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THE EFFECT OF ESTROGEN ON SUGAR TRANSPORT IN THE RAT UTERUS

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SUMMARY

1. The characteristics of sugar transport in the isolated rat uterus were studied using the non-metabolized glucose analogue 3-*O*-methyl-D-glucose. The effect of estrogen injection into ovariectomized rats on the transport rate was also determined.

2. Estrogen injection increased the initial rate of 3-*O*-methylglucose transport into uterine cells. The effect was evident at 2 h, and maximal at 4 h. After 12 h the control value was attained again.

3. Sugar transport obeyed Michaelis–Menten kinetics. Estrogen increased the v_{max} 2-fold, but did not change the K_m.

4. Sugar transport exhibited substrate specificity, competition between sugar pairs, and countertransport.

5. Estrogen increased the rate of sugar efflux from the uterus.

6. Estradiol, insulin, *N*-ethylmaleimide, NaCN, diisopropylphosphorofluoridate, *p*-chloromercuribenzoate and dinitrophenol did not alter the transport rate when added *in vitro*.

7. It is concluded that estrogen increases the rate of sugar transport in the rat uterus and that sugar transport takes place by means of a specific mobile carrier transport system.

INTRODUCTION

The stimulation of uterine anabolic activity by estrogenic hormones is accompanied by significant early changes in the carbohydrate metabolism of this tissue. These include: an increase in glucose uptake in isolated rat uteri 4 h after estrogen injection[1], an increase in glycogen content[2,3] which is detectable after 2 h of estrogen treatment[4], and an increase in the incorporation of uniformly labeled [^{14}C]glucose

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into uterine lipid, RNA, protein and CO_2 within 1–2 h after estrogen treatment[5]. SZEGO AND ROBERTS[1] and NICOLLETTE AND GORSKI[5] have suggested that glucose transport may be stimulated by estrogen administration. However, HALKERSTON *et al.*[6] found no significant changes in the distribution of D-xylose in uteri from ovariectomized rats 1.5 and 6.0 h after estrogen injection, and concluded that estrogen does not alter sugar permeability in uterus. The available literature on effects of estrogen on sugar and amino acid transport in the uterus has been reviewed recently[7–9].

In the present study the rate of transport of the non-metabolized glucose analogue 3-*O*-methylglucose was measured. This compound is transported into erythrocytes at a rate that very closely approximates that of glucose[10]. These studies differ from those of HALKERSTON *et al.* in that short incubations *in vitro*, and not administration of glucose analogues *in vivo*, were employed to measure the transport rate. The present study demonstrates the existence in the uterus of a sugar transport system with many characteristics similar to transport systems of other tissues, but also responsive to estrogen.

METHODS AND MATERIALS

Chemicals

The 3-*O*-methyl-D-glucopyranose, D-mannose, D-galactose, D-xylose, L-arabinose, and D-sorbitol were obtained from Calbiochem Corp. The D-lyxose and 2-deoxy-D-glucose were obtained from Nutritional Biochemicals Corp. The D-glucose was purchased from Pfanstiehl Laboratories, Inc. The 3-*O*-[^{14}C]methyl-D-glucose (2.72 mC/mmole) and D-[1-^{3}H]sorbitol (168 mC/mmole) were obtained from New England Nuclear Corp. Crystalline 17β-estradiol was purchased from the Sigma Chemical Co.

Incubation medium

Incubations were carried out at 37° in Krebs–Ringer bicarbonate previously equilibrated with O_2–CO_2 (95:5, v/v) (pH 7.4)[11]. The incubation medium contained 0.10 μC of 3-*O*-[^{14}C]methyl-D-glucose and 0.50 μC of D-[1-^{3}H]sorbitol per ml. Carrier 3-*O*-methyl-D-glucose and D-sorbitol were added to a final concentration of 1.0 mM except where otherwise specified. All additions to the buffer were made by adding 5 or 10 μl of concentrated solution per ml of buffer or by dissolving the substance directly in freshly prepared buffer to give the desired final concentration. Each uterine horn was incubated in 0.5 ml of the medium for the time specified.

Animals

Sprague–Dawley rats weighing about 110–130 g were ovariectomized by the lateral approach using ether anesthesia. Animals operated on the same day were used for a given experiment 7–30 days postoperatively. The animals were allowed access to Rickland rat/mouse diet and water at all times.

