and on the rapid-uptake phase of 3-*O*-methylglucose transport. 5. It was estimated that about 70% of the pH effect on the rates of glucose consumption may be due to the effect on sugar transport and the remainder to the effect on the activities of glycolytic enzymes.

Rates of glycolysis increase with the rise in extracellular pH in a wide variety of cells and tissues (Zwartouw & Westwood, 1958; Halperin *et al.*, 1969; Wilhelm *et al.*, 1971). The pH-induced changes in glycolytic rates are large, and may constitute a major cellular defence mechanism against alkalosis and acidosis. The effect of pH has been generally ascribed to changes in activities of several key glycolytic enzymes, in particular phosphofructokinase (Delcher & Schipp, 1966; Ui, 1966; Halperin *et al.*, 1969; Wilhelm *et al.*, 1971; Fodge & Rubin, 1973).

The present study shows that sugar transport is markedly pH-dependent and may be the single most important factor determining the pH-dependence of glycolysis. Rates of sugar transport were measured by using 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose, which are transported by the same system as D-glucose at the concentrations used in the present study (Hatanaka, 1974; Plagemann & Richey, 1974; Kletzien & Perdue, 1974; Christopher *et al.*, 1976). After the transport step 3-*O*-methylglucose is not further metabolized, and 2-deoxyglucose is phosphorylated but not further metabolized in the glycolytic pathway (Schmidt *et al.*, 1974).

The study was carried out with Ehrlich ascites-tumour cells that had been adapted to grow in continuous-suspension cultures (Van Venrooij *et al.*, 1970; Kaminskas, 1973; Kaminskas *et al.*, 1976). The conditions of culture permitted exponential growth with cell doubling times of 12–15h. The cells were maintained in good nutritional state, characterized by high rates of protein and RNA synthesis and by a high-energy charge of the adenylate pool (Live & Kaminskas, 1975).

Experimental

Materials

Eagle Minimal Essential Medium with Earle's salts, phosphate-buffered saline and foetal calf serum were purchased from Grand Island Biological Co., Grand Island, NY, U.S.A. Morpholinopropanesulphonic acid, bovine serum albumin, 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Omnifluor, D-[G-^{14}C]glucose (5 mCi/mmol), 2-deoxy-D-[G-^{3}H]glucose (7.2 Ci/mmol) and 3-*O*-[^{3}H]methyl-D-glucose (4.2 Ci/mmol) were purchased from New England Nuclear Corp., Boston, MA, U.S.A. GOD–PERID test kits and lactate test kits were purchased from Boehringer-Mannheim Corp., NY, U.S.A. Sodium dodecyl sulphate was obtained from Bio-Rad, Richmond, CA, U.S.A.

Methods

Cell cultures. Ehrlich ascites-tumour cells were routinely grown in spinner flasks (Bellco Glass, Inc.,

Vineland, NJ, U.S.A.) at 37°C in an incubator. Experimental cultures were incubated in water baths equipped with B. Braun Thermomix II heater-circulators. The growth medium consisted of Eagle Minimal Essential Medium with $NaHCO_3$ concentration decreased to 13mM and supplemented with 25mM-morpholinopropanesulphonic acid, 5.5mM-glucose, heat-inactivated 2.5% (v/v) foetal calf serum, 75 units of penicillin G/ml and 50μg of streptomycin/ml; pH was adjusted to 7.4 with NaOH. The cell concentration was maintained at 2×10^5–1×10^6 cells/ml by resuspending the cultures daily in fresh medium. Cells were not inadvertently starved of glucose, since the growth medium after 24h of cultivation contained at least 2mM-glucose. Before being used for experiments, cells were resuspended in fresh medium and incubated for at least 2h. The viability of cells, as determined by Trypan Blue exclusion, under all experimental conditions was over 95%.

