50 μg of III/ml and 5 μg of IV and V/ml. These concentrations greatly exceed those expected in plasma. None of the three metabolites was recorded.

Application—The technique was applied to study phenylbutazone elimination after oral administration to humans. The mean plasma phenylbutazone concentration–time curve is depicted in Fig. 2. The mean elimination half-life was 68.5 hr.

The technique has been applied in studying the pharmacokinetics of phenylbutazone in humans after the administration of single and multiple doses[12].

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Effect of Enzyme-Inducing and Enzyme-Inhibiting Agents on Drug Absorption I: 3-*O*-Methylglucose Transport in Rats

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Abstract □ The influence of preadministration of phenobarbital, benzo[*a*]pyrene, and proadifen hydrochloride on 3-*O*-methylglucose transfer across the everted rat small intestine was examined. The active and passive components of the sugar transport mechanism were evaluated using phlorizin, a potent inhibitor of active transport of sugars. Pretreatment of the animals with phenobarbital did not alter 3-*O*-methylglucose transfer characteristics. Pretreatment with intraperitoneally administered benzo[*a*]pyrene increased mucosal to serosal transfer of the sugar at low (0.1 m*M*) sugar concentrations. Enhancement of the active transfer of 3-*O*-methylglucose by pretreatment with proadifen hydrochloride was noted at low sugar concentrations. The passive transfer of the sugar was reduced after pretreatment with proadifen hydrochloride.

Keyphrases □ 3-*O*-Methylglucose—transfer across everted rat small intestine, effect of preadministration of phenobarbital, benzo[*a*]pyrene, and proadifen hydrochloride □ Transport, drug—3-*O*-methylglucose, transfer across everted rat small intestine, effect of preadministration of phenobarbital, benzo[*a*]pyrene, and proadifen hydrochloride □ Enzyme inducers—phenobarbital, benzo[*a*]pyrene, and proadifen hydrochloride, effect on transfer of 3-*O*-methylglucose across everted rat small intestine □ Phenobarbital—effect of preadministration on transfer of 3-*O*-methylglucose across everted rat small intestine □ Benzo[*a*]pyrene—effect of preadministration on transfer of 3-*O*-methylglucose across everted rat small intestine □ Proadifen hydrochloride—effect of preadministration on transfer of 3-*O*-methylglucose across everted rat small intestine

Intestinal glucose absorption is an active energy-expending process (1) believed to be controlled enzymatically and susceptible to alteration by chemical agents known to affect cell metabolism (2–4). The structural specificity of this particular mechanism has been well documented (5, 6). The 3-*O*-methyl derivative possesses transport characteristics similar to glucose; however, it is not metabolized by animal tissue (7). Thus, this sugar is useful for studying the sugar transport process across intestinal tissue.

Recent investigations showed the ability of various hepatic enzyme-inducing agents to enhance drug metabolism in the intestinal mucosa (8). Phenobarbital produced a striking hypertrophy of the small intestinal endoplasmic reticulum with an increase in this tissue's *N*-demethylase activity (9). However, no difference in mucosal to serosal transfer of ^{14}C-3-*O*-methylglucose or ^{14}C-palmitic acid was observed compared to controls. Previously, evidence that phenobarbital pretreatment heightened the mucosal transfer rate of ^{59}Fe-iron sulfate in duodenal rat intestinal sacs was reported (10). That effect may be due, in part, to an increased synthesis of a carrier molecule. In addition, subcutaneous phenobarbital injections enhanced active bile salt ileal transport (11).

It was of interest, therefore, to examine the possible effect of pretreatment with known hepatic enzyme-inducing agents on the active transport of 3-*O*-methylglucose

Table I—3-*O*-Methylglucose Transfer across the Everted Rat Small Intestine as a Function of Concentration and Time

