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DEMONSTRATION OF A D-GLUCOSE TRANSPORT SYSTEM IN THE BILIARY TREE OF THE RAT BY USE OF THE SEGMENTED RETROGRADE INTRABILIARY INJECTION TECHNIQUE

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Abstract—Using the segmented retrograde intrabiliary injection (SRII) technique, a 40-μl segment of radioactive D-glucose solution was washed into the biliary tree with 100 μl saline. The total volume exceeded the distended biliary tree capacity of the rat; nevertheless, about 18 per cent of the administered dose of glucose was retained by the membranes of the biliary tree and was recovered in recollected bile. Changes in this retained fraction were used to demonstrate the presence of a glucose transport system in the hepatobiliary system of the rat. The intraportal administration of phlorizin enhanced the biliary retention of [^{14}C]-D-glucose and [^{14}C]methyl-α-D-glucopyranoside given by SRII. Phlorizin treatment had no effect on the biliary retention of [^{3}H]mannitol, [^{3}H]-3-*O*-methyl glucose or [^{3}H]sucrose. The i.v. administration of a large loading dose of D-glucose also produced an increase in the hepatobiliary retention of [^{14}C]-D-glucose given by SRII. Furthermore, if D-glucose or methyl-α-D-glucopyranoside was added to the SRII solution of [^{14}C]-D-glucose, the retention of labeled glucose was enhanced. Mannitol and 3-*O*-methyl glucose had no such effect. Therefore, the experimental results demonstrated that, in the biliary tree of the rat, there existed a system which shows high selectivity for transferring D-glucose from bile to liver.

Intracellular concentrations of free glucose in liver have been found to parallel plasma concentrations [1,2]. In contrast to this relation, glucose concentrations in bile were far less than that in plasma [3–5]. In an attempt to explain this latter finding, Jenner and Symth [3] studied the biliary excretion of D-glucose in dogs treated with phlorizin, an inhibitor of D-glucose transport in the gastrointestinal mucosa and the proximal tubule of the kidney [6–8]. Following treatment with phlorizin, the concentration of glucose in bile rose to a value approaching the plasma concentration. They proposed that phlorizin might be blocking a transport process which reabsorbed glucose from bile. In a more recent study, Guzelian and Boyer [5] reported a similar increase in bile glucose concentration following the intraportal administration of phlorizin or methyl-α-D-glucopyranoside. These results, along with some experiments with a biliary stop flow system, indicated that glucose was reabsorbed from bile.

The purpose of this paper was to provide further insight into the existence of a system which reabsorbs glucose from bile, using a new technique called segmented retrograde intrabiliary injection (SRII). The general approach in the use of SRII has been presented [9]. The preliminary results described in that paper indicate that [^{14}C]-D-glucose, in contrast to [^{3}H]-3-*O*-methyl glucose and [^{3}H]mannitol, was taken up from bile more rapidly than expected. The present study expands and builds upon those preliminary results.

METHODS

Isotopes and chemicals. The following compounds were administered by the SRII procedure: [1-^{3}H]-D-mannitol (24 Ci/mmole), [*methyl*-^{3}H]-3-*O*-methyl-D-glucose (3.62 Ci/mmole), [D-glucose-^{14}C-(u)]methyl-α-D-glucopyranoside (275 mCi/mmole) (New England Nuclear Corp., Boston, MA); [6,6′-(*n*)-^{3}H]sucrose (2.0 Ci/mmole) (Amersham/Searle Corp., Arlington Heights, IL); and [U-^{14}C]-D-glucose (10.98 mCi/mmole) (ICN Pharmaceuticals, Inc., Irvine, CA). Phlorizin, 3-*O*-methyl glucose and methyl-α-D-glucopyranoside (Sigma Chemical Co., St. Louis, MO) were administered along with the radioactive compounds in other experiments.

Animal preparation. Male Sprague–Dawley rats (ARS Sprague–Dawley, Madison, WI), weighing 298–410 g, were anesthetized and surgically prepared with a femoral vein and common bile duct cannula, as described previously [9]. The rats were maintained at 37 ± 0.5°.