Rates of glucose consumption and of lactate production. The rates of decrease of glucose concentration in the media were used to measure the rates of glucose consumption by cells. For this purpose, cells were suspended in complete medium at the pH as stated and containing 5.5mM-glucose, placed into spinner flasks and incubated for a time period sufficient for cells to consume at least 1μmol of glucose/ml (3–6h). In the pH range of 6.1–6.8, cells were incubated at densities of 3.5×10^6 cells/ml or higher in tightly stoppered spinner flasks. In the pH range of 7.4–8.5 cells were incubated at densities of about 1×10^6 cells/ml and the cultures allowed to equilibrate with room air. The pH did not change by more than 0.2 unit during the course of each incubation. At hourly intervals 1.0ml samples of cultures were removed with an Eppendorf pipette and centrifuged at 300*g* for 3min at 18°C. Glucose and lactate concentrations in the medium were determined in duplicate by the glucose oxidase (GOD–PERID) method (Bernt & Lachenicht, 1974) and by the lactate dehydrogenase method (Gutmann & Wahlefeld, 1974) respectively; pH of the medium was determined with a Radiometer model 62 pH-meter at room temperature. The cell pellets were washed once with 1.0ml of phosphate-buffered saline, solubilized with 1% sodium dodecyl sulphate and their A_{260} determined. The A_{260} of cell lysates was found to be an accurate measure of cell number (Kaminskas *et al.*, 1976); in 100 determinations in triplicate, single determinations deviated from the mean by an average of 3.5%. A lysate of 9.6×10^5 exponentially growing cells has an A_{260} of 1.0 (Kaminskas *et al.*, 1976) and contained 0.24mg of cell protein. Cells depleted glucose from the medium as a function of cell density and of time of incubation in a linear fashion in the pH range 6.1–8.5 and at glucose concentrations between 1 and 5.5mM. Glucose-consumption rates were calculated as nmol of glucose depleted/min per 10^6 cells. Increases in lactate concentration in the medium were used to measure lactate-production rates by cells; the rates were calculated as nmol of lactate produced/min per 10^6 cells.

Rates of 2-deoxyglucose and of 3-O-methylglucose uptake. For determinations of 2-deoxyglucose-uptake rates, 2×10^6 cells were washed once with 5ml of phosphate-buffered saline, suspended in 1.0ml of assay medium, placed in a stoppered 25ml Erlenmeyer flask and incubated at 37°C in a gyrorotary shaker bath. The assay medium was the above culture medium formulated without glucose or serum and containing 1mg of bovine serum albumin/ml to minimize cell adhesion to glass (Kaminskas, 1972); pH of the medium was adjusted with NaOH or with HCl shortly before use. Changes in pH did not exceed 0.02 unit during the assays. After 10min of incubation, 2-deoxy-D-[^{3}H]glucose (1μCi/μmol) was added to the assay flask, mixed rapidly and the incubation continued. Samples of 200μl were removed with a Pipetman after 15s, 2min 15s and 5min 15s, placed into test tubes kept at 2°C containing 3ml of cold phosphate-buffered saline supplemented with 11mM-2-deoxyglucose, and mixed rapidly by inversion. Cells were collected by centrifugation at 400*g* for 1min at 4°C and washed once with 3ml of the above buffer. The supernatant was carefully removed by aspiration and discarded, and the cell pellet was lysed by the addition of 200μl of 1% sodium dodecyl sulphate and vortex-mixing. A sample (100μl) of the lysate was spotted on to a Reeve–Angel glass-fibre disc, which was dried and placed into Omnifluor/toluene; its radioactivity was determined in a Beckman LS-100 liquid-scintillation counter. The remainder of the lysate was mixed with 900μl of water and its A_{260} determined in a Zeiss PM2D spectrophotometer. Uptake of 2-deoxy[^{3}H]-glucose by cells was linear with time between 15s and 10min 15s at 2-deoxyglucose concentrations of 0.2–2mM and in the pH range of 6.3–8.5 (Figs. 2(*a*) and 2(*b*)). Rates of 2-deoxyglucose uptake were determined at 1mM-2-deoxyglucose and were calculated as nmol/min per 10^6 cells. This method is similar to the one used by Renner *et al.* (1972), except that by placing the assay samples into cold buffer containing a high concentration of unlabelled sugar we chilled the cells rapidly and at the same time diluted the specific activity of the labelled sugar.

