Intestinal Transport of Sugars and Amino Acids in Diabetic Rats

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ABSTRACT The specificity and mechanism of altered intestinal transport of diabetic rats was studied with an everted ring technique. Increased intracellular accumulation of amino acids, as well as galactose and 3-*O*-methylglucose, was demonstrated in diabetes. The greater accumulation by diabetic intestine could not be attributed to a direct effect of the agent used to induce diabetes or to an alteration in food consumption. Although the changes were related to the severity of diabetes and could be reversed with treatment with insulin, they could not be modified by addition of insulin in vitro. The changes could not be induced in control intestine either with hyperglycemia from glucose infusion or preincubation with glucose in vitro.

Although the higher concentration gradients of amino acids, galactose, and 3-*O*-methylglucose could result from increased energy utilization by diabetic intestine, an alteration of cell membrane function, as well, is suggested by the demonstration with kinetic studies of increased influx with an increase in V_{max}.

INTRODUCTION

Although insulin increases the rate of entrance of glucose and amino acids into cells of a variety of tissues (1), the intestine appears not to share in this effect (2, 3). In fact, intestinal glucose transport is enhanced in two conditions characterized by insulin deficiency, juvenile onset diabetes of man (4) and alloxan-induced diabetes of rats (5–7, 2). This increased rate of sugar uptake by diabetic intestine does not depend on its metabolic fate because both 6-deoxyglucose, a non-metabolized analog of glucose, and D-galactose, a sugar which is poorly metabolized by the intestine, are also transported at faster rates in alloxan diabetes (2). Since little is known of the nature of this abnormality of intestinal function, we have studied both monosaccharide and amino acid transport by intestine from rats made diabetic with alloxan to define the specificity of the diabetic effect and to make some observations on its mechanism.

METHODS

Sprague-Dawley rats weighing between 150 and 200 g were fasted for 48 hr; while under light ether anesthesia, they were injected with either alloxan, 80 mg/kg, or saline via the tail vein. Some animals received 65 mg/kg of streptozotocin[1] as an alternative method of inducing diabetes (8). Control and diabetic animals were pair fed until sacrifice. After injection with saline and pair feeding of control animals were shown to have virtually no effect on transport characteristics, these steps were omitted and all rats were allowed free access to water and standard laboratory food. The rats were housed in individual metabolic cages so that urine collections could be tested for the presence of glucose with glucose oxidase paper.[2] To ensure comparability only those diabetic animals whose urines gave heavy (500 mg/100 ml or more) daily tests for glucose were used for study. This degree of glucosuria corresponded to a blood sugar at the time of sacrifice of 300–500 mg/100 ml.

5 days after administration of alloxan or streptozotocin, the rats were sacrificed by stunning and decapitation; in some cases blood was collected in heparinized containers for plasma glucose determination. Usually three diabetic and three control animals were used for each study. Proximal jejunum was quickly removed, everted, washed with ice-cold saline, and cut into 10–25 rings of 3–5 mm width, excluding those segments with visible lymph nodules (9). The rings were kept in chilled Krebs-Ringer bicarbonate buffer which had been gassed for 10 min with 95% O_2–5% CO_2, until incubation.

A part of this work appeared in abstract form in 1968 *J. Clin. Invest.* 47: 74a.

Received for publication 5 August 1968 and in revised form 5 May 1969.

[1] Courtesy of The Upjohn Co., Kalamazoo, Mich.

[2] Labstix, Ames Co., Inc., Elkhart, Ind.

The rings were then placed in 25-ml Erlenmeyer flasks containing ^{14}C-labeled sugar or amino acid in 2 ml of Krebs-Ringer bicarbonate buffer which had been gassed with 95% O_2–5% CO_2; the flasks were again gassed and incubated at 37°C for varying times in a Dubnoff shaking incubator. To terminate incubation, the rings were removed from the flasks, washed twice in isotonic saline, and blotted gently on filter paper. Wet weight was measured with a torsion balance after which the rings were placed in test tubes containing 2 ml of water which were then placed in a boiling water bath for exactly 6½ min to allow equilibration with intracellular isotope (10). No difference was found between extraction in this fashion and extraction in 0.1 N nitric acid at 100°C for 6½ min.

To minimize the effects of variation between individual rings from the same animal and between individual rats in each group, three rings were placed in each flask, one ring from each diabetic animal and one ring from each control animal studied. Tissue from each flask was pooled for weighing and extraction of isotope. The number of identical flasks used to determine each data point as well as the number of animals used is given for each experiment in the section on results; incubations of at least three flasks were used for each value.

Radioactivity measurements were made on 0.2 ml aliquots of tissue extract or medium fluid. To the aliquots were added 2.8 ml of absolute alcohol and 7.0 ml of 0.4% 2,5-diphenyloxazole (PPO) and 0.005% *p*-bis-[2-(5-phenyloxazolyl)]-benzene (POPOP)[8] in toluene. The vials were counted in a liquid scintillation spectrometer with an efficiency of 67% for ^{14}C. Since there was no quenching in buffer or aqueous tissue extract, the radioactivity is expressed in counts per minute.