Segmented retrograde intrabiliary injection (SRII) procedure. The general procedure for SRII was described previously [9]. The details specifically pertinent to the present experiment were as follows. The SRII consisted of a retrograde intrabiliary injection of an initial 40 μl "segment" of solution containing a given radioactive marker compound (5–10

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μCi/ml in 0.9 per cent saline), followed by a 110 μl wash in with 0.9 per cent saline. The SRIIs were given at rates of 2.3, 5.7, 11.3 or 22.7 μl/sec. Within 5 sec after the SRII injection, individual bile drops were collected and the radioactivity was counted as before. The radioactive content in each drop of bile was expressed as the percentage of the total counts administered by SRII.

Treatment. All but one group of rats received several SRIIs prior to (control) and following treatment with 30 mg phlorizin. The phlorizin was dissolved in propylene glycol. This solution (0.1 ml) was slowly injected into the portal vein through a 30 gauge needle. Following injection and withdrawal of the needle, gentle pressure was applied upon the vessel for about 30 sec to prevent bleeding. For each rat control, SRIIs were made 10 min after the intraportal injection of 0.1 ml propylene glycol, and then the SRIIs were repeated 10 min after phlorizin treatment. A period of 10 min was allowed between collection of the last sample of bile and initiation of the next treatment.

One group of rats was given three SRIIs prior to and following the systemic administration of D-glucose. In this experiment, an aqueous solution of D-glucose (0.8 g) was injected into a femoral vein cannula (PE 50). Ten min after glucose loading, the three SRIIs were repeated.

Competition experiments. In two experiments, the 40 μl volume with the initial [^{14}C]-D-glucose for the SRII was dissolved in a solution containing a nonradioactive sugar or sodium chloride. These solutions contained 300 mosmolal mannitol, methyl-α-D-glucopyranoside, D-glucose, 3-*O*-methyl glucose or sodium chloride. The experiments were designed to determine what effect a nonradioactive sugar would have on the recovery of [^{14}C]-D-glucose in bile, when both were given by SRII.

Analytical procedures. The ^{3}H and ^{14}C contents of bile samples were estimated by counting in a liquid scintillation spectrometer (ISOCAP 300, Searle Analytic Inc., Des Plaines, IL). The scintillation medium consisted of 4 g of 2,5-diphenyloxazole (PPO) and 50 mg of 1,4-bis[2-(5-phenyloxazolyl)]-benzene (POPOP) dissolved in 1.0 liter toluene and 0.5 liter Triton X-100.

Statistical analysis. The paired *t*-test [10] was used in Figs. 1, 2, 5 and 6 to compare control results with those after treatment with phlorizin. One-way analysis of variance and Dunnett's test [11] were used to compare recoveries in Figs. 3 and 4. For both tests significance was set at $P < 0.05$.

RESULTS

Figure 1 shows the mean SRII recollection curves for labeled D-glucose, methyl-α-D-glucopyranoside and 3-*O*-methyl glucose that were obtained from groups of rats before and after treatment with phlorizin. The structural formula of the sugar in each panel designates the radioactive marker compound which was given by SRII. In the top panel, a 17.6 ± 2.0 per cent (mean ± S.E.) recovery of [^{14}C]-D-glucose in bile was obtained for the control. This value corresponded to the area under the control SRII recollection curve. Also, this recovery value represented that portion of the administered dose which was retained within the biliary system following administration by SRII. Following treatment with 30 mg phlorizin, the same [^{14}C]-D-glucose SRII gave a significantly larger per cent recovery. Thus, treatment with phlorizin increased the amount of [^{14}C]-D-glucose that was retained in the biliary tree and thereby recollected in the bile. This sensitivity to phlorizin indicated the presence of a glucose carrier system. In the middle panel, the marker compound given by SRII was [^{14}C]methyl-α-D-glucopyranoside, the 1-methyl derivative of D-glucose. The SRII recollection curves for the 1-methyl derivative were