Hormone administration

A stock solution of 2.0 mg/ml 17β-estradiol in 95% ethanol was diluted to 20 μg/ml in 20% ethanol before each experiment. 1 μg (0.05 ml) was injected subcutaneously at the time indicated.

Preparation and incubation of the uterus

The animals were sacrificed by decapitation. The abdomen was opened and the uterine horns were excised and placed in 0.15 M NaCl at ambient temperature. The uterine horns were transferred to a filter paper moistened with normal saline and the excess connective tissue was removed. Each uterine segment was blotted on dry filter paper and transferred to 0.5 ml incubation medium in a round-bottom plastic tube in a Dubnoff metabolic shaking incubator under an atmosphere of O_2–CO_2 (95:5, v/v). After the incubation period, the segments were immersed briefly in physiologic saline at room temperature to remove adhering drops of medium, incised longitudinally, blotted on dry filter paper, and weighted on a Roller Smith torsion balance. Each uterine horn was transferred to a 12-ml graduated conical centrifuge tube containing about 0.5 ml of water. To prepared controls unincubated uterine segments were added to conical centrifuge tubes with a small aliquot of the incubation medium and standard amounts of 3-*O*-[^{14}C]methyl-D-glucose and D-[1-^{3}H]-sorbitol in 0.5 ml of water. All of the tubes were placed in a boiling-water bath for 15 min, and then allowed to cool to room temperature. The contents of each tube were transferred to a small, conical, glass tissue grinder (Duall tissue grinder, Kontes Glass Co.), homogenized for about 15 sec and returned to the graduated tube. The homogenizer was rinsed twice with 0.5-ml portions of distilled water which were also transferred to the conical tube. The homogenates were deproteinized by adding 0.10 ml 0.15 M $Ba(OH)_2$, followed after 10 min by 0.10 ml 5% $ZnSO_4$, and centrifuged at 750 × *g* for 15 min. The solution volumes were recorded and 1.0-ml aliquots were added to 15 ml of BRAY's solution[12] in a plastic vial for ^{14}C and ^{3}H determination in a Packard Tri-Carb liquid-scintillation counter. The counts due to each isotope were calculated by the method of OKITA *et al.*[13].

Definitions

3-O-Methyl-D-glucose space. This is defined as the volume of tissue water necessary to contain the 3-*O*-methyl-D-glucose of the tissue at the concentration of the incubation medium.

$$\text{3-}O\text{-Methyl-D-glucose space } (\mu\text{l/g}) = \frac{\text{counts/min 3-}O\text{-[}^{14}\text{C]methyl-D-glucose per g tissue}}{\text{counts/min 3-}O\text{-[}^{14}\text{C]methyl-D-glucose per } \mu\text{l incubation medium}}$$

D-*Sorbitol space.* This is defined and calculated in a manner analogous to that for the 3-*O*-methyl-D-glucose space.

Intracellular 3-O-methyl-D-glucose space. This is the difference between the 3-*O*-methyl-D-glucose space and the D-sorbitol space:

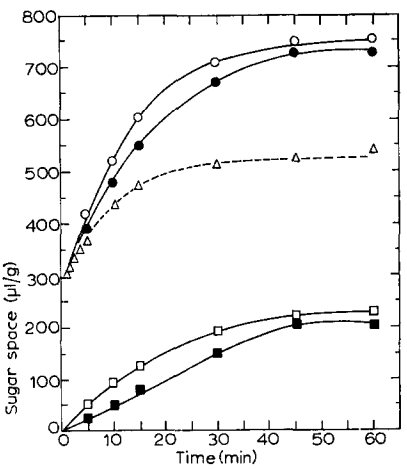
Intracellular 3-*O*-methyl-D-glucose space = 3-*O*-methyl-D-glucose space − sorbitol space

RESULTS

Sugar uptake in uterine horns from untreated and 4-h estrogen-treated ovariectomized rats