Concentration, m*M*	*n*	Cumulative 3-*O*-Methylglucose Transferred with Time, m*M* × 10^3			
		30 min	60 min	90 min	120 min
1	10	1.40	2.92	4.42	5.81
2	6	2.31	4.74	6.72	8.29
3.5	3	3.00	7.41	11.7	15.7
5	8	5.54	11.1	15.7	19.7
6.25	3	5.00	11.0	16.4	21.4
7.5	10	5.27	13.0	19.8	26.3
8.5	2	5.28	12.2	19.4	27.1
10	8	7.56	17.3	25.8	33.9
		With Phlorizin (0.08 m*M*)			
1	4	0.42	0.61	0.86	1.22
2	4	0.51	0.95	1.61	2.69
5	4	0.61	1.74	3.57	6.08
10	3	2.25	6.09	10.73	15.78

across rat jejunal sacs. The agents examined were phenobarbital, proadifen hydrochloride (I), and benzo[*a*]pyrene (II); all previously were found to alter hepatic drug metabolism. The experimental drug, I, is well documented as a rather nonspecific enzyme inhibitor (12) but also was found to induce microsomal enzyme activity at later periods following administration (13).

EXPERIMENTAL

Animals and Treatment—Male Sprague–Dawley rats[1], 176–250 g, were maintained on regular rat chow and water *ad libitum*. The rats were fasted 24 hr prior to the experiment. The animals were given 80 mg of phenobarbital sodium[2]/kg ip or po for 3 consecutive days before the experiment.

Compound II[3] was administered intraperitoneally or orally dissolved in corn oil at a dosage of 20 mg/kg 48 hr prior to the experiment. The last group received I[4] either orally or intraperitoneally dissolved in water at a dose of 100 mg/kg 24 hr prior to the experiment. Control animals were dosed similarly with the appropriate vehicle.

Preparation of Jejunal Sacs—Under ether anesthesia, a midline incision was made and the entire small intestine was removed. The intestine was immediately rinsed with cold saline, and the first 15 cm distal to the pylorus was discarded. The remaining intestine was everted over a glass rod. Either the Wilson and Wiseman (14) technique of everted sacs or a method described by Feldman and Gibaldi (15) utilizing cannulated sacs was used.

For both preparations, the mucosal bathing solution and the serosal solution consisted of pH 7.4 Krebs–Henseleit buffer. The buffer pH was checked at the beginning and end of the experiments. Prior to use, all serosal solutions were bubbled with 95% oxygen–5% carbon dioxide; the mucosal solutions were bubbled continuously throughout the experiments. In all studies, the temperature of the preparations was maintained at 37 ± 0.2°.

Serosal to Mucosal Concentration Ratio Experiment—For these studies, 5-cm sacs were prepared as described by Wilson and Wiseman (14). After filling the sacs with 0.5 ml of buffer containing 0.78 m*M* 3-*O*-methylglucose[5], the sacs were incubated in 15 ml of mucosal buffer of the same sugar concentration for 0.5 hr. After incubation, the contents of the everted intestinal sacs and mucosal solution were analyzed for the sugar.

Cannulated Everted Intestinal Sacs—Everted intestinal segments, 10 cm, were hung from glass cannulas, as described by Feldman and Gibaldi (15), and were suspended in 60 ml of mucosal buffer containing varying concentrations of 3-*O*-methylglucose. After placement, 2 ml of buffer, free of the sugar, was instilled into the sacs. The serosal fluid was withdrawn at predetermined intervals, followed by rinsing of the serosa with 2 ml of buffer which was then combined with the initial sample for that time period. Another 2 ml was then placed into the sac for the next interval. Intervals were chosen such that the accumulated serosal concentration of 3-*O*-methylglucose never exceeded 30% of that of the mucosal solution.

Table II—Estimates of the Transfer Rate Constant for 3-*O*-Methylglucose Transport

	Graphical Analysis	Computer Fit
K_D, liters/min	8.8×10^{-6}	9.26×10^{-6}
K_m, m*M*	5.45	5.32
V_{max}, m*M*/min	2.50×10^{-4}	2.44×10^{-4}

In certain experiments, 0.08 m*M* phlorizin[6] was dissolved in both serosal and mucosal buffers. Phlorizin is a potent inhibitor of active sugar absorption (16). This concentration of phlorizin completely inhibited active sugar transport as determined by transfer experiments at a 10 m*M* sugar concentration.