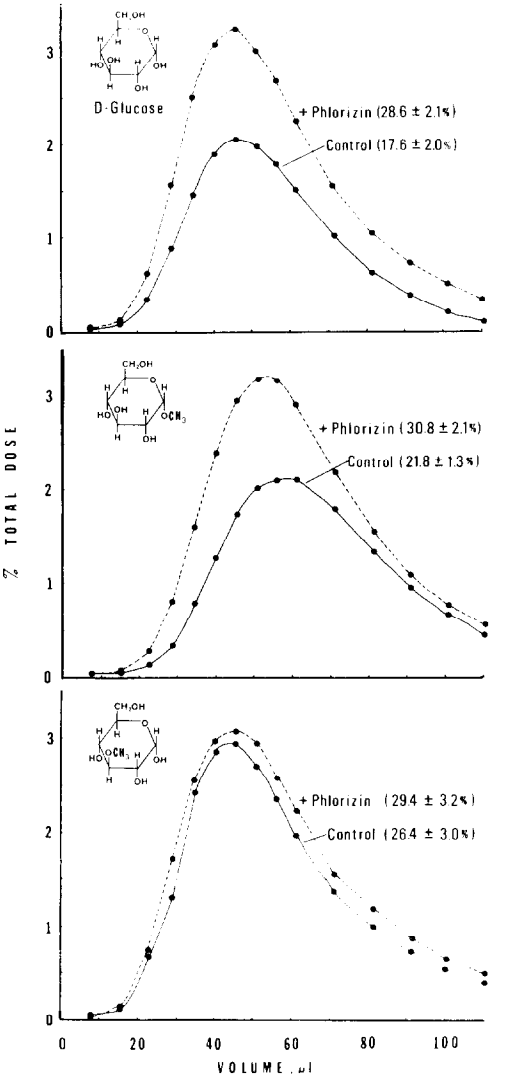

Fig. 1. Mean SRII recollection curves of radioactivity recovered in bile drops after SRII. An SRII rate of 23 μl/sec was used in the three groups of five or more rats each. Each group was given an SRII of [^{14}C]-D-glucose (top panel), [^{14}C]methyl-α-D-glucopyranoside (middle panel), or [^{3}H]-3-*O*-methyl glucose (bottom panel), before (control) and 10 min after the intraportal administration of 30 mg phlorizin. The values in parentheses are the mean ± S.E. per cent recovery of each marker compound, obtained from calculating the area under the respective SRII recollection curve.

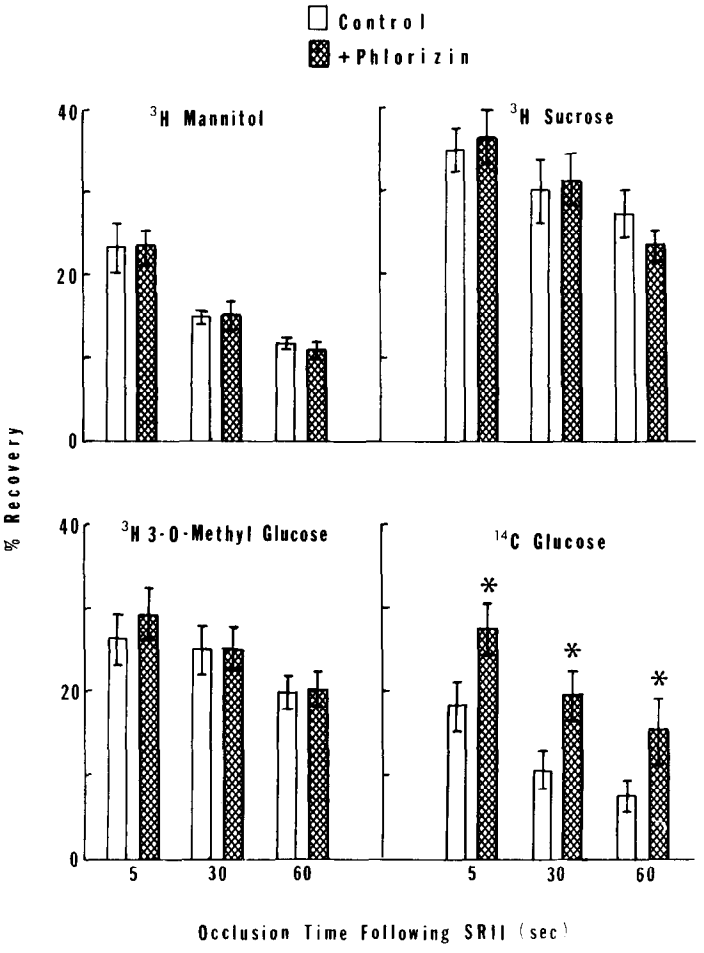

Fig. 2. Effect of phlorizin treatment and increasing occlusion time on the recovery of four radioactive marker compounds given by SRII (23 μl/sec). Each panel gives the mean ± S.E. per cent recovery of a marker compound before (control, open bars) and after administration of 30 mg phlorizin (hatched bars) to a group of four rats each. The asterisks (*) indicate the phlorizin treatments which produced a significant alteration from the control recovery value (t-test).