Extracellular fluid spaces were measured as a percentage of wet weight by similar incubations with inulin-^{14}C (10). Total tissue water, expressed as a percentage of weight was determined by the difference between wet weight and the weight after drying for 24 hr at 105°C (11). Although total tissue water of intestinal rings was not altered by alloxan diabetes, extracellular space was increased somewhat; for example, the total tissue water for both diabetic and control intestine with 60-min incubations was 83 ±1% (SE) while extracellular fluid space was 20 ±1% (SE) for diabetic tissue and 17 ±1% (SE) for control tissue. Extracellular fluid and total tissue water were determined with both diabetic and control intestine at all time points used and the appropriate data were used in calculation of intracellular concentration of sugars and amino acids.

Calculations. Extracellular space was calculated using the following formula:

$$\text{extracellular space (as per cent wet weight)} = \frac{\text{cpm inulin-}^{14}C \text{ in total tissue water} \times 100}{\text{cpm inulin-}^{14}C\text{/ml medium} \times \text{wet tissue weight}}$$

(12). Tissue concentration of ^{14}C was calculated as cpm/ml intracellular fluid as follows:

$$\text{cpm/ml intracellular fluid} = \frac{\text{cpm in total tissue water} - \text{cpm/ml medium} \times \text{wet tissue weight} \times \text{extracellular space}}{\text{wet tissue weight} \times \text{total water space} - \text{wet tissue weight} \times \text{extracellular space}}$$

[8] Obtained as Liquifluor, T. M. Pilot Chemicals, Inc., Watertown, Mass.

(12). Most results are expressed as the following distribution ratio (ICF : ECF):

$$\frac{\text{cpm/ml intracellular fluid}}{\text{cpm/ml incubation fluid}}.$$

For kinetic studies the concentration of sugar or amino acid/ml intracellular fluid was calculated as:

$$\frac{\text{cpm/ml intracellular fluid}}{\text{specific activity of isotope in incubation fluid}}.$$

Compounds. 0.1–0.2 μc of the following labeled sugars and amino acids was added to each flask: 3-*O*-methyl-^{14}C-D-glucose, 5.1 mc/mmole;[4] D-galactose-1-^{14}C, 3.4 mc/mmole;[5] D-arabinose-1-^{14}C, 2.05 mc/mmole;[5] D-ribose-^{14}C, 8.2 mc/mmole;[5] α-amino-isobutyric-1-^{14}C acid (AIB), 4 mc/mmole;[4] and L-lysine-^{14}C (U), 220 mc/mmole.[4] Inulin-carboxyl-^{14}C, mol wt 5000–5500, 1–3 mc/g[4] was used for determination of extracellular space. To ensure that tissue radioactivity was a valid measure of original substrate within tissue in the case of metabolizable substances, tissue extracts from both diabetic and control intestine after 60-min incubations with D-galactose, D-ribose, and L-lysine were chromatographed using descending paper chromatography. In the case of L-lysine, more than 95% of the radioactivity migrated at the R_f of the compound originally added to the incubation fluid. Greater than 90% of the radioactivity in the case of D-galactose and D-ribose migrated at the appropriate R_f.

In some experiments the following unlabeled compounds were added to the incubation fluid: D-galactose,[6] 3-*O*-methyl-D-glucose,[7] 1-amino-cyclopentane-1-carboxylic acid (ACPC),[7] D-arabinose,[6] and D-ribose.[6]

Experimental design. The individual conditions are given with each experiment. Usually tracer concentrations of ^{14}C substrate were used but higher concentrations were also studied (1 or 5 mmoles/liter) to ensure that the effects of diabetes included changes within a wide range of physiologic concentrations. Time curves were carried out at these higher concentrations so that a time interval suitable for subsequent studies of the relationship of concentration to initial transport rate could be determined.

Because initial studies demonstrated that intracellular substrate concentration approached maximum levels by 60 min of incubation, this time period was used for most studies. In those experiments where preincubations were carried out, the incubation time after addition of isotope was limited to 40 min in order that the total incubation time would not exceed 70 min and thus remain well within the limits of tissue viability in this preparation. For data on initial rates, a time interval was chosen from the early linear portion of the time curve, i.e., 10 min or less.

Variation of the method. In order to assess the degree of variation in calculated ICF/ECF from one animal to another, uptake of 3-*O*-methyl-^{14}C-D-glucose was measured in each of a group of six control rats. The mean ICF/ECF of two series of eight flasks containing rings from the same animal was 2.0; the SE was 0.1.

[4] New England Nuclear Corp., Boston, Mass.

[5] Nuclear-Chicago Corporation, Des Plaines, Ill.

[6] Pfanstiehl Labs., Inc., Waukegan, Ill.

[7] California Corp. For Biochemical Research, Los Angeles, Calif.