Tissue Uptake and Transfer of ^{14}C-3-*O*-Methylglucose—Mucosal solutions were prepared such that they contained 0.03 μCi of ^{14}C-3-*O*-methylglucose[7] with appropriate addition of unlabeled sugar to assure a final concentration of 0.1 m*M*. Everted ligated sacs, 10 cm, prepared according to Wilson and Wiseman (14), were filled with 1.0 ml of buffer free of sugar and incubated for 10 min in 15 ml of mucosal solution. The sac contents and mucosal fluid were saved for liquid scintillation analysis. The sac tissue was rinsed quickly with 2 ml of water and then homogenized[8] in 2 ml of methanol. The homogenate was then centrifuged, and the methanol supernate was counted. Approximately 89% of all labeled compound added to the preparation was accounted for.

Assay—A colorimetric method (17) was employed for the quantitation of unlabeled 3-*O*-methylglucose. All solutions were then filtered[9] and analyzed spectrophotometrically[10] at 500 nm. The 3-*O*-methylglucose concentration was calculated using standard curves, taking into account blank tissue values.

Final solutions from ^{14}C-3-*O*-methylglucose experiments were analyzed for radioactivity with a liquid scintillation spectrometer[11]. From volume-adjusted samples, 0.2-ml aliquots were combined with 11 ml of scintillation cocktail[12] for counting both the aqueous and methanol solutions.

Data Evaluation—Transfer rates for each segment were calculated by averaging the amounts appearing in the serosal fluid for each time interval or from the slope of the cumulative amount as a function of time plot. With repeated experiments, rate determinations by either method yielded identical values. Statistical analysis of the data was evaluated by the Student *t* test (18). In all experiments, the two intestinal sacs prepared from each animal revealed no significant difference in transfer capabilities.

RESULTS AND DISCUSSION

To ensure the integrity of the transport mechanism and to characterize this process under the experimental conditions, sugar transfer rates were determined at various mucosal drug concentrations. Table I lists the average cumulative millimoles of 3-*O*-methylglucose transferred at the end of each time period with respect to the mucosal concentration. The amounts in the lower portion of the table are cumulative millimoles of 3-*O*-methylglucose transferred for experiments performed in the presence of 0.08 m*M* phlorizin. Sugar transfer with phlorizin would be indicative of the passive transfer rate of 3-*O*-methylglucose across the everted rat small intestine. Inspection of the rates for each concentration during each individual time period shows that the fluxes were similar up to 90 min, thus giving reason to believe that a steady state was obtained and that the integrity of the active transport process was maintained.

The 90–120-min interval is somewhat inconclusive because of the accelerated passive component at higher 3-*O*-methylglucose concentrations (Table I). The low calculated passive clearance may suggest a membrane-limited passive diffusion process as reported (19) for drugs with similarly low clearance values across rat intestinal sacs. Such drugs would display increases in their diffusion in the later stages of incubation, resulting from a loss of the functional integrity of the epithelial mucosal barrier. However, a tissue accumulation phenomenon as described for

[1] Texas Inbred Mice Co.
[2] Merck and Co., Rahway, N.J.
[3] Eastman-Kodak Co., Rochester, N.Y.
[4] Smith Kline and French Laboratories, Philadelphia, Pa.
[5] Calbiochem, Los Angeles, Calif.
[6] Sigma Chemical Co., St. Louis, Mo.
[7] New England Nuclear, Boston, Mass.
[8] Polytron model 125-C, Brinkmann Instruments.
[9] Type HA, 0.45 μm, Millipore Corp., Bedford, Mass.
[10] Coleman model 124.
[11] Packard model 3326 Tri-Carb.
[12] PCS, Amersham/Searle Corp., Arlington Heights, Ill.

Table III—Ratio of Serosal to Mucosal (S/M) Concentration of 3-*O*-Methylglucose[a] after 30 min of Incubation

Treatment	*n*	Ratio (S/M)[b]
Control	44	1.57 ± 0.25
Phenobarbital[c]	20	1.58 ± 0.23
II[d]	16	1.53 ± 0.32
I[e]	8	1.95 ± 0.29[f]

[a] Initial mucosal and serosal concentration = 0.78 m*M*. [b] Mean ± *SD*. [c] Given 80 mg/kg ip for 3 days prior to experiment. [d] Given 20 mg/kg ip for 48 hr prior to experiment. [e] Given 100 mg/kg ip for 24 hr prior to experiment. [f] $p < 0.05$.

methyl orange (20) cannot be ruled out. For this reason, only data up to 90 min were considered for the calculation of transfer rates.

