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INFLUENCE OF DIMETHYLSULFOXIDE ON TRANSMEMBRANE TRANSPORT

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Abstract—The influence of dimethylsulfoxide in low concentrations on sugar transport in yeast cells and in rat intestine was investigated. Transport of the non-metabolizable sugars sorbose and galactose in yeast was inhibited by dimethylsulfoxide in the concentration range 0–1·5 M. It was shown that this inhibition is presumably caused by a direct influence of the drug on the transport system. Glucose absorption from rat intestine was studied *in vivo*, by perfusion of intestinal segments. Dimethylsulfoxide in a concentration of 0·5 M appeared to have no influence on intestinal glucose transport. In contrast, a pronounced stimulation of intestinal glucose transport by dimethylsulfoxide in this concentration has been described in recent literature. The background and obvious solution of this apparent contradiction is discussed.

THE INFLUENCE of dimethylsulfoxide (DMSO) on transmembrane solute transport has been studied in a variety of cells and tissues. In the concentration range from 4 to 11 M DMSO increases the permeability of skin with respect to various solutes.[1–5] At lower concentrations (0·3–1 M) DMSO inhibits glycine transport in *Staphylococcus aureus*[6] and alanine transport in hamster intestine.[7] Contrary to these results Csáky and Ho described in rats the stimulation of intestinal glucose transport at a DMSO concentration of 0·5 M.[8]

In connection with investigations on the influence of DMSO on biomembranes the influence of this drug in low concentrations on sugar transport in yeast cells and in rat intestine was studied. The results of these studies are presented in the present communication.

METHODS

In these experiments two yeast strains were used: commercial bakers yeast ("Koningsgist", obtained from the Gist en Spiritusfabriek, Delft) and strain NCYC 240. The latter yeast strain was grown and harvested as described previously.[9] Uptake of ^{14}C-labelled sugars in yeast cells was measured as described in a previous paper.[10] Respiration of yeast cells was measured with the standard Warburg technique.

Intestinal transport studies were carried out *in vivo*, with unstarved rats of 220–250 g. The animals were anesthetized with urethane (1·5 g/kg body weight). An intestinal segment, corresponding to 20 vascular arcades, counted from the plica of Treitz, was perfused *in situ*, as described by Csáky and Ho.[11] The perfusion fluid was 150 mM in glucose, 66·7 mM in Na_2SO_4, 3 mM in K_2SO_4 and 0 or 0·5 M in DMSO, with an initial volume of 25 ml. The perfusion fluid was pumped through the intestinal segment at a speed of 15 ml/min, utilizing a Bühler mp 1 micropump. At intervals samples were analysed for glucose, Na^+, K^+ and DMSO.

Possible volume changes, caused by water shifts between the intestinal lumen and the body fluids of the rat, were measured in separate experiments with ^{14}C-inuline as volume marker. In control experiments no measurable absorption of ^{14}C-inuline was found.

Based on the measured concentrations in the perfusion fluid, calculations of absorption from, or excretion into the intestinal lumen were performed in two different ways. The first method of calculation was based on the assumption of a constant volume of the perfusion fluid, except for the volume decrease due to withdrawal of samples. This method of calculation was applied by Csáky and Ho.[8] The second way of calculation also took into account the additional volume changes, as indicated by the changing ^{14}C-inuline concentration in the perfusion fluid. In these calculations the mean value of the volume changes at each time, measured in the ^{14}C-inuline experiments, was adopted as correction factor.

After the experiments the animals were sacrificed. The perfused intestinal segments were excised, freed from peritoneal tissue and fat, and dried at 95° to constant weight. Absorption and excretion were expressed in m-moles per gram intestinal dry weight (d.w.).