The upper two curves (Fig. 1) show the rate of 3-*O*-methyl-D-glucose uptake in the estrogen-treated and untreated cases, the middle curve shows the rate of

sorbitol uptake in both cases, and the lower two curves show the rate of intracellular 3-*O*-methyl-D-glucose uptake. The latter curves indicate that the initial rate of 3-*O*-methyl-D-glucose appearance in the intracellular space was increased about 100% with estrogen treatment. The rate of sorbitol uptake was the same in both the control and estrogen-treated tissues. This indicates that the rate of appearance of incubation medium in the extracellular space was the same in each case. Therefore, the increased rate of transport of 3-*O*-methyl-D-glucose cannot be ascribed to any



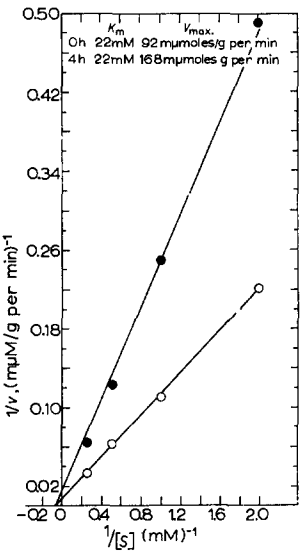


Fig. 1. Sugar uptake in uterine horns from estrogen-treated (4 h) and untreated ovariectomized rats. The sugar spaces were determined as described in METHODS AND MATERIALS. Open symbols represent data from estrogen-treated animals, and solid symbols represent data from untreated animals. Each point represents the mean of eight determinations. The standard error of the mean of each point is ± 4.0 µl or less. ○—○, 3-*O*-methyl-D-glucose space; △—△, D-sorbitol space; □—□, intracellular 3-*O*-methyl-D-glucose space.

Fig. 2. Determination of K_m and v_{max} of 3-*O*-methyl-D-glucose transport in uterine horns from estrogen-treated (4 h) and untreated ovariectomized animals. Initial rates were determined during a 5-min incubation for the estrogen-treated group and a 10-min incubation in the control group. Each point represents the mean of eight determinations. ○—○, treated; ●—●, untreated.

difference in the rate of transfer of 3-*O*-methyl-D-glucose from the medium to the site of transport.

The water content of the uterus was 79% in the control uterus (790 µl/g) and 83% in the uterus from the 4-h estrogen-treated animal (830 µl/g). The 3-*O*-methyl-D-glucose space corresponded to 96% of the total cell water in the control (760 µl per g: 790 µl per g) and to 95% of the total cell water in the treated case (775 µl per g: 830 µl per g). Thus, the uterus did not concentrate 3-*O*-methyl-D-glucose relative to the medium. Although these results could be explained by the simple diffusion of 3-*O*-methyl-D-glucose from the medium into the tissue cells, evidence to be presented indicates the existence of a specific sugar transport system.

Determination of K_m and v_{max} of transport in uteri from untreated and estrogen-treated animals

The K_m and v_{max} for 3-*O*-methyl-D-glucose transport were determined by means of double reciprocal plots ($1/v$ *vs.* $1/[S]$ as shown in Fig. 2. Initial velocity of transport was approximated by using short incubation periods (*i.e.*, influx $\gg$ efflux). The uteri from untreated animals were incubated for 10 min, and those from 4-h estrogen-treated animals were incubated for 5 min in order to utilize the more linear portion of the uptakes curves (Fig. 1). As a result of this restriction 3-*O*-methyl-D-glucose and sorbitol did not equilibrate completely with the extracellular tissue fluid and therefore the cells of the uterus were exposed to a lower average concentration of the 3-*O*-methyl-D-glucose than that in the medium. This undoubtedly produced a systematic error such that the observed K_m of approx. 22 mM in both cases represents a maximum estimate only. Correction for this error was not attempted because it would require several assumptions concerning the diffusion of the substrate into the tissue, and it would not alter the interpretation with regard to either the mechanism of transport on the effect of estrogenic hormone. The calculated v_{max} in the estrogen-treated group was about twice that in the untreated group (168 mμmoles/g·min *vs.* 92 mμmoles/g·min). These results can be interpreted as indicating saturation of a finite number of sites of diffusable membrane carriers; they cannot be reconciled with the kinetics of simple diffusion which would not exhibit saturation[14].

Countertransport and competition between 3-O-methyl-D-glucose and glucose

The upper and lower curves (Fig. 3) were determined using uteri from untreated ovariectomized animals incubated without glucose or with 20 mM glucose, respectively. Glucose inhibited 3-*O*-methyl-D-glucose transport into the uterine cells. Presumably this is due to mutual competition for transport sites, and is consistent with a carrier-mediated transport mechanism.