similar to that for D-glucose in the top panel. Phlorizin treatment again increased the amount of the marker retained within the biliary system. On the other hand, the SRII recollection curves of [^{3}H]-3-*O*-methyl glucose in the bottom panel gave results which were different from D-glucose in two ways. First, the control recovery for this derivative, 26.4 ± 3.0 per cent was significantly greater than the control recovery of labeled D-glucose (17.6 ± 2.0 per cent). Second, phlorizin treatment did not increase significantly the recovery of this derivative in contrast to the above sugars. It should be noted that phlorizin treatment increased the recovery of [^{14}C]-D-glucose (and also of [^{14}C]methyl-α-D-glucopyranoside) to a value coincident to that of [^{3}H]-3-*O*-methyl glucose.

The experiment shown in Fig. 2 was designed to assess the effect of increasing the period of biliary occlusion on the recovery of four marker compounds. Increase in occlusion time, after the SRII, was accomplished by increasing the usual time interval of 5 sec between completion of the SRII and start of the recollection of bile drops to 30 and 60 sec. In this experiment, groups of rats were given SRIIs of either labeled mannitol, sucrose, 3-*O*-methyl glucose or D-glucose at the three occlusion times, before and after treatment with 30 mg phlorizin. With all four of the marker compounds, an increase in occlusion time from 5 to 30 and 60 sec produced a decrease in the per cent recovery both during the control and the phlorizin treatment situations. This decrease in recovery arose from the increase in duration of contact of the SRII solution with biliary tree, which was produced by prolonging the occlusion time. With labeled mannitol, sucrose and 3-*O*-methyl glucose as the marker compounds, no differences were found at the three occlusion times between each of the controls and phlorizin treatments. Phlorizin had no effect on these marker compounds. In contrast, phlorizin treatment significantly increased recoveries at the three occlusion times when D-glucose was the marker compound in the SRII.

As seen from the results in Figs. 1 and 2, the phlorizin sensitive sugars were D-glucose and methyl-α-D-glucopyranoside. Therefore, these two sugars were studied further in Fig. 3 to assess the effect of changing another variable of the SRII, the rate of

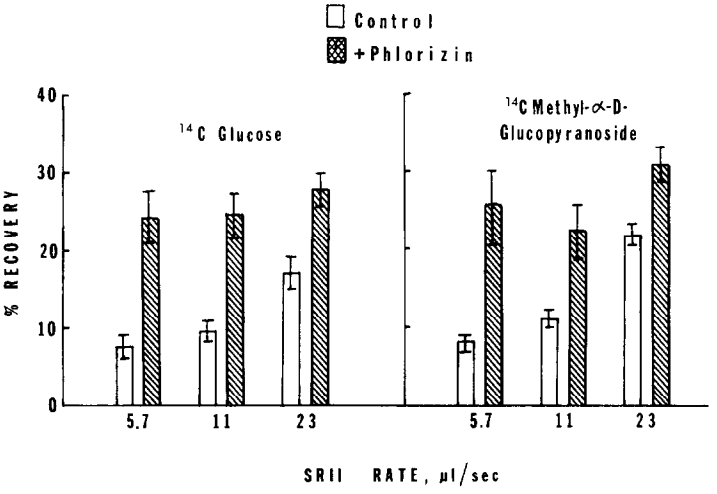

Fig. 3. Effect of the rate of administration of the SRII of [^{14}C]-D-glucose and [^{14}C]methyl-α-D-glucopyranoside in relation to phlorizin treatment. Each panel gives the mean ± S.E. of recoveries in six rats before (control) and after treatment with 30 mg phlorizin. In each panel, one-way analysis of variance followed by Dunnett's test, using the response at 23 µl/sec as the standard, showed a significant difference in the control recoveries obtained at the other two SRII rates, while no significant differences were obtained with the different rates after phlorizin treatment.

administration of the SRII. The control SRII recoveries of both compounds increased as the rate was increased from 5.7 to 23 µl/sec, while the recoveries within the phlorizin treatment groups showed no significant changes with increase in SRII rate (one-way analysis of variance). This experiment was instructive in showing that the greatest difference between control and phlorizin treatment could be obtained by using the slower rate of SRII administration.