Na^+ and K^+ concentrations were assayed with a flame photometer. Glucose was measured with the glucose oxidase method, as modified by Washko and Rice.[12] ^{14}C-labelled substrates were measured in a liquid scintillation counter, with the liquid scintillator described by Bray.[13]

RESULTS

The influence of DMSO on the transport of the non-metabolizable sugars galactose and sorbose in commercial bakers yeast is depicted in Fig. 1. As shown, increasing DMSO concentrations caused an increasing inhibition of transport. The inhibition was not time-dependent; immediately after drug addition the inhibition reached a constant value. Glucose and alcohol respiration were inhibited at slightly higher

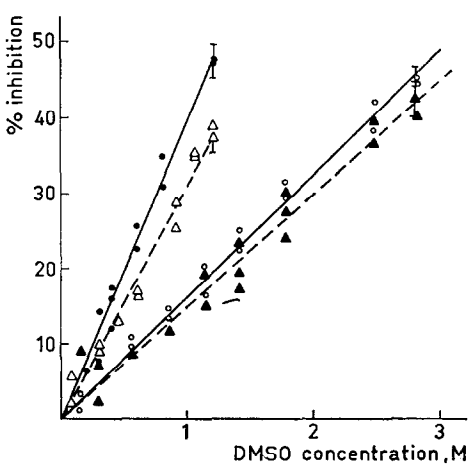

FIG. 1. Inhibition of sorbose transport (●—●), galactose transport (△—△), glucose respiration (○—○) and alcohol respiration (▲—▲) by DMSO in commercial bakers yeast at 25°. Substrate concentrations: 200 mM. At substrate concentrations of 100 and 300 mM identical results were obtained. Vertical bars represent the S.E.M. of four experiments.

DMSO concentrations (Fig. 1). Similar results with respect to transport of non-metabolizable sugars and respiration were obtained with the yeast strain NCYC 240.

The influence of DMSO on intestinal glucose transport was studied with the *in vivo* procedure, described under methods. Histological control of the intestinal tissues after a perfusion period of 90 min revealed a perfectly normal appearance both with and without 0·5 M DMSO in the perfusion fluid.

Assuming constant volume of the perfusion fluid, calculation of glucose absorption gave results as shown in Fig. 2, in good agreement with the results of Csáky and Ho,[8] calculated via the same procedure. Simultaneous measurements of the Na^+ concentration in the perfusion fluid revealed an unexpected phenomenon, as shown in Fig. 3. In the absence of DMSO a slight increase of the Na^+ concentration could be observed during the experimental period. In the presence of DMSO, however, an initial decrease of the Na^+ concentration, followed by a subsequent increase was

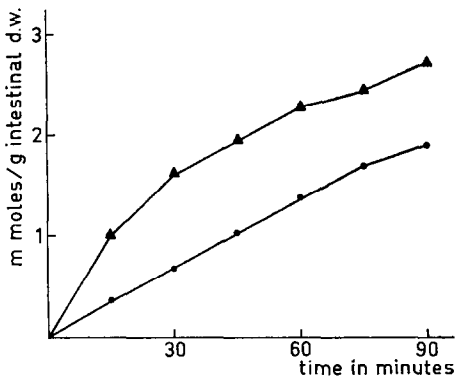

FIG. 2. Absorption of glucose in m-moles/g intestinal dry weight in the absence (●—●) and in the presence (▲—▲) of 0·5 M DMSO.

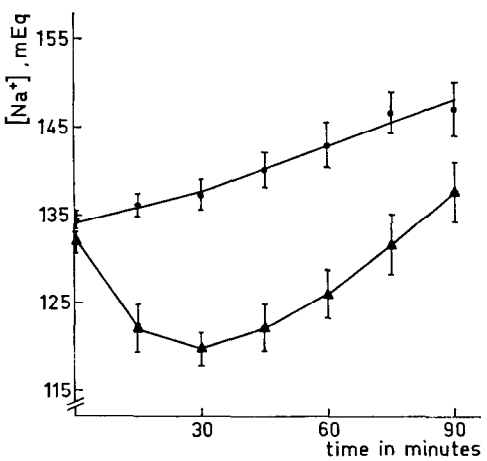

FIG. 3. Na^+ concentration in the perfusion fluid in the course of time, in the absence (●—●) and in the presence (▲—▲) of 0·5 M DMSO. The vertical bars show the S.E.M. of four experiments.

found in all experiments. To elucidate the background of this phenomenon, the total volume of the perfusion solution was measured in separate experiments, utilizing ^{14}C-inuline as volume marker. The results are shown in Fig. 4. Without DMSO a slow decrease of the volume was observed in the course of time. In the presence of DMSO, however, a similar decrease was preceded by an initial increase of the volume. Correction of the glucose absorption data for these volume changes yielded the results shown in Fig. 5. Apparently there was no significant difference between glucose absorption in the presence and in the absence of 0·5 M DMSO.