TABLE I

Effect of Alloxan Diabetes on Uptake of Actively Transported Monosaccharides with 60-min Incubations*

	Diabetes			Control			
	$\frac{[\text{Intracellular}]}{[\text{Extracellular}]}$ Ratio‡	No. of flasks	No. of animals	$\frac{[\text{Intracellular}]}{[\text{Extracellular}]}$ Ratio‡	No. of flasks	No. of animals	Significance of difference§
3-*O*-methylglucose	5.9 ±0.4	63	12	3.6 ±0.2	55	15	$P<0.001$
D-galactose	5.3 ±0.4	32	10	2.5 ±0.2	38	13	$P<0.001$

* ^{14}C sugars were added in tracer quantities (<0.3 μmoles/ml).
‡ Mean ±SE.
§ Calculated using Student's *t* test.

RESULTS

Effect of alloxan diabetes upon uptake of actively transported sugars. Galactose and 3-*O*-methylglucose are actively transported monosaccharides believed to share the glucose transport mechanism. Galactose is only minimally metabolized by intestine, and 3-*O*-methylglucose is not metabolized at all. Table I shows the distribution ratios developed by diabetic and control intestinal rings during 60-min incubations with tracer amounts of galactose and 3-*O*-methylglucose. The differences between control and diabetic tissue are significant for both galactose and 3-*O*-methylglucose.

Effect of streptozotocin diabetes upon galactose uptake. In order to investigate the possibility that the increased distribution ratios of actively transported sugars seen in diabetic intestine were due to a primary effect of alloxan upon the intestine rather than to the diabetes, rats made diabetic with streptozotocin were studied. Table II shows the distribution ratios of galactose using intestine from streptozotocin-induced diabetics and controls. Enhanced transport in diabetes was again demonstrated. It is clear that the effect of diabetes upon active sugar transport was independent of the drug used to induce diabetes.

The relationship of the transport alteration in alloxan diabetes to hyperglycemia and insulin deficiency. The increased uptake of actively transported sugars by diabetic intestine was related to the severity of diabetes. This is illustrated in Table III where the 3-*O*-methylglucose distribution ratios appeared to correlate directly with the severity of diabetes as judged by plasma and urine glucose concentrations.

Evidence that these results could not be attributed to hyperglycemia per se was obtained from two experiments. 5 days of intravenous infusion of 50% glucose into normal rats resulted in plasma glucose concentrations of about 100 mg/100 ml, but failed to alter uptake of 3-*O*-methylglucose by the intestine of these animals after sacrifice, as shown in Fig. 1. Preloading of normal intestinal rings by preincubation with 20 mM glucose for 30 min also did not alter subsequent 3-*O*-methylglucose-^{14}C uptake, as seen in Fig. 2.

Control of diabetes with insulin, however, did modify the intestinal effect. Two groups of 10 rats each were given alloxan as usual. In one group, rats received 3 U of NPH insulin subcutaneously each day. The other group was not treated. Fig. 3 demonstrates the reversal of the increased galactose transport of diabetes with insulin therapy. This experiment provides further evidence that the observed transport changes resulted from diabetes and not from some effect of the mode of induction of diabetes. Insulin added in vitro, on the other hand, had no effect on transport. Table IV shows that the uptake of galactose by diabetic or control intestine was not affected by a large concentration of insulin in vitro.

Effect of alloxan diabetes upon uptake of amino acid and nonactively transported sugar. Transport in diabetic intestine has previously been studied only with

TABLE II

Effect of Streptozotocin Diabetes on Uptake of Galactose with 60 min Incubation*

Diabetes			Control			
$\frac{[\text{Intracellular}]}{[\text{Extracellular}]}$ Ratio‡	No. of flasks	No. of animals	$\frac{[\text{Intracellular}]}{[\text{Extracellular}]}$ Ratio‡	No. of flasks	No. of animals	Significance of difference§
4.2 ±0.1	8	3	1.9 ±0.1	8	3	$P<0.001$

* Galactose-^{14}C was added in tracer quantity (0.15 μmoles/ml).
‡ Mean ±SE.
§ Calculated using Student's *t* test.

TABLE III

Relationship of Severity of Diabetes to Uptake of 3-O-methylglucose with 60 min Incubation*

No. of animals	Plasma glucose‡	Glucosuria§	$\frac{[\text{Intracellular}]}{[\text{Extracellular}]}$ Ratio‖
	mg/100 ml		
Controls (3)¶	104	None	3.1 ±0.4
Diabetic (1)	225	Moderate	5.6 ±0.3
(1)	351	Moderate	8.4 ±0.8
(1)	559	Heavy	12.8 ±0.3

* 3-*O*-methylglucose-^{14}C was present in tracer quantities (0.1 μmoles/ml).
‡ Collected at time of sacrifice.
§ Determined on urine collected on the day before sacrifice.
‖ The mean ±SE of four incubations is given.
¶ Tissues from all three animals were pooled for study.

actively transported sugars. To examine the specificity of the transport abnormality, intestinal uptake of several amino acids including the nonmetabolizable amino acids, ACPC and AIB, and the two passively transported sugars, arabinose and ribose, was measured.

