Effect of Dimethylsulfoxide on the Intestinal Sugar Transport.*† (31271)

T. Z. Csáky and P. M. Ho
Department of Pharmacology, University of Kentucky, College of Medicine, Lexington

According to presently prevailing views, the lumen-facing membrane of the intestinal epithelium, the so-called "brush border," is the primary osmotic barrier between the lumen of the intestine and the blood stream. Because of its lipid nature and because of the intimate fusion between adjacent cells(1), this membrane is essentially a continuous hydrophobic sheet impermeable for polar molecules, except for those which are very small (<4.0 Å radius(2)) and can pass through the pores. Larger hydrophilic molecules, like simple sugars, are too big for such passage; therefore these permeate essentially exclusively by carrier-mediation. Although the exact molecular mechanism of the carrier function is not known, it is assumed that it causes the highly hydrophilic sugar to break its hydrogen bonding with water so as to become temporarily fat soluble. In the course of this process the substrate combines with the carrier and, as the available carrier sites are apparently limited, a saturation-type kinetics takes place; *viz.*, with the increase of the sugar concentration in the lumen a limit is reached above which the rate of absorption does not increase, despite a further hiking of the sugar concentration. The carrier-mediated transport of the aldohexoses is competitively inhibited by phlorizin(3).

If the primary function of the carrier is to render the sugar molecule fat soluble, then another manipulation which achieves the same result should enable the sugar to by-pass carrier-mediation and be absorbed directly by diffusion through the lipid membrane. DMSO is capable of strong hydrogen bonding with alcohols as manifested by the considerable heat which develops upon mixing this solvent with alcohols(4). That hydrogen bond formation applies to the alcoholic groups of glucose as well is indicated by the unusually high solubility of this sugar in DMSO(5). It was therefore reasonable to assume that DMSO may enable the glucose molecule to break its hydrogen bonding with the water and enter the lipid phase of the membrane without the necessity of carrier-mediation. The purpose of this communication is to describe and discuss experiments concerning the effect of DMSO upon the transport of glucose, galactose, and 3-0-methylglucose in the small intestine of the rat.

Materials and methods. All the reagents used in this study were of analytical grade. DMSO, purified by repeated fractional freezing until a constant maximum freezing point of 18.50°C was obtained, was a gift of Dr. Paul Sears of the Chemistry Department. 3-0-Methylglucose (3-methylglucose) was a gift of Ayerst Laboratories, New York. Glucose and galactose were D-glucose and D-galactose, respectively.

Reducing sugars were determined by the Nelson method(6), glucose with glucose oxidase(7). Preliminary experiments showed that the presence of DMSO, in concentrations employed in this study, did not affect these analytical procedures.

The experiments were carried out in urethan-anesthetized male Sprague-Dawley rats. A loop of the small intestine was cannulated and perfused with 50 ml of the appropriate solution, using Method I as described earlier(8). Every 15 minutes a sample was analyzed for sugar. At the end of the experiment the animal was killed, the perfused loop excised, carefully freed from excess peritoneal tissue and fat, and its dry weight determined after drying at 95°C. The sugar absorption was calculated as μmol sugar disappeared from the perfusate per gram gut dry weight. Thereby the assumption was made that the surface of the mucosa in a loop

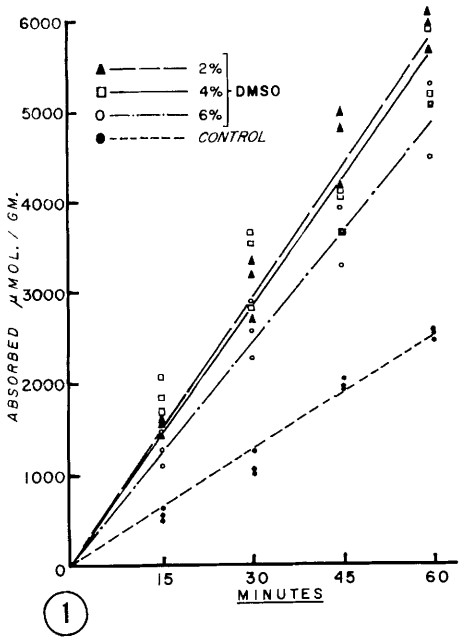
* Aided by grants from U.S.P.H.S. and the American Heart Association.