Figure 4 shows the results obtained from competition experiments which were done at an even slower SRII rate of 2.3 µl/sec. In this study, the initial 40-µl segment of the SRII contained [^{14}C]-D-glucose in a 300 mosmolal solution of nonradioactive NaCl (saline), mannitol, methyl-α-D-glucopyranoside, D-glucose or 3-0-methyl glucose. Panel A gives the results from one experiment, where the control [^{14}C]-D-glucose recoveries were very similar, with sodium chloride and mannitol in the 40-µl segment.

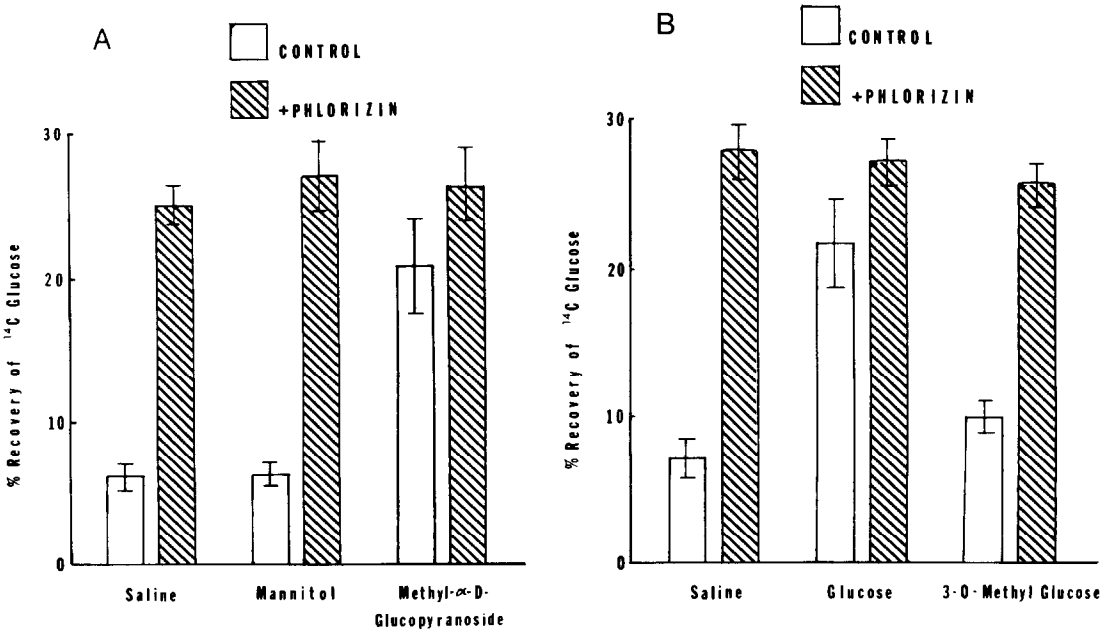

Fig. 4. Effect of adding various sugars to [^{14}C]-D-glucose in the SRII. An SRII rate of 2.3 µl/sec was used. The initial 40 µl segment of the SRII contained [^{14}C]-D-glucose in a 300 mosmolal solution of nonradioactive NaCl (saline), mannitol, methyl-α-D-glucopyranoside, D-glucose or 3-*O*-methyl glucose (indicated on abscissa). Each panel gives the mean ± S.E. (N = 6) recovery for [^{14}C]-D-glucose in the SRII experiments before (control) and after the intraportal administration of 30 mg phlorizin. The respective responses in the saline groups served as the standard in Dunnett's test.