To further distinguish a mobile carrier transport system from simple diffusion or other possible types of facilitated diffusion, countertransport experiments were

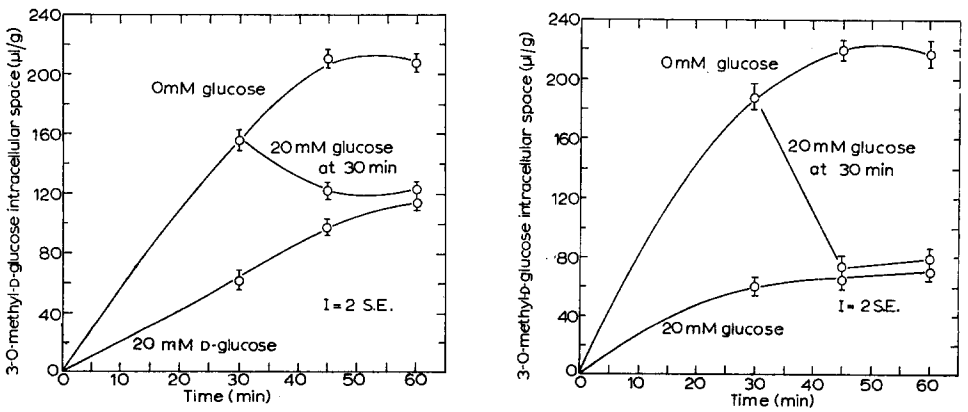


Fig. 3. Countertransport of 3-*O*-methyl-D-glucose in uterine horns from untreated (left) and estrogen-treated (4 h, right) ovariectomized rats. The experiment is described under RESULTS. Each point represents the average of six determinations. The vertical lines represent 2 standard errors of the mean.

carried out *in vitro*[10,14,15]. Countertransport is defined as a shift in the distribution of a sugar between the intracellular and extracellular compartments induced by a difference in concentration across the cell membrane of another sugar that competes for the same carrier site.

Equilibration of 3-*O*-methyl-D-glucose was allowed to occur by incubating uterine horns in glucose-free medium for 30 min. They were then transferred to a second medium containing 20 mM glucose. The intracellular 3-*O*-methyl-D-glucose space decreased when glucose was added, as shown in Fig. 3. This reflects a decrease in the intracellular concentration of 3-*O*-methyl-D-glucose which, under these conditions, was transported from the region of lower concentration (intracellular) to the region of higher concentration (extracellular) against a concentration difference. These results are best explained by a mobile carrier transport system[4,10,15].

Substrate specificity

Various sugars were examined for their ability to produce countertransport of 3-*O*-methyl-D-glucose, as a means of determining the substrate specificity of the glucose transport system. Addition of a substrate that competes with 3-*O*-methyl-D-glucose for its carrier will produce a net decrease in the 3-*O*-methyl-D-glucose space. Other substrates should not influence the system. Uterine horns were incubated for

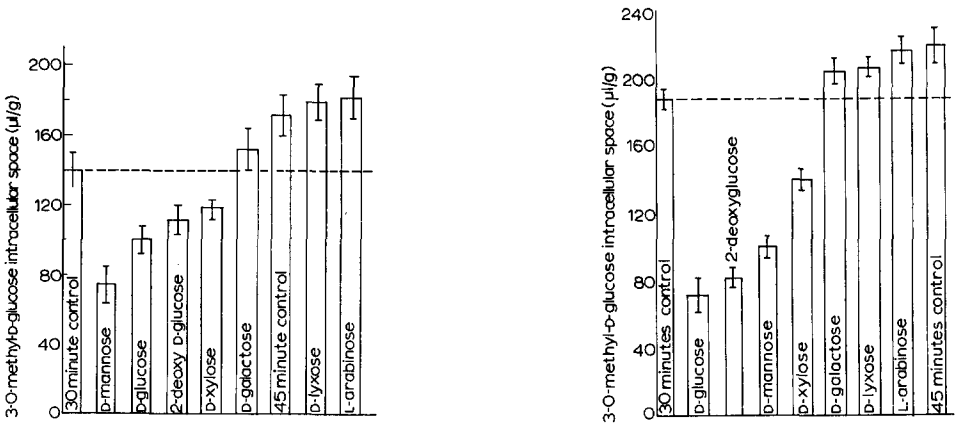


Fig. 4. Substrate specificity of sugar transport in uterine horns from untreated (left) and estrogen-treated (4 h, right) ovariectomized rats. Each uterine horn was incubated in medium containing 1 mM 3-*O*-methyl-D-glucose for 30 min. It was then transferred to medium that contained the test sugar at a concentration of 20 mM and incubated 15 min. The bar represents the mean of six determinations, and the vertical line through the bar represents 2 standard errors of the mean.