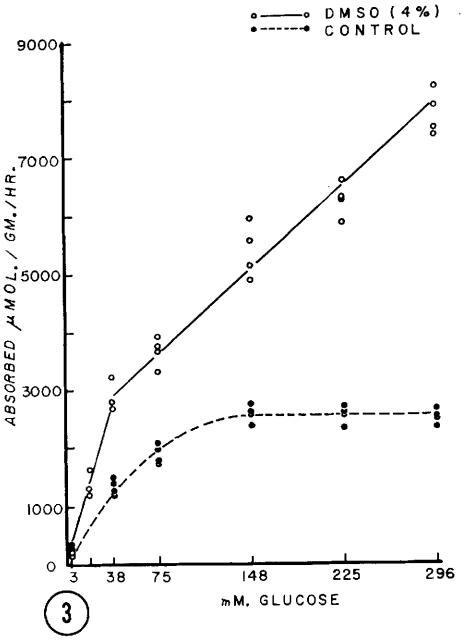
† Abbreviations: DMSO = dimethysulfoxide; ATP = adenosine triphosphate; ATPase = adenosine triphosphatase.

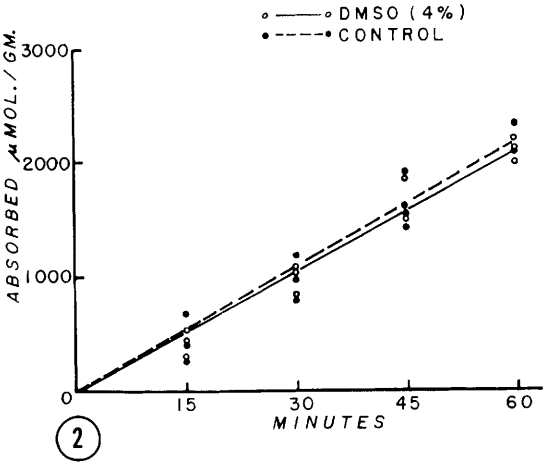
of small intestine is proportional to the dry weight.

When the concentration of DMSO is given in % it means v/v.

Results. 1. *Effect of DMSO upon the absorption of glucose, galactose, and 3-methylglucose at high initial sugar concentrations.* The intestine was perfused in these experiments with a 1:1 mixture of isosmotic sugar and isosmotic Na_2SO_4 (initial sugar concentration 148 mM) with or without varying concentrations of DMSO. Fig. 1 shows that the







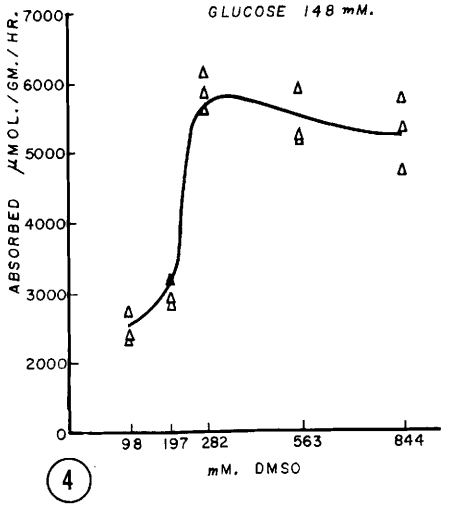


FIG. 1. Effect of DMSO on rate of absorption of glucose (148 mM isosmotic with Na_2SO_4).

FIG. 2. Rate of absorption of 3-0-methylglucose (148 mM isosmotic with Na_2SO_4) with and without 4% DMSO.

FIG. 3. Absorption of glucose (1 hr) at various initial concentrations with and without 4% DMSO. Perfusing solution was isosmotic with Na_2SO_4.