Uptakes of 3-*O*-methylglucose and of glucose were performed similarly. Assay samples were removed at 15, 45, 75 and 195s after the addition of 3-*O*-[^{3}H]-methyl-D-glucose (2μCi/μmol) and 15, 45, 75 and 135s after the addition of D-[^{14}C]glucose (0.1μCi/μmol). Cells were washed with phosphate-buffered saline. Adenylate concentrations in cell extracts were determined by t.l.c. as previously described (Live & Kaminskas, 1975).

Results

pH-dependence of glycolysis

Glycolytic rates of cultured Ehrlich ascites-tumour cells were determined in the pH range of 6.1–8.5. Fig. 1 shows the rates of glucose consumption and of lactate production in a set of parallel cultures incubated at the mean pH values as shown. Glucose-consumption rates increased as a linear function of pH of the incubation medium. From these results it was calculated that glucose consumption rates increased at a rate of 3.3nmol/min per 10^6 cells with each increase of 1 pH unit. Increases of 2.8–3.6nmol/min per 10^6 cells per 1 pH unit were recorded in other such experiments. It should be noted that the rate of increase is quite high, i.e. glucose consumption rate was about 8-fold higher at pH8.5 than at pH6.4. About 65–85% of glucose was converted into lactate. In this and other such experiments proportionately less lactate was excreted into the medium by cells incubated at pH8.5 than by cells incubated at lower pH values. Lactate could not be detected in glucose-free medium in which 5×10^6 cells/ml were incubated for 6h.

pH-dependence of 2-deoxyglucose uptake. Cells transported and phosphorylated 2-deoxyglucose at a constant rate starting at 15s after the addition of the labelled sugar (Fig. 2*a* and 2*b*). The values obtained in the first 15s represent an unsaturable component of the transport process, probably simple diffusion, and have to be subtracted to obtain linear rates of transport and phosphorylation (Plagemann & Richey, 1974). Fig. 2(*c*) shows the increase in the rates of 2-deoxyglucose uptake and phosphorylation as the pH of the assay medium was increased from pH6.3 to 8.5. The increase in rates was linear and was calculated as 2.5nmol/min per 10^6 cells with each increase of 1 pH unit. The uptake rate at pH8.5 was about 2.5-fold higher than at 6.3.

The effect of pH on 2-deoxyglucose transport and phosphorylation was mainly on the $V_{max.}$, K_m values were lower at pH6.3 and at pH8.5 than at pH7.4. In the experiment shown in Fig. 2(*d*), cells from one culture were assayed at the three pH values and at the substrate concentrations shown. The increases in uptake rates were linear up to 0.2mM-2-deoxyglucose at pH6.3 and at pH7.4, and up to 0.3mM at pH8.5. The rates then increased in a manner consistent with Michaelis–Menten kinetics up to 1mM-2-deoxyglucose at all three pH values. When plotted by the method of Lineweaver & Burk (1934) (Fig. 2*e*), $V_{max.}$ values of 7.7nmol/min per 10^6 cells, 14.1nmol/min per 10^6 cells and 16.2nmol/min per 10^6 cells were obtained for cells incubated at pH6.3, 7.4 and 8.5 respectively. The K_m values were 0.27, 0.66 and 0.30mM at pH6.3, 7.4 and 8.5 respectively. Other such determinations yielded similar data.

pH-dependence of 3-O-methylglucose uptake. Uptake of 3-*O*-methylglucose was very rapid, and a linear portion of this process could not be detected (Fig. 3*a*). This finding is consistent with the results of Renner *et al.* (1972) with Novikoff rat hepatoma cells and of Hatanaka *et al.* (1969) and of Venuta & Rubin (1973) with transformed mouse and chick fibroblasts. In contrast, untransformed fibroblasts transported 3-*O*-methylglucose at a slower and initially linear rate (Hatanaka *et al.*, 1969; Venuta & Rubin, 1973; Christopher *et al.*, 1976). Fig. 3(*a*) also shows that the uptake process was saturable, in that the uptake rates at all time intervals became near-maximal at 30mM-3-*O*-methylglucose.