Table V demonstrates the higher distribution ratios of ACPC, AIB, and lysine in diabetic intestine. Thus, alloxan diabetes appears to affect intestinal transport of not only actively transported sugars but also at least three amino acids representing differing transport systems.

Table VI shows that diabetes had no effect upon the distribution ratios of arabinose and ribose after 60-min incubations. Studies of arabinose and ribose uptake over a wide concentration range and at different incubation times also failed to demonstrate a difference between

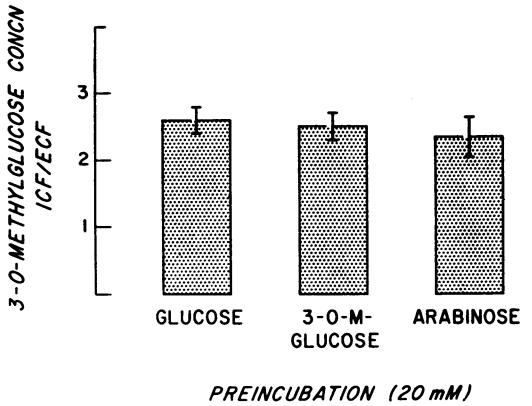

FIGURE 2 Effect of preloading control intestinal rings with glucose on accumulation of tracer quantities of 3-*O*-methylglucose. For comparison, rings were also preincubated with 3-*O*-methyl-D-glucose and D-arabinose at a 20 mM concentration so that osmolality was identical. One control sugar is actively transported and believed to use the glucose transport system, the other is passively transported. Rings were preincubated with one of the sugars for 30 min at a 20 mM concentration. They were then removed, washed, and transferred into fresh buffer containing only 3-*O*-methylglucose-^{14}C and accumulation measured with 40 min of incubation. The mean ±SE of six flasks in each group is shown.

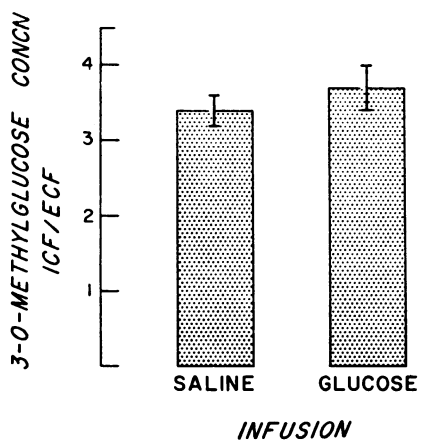

FIGURE 1 Influence of nondiabetic hyperglycemia on intestinal accumulation of 3-*O*-methylglucose. Control rats received an intravenous infusion by pump of either 50% glucose at a rate sufficient to produce marked and constant glucosuria and plasma glucose concentrations in excess of 1000 mg/100 ml or isotonic saline at the same rate. After 5 days of infusion, intestinal accumulation of 3-*O*-methylglucose over 60 min was measured using tracer quantities (0.1 μmoles/ml) of 3-*O*-methylglucose-^{14}C. Both total water and inulin spaces were determined and used in the calculations. The mean ±SE of 12 flasks containing tissue from two animals is shown for each group.

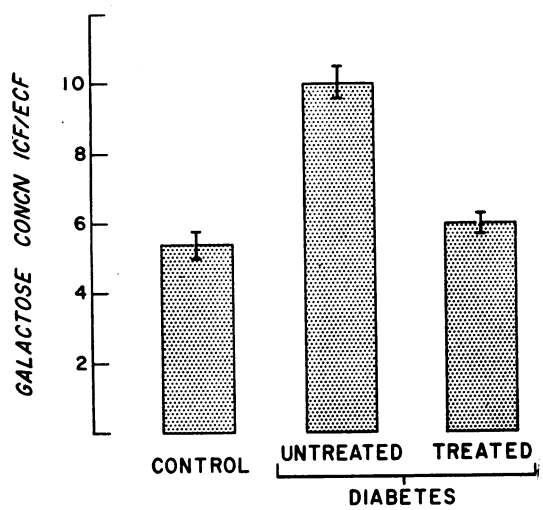

FIGURE 3 Relationship of insulin therapy to galactose uptake. Rats with comparable diabetes were either not treated or given 3 U of NPH insulin subcutaneously each day for 4 days. Accumulation of galactose initially present at 1 mM concentration is shown for these animals as well as control litter mates. Incubation time was 60 min. The mean ±SE of five flasks, each with tissue from four rats, is shown for each group.

TABLE IV

Effect of Insulin Added In Vitro on Uptake of Galactose by Diabetic and Control Intestine*

	Intracellular/extracellular ratio‡	
	Diabetes	Control
No insulin	2.6 ±0.3	1.0 ±0.3
Insulin added (0.1 U/cc), no preincubation	2.7 ±0.2	1.0 ±0.3
Insulin added (0.1 U/cc), 45 min preincubation	2.4 ±0.4	1.0 ±0.4

* Galactose concentration was 1 mmole/liter.
‡ 10-min incubations.