FIG. 4. Effect of varying concentrations of DMSO upon absorption (1 hr) of glucose (148 mM isosmotic with Na_2SO_4).

presence of DMSO more than doubled the rate of glucose absorption. Identical results were obtained in similar experiments if glucose was replaced by galactose; however, the absorption of 3-methylglucose was not affected by DMSO (Fig. 2).

2. *Kinetics of the DMSO-stimulated glucose transport.* Fig. 3 shows the results of experiments in which the average 1-hour absorption was examined at increasing initial glucose concentrations. Without DMSO the curve shows the well-known "saturation-type" kinetics. In the presence of 4% DMSO the asymptotic curve disappears, and the transport rate is directly proportional to the initial sugar concentration. Such a relationship is an indication of simple diffusion, apparently not dependent upon a saturable carrier.

3. *Reversibility of the DMSO effect.* In these experiments the gut was perfused with an equal mixture of isosmotic glucose and isosmotic sodium sulfate containing 4% DMSO. After 1 hour perfusion, the perfusate was changed to a similar composition but without DMSO. These loops then absorbed glucose at the same rate as an intestinal loop does without the presence of DMSO, indicating that the DMSO effect was fully reversible (Table I).

4. *Effect of the relative sugar-DMSO ratio upon glucose transport.* Fig. 4 shows that, if the relative DMSO-glucose mol. ratio was 1.0 or less, no effect of DMSO was apparent. However, by increasing the relative concentration of DMSO, the maximum effect of the solvent became apparent at the DMSO-glucose mol. ratio of approximately 2.

TABLE I. Reversibility of DMSO Effect. Intestines of 2 groups of rats were perfused with an isosmotic solution containing 148 mM glucose + Na_2SO_4. Rate of glucose absorption is recorded as μmol/g dry gut/hr. Group A: Control. Perfused for 1 hr. Group B: 4% DMSO added to 1st hr perfusate followed by a 1 hr perfusion without DMSO.

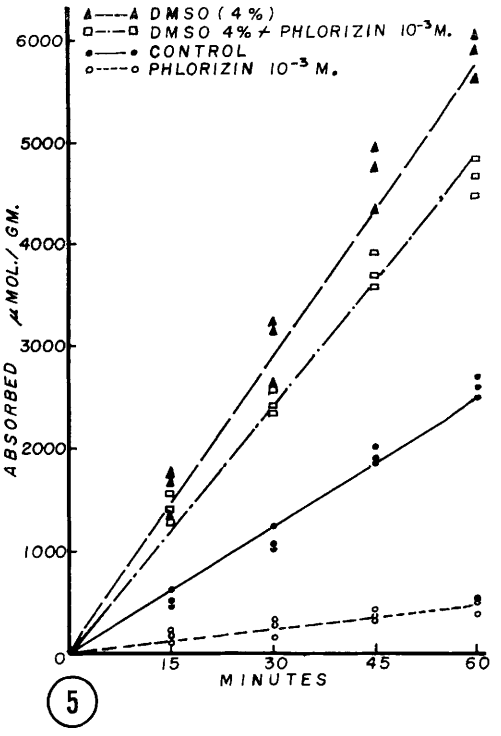
Group A		Group B		
Animal No.		Animal No.	With DMSO	Without DMSO
1	2550	5	5659	2804
2	2581	6	4706	2731
3	2731	7	5901	2306
4	2560	8	5640	2580
Avg	2606	Avg	5477	2605

5. *Effect of phlorizin upon the DMSO-stimulated glucose transport.* In these experiments the intestine was perfused with 148 mM glucose solution rendered isosmotic with sodium sulfate. 4% DMSO was present in the perfusate. Phlorizin was added to the perfusate at the beginning of the experiment in 10^{-3} M concentration. Fig. 5 shows that phlorizin only very slightly inhibited the DMSO-stimulated transport of glucose. The same concentration of phlorizin almost completely inhibited the absorption of glucose without DMSO.