The experiments shown in Figs. 3(*a*), 3(*b*) and 3(*c*) were performed with cells from one culture and results obtained are directly comparable. Other such experiments yielded very similar results. Uptake rates were lower at pH 6.3 than at pH7.4 at all time intervals; the uptake process was saturable at 30mM-3-*O*-methylglucose. Uptake rates were higher at pH8.5 than at pH 7.4 (the 15 mM-3-*O*-methylglucose uptakes were unusually low in this experiment). The effect of high pH was especially prominent at higher 3-*O*-methylglucose concentrations and at later periods in the uptake curves. The uptake process was saturable at 20mM-3-*O*-methylglucose. Maximal uptakes were reproducibly recorded at 75s, whereas the uptakes at

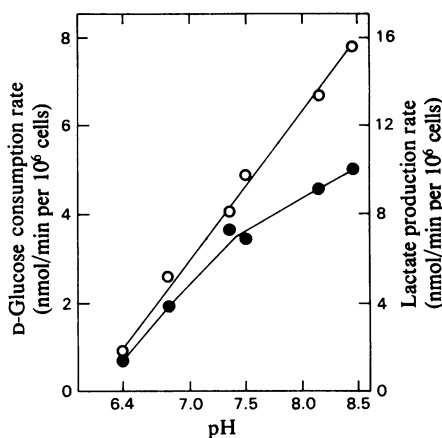

Fig. 1. *Glucose consumption and lactate production rates as a function of pH*

Cells were incubated for 3–6h in parallel spinner flasks at the mean pH values shown. Cell concentrations were 3.5×10^6/ml and 1×10^6/ml in the pH ranges 6.3–7.4 and 7.4–8.5 respectively. Glucose consumption (○) and lactate production (●) rates were calculated and plotted as a function of the mean pH values recorded in each culture during the course of the experiment. The pH of the medium did not change by more than 0.2 pH unit in the course of the experiment.