By means of the ^{14}C-inuline measurements of volume, the mean values of the total Na^+ and K^+ contents of the perfusion solution could be calculated. This revealed a gradual leakage of these ions into the intestinal lumen. As shown in Fig. 6, DMSO enhanced Na^+ leakage, but did not affect K^+ leakage.

The absorption of DMSO from the intestinal lumen, as compared to the absorption of glucose, is depicted in Fig. 7.

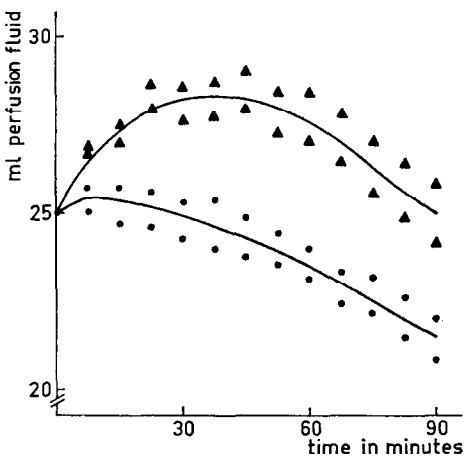

FIG. 4. Volume of the perfusion fluid in the course of time in two sets of control experiments in the absence (●—●) and in the presence (▲—▲) of 0·5 M DMSO.

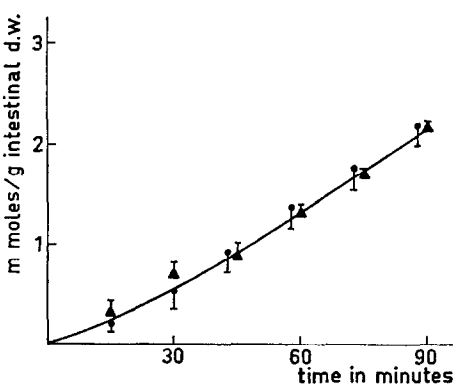

FIG. 5. Absorption of glucose, in m-moles/g intestinal dry weight, corrected by means of the ^{14}C-inuline measurement of volume, in the absence (●—●) and in the presence (▲—▲) of 0·5 M DMSO. Vertical bars represent the S.E.M. of four experiments.

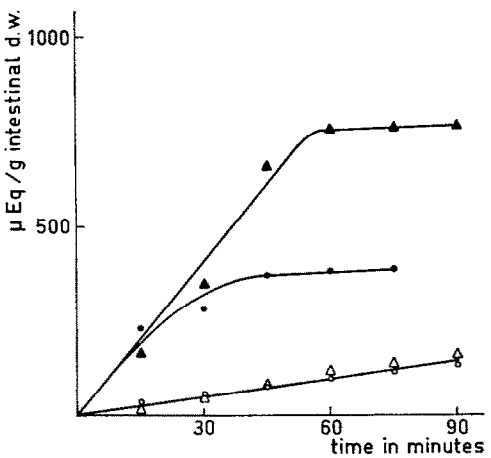

FIG. 6. Mean value of Na^+ and K^+ excretion into the intestinal lumen, in µequiv./g intestinal dry weight. These mean values were calculated from the measured Na^+ and K^+ concentrations in the perfusion fluid and corrected for the volume changes, determined with ^{14}C-inuline. ●—●: Na^+ excretion without DMSO; ○—○: K^+ excretion without DMSO; ▲—▲: Na^+ excretion in the presence of DMSO; △—△: K^+ excretion in the presence of DMSO.