6. *Effect of DMSO upon the up-hill transport of glucose.* In these experiments the intestine was perfused with an isosmotic Na_2SO_4 containing 50 mg% glucose. Fig. 6 shows that the presence of 0.04% DMSO was without effect and 4% DMSO did not enhance the transport of glucose; on the contrary, it inhibited it slightly. The apparent inhibition was not due to an increased outflux of glucose from blood into lumen, as no glucose could be detected in the solution of isosmotic sodium sulfate with 4% DMSO after it was used to perfuse a segment of the small intestine.

The possibility of an inhibition by DMSO of the sodium-potassium-dependent intestinal ATPase was eliminated by direct experimentation: The intestinal mucosa of a rat was scraped, homogenized in sucrose and the "microsome" fraction incubated with ATP and appropriate amounts of potassium and sodium, using the method described by Järnefelt(9). There was no change of ATPase activity in the presence of either 2% or 4% DMSO.

7. *Water-lipid distribution of glucose in the presence of DMSO.* A solution containing glucose (50 mg%) and DMSO (4%) was gently shaken by inversion in a large tube 60-80 times with equal volumes of hexane or peanut oil. After the phases separated, a sample of the water phase was analyzed for glucose. A sample of the hexane was removed, evaporated, and the residue also assayed for glucose. 100 $\pm$ 2% of the glucose was recovered in the water phase in both hexane and peanut oil experiments. No reducing sugar was detected in the hexane.



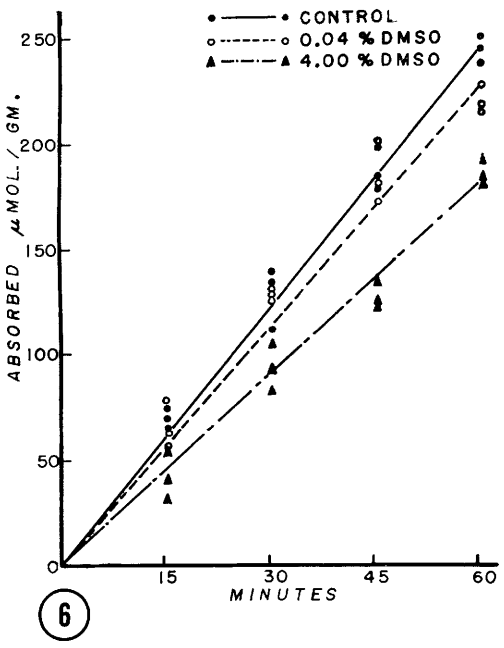


FIG. 5. Effect of phlorizin upon normal and DMSO-stimulated absorption of glucose (148 mM isosmotic with Na_2SO_4).

FIG. 6. Effect of DMSO upon up-hill transport of glucose (0.05% in isosmotic Na_2SO_4).

Discussion. The effect of DMSO on sugar absorption varies, depending upon whether the sugar is present in high or low concentration in the lumen. In the former case, carrier-mediated diffusion, in the latter, active transport is primarily involved in the process of absorption. At high glucose concentration 4% DMSO not only increases the rate of sugar absorption but produces a direct proportional relationship between the concentration and transport rate. Such kinetics indicates a simple diffusion without involvement of the carrier. This assumption is further supported by the observation that DMSO-stimulated glucose absorption is practically not affected by phlorizin which is otherwise a strong inhibitor of the carrier-mediated aldose transport. The increase in the absorption rate could be due to either a diffusion through the lipid layer or to an increase of the membrane pore size. The latter however is rather unlikely, as the absorption of 3-methylglucose, which is approximately the same size molecule as glucose, is not affected by DMSO. Furthermore, because the transport of glucose and galactose is altered by DMSO but not the transport of 3-methylglucose, which otherwise shares the intestinal transport mechanism with the two other hexoses, it appears that DMSO changes primarily the sugar molecules and not the permeability of the membrane.