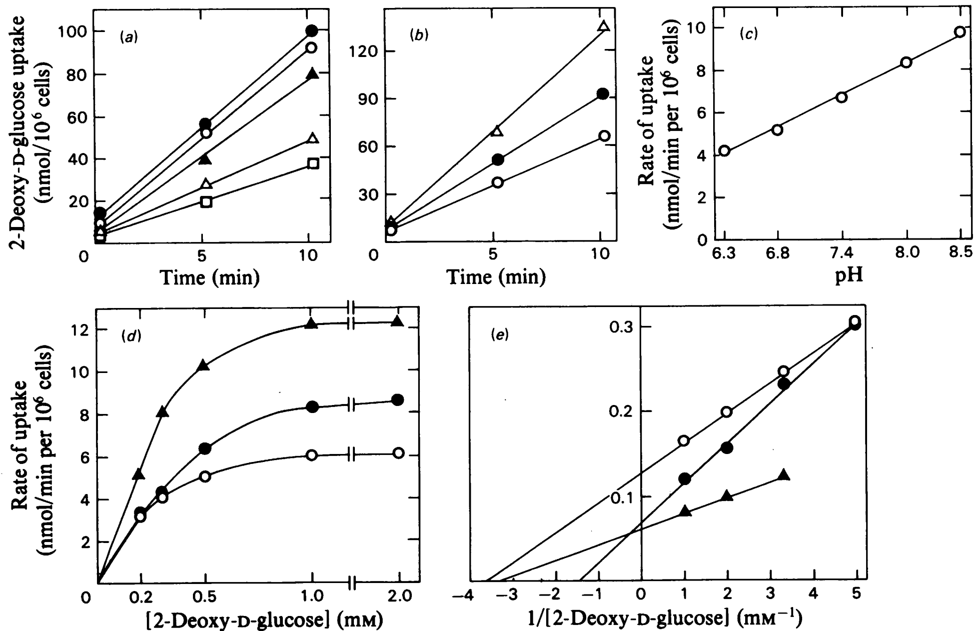

Fig. 2. *Effects of 2-deoxyglucose concentration and of pH on the rates of 2-deoxyglucose uptake*
(*a*) Linearity of 2-deoxyglucose uptake in assay media containing 0.2mM- (□), 0.3mM- (△), 0.5mM- (▲), 1mM- (○) and 2mM- (●) 2-deoxyglucose. Cells from one culture were assayed sequentially in the above media. (*b*) Linearity of 2-deoxyglucose uptake in 1mM-2-deoxyglucose-containing media at pH6.3 (○), pH7.4 (●) and pH8.5 (△). Cells from one culture were assayed sequentially in the above media. (*c*) Effect of pH on the rates of 2-deoxyglucose uptake. Cells from one culture were assayed in 1mM-2-deoxyglucose media at the pH values as shown. (*d*) Effect of 2-deoxyglucose concentration on the rates of 2-deoxyglucose uptake in assay media at pH6.3 (○), pH7.4 (●), and pH8.5 (▲). Cells from one culture were assayed sequentially in assay media at the above pH values and at 2-deoxyglucose concentrations as shown. (*e*) Data from (*d*) plotted by the double-reciprocal method of Lineweaver & Burk (1934).

pH6.3 and 7.4 continued to increase up to 195s. This prolongation of the rapid-uptake phase at high pH is better illustrated in the experiment shown in Fig. 3(*d*), in which cells from one culture were assayed at five pH values between pH6.3 and 8.5 in 20mM-3-*O*-methylglucose medium. The differences in uptakes at different pH values became fully apparent only after 75s of incubation.

The lack of linearity of the uptake process made accurate estimations of the initial rates of uptake difficult. The finding that uptakes were most rapid in the first 15s is consistent with the results of Coe & Saha (1966) on glucose uptake by Ehrlich ascites cells. However, the use of 15s-uptake values as the initial rates of uptake would lead to an underestimation of the effect of high pH. The saturability of the uptake process at a lower 3-*O*-methylglucose concentration at pH8.5 suggests that the K_m for 3-*O*-methylglucose is slightly lower at pH8.5 than at pH7.4.

Contribution of transport to pH-dependence of glycolysis. To what extent may sugar transport contribute to the pH-dependence of glucose consumption and glycolysis? Direct comparisons of the increases in uptake rates of each sugar as a function of pH are not possible, since their absolute uptake rates are different. The present data on the differences in the uptake rates of glucose and of 2-deoxyglucose are consistent with the previously published findings with Ehrlich ascites-tumour cells (McComb & Yushok, 1964). The 3-*O*-methylglucose-uptake rates, even though estimated with the above expressed reservations, are clearly much higher than those of glucose or of 2-deoxyglucose. One method of determining the effect of pH on the uptake of each sugar is to plot the rates at each pH value as a percentage of the rate obtained at pH8.5. Such a plot (Fig. 4) derived from the results of Fig. 1, Fig. 2(*c*) and Fig. 3(*d*) (75s values) shows that the uptake of glucose is more pH-dependent than the uptakes of 2-deoxyglucose and of 3-*O*-methylglucose, which are very similar. At pH7.5 the rates of uptake of glucose, of 2-deoxyglucose and of 3-*O*-methylglucose were lower by 41, 27 and 30% respectively than at pH8.5. This calculation suggests