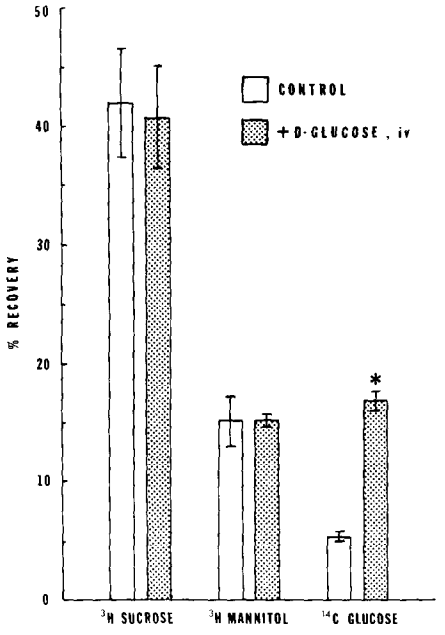

Fig. 5. Recovery of [^{3}H]sucrose, [^{3}H]mannitol and [^{14}C]-D-glucose given by SRII before (control, open bars) and after (stippled bars) the femoral vein administration of 0.8 g of D-glucose. The recoveries are the mean ± S.E. of a group of six rats. The SRIIs were given at a rate of 2.3 μl/sec. An asterisk (*) indicates that the treatment was significantly different from the corresponding control value (*t*-test).

In contrast, the control [^{14}C]-D-glucose recovery increased significantly when methyl-α-D-glucopyranoside was included in the 40-μl segment with the [^{14}C]-D-glucose. Results from another experiment are given in panel B. The control [^{14}C]-D-glucose recoveries with sodium chloride and 3-*O*-methyl glucose were similar to the results for sodium chloride and mannitol in panel A. A significant increase in control [^{14}C]-D-glucose recovery was seen when nonradioactive D-glucose was placed in the 40 μl segment with [^{14}C]-D-glucose. Thus, the addition of nonradioactive D-glucose or methyl-α-D-glucopyranoside to the 40-μl segment resulted in a significant increase in the control [^{14}C]-D-glucose per cent recovery. Now examine the effect of phlorizin treatment. Because the inclusion of glucose and methyl-α-D-glucopyranoside had already raised the recovery of [^{14}C]-D-glucose, phlorizin treatment in this situation had little effect in raising the recovery further. In the case of 3-*O*-methyl glucose, mannitol and sodium chloride, which did not alter [^{14}C]-D-glucose recovery, the intraportal administration of phlorizin had the expected effect of raising [^{14}C]-D-glucose recovery. The magnitude of the effect of phlorizin treatment appeared to be the same at this 2.3 μl/sec rate, as the 5.7 μl/sec results in Fig. 3.

The experiments in Fig. 5 illustrate further the competitive processes for the biliary uptake of D-glucose. The biliary uptake of three marker compounds was studied by SRII before and after femoral vein administration of 0.8 g of D-glucose. The SRII recovery of [^{3}H]sucrose and [^{3}H]mannitol was unaffected by systemic loading with D-glucose. On the other hand, the recovery of [^{14}C]-D-glucose was increased significantly following D-glucose. Thus, loading with D-glucose produced a specific effect.

Additional factors in the action of phlorizin were considered in the experiment in Fig. 6. The bile flow rate and SRII per cent recovery of [^{14}C]-D-glucose and [^{3}H]sucrose are shown following intraportal injections of 0.1 ml saline, 0.1 ml propylene glycol (PG) and 30 mg phlorizin. The control results indicated that propylene glycol, the solvent for phlorizin, had no effect on the variables measured. Phlorizin treatment significantly increased bile flow rate during the initial 60 min following administration. During this period the characteristic effect of increased [^{14}C]-D-glucose SRII recovery was obtained. Furthermore, at 150 min following treatment, when the choleretic effect was gone, the effect of phlorizin of increasing [^{14}C]-D-glucose recovery still persisted. Over the duration of the experiment, phlorizin treatment produced no effect on [^{3}H]sucrose SRII recovery. Thus, the effect of phlorizin on [^{14}C]-D-glucose recovery was not directly related to the choleretic effect, and it was probable that the nonresponsiveness of sugars, such as sucrose, to phlorizin was unrelated to the choleresis.