It was not the purpose of the present study to examine in detail the possible molecular reactions between sugar and DMSO. Nevertheless, if the relationship between the relative concentrations of sugar and DMSO is examined, it becomes apparent that approximately 2 moles of DMSO have to be present for each mole of sugar to produce a change in the sugar permeability. Below this DMSO concentration, little or no action was found on the sugar; at the above ratio the effect became suddenly maximal. This would indicate that approximately 2 moles of DMSO react with each mole of sugar. Finding that the transport of 3-methylglucose is not affected by DMSO may indicate that each

DMSO molecule reacts with 2 hydroxyls which have to be attached to adjacent carbon atoms. Inspection of the formulae of glucose, galactose and 3-methylglucose indicates that the latter possesses only one pair of adjacent OH's (on C1-C2); whereas, glucose and galactose have 2 pairs (C1-C2 and C2-C4). The reaction between DMSO and sugar is most probably hydrogen-bonding; thus DMSO effectively competes with the hydrogen-bonding properties of water for the sugar. The possibility of a covalent linkage is unlikely because the sugar does not become fat soluble *in vitro* in the presence of DMSO. It should be emphasized that the experiments described under Paragraph 7 above were carried out for the sole purpose of examining this point. Peanut oil and hexane were chosen as a lipid and a lipid solvent in which a hypothetical hydrophobic, "stable" glucose-DMSO compound would be soluble. The physicochemical properties of peanut oil and hexane are not necessarily identical with those of the lipids in the cell membrane; consequently, it would be inexpedient to speculate about the apparent differences in the solubility of the DMSO-glucose complex in the membrane and in the peanut oil and hexane.

When glucose and galactose are present in the gut in a concentration higher than that in the blood, DMSO causes a rapid influx of the sugar according to diffusion kinetics. If the sugar concentration in the lumen is low, no diffusion can take place; thus, it is expected that the rate of sugar transport will not be increased by DMSO. The experimental finding corroborates this expectation. Moreover, at sugar concentration levels where active transport prevails normally, the presence of DMSO is slightly inhibitory. At present it is not clear what is the mechanism of the inhibition. It is unlikely that, at the concentration employed, the solvent acted as a metabolic inhibitor, as the concentration was far from what could be considered toxic(10). A digitalis-like inhibition of the transport ATPase was excluded by direct experiments.

The present study was conducted to test the validity of the assumption that the primary function of the carrier is to make the sugar temporarily fat-soluble by enabling it to break its strong bonding with the water phase and to enter the lipid phase of the membrane. This assumption was verified in those experiments where the lumen-to-cell concentration difference of the sugar was sufficiently high to provide the primary driving "force" for transport. In these experiments DMSO provided the proper medium to facilitate the entry of sugar into the lipid phase of the membrane, and no carrier was needed for the process. Moreover, in this case the "by-passing" of the carrier with the help of DMSO not only caused the absorption of glucose and galactose by diffusion but also increased the rate of their absorption. Thus not only does the membrane represent a true osmotic barrier for diffusion, but also the carrier does not really "facilitate (*viz.*, enhance)—but rather "mediates"—the transport. Consequently, it is more proper, in the case of the gut, to speak of "carrier-mediated diffusion" than of "facilitated diffusion."

If the sugar concentration in the lumen of the gut was so low that the transport proceeded by active pumping, there was no evidence that DMSO caused the by-passing of the carrier, and it did not increase but slightly decreased the rate of transport. It would be too early to speculate about the reason for the difference between the two transports as far as the effect of DMSO is concerned. It is clear, however, that in case of active transport another factor besides the carrier is also involved in the process. This other factor is the "pump," a transducer responsible for converting chemical into osmotic energy. Presently little is known about the relationship between the carrier and this "pump"; consequently, an attempt to analyze the effect of DMSO upon this intricate mechanism would be only speculation.

Summary. The effect of DMSO upon intestinal transport of glucose, galactose, and 3-0-methylglucose was examined. If glucose or galactose are present in the lumen in high initial concentrations, DMSO enhances the rate of absorption, and also changes the kinetics of transport from a saturation type (typical for carrier-mediated transport) to a diffusion type. Intestinal absorption of 3-0-methylglucose is not influenced by DMSO. If the sugar absorption is primarily an active transport, DMSO does not enhance it. It is

concluded that DMSO causes glucose and galactose to become sufficiently fat-soluble to bypass carrier mediation.

1. Farquhar, M. G., Palade, G. E., J. Cell. Biol., 1963, v17, 375.
2. Lindemann, B., Solomon, A. K., J. Gen. Physiol., 1962, v45, 801.
3. Alvarado, F., Crane, R. K., Biochim. Biophys. Acta, 1962, v56, 170.
4. Sears, P. G., Siegfried, W. D., Sands, D. E., J. Chem. Eng. Data, 1964, v9, 261.
5. Lindberg, J. J., Pietilä, I., Suomen Kemistilehti, 1962, vB35, 30.
6. Nelson, N., J. Biol. Chem., 1944, v153, 375.
7. Teller, J. D., 130th Meeting, A.C.S., 1956, 69c.
8. Csáky, T. Z., Ho, P. M., Proc. Soc. Exp. Biol. & Med., 1965, v120, 403.
9. Järnefelt, J., Biochim. Biophys. Acta, 1961, v48, 104.
10. Brown, V. K., Robinson, J., Stevenson, D. E., J. Pharmacy & Pharmacol., 1963, v15, 688.

Received March 31, 1966. P.S.E.B.M., 1966, v122.

Dimethyl Sulfone: Isolation from Cows' Milk.* (31272)

KENNETH I. H. WILLIAMS, SUMNER H. BURSTEIN, AND DONALD S. LAYNE
(Introduced by G. Pincus)
Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

Recently, we observed that normal human urine contained 5-10 mg/24 hr of dimethyl sulfone ($DMSO_2$)(1). It occurred to us that cows' milk might be a source of some of this sulfone. Patton *et al*(2) reported that dimethyl sulfide (DMS) is one of the flavor constituents of cows' milk. $DMSO_2$ has been isolated from dried cows' blood(3) and from beef adrenals(4). We have observed that DMS is oxidized to $DMSO_2$ *via* dimethyl sulfoxide (DMSO) in the rabbit. Thus we thought it would be interesting to see if cows' milk contains DMSO and/or $DMSO_2$.

One liter samples of pasteurized cows' milk, obtained from local markets, were treated with 2 volumes of acetone to precipitate the proteins. The filtrates were freed of acetone with a rotating film evaporator and were then extracted with 3 × 250 ml of hexane to remove most of the fats.

The aqueous residues were extracted continuously with methylene chloride for 24 hours and the extracts were analyzed by gas chromatography as previously described(1). The results are tabulated in Table I. The extraction efficiency was tested by addition of 3500 cpm of ^{14}C $DMSO_2$ to one sample of milk prior to the acetone treatment. All of the radioactivity was present in the final extract.

TABLE I. Dimethyl Sulfone in Samples of Pasteurized Cows' Milk.

Sample	mg $DMSO_2$/liter*
1	8.2
2	7.6
3	6.1
4†	7.8
5	7.1

* By gas chromatographic analysis.
† Taken from the same container as sample #2 but allowed to sour before workup.

Combined extracts containing 38 mg of $DMSO_2$ (by gas chromotographic analysis) were applied to a 13 × 1.8 cm activity grade III neutral alumina column and the column was eluted with 100 ml of methylene chloride. Concentration of the eluent gave 1-2 ml of oil from which needles crystallized. These needles were collected and washed with a little 1:1 benzene:hexane to give 7 mg of colorless crystalline material with mp 108-109°. The infrared spectrum of the crystals in KBr was identical to that of an authentic sample of $DMSO_2$. An additional 11 mg of $DMSO_2$ was recovered from the mother liquors. If any DMSO was in the extract it was below our limits of detectibility (0.5 mg/liter of milk).

After removing one liter of milk for analysis from sample #2 the remainder was allowed

* Supported in part by grant AM-06294 from Nat. Inst. of Arthritis & Metab. Dis.