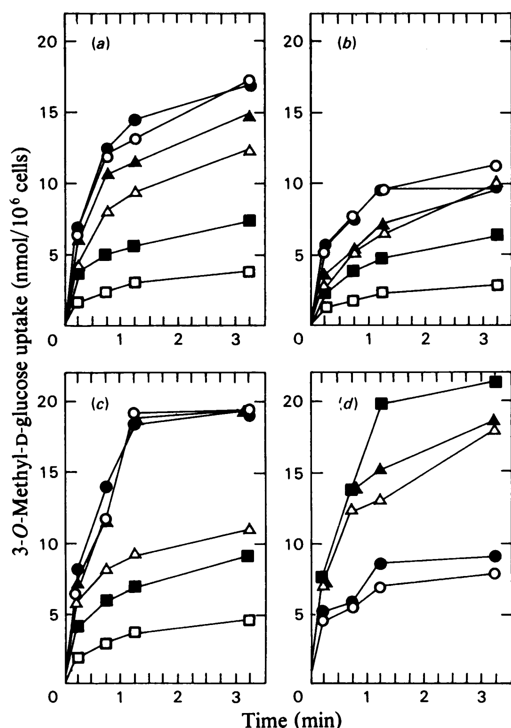

Fig. 3. *Effects of* 3-*O*-*methylglucose concentration and of pH on the rates of* 3-*O*-*methylglucose uptake*

Cells from one culture were assayed sequentially for their rates of 3-*O*-methylglucose uptake in assay media at pH7.4 (*a*), pH6.3 (*b*) and pH8.5 (*c*) at 5mM- (□), 10mM- (■), 15mM- (△), 20mM- (▲), 30mM- (○) and 50mM- (●) 3-*O*-methylglucose. Sampling times were 15s, 45s, 75s, and 195s. (*d*) Cells from one culture were assayed sequentially for their rates of 3-*O*-methylglucose uptake in assay media containing 20mM-3-*O*-methylglucose at pH6.3 (○), pH6.8 (●), pH7.4 (△), pH8.0 (▲) and pH8.5 (■).

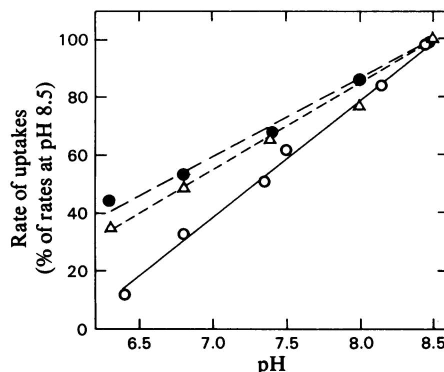

Fig. 4. *Increases in the rates of glucose,* 2-*deoxyglucose and* 3-*O*-*methylglucose uptakes as a function of pH*

The data on sugar-uptake rates contained in Fig. 1, Fig. 2(*c*) and Fig. 3(*d*) (75s values) were plotted as a percentage of the uptake rates at pH8.5. ○, Glucose; ●, 2-deoxyglucose; △, 3-*O*-methylglucose.

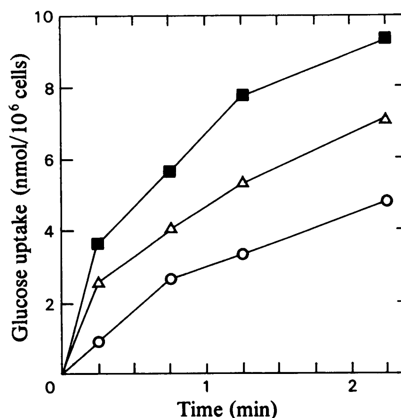

Fig. 5. *Glucose uptake in glucose-starved cells as a function of pH*

Cells were incubated for 5h in glucose-free medium and then assayed sequentially for their rates of glucose uptake in assay media containing 5mM-glucose at pH6.3 (○), pH7.4 (△) and pH8.5 (■).

that as much as 70% of the pH-dependence of glucose consumption may be due to the pH-dependence of sugar transport.