30 min in a medium containing 1 mM labeled 3-*O*-methyl-D-glucose. The intracellular 3-*O*-methyl-D-glucose space was determined in control uteri, and others were transferred to the same medium containing 20 mM of the test sugar and were incubated for 15 min longer. The results obtained with uterine horns from untreated and 4-h estrogen-treated ovariectomized rats were similar qualitatively (Fig. 4). D-Glucose, D-mannose, 2-deoxy-D-glucose, and D-xylose decreased the intracellular 3-*O*-methyl-D-glucose space, indicating competition with 3-*O*-methyl-D-glucose for its carrier. On the other hand, D-galactose and its C_5 analogue L-arabinose

did not compete with 3-*O*-methyl-D-glucose transport. The reason for the difference in behavior of D-mannose and its C_5 analogue, D-lyxose, is not known. BATTAGLIA AND RANDLE[16] have reported that D-galactose and L-arabinose, are transported independently in the rat diaphragm.

Sugar efflux from uterine horns from untreated and estrogen-treated animals

Both uterine horns from estrogen-treated (4 h) and untreated animals were incubated in 1 mM 3-*O*-methyl-D-glucose for 45 min. The 3-*O*-methyl-D-glucose space was determined in one horn from each animal. The other horn was then transferred to successive 0.6-ml aliquots of fresh sugar-free Krebs–Ringer bicarbonate solution at 0, 5, 10, and 15 min. The 3-*O*-methyl-D-glucose space of this horn was determined after incubation for a total period of 30 min in the sugar-free medium, 3-*O*-methyl-D-glucose efflux (3-*O*-methyl-D-glucose space — sorbitol space) was calculated after 5, 10, 15, and 30 min of incubation in the sugar-free medium. As shown in Fig. 5 the initial efflux from the uteri of the estrogen-treated animals was about

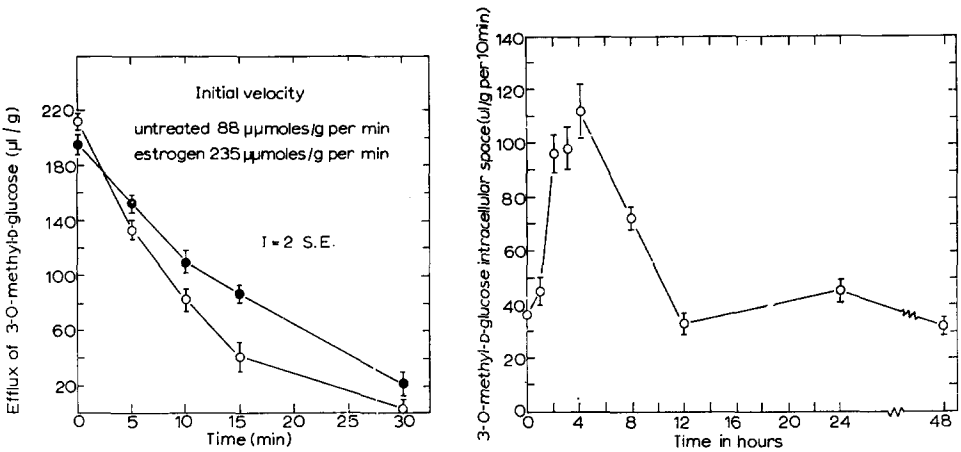


Fig. 5. 3-*O*-Methyl-D-glucose efflux from 4-h estrogen-treated and untreated ovariectomized rats. The experimental procedure is described under RESULTS. Each point represents the mean of six determinations, and the vertical line represents 2 standard errors of the mean. ○—○, untreated; ●—●, 4-h estrogen-treated ovariectomized rats.