Figure 1 shows the relationship between transfer rates obtained from the data in Table I and the 3-*O*-methylglucose mucosal concentration. The total active and passive transfer and the passive diffusion are illustrated separately.

The apparent active component can be evaluated from the observed differences in comparing the two sets of data. The application of Michaelis–Menten kinetics to a number of active intestinal transport systems was documented (21, 22) and yielded useful information regarding the characteristics of these transport processes. This fairly simple model was imposed on the apparent active transport, thus allowing estimation of the parameters descriptive of this treatment. Values for V_{max} of 2.50 $\times 10^{-4}$ m*M*/min and for K_m of 5.45 m*M* were calculated from linear regression analysis under the conditions of the experiments, and the representative Lineweaver–Burke plot is illustrated in Fig. 2.

For purposes of comparison, overall transfer rates for the case where the inhibitor was not present were again utilized for reestimation of parameters by a nonlinear least-squares computer program. The equation for the iterations was:

$$R = K_D[C] + \frac{V_{max}[C]}{K_m + [C]} \qquad \text{(Eq. 1)}$$

where R refers to the observed sugar transfer rate, K_D is the constant for passive clearance, C is the 3-*O*-methylglucose concentration in the mucosal buffer, and V_{max} and K_m are Michaelis–Menten kinetic constants. Computer estimates of V_{max}, K_m, and K_D appear in Table II. Original estimates were determined from linear regression analysis and the passive rate (from data presented in Fig. 1 and Table I). Data points at each concentration were weighted with the reciprocal of the variances. Comparatively, both methods of approximation gave fairly similar values for the parameters for the experiments (Table II).

The results of studies employing the Wilson and Wiseman (14) technique for comparing differences in intestinal sugar transport in treated and control animals appear in Table III. All segments were incubated for 1 hr, and pretreatments were performed as described under *Experimental*. These experiments were designed to provide a means for preliminary screening for the effects of pretreatment. Segments from control animals had final serosal to mucosal concentration ratios of 1.57 ± 0.25.

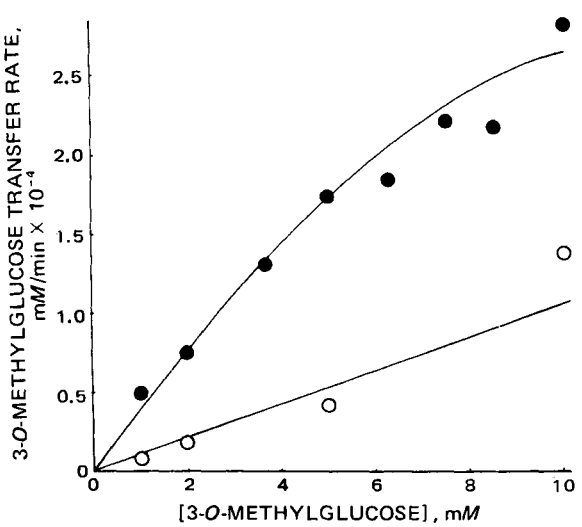

Figure 1—*Plot of the 3-O-methylglucose transfer rate across the everted rat small intestine* versus *3-O-methylglucose concentration alone (●) and in the presence of 0.08 m*M *phlorizin (○).*

Table IV—Effect of Pretreatment on the Rate and Amount of 3-*O*-Methylglucose[a] Transferred across the Everted Rat Small Intestine

Treatment	*n*	Rate of 3-*O*-Methylglucose Transfer, m*M*/min × 10^{-6} ± *SD*	Amount Transferred in 2 hr, m*M*
Control	10	49.2 ± 10.1	5.81 ± 0.99
Phenobarbital, oral	2	44.3	5.67
Phenobarbital, intraperitoneal	4	40.7 ± 5.4	4.73 ± 0.76
II, oral	4	45.2 ± 10.6	5.63 ± 1.07
II, intraperitoneal	4	37.5 ± 3.7	5.09 ± 0.59
I, oral	8	45.9 ± 12.8	5.49 ± 1.46
I, intraperitoneal	8	42.3 ± 9.4	5.37 ± 1.04

[a] Initial mucosal concentration = 1 m*M*.

Intestinal sacs prepared from animals pretreated with phenobarbital and II