Direct evidence of pH-dependence of glucose transport was sought by depleting cells of ATP (and thus inhibiting the hexokinase reaction) and then measuring labelled glucose uptake. ATP depletion was achieved by incubating cells in glucose-free medium for 5h. Previous studies (Live & Kaminskas, 1975) showed, and present determinations confirmed, that ATP concentrations in these cells were decreased by 80–85%. Their rates of 2-deoxyglucose uptake and phosphorylation were decreased by 75% in the first 75s and by over 90% in the subsequent 60s, indicating a severe inhibition of the hexokinase reaction. Uptake of 3-*O*-methylglucose was decreased by 40–50%. Uptake of labelled glucose by these cells was clearly pH-dependent (Fig. 5). When 45, 75 and 135s uptake values were plotted as in Fig. 4, they fitted the data for pH-dependence of 2-deoxyglucose uptake. When rates of uptake (15–75s at pH7.4 and 8.5, and 45–135s at pH6.3) were calculated and plotted as in Fig. 4, they fitted the pH-dependence data of 3-*O*-methylglucose.

Discussion

The present study showed that, in cultured Ehrlich ascites cells, glycolysis, transport of 3-*O*-methylglucose, transport and phosphorylation of 2-deoxyglucose and transport of glucose (as tested in ATP-depleted cells) were all pH-dependent processes. The range of pH chosen in the present study was compatible with high degree of cell viability (cells could be cultivated for at least 3 days at each extreme of pH). Rates of glucose consumption were found to increase as a linear function of pH and therefore it was possible to quantify the pH effect. A linear response of the glycolytic rate to changes in pH is evident in the data of Delcher & Schipp (1966), who studied this effect in perfused rat hearts. In contrast with our findings, Ibsen *et al.* (1960) found that Ehrlich ascites cells obtained from animals increased glucose consumption rates as pH was increased from pH 6.3 to 7.2, but not on further increases to pH 7.6 (effects of pH above 7.6 were not tested). The difference between these results and ours may be due to different nutritional state of cells.

After the suggestions by Delcher & Schipp (1966) and by Ui (1966), the effect of pH on glycolysis has been thought to be due to its effect on phosphofructokinase and other glycolytic enzymes. Halperin *et al.* (1969) and Wilhelm *et al.* (1971) concluded that phosphofructokinase, glyceraldehyde 3-phosphate dehydrogenase or phosphoglycerate kinase, and pyruvate kinase were the main control points in pH-dependence of glycolysis. The possibility that glucose transport may be responsible for a substantial part of the pH effect had not been, to our knowledge, previously considered. However, evidence of pH-dependence of 2-deoxyglucose uptake in chick embryo fibroblasts has been presented by Rubin (1971). Only dense cultures, but not sparse ones, were thought to exhibit pH-dependence of 2-deoxyglucose uptake and of thymidine incorporation into DNA, and these phenomena were related to regulation of cell growth by pH. The data for sparse cells (Rubin, 1971; his Fig. 11), when replotted as in Fig. 2(*c*), are very similar to our own. The results for dense cells show a biphasic curve, linear between pH 6.9 and 7.4 and between pH 7.4 and 8.0, the former component increasing slowly, and the latter very rapidly. This complex pattern may well be related to growth stimulation of dense cells in high pH media; however, it is also difficult to be certain of the actual pH values in the microenvironment of dense cultures.

Transport of the three sugars was pH-dependent to about the same degree, and the linear relationship between transport rates and pH permitted a quantification of the pH effect. Both simple diffusion (unsaturable component), as measured in the first 15 s after sugar addition, and facilitated diffusion (saturable component) were affected by changes in pH, as best shown in Figs. 3(*d*) and 5. Studies with 2-deoxyglucose showed that pH affected mainly the $V_{max.}$, the K_m values being slightly lower at both extremes of pH. Studies with 3-*O*-methylglucose showed that at high pH, sugar influx was accelerated, and the equilibrium between the extracellular and intracellular sugar concentrations was reached faster and at a lower sugar concentration than at pH 7.4. At low pH, sugar influx was depressed at all concentrations in proportion. Experiments on uptake of labelled glucose confirmed the data obtained with the non-metabolizable sugars. Since there are no specific inhibitors of hexokinase, depleting cells of ATP may be the only method of inhibiting the hexokinase reaction and measuring glucose transport. This was accomplished by incubating Ehrlich ascites-tumour cells in glucose-free medium. Other cells do not decrease their ATP concentrations on glucose starvation, but do so on anaerobiosis (Freudenberg & Mager, 1971). ATP may also be depleted by incubating cells with 2-deoxyglucose (Letnansky, 1964). However, this procedure may not be appropriate for sugar-transport studies, since influx of added sugar would be complicated by efflux of non-phosphorylated 2-deoxyglucose.