Fig. 6. Time course of the estrogen-induced sugar transport increase. Experimental animals received 1.0 μg of 17β-estradiol in 20% ethanol at zero time. They were sacrificed at the stated time, and the rate of uterine 3-*O*-methyl-D-glucose transport was determined during a 10-min incubation period. Controls received vehicle only for 4 h. Each point represents the mean of five determinations. The vertical lines represent 2 standard errors of the mean.

twice as great as that from the uteri of the untreated animals (235 μμmoles/g·min *vs.* 88 μμmoles/g·min). Thus, estrogen produces both an increase in 3-*O*-methyl-D-glucose influx as well as efflux, as would be expected from the mobile carrier model.

Time course of the transport response to estrogen

3-*O*-Methyl-D-glucose transport was determined in ovariectomized rats sacrificed after various periods of estrogen treatment. Incubation periods of 10 min were employed in order to determine the initial transport velocities. After 1 h of estrogen

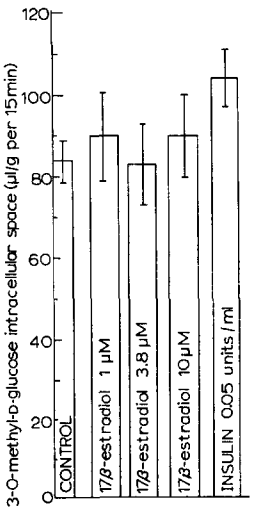
treatment, the transport rate was increased a slight, but not significant, amount ($P < 0.2$) (Fig. 6). A 3-fold increase occurred by 2 h ($P < 0.01$). The maximal response occurred at 4 h and thereafter the rate decreased to the initial value by 12 h.

Effect of 17β-estradiol and insulin in vitro on sugar transport

Uterine horns from untreated ovariectomized rats were preincubated in various concentrations of 17β-estradiol in Krebs–Ringer bicarbonate for 15 min at 37°. 3-*O*-Methyl-D-glucose transport was measured during a subsequent 15-min incubation period in a medium containing the same amount of 17β-estradiol. Contralateral horns were treated in a similar manner in estrogen-free media. The results plotted in Fig. 8 show that 1 μM, 3.8 μM and 10 μM 17β-estradiol does not affect transport under these conditions *in vitro*. Insulin, which increases glucose transport *in vitro* by diaphragm[17,18] and heart[19], did not significantly increase 3-*O*-methyl-D-glucose transport in the isolated uterus (Fig. 7).

Effect of various metabolic inhibitors on sugar transport

Uterine horns from untreated ovariectomized animals were preincubated for 15 min in Krebs–Ringer bicarbonate containing the specified compound. Transport was measured during a subsequent 15-min incubation period in the presence of the inhibitor. Control horns were treated similarly in the absence of inhibitor. 2,4-Dinitrophenol, *N*-ethylmaleimide, iodoacetate, *p*-chloromercuribenzoate, diisopropylphosphorofluoridate and NaCN, at the concentrations indicated, did not affect the rate of 3-*O*-methyl-D-glucose transport significantly (Fig. 8). Under similar



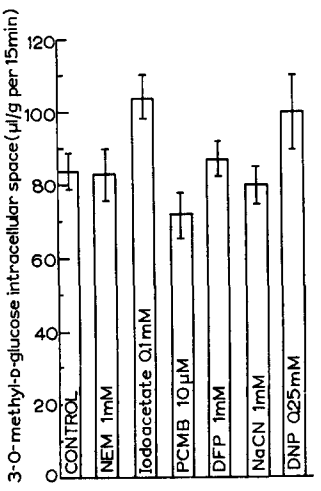


Fig. 7. Effect of 17β-estradiol and insulin *in vitro* on sugar transport. The experiment is described under RESULTS. Each bar represents the mean of five determinations and the vertical lines represents 2 standard errors of the mean.