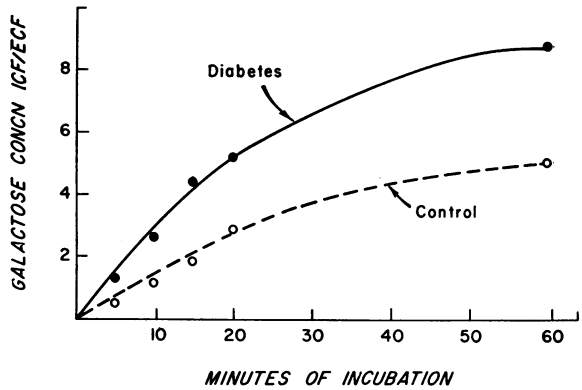

FIGURE 4 Galactose accumulation with time. Initial concentration was 5 mmoles/liter. Means of values from three flasks, each containing tissue from three animals, are given.

diabetic and control intestine. Thus, passive sugar transport was shown not to be affected by diabetes.

Kinetic experiments. Figs. 4 and 5 present data on accumulation of galactose and ACPC by diabetic and control intestine with time when the initial concentration was 5 mmoles/liter. Similar curves were found when 3-*O*-methylglucose was studied. Tissue accumulation was greater in diabetic than in control intestine as early as 5 min after the start of incubation. Early time points, on the linear portion of the curve, largely reflect initial rates of entry, i.e., efflux has a relatively insignificant effect on rate of accumulation at these early points. The greater accumulation in diabetic intestine after 5 min, therefore, indicates enhanced rate of entry (influx) of sugar and amino acid in this condition.

Theoretically, the higher distribution ratio of sugars and amino acids in diabetes at later time points could be the result of slower efflux in diabetes in addition to faster influx. To determine the effect of diabetes upon efflux, diabetic and control rings were loaded with galactose-^{14}C to approximately the same concentration by incubation for 10 and 20 min respectively in Krebs-Ringer bicarbonate buffer containing D-galactose-1-^{14}C at a concentration of 0.05 μc/ml. The rings were then washed in isotonic saline and transferred into buffer containing no galactose, and the decline of residual intracellular radioactivity with time was measured. The large buffer volume (50 ml) was chosen to minimize any effect of reentry on the tissue radioactivity. Fig. 6 shows the results of that experiment. A Student's *t* test indicated that the slope of the line representing efflux in diabetes was not significantly different from that representing efflux in controls.

To determine the nature of the increased influx in diabetes, the relationship of substrate concentration to initial transport rates was studied. Fig. 7 shows such a study using galactose. As higher concentrations were reached, the curves began to flatten, consistent with the presence of a saturable transport mechanism. Fig. 8 is a Lineweaver-Burk plot of the same data. Similar plots were constructed for ACPC transport. Kinetic constants were calculated from these experiments using the logarithmic transformation described by Barber, Welch, and Mackay to reduce the effects of variation of V_{max} between individual tissue specimens (13). These are shown in Table VII. It is clear that diabetes increased the maximal velocity of entry (V_{max}) of both galactose

TABLE V

Effect of Alloxan Diabetes on Uptake of Amino Acids with 60-min Incubations*

	Diabetes			Control			
	$\frac{[\text{Intracellular}]}{[\text{Extracellular}]}$ Ratio‡	No. of flasks	No. of animals	$\frac{[\text{Intracellular}]}{[\text{Extracellular}]}$ Ratio‡	No. of flasks	No. of animals	Significance of difference§
ACPC	11.2 ±0.8	24	14	6.8 ±0.4	24	13	$P < 0.001$
AIB	2.0 ±0.1	24	14	1.6 ±0.1	23	13	$P < 0.01$
Lysine	10.6 ±1.1	17	7	3.7 ±0.9	17	7	$P < 0.001$

* ^{14}C amino acids were added in tracer quantities (<0.3 μmoles/ml).
‡ Mean ±SE.
§ Calculated using Student's *t* test.

TABLE VI

Effect of Alloxan Diabetes on Uptake of Passively Transported Monosaccharides with 60-min Incubations*

	Diabetes			Control			
	[Intracellular]/[Extracellular] Ratio‡	No. of flasks	No. of animals	[Intracellular]/[Extracellular] Ratio‡	No. of flasks	No. of animals	Significance of difference§
D-arabinose	0.4 ±0.03	19	6	0.5 ±0.06	10	8	NS
D-ribose	0.5 ±0.07	5	3	0.6 ±0.08	6	3	NS

* ^{14}C sugars were added in tracer quantities (<0.3 μmoles/ml).
‡ Mean ±SE.
§ Calculated using Student's *t* test.

and ACPC. Although most studies also demonstrated a decrease in affinity constant (K_t), the differences were not statistically significant.

Because diabetes specifically affected transport of sugar and amino acids whose uptake is dependent upon extracellular sodium concentration, we examined the possibility that the effect of diabetes on transport depended on the presence of sodium in the incubation medium. Galactose uptake was studied in buffer in which sodium was replaced by Tris (hydroxymethyl) aminomethane and choline. Fig. 9 shows that complete replacement of sodium decreased galactose uptake markedly in both diabetic and control intestine and abolished the difference between them.