The analysis in Fig. 4 of the contribution of sugar transport to pH-dependence of glucose consumption presents the probable maximum. On the basis of these data and the results of Halperin *et al.* (1969) and of Wilhelm *et al.* (1971) the simplest view is that glucose transport is the major pH-dependent step in the glycolytic sequence and that changes in the activities of glycolytic enzymes further enhance the pH-dependence of glycolysis.

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References

Bernt, E. & Lachenicht, R. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1215–1216, Academic Press, New York

Christopher, C. W., Kohlbacher, M. S. & Amos, H. (1976) *Biochem. J.* **158**, 439–450

Coe, E. L. & Saha, J. (1966) *Arch. Biochem. Biophys.* **114**, 282–287

Delcher, H. K. & Schipp, J. C. (1966) *Biochim. Biophys. Acta* **121**, 250–260

Fodge, D. W. & Rubin, H. (1973) *Nature (London) New Biol.* **246**, 181–183

Freudenberg, H. & Mager, J. (1971) *Biochim. Biophys. Acta* **232**, 537–555

Gutmann, I. & Wahlefeld, A. W. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1464–1468, Academic Press, New York

Halperin, M. L., Connors, H. P., Relman, A. S. & Karnovsky, M. L. (1969) *J. Biol. Chem.* **244**, 384–390
Hatanaka, M. (1974) *Biochim. Biophys. Acta* **355**, 77–104
Hatanaka, M., Huebner, R. J. & Gilden, R. V. (1969) *J. Natl. Cancer Inst.* **45**, 1091–1096
Ibsen, K. H., Coe, E. L. & McKee, R. W. (1960) *Cancer Res.* **20**, 1399–1407
Kaminskas, E. (1972) *J. Biol. Chem.* **247**, 5470–5475
Kaminskas, E. (1973) *J. Cell. Physiol.* **82**, 475–488
Kaminskas, E., Field, M. & Henshaw, E. C. (1976) *Biochim. Biophys. Acta* **444**, 539–553
Kletzien, R. F. & Perdue, J. F. (1974) *J. Biol. Chem.* **249**, 3366–3374
Letnansky, K. (1964) *Biochim. Biophys. Acta* **87**, 1–8
Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **51**, 426–430
Live, T. R. & Kaminskas, E. (1975) *J. Biol. Chem.* **250**, 1786–1789
McComb, R. B. & Yushok, W. D. (1964) *Cancer Res.* **24**, 193–197
Plagemann, P. G. W. & Richey, D. P. (1974) *Biochim. Biophys. Acta* **344**, 263–305
Renner, E. D., Plagemann, P. G. W. & Bernlohr, R. W. (1972) *J. Biol. Chem.* **247**, 5765–5776
Rubin, H. (1971) *J. Cell Biol.* **51**, 686–702
Schmidt, F. G., Schwartz, R. T. & Scholtissek, C. (1974) *Eur. J. Biochem.* **49**, 237–247
Ui, M. (1966) *Biochim. Biophys. Acta* **124**, 310–322
Van Venrooij, W. T. W., Henshaw, E. C. & Hirsch, C. A. (1970) *J. Biol. Chem.* **245**, 5947–5953
Venuta, S. & Rubin, H. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 653–657
Wilhelm, G., Schultz, J. & Hofmann, E. (1971) *FEBS Lett.* **17**, 158–162
Zwartouw, H. T. & Westwood, J. C. N. (1958) *Br. J. Exp. Pathol.* **39**, 529–539