Fig. 8. Effect of various metabolic inhibitors on sugar transport. The experiment is described under RESULTS. Each bar represents the mean of five determinations and the vertical lines represent 2 standard errors of the mean. Abbreviations: NEM, *N*-ethylmaleimide; PCMB, *p*-chloromercuribenzoate; DFP, diisopropylphosphorofluoridate and DNP, 2,4-dinitrophenol.

conditions 2,4-dinitrophenol and NaCN have been reported to increase sugar transport in the rat diaphragm[17,18] and the perfused isolated rat heart[19]. BATTAGLIA AND RANDLE[16] have reported that *N*-ethylmaleimide and *p*-chloromercuribenzoate inhibit D-xylose accumulation in the rat diaphragm, but that diisopropylphosphorofluoridate is without effect.

DISCUSSION

The following observations support the theory that a specific diffusable carrier transport system for sugars exists in rat uterus: (1) 3-*O*-Methyl-D-glucose transport exhibits saturation kinetics. This behavior can be interpreted most readily in terms of saturation of a finite number of combining sites necessary for transport. (2) The system exhibits substrate specificity as evidenced by the competitive inhibition of 3-*O*-methyl-D-glucose influx by D-mannose, D-glucose, 2-deoxy-D-glucose, and D-xylose, whereas D-galactose, L-arabinose, and D-lyxose did not compete. (3) The demonstration of countertransport establishes the existence of a specific mobile carrier type of transport system. It rules out simple diffusion and irreversible transport mechanisms. (4) The sugar is not concentrated intracellularly.

HALKERSTON *et al.*[6] have reported that D-xylose distributes to almost all of the cell water (92%) in the castrate uterus, and that estrogen treatment produces no change. In their experiments D-[^{14}C]xylose was injected into the rats 1.5 h before sacrifice. The 92% figure for distribution indicates that equilibration had nearly occurred and, therefore, their experiments gave no measure of the rate of entry of the sugar into the uterine cell water. We have found also that uteri from castrate and estrogen-treated animals transport sugars to approximately the same volume of uterine cell water after incubation for 1 h. However, the rate of transport in the latter case clearly is increased when measured after short inbation periods.

In this study the sorbitol space was defined as the extracellular space. It was found to be about 550 μl/g or 55%. HALKERSTON and co-workers[6-8] found similar values for the uterus using inulin, sucrose, and Na^+ distribution, but found values of approx. 20% for other tissues (adrenal, thymus, liver diaphragm and gastrocnemius). Because of the discrepancy between the uterus and other tissues, these authors concluded that inulin and sucrose penetrate uterine cells. This assumption is unnecessary since the castrate uterus obviously does contain an exceptionally large extracellular space. This is borne out by the high collagen content of the tissue. The uterus from an ovariectomized rat contains about 60 mg of collagen per g of wet tissue[21], whereas rabbit striate muscle has only 4 mg of collagen per g wet weight[20], corresponding to its extracellular space of about 20%.

The administration of physiologic doses of 17β-estradiol causes a 2-fold increase or more in the initial velocity of sugar transport (both influx and efflux) (Figs. 1, 2, 6) without any measurable change in the apparent K_m of transport (Fig. 2). The response appeared between 1 and 2 h. Incubation in the presence of 17β-estradiol was without effect *in vitro*. The fact that more than 1 h after administration of estrogen is required to produce an effect suggests that the increase is not due to a primary physicochemical interaction of the hormone with the transport system in the cell membrane. The studies of JENSEN AND JACOBSON[22] with immature rats indicate that 17β-estradiol is already present in the uterus within 20 min after

subcutaneous administration. Therefore, it seems likely that the estrogen-induced increase in transport depends upon antecedant metabolic changes.

Enhanced transport occurs as early as the increased glucose utilization *in vitro* demonstrated by SZEGO AND ROBERTS[1]. It occurs within the same time period as the increase in the incorporation of glucose into CO_2, RNA, lipid, and protein which was demonstrated by NICOLLETTE AND GORSKI[5]. It is not possible to determine whether enhanced transport contributes to the increase they observed or whether the changes simply occur simultaneously. Differences due to the animal age and strain, as well as to the dosage of estrogen also must be considered.

Increased transport precedes the estrogen-induced accumulation of glycogen in the uterus reported by BITMAN *et al.*[4]. They found only a small increase in glycogen at 2 h and a steady increase until 12–14 h. Our results show that by 2 h after administration of estrogen the rate of transport already is increased 2–4-fold. The accumulation of glycogen may result indirectly from increased transport of sugar, but a direct effect of estrogen upon reactions involved in glycogen synthesis or degradation also must be considered[23,24].

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