DISCUSSION

The preceding paper [9] presented the SRII method as an approach for assessing the filtering properties of the rat biliary tree. It was proposed that, during the SRII, a portion of the administered marker compound was retained at filtration sites of the biliary tree, which may include the bile canaliculi. The SRII recollection curve represented the distribution of the radioactive marker which was retained in the biliary tree, prior to its recovery in bile. The area under this curve corresponded to the per cent recovery of the compound in bile. Studies with compounds of a similar molecular weight range indicated that mannitol and 3-*O*-methyl glucose had very similar SRII per cent recoveries, while D-glucose had a significantly lower recovery. Thus, D-glucose appeared to be taken up from the biliary tree more rapidly than other compounds of similar molecular weight.

The results in Fig. 1 confirm and extend these findings. The control SRII per cent recoveries (area under curve) of [^{14}C]-D-glucose and [^{14}C]methyl-α-D-glucopyranoside (1-methyl glucose) were significantly less than the per cent recovery for [^{3}H]-3-*O*-methyl glucose. The SRIIs were then repeated following the intraportal administration of 30 mg phlorizin, a competitive inhibitor of glucose transport. Phlorizin treatment significantly increased the amounts of D-glucose and methyl-α-D-glucopyranoside recovered, while having no effect on the recovery of 3-*O*-methyl glucose. In fact, the phlorizin-induced block resulted in similar SRII per cent recoveries for all three marker compounds. Thus, it appears that phlorizin blocked the uptake of D-glucose and methyl-α-D-glucopyranoside from the hepatobiliary system to a point where the recoveries were like those of 3-*O*-methyl glucose and mannitol (nontransported sugars). This finding indicates that phlorizin specifically blocked a carrier system for D-glucose and methyl-α-D-glucopyranoside.

Further support of the specificity of inhibition of D-glucose uptake by phlorizin is illustrated in Fig. 2.

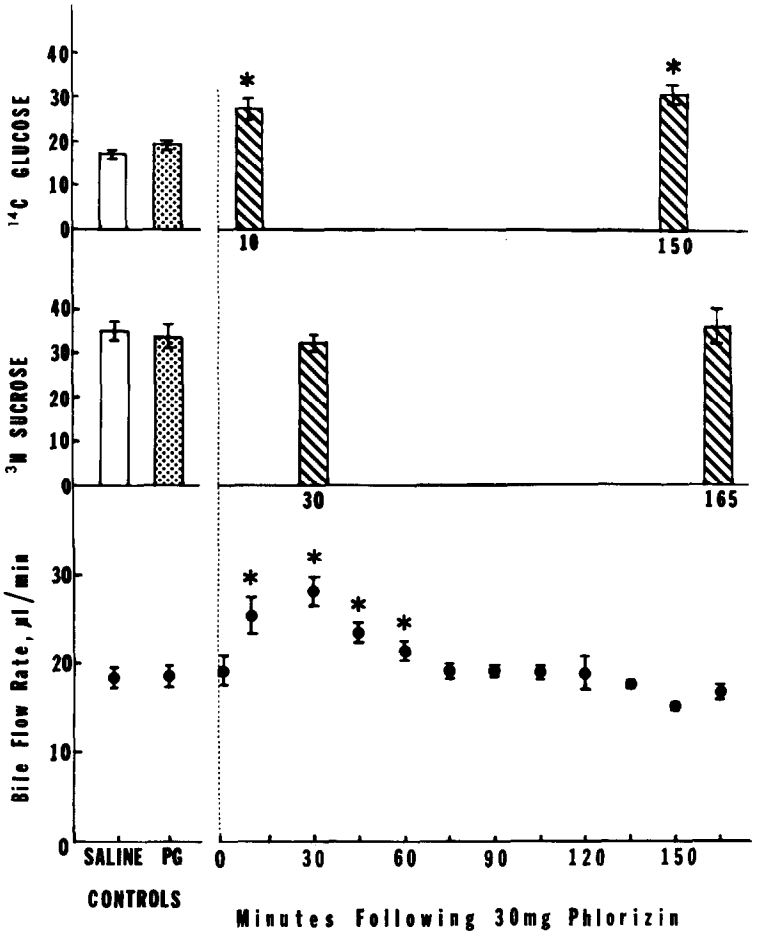

Fig. 6. Effect of phlorizin on bile flow rate in relation to the percent recovery of [^{3}H]sucrose and [^{14}C]-D-glucose given by SRII. The sugars were given by SRII at a rate of 22.7 µl/sec. Each rat was given the sequence of treatments shown on the abscissa, consisting of the intraportal administration of 0.1 ml saline, propylene glycol (PG) and phlorizin (30 mg). The results represent the mean ± S.E. of a group of seven rats. An asterisk (*) indicates that the phlorizin result was significantly different from the propylene glycol control (*t*-test).