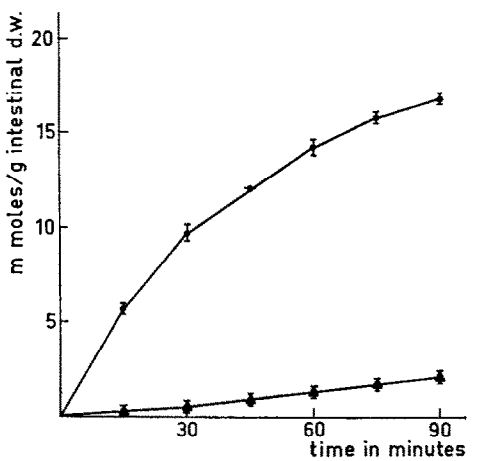

FIG. 7. Absorption of DMSO and of glucose, in m-moles/g intestinal dry weight. ●—●: DMSO absorption; ▲—▲: glucose absorption. The vertical bars represent the S.E.M. of four experiments.

DISCUSSION

The experimental results show an inhibition of transmembrane sugar transport in yeast cells by DMSO in concentrations up to 3 M. At these concentrations DMSO does not cause membrane deterioration. Increase of passive cation permeability and cytolysis are only evoked by DMSO concentrations of 5 M and higher.[14] At a fixed drug concentration the inhibition of transport of the non-metabolizable sugars galactose and sorbose exceeded the inhibition of respiration (Fig. 1). This indicates that the inhibition is the consequence of a direct influence of the drug on the transport

system and not, e.g. of a primary metabolic inhibition. This is also indicated by the immediate onset of inhibition after drug addition. If DMSO would act primarily on an intracellular metabolic system, the inhibition of transport should be expected to develop gradually, as complete intracellular equilibration with DMSO takes about 120 min at 25°, with a half-time of 20 min.[15] These results are analogous with those of Ghajar and Harmon,[6] concerning glycine transport in *Staphylococcus aureus.*

It cannot be precluded *a priori* that solute transport into single cells and transcellular, e.g. intestinal, transport would be influenced by DMSO in a different fashion. In this respect the apparent discrepancy between the inhibition of alanine transport in hamster intestine[7] and the stimulation of glucose transport in rat intestine[8] by low concentrations of DMSO was of interest. As the present studies concerned the susceptibility of sugar transport to DMSO, it seemed worthwhile to reinvestigate the influence of this drug on intestinal sugar transport. The described results, indicating no influence of 0·5 M DMSO on intestinal glucose transport, contradict the results of Csáky and Ho. These authors, however, did not consider volume changes of the perfusion fluid (cf. Figs. 2 and 4). If the perfusion solution is not isotonic, an osmotic water shift can be anticipated. Further, if one or more solutes are absorbed from an initially isotonic perfusion fluid, a concomitant osmotic water uptake should be expected. This explains both the initial volume increase in the presence of DMSO, where the perfusion fluid was initially hypertonic, and the gradual volume decrease, associated with glucose and DMSO absorption (Fig. 4).

Csáky and Ho suggested a transmembrane transport of a molecular complex, consisting of one glucose and two DMSO molecules. The experimental results shown in Figs. 5 and 7 contradict this assumption. If extra glucose uptake would occur, in which one molecule of glucose would be transported in complex association with two molecules of DMSO, the relatively fast absorption of DMSO should account for an additional uptake of 7 m-moles glucose/g intestinal d.w. in the first hour. This would give a 6-fold increase of glucose absorption, as compared to the control in the absence of DMSO, under the present experimental conditions. In fact, however, the DMSO absorption from the intestinal lumen was not associated with any increase of glucose uptake.

As shown in Fig. 6, Na^+ and K^+ ions are leaking into the intestinal lumen during the experimental period. In the presence of DMSO the leakage of Na^+ ions is larger than in the absence of this drug. Possibly this is the result of the initial dilution of the perfusion fluid, caused by the described osmotic water shift, changing the Na^+ concentration gradient. No explanation is available yet for the unchanged K^+ leakage.

The experimental procedure with intestine perfused *in vivo*, has several advantages as compared to the inverted sac method. It is possible to analyse several samples in the course of time. Histological control revealed a perfectly normal appearance of the tissues after an experimental period of 90 min. In contrast, serious deterioration of the intestinal tissues has been described with the inverted sac method, after incubation periods of 30 min at 37°.[16]

Especially if the perfusion fluid is not isotonic, or if osmotically significant amounts of solutes are taken up from the perfusion solution during the experimental period, volume control with a volume marker like inuline is absolutely necessary, to avoid faulty conclusions.

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