DISCUSSION

The early studies demonstrating increased rates of glucose disappearance from the intestinal lumen of animals with alloxan diabetes did not distinguish between changes in membrane transport and alterations in the cellular and peripheral disposition of glucose (5–7). Crane used in vitro methods to show that diabetes increased the accumulation of sugar in the intestinal cell against concentration gradients and that this increased cellular concentration could not be explained by diminished metabolism of the sugar since cellular concentration of 6-deoxyglucose, an actively transported but unmetabolizable analog of glucose, was also increased in diabetes (2). He suggested that an alteration in functional characteristics of the intestine occurred in the diabetic state produced by alloxan. The relevance of this change to human disease became apparent when Vinnik, Kern, and Sussman (4) demonstrated increased rates of intestinal glucose absorption in juvenile onset diabetes of man. We have studied intestinal transport in alloxan

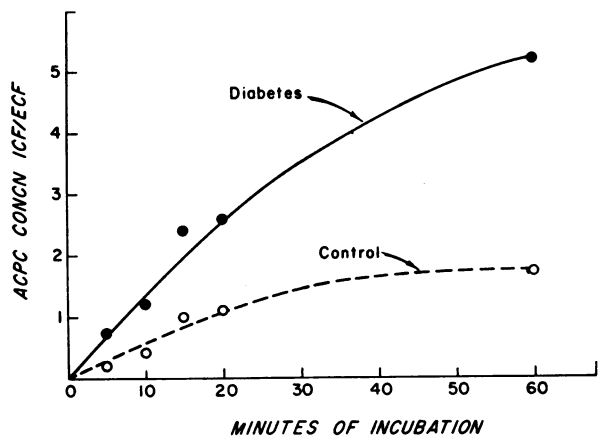

FIGURE 5 ACPC accumulation with time. Initial concentration was 5 mmoles/liter. Means of values from three flasks, each containing tissue from three animals, are given.

TABLE VII

*Kinetic Constants**

Substrate	K_t			V_{max}		
	Controls	Diabetics	Significance of difference	Controls	Diabetics	Significance of difference‡
	mmoles/liter			*μmoles/ml per 7 min*		
Galactose	43 ±9 (5)	23 ±3 (5)	NS	28 ±5 (5)	34 ±4 (5)	P <0.02
ACPC	18 ±5 (4)	12 ±1 (4)	NS	13 ±2 (4)	26 ±3 (4)	P <0.01

* Mean ±SE is given followed by the number of kinetic studies in parentheses. Each study was done in triplicate with each flask containing pooled tissue from three animals.
‡ Calculated using the *t* test for paired experiments.

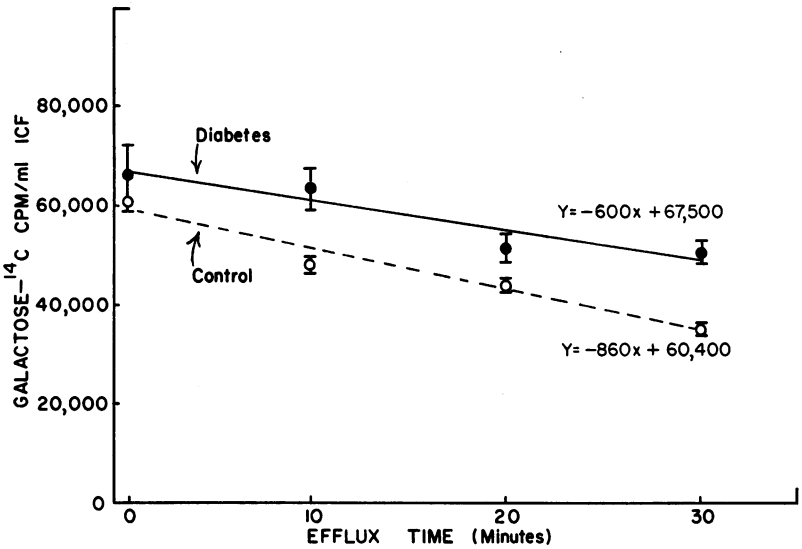

FIGURE 6 Efflux of galactose. The mean ±SE of eight flasks is shown. Five diabetic and five control rats were studied. The lines were drawn by the method of least squares.

diabetes in an attempt to further characterize the altered function of the intestine in this disease. Our studies confirm Crane's observations with sugars and demonstrate, in addition, that accumulation of utilizable and nonutilizable amino acids is increased in diabetes. Our results indicate that these increased rates of intestinal absorption involve only substances that undergo active transport as accumulation of D-arabinose and D-ribose was not affected.

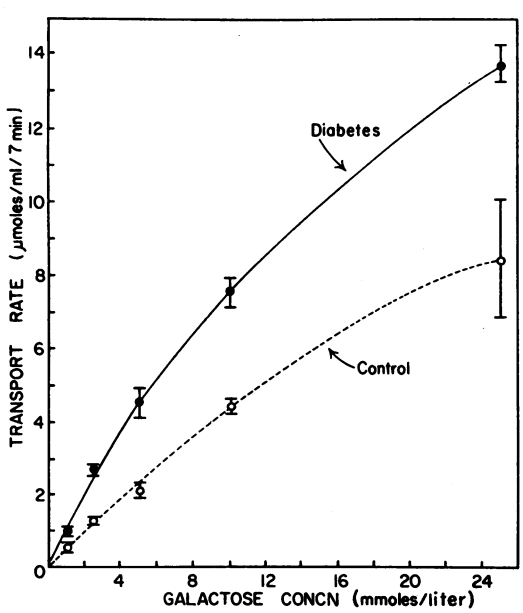

FIGURE 7 Relationship of galactose transport to initial concentration. Incubation time was 7 min. The mean ±SE of three flasks, each with tissue from three animals, is shown.

Before analyzing the mechanism of the diabetic effect, we attempted to exclude any possibility that our data reflected some artifact of technique. Two observations suggest that our results were not secondary to a direct effect of aloxan on intestinal mucosa. First, intestine from animals made diabetic with streptozotocin, a drug structurally unrelated to alloxan, also accumulated galactose at higher rates. Second, control of alloxan diabetes with exogenous insulin prevented the alteration of galactose transport. Since it is unlikely that insulin would reverse a direct effect on intestinal cells, we conclude that the effect of alloxan on intestinal transport was the result of the induction of diabetes.

It is also evident that our observations are not the result of differences in weight, growth, or food consumption. Crane showed increased sugar transport in diabetes whether age- or weight-matched controls were used (2). We have confirmed his experience and further shown by pair-feeding experiments that differences in food consumption do not account for the diabetic effect.

Since small bowel dilatation and hypertrophy have been observed in rats after several months of alloxan diabetes (14), it has been suggested that the increased rates of glucose absorption in diabetes are secondary to a greater mucosal absorptive surface area (15). It is unlikely that this interpretation explains our results. Our studies were done 5 days after administration of alloxan before any morphologic change was discernible with the light microscope.[8] Also, since we measured cellular uptake rather than transmural transport, a simple

[8] Olsen, W. A., and I. H. Rosenberg. Unpublished observations.

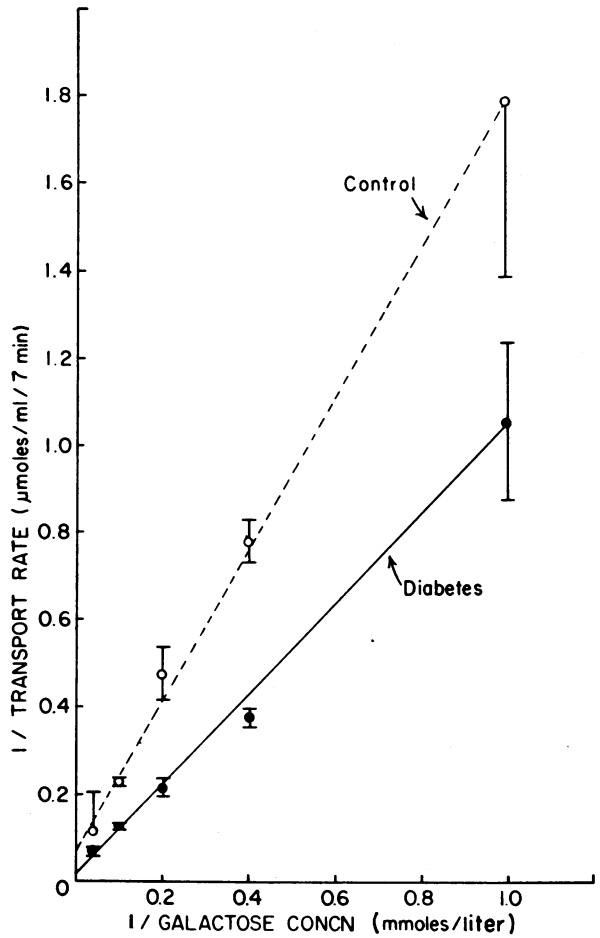

FIGURE 8 Lineweaver-Burk plot of data shown in Fig. 7. Mean ±SE is shown. Lines were drawn by the method of least squares.

change in surface area would not explain increased concentration gradients. To exclude the possibility that our results were due to a change in the ratio of epithelial cells to submucosa and muscular coats, we also measured uptake of galactose in scrapings of mucosa from diabetic and control intestine; again this technique demonstrated greater uptake in diabetic intestine.[8]

It is evident that a more specific change in transport is present in diabetes. In an effort to define the determinants of this change, a number of studies were done. Because concentration gradients in diabetes bore a direct relationship to the blood sugar concentration at the time of sacrifice, we explored the possibility that either hyperglycemia per se or increased tissue glucose concentrations increased active transport. However, intestine from control animals which had been maintained in a hyperglycemic state for 5 days by intravenous glucose infusion and control intestine preincubated in 20 mM glucose for 30 min were found to have normal transport rates of 3-*O*-methylglucose. We also considered the possibility that low levels of circulating insulin could explain our results especially when we were able to demonstrate that control of diabetes with insulin prevented the diabetic effect. However, we were unable to demonstrate any effect of insulin in vitro even after a 45 min preincubation thus confirming the refractoriness of the intestine to insulin in vitro shown by other investigators. In addition, Crane was unable to modify the diabetic effect by administration of insulin to animals just before sacrifice (2). We conclude, therefore, that the transport changes in diabetes are neither related to hyperglycemia or to increased tissue glucose, nor are they directly related to an acute loss of a pharmacologic effect of insulin. The changes could, however, be secondary to a mucosal alteration induced by chronic insulin deficiency or changes in other humoral factors in diabetes. Whatever the mechanism, diabetes appears to produce functional alterations in the intestinal mucosa which may be related to subtle structural or biochemical changes in the epithelial cell.

The cellular localization of the functional alterations is uncertain. Since there is greater accumulation against a concentration gradient, it appears that more energy is being utilized for transport. We have preliminary data, in fact, suggesting that the diabetic intestine is a metabolically more active organ even when it is not transporting sugar or amino acid. Oxygen consumption by mucosal scrapings incubated with or without added substrate is greater in diabetes.[9] Thus it may be that the transport changes in the diabetic intestine are secondary to an increase in intracellular energy metabolism. However, the finding of increased rates of entry at the earliest times studied in addition to evidence for a functional increase in transport capacity in diabetes (increased V_{max}) suggests an effect upon the cell membrane. These effects need not be unrelated; Leaf and Dempsey (16) and Leaf and Renshaw (17) have shown that energy utilization by the cell is responsive to changes in sodium transport, and Whittam has proposed that sodium-potassium adenosine triphosphatase which appears to control active transport of sodium may be the "pacemaker" of cell respiration (18). Since the diabetic effect on transport is limited to those sugars and amino acids where cellular uptake is believed to occur in complex with sodium and "carrier" (19) and since the effect is abolished in the absence of sodium, it is conceivable that an increased activity of the "sodium pump" in diabetes could account for the observed changes in nonelectrolyte transport and cell respiration. Some additional support for this suggestion is the re-

[9] Rosenberg, I. H., and W. A. Olsen. Unpublished observations.

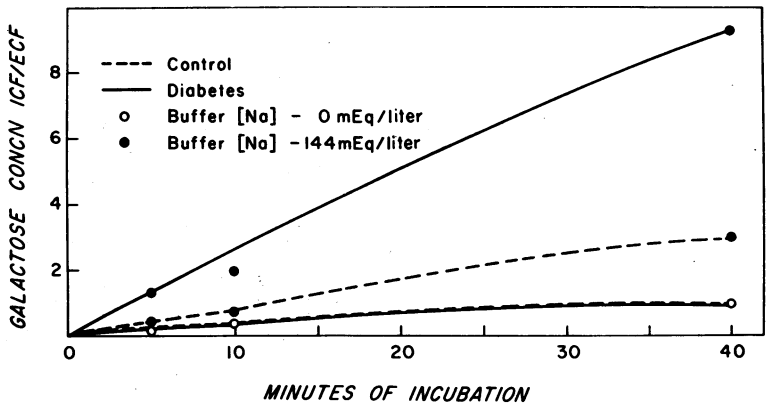

FIGURE 9 Effect of replacement of sodium in medium on uptake of galactose. Initial galactose concentration was 1 mmole/liter. Means of values from three flasks, each containing tissue from three animals, are given.

cent demonstration of increased net transmucosal sodium movement in gut sacs from diabetic rats (20).

Analysis of these relationships in diabetes may permit further evaluation of currently accepted hypotheses of the relationship of membrane function and cell respiration. In addition, further information may be obtained on the mechanism of sodium dependent nonelectrolyte transport.

Finally, it should be noted that altered active transport in diabetes may involve other organs as well. It has been recently shown that amino acid transport into renal medullary slices is faster in diabetic rats.[10] Also, the transport of at least some amino acids into both cardiac and striated muscle proceeds at a faster than normal rate in diabetes (21, 22). Since one of the known actions of insulin is facilitation of amino acid uptake by muscle cells (1), the functional changes in these tissues with diabetes appear to compensate for insulin lack. Diabetes may, therefore, induce a widespread change in the cellular membrane which acts to compensate at least partially for some of the effects of insulin lack.

ACKNOWLEDGMENTS

We are grateful to Doctors Charles S. Davidson, Ronald A. Arky, and Leah M. Lowenstein for their interest and helpful discussions and to Miss Anitra Bard, Miss Nancy Grimme, and Miss Lydia Rodgers for their technical assistance.

This work was supported in part by Public Health Service Grant Nos. AM-09115, AM-5413, and FR-0076 from the National Institutes of Health, Bethesda, Md.; a General Research Support Grant to the University of Wisconsin Medical School from the National Institutes of Health, Division of Research Facilities and Resources, Bethesda, Md.; and the Veterans Administration Medical Research Funds.

[10] Lowenstein, L. M., and W. A. Olsen. Unpublished observations.

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