Phlorizin treatment produced no change in the recovery of [^{3}H]mannitol, [^{3}H]sucrose and [^{3}H]-3-*O*-methyl glucose given by SRII, while it significantly increased the recovery of [^{14}C]-D-glucose. Furthermore, these results indicate that, even with prolonged occlusion time or increased biliary contact time, the effect of phlorizin remained specific for D-glucose.

The larger difference between control and phlorizin groups at the slower SRII rate in Fig. 3 could be due to the longer duration of the slower SRII. The slower SRII rate may have allowed more time for the glucose to be transported and a greater blocking effect of phlorizin to be manifested than at the fast SRII rate. Similarly, the results in Fig. 2 may indicate that the blocking effect of phlorizin was relatively greater (*ca.* 50 per cent) at the longer occlusion time than at the shorter occlusion time (*ca.* 25 per cent). That is, the longer contact time between the glucose and the biliary tree in both sets of experiments leads to a relatively greater blocking effect of phlorizin on glucose transport. However, this conclusion is tenuous because it could be argued that in the occlusion time experiments (Fig. 2) the absolute effect of phlorizin was no greater at 60 sec than at 5 sec of occlusion.

The experiments in Fig. 4 were designed to assess what effect various nonradioactive sugars would have on the recovery of [^{14}C]-D-glucose when both were given together in the same SRII solution. From the control SRIIs it appears that methyl-α-D-glucopyranoside and D-glucose interfered with the hepatobiliary uptake of [^{14}C]-D-glucose and produced a significantly larger recovery of [^{14}C]-D-glucose. In Fig. 5, the intravenous administration of nonradioactive D-glucose produced an increase in the recovery of [^{14}C]-D-glucose given by SRII, while having no effect on the recovery of [^{3}H]sucrose or [^{3}H]mannitol. Thus, it was possible to arrive at the same kind of specificity for labeled D-glucose, whether the blocking agents were given along with the labeled D-glucose in the SRII solution, or whether the blocking agent was given systemically.

Whenever enhanced labeled D-glucose recoveries were produced by the different blocking agents, the recoveries approached, but never surpassed, values found for nontransported sugars, such as mannitol. Thus, the drug-induced block in the biliary absorp-

tion of [^{14}C]-D-glucose had no effect on the glucose which entered the liver by solvent drag.

In addition to blocking the hepatobiliary uptake of D-glucose and methyl-α-D-glucopyranoside, phlorizin is shown in Fig. 6 to produce a moderate increase in bile flow rate of rats. Phlorizin has been shown previously by Jenner and Smyth [12] to have a choleretic effect in the dog, while Guzelian and Boyer [5] reported no choleretic effects in rats treated with phlorizin. Regardless of these divergent findings, our results in Fig. 5 indicate that phlorizin continued to specifically block the uptake of D-glucose long after the bile flow rate returned to the control level. Thus, the initial phlorizin-induced choleresis was not responsible for its effects on SRII D-glucose recovery.

The results reported in the present paper support the concept that there exists in the hepatobiliary system of the rat a specific system for absorbing D-glucose from bile. The specificity of phlorizin in increasing D-glucose recovery in these experiments was similar to that reported for phlorizin in the dog kidney [8]. Silverman and Black [8] found that D-glucose and methyl-α-D-glucopyranoside demonstrated very high affinity for the phlorizin receptors on the brush border of the proximal tubule *in vivo* and *in vitro*, while 3-*O*-methyl glucose demonstrated no significant affinity. They further concluded that these phlorizin receptor sites were either in close proximity or identical to the glucose transport receptor.

We believe that the results of the present experiments demonstrate the kind of utility the technique of SRII has in studying hepatobiliary function. In a broader sense, the present SRII approach should be applicable to studying other transport processes in the hepatobiliary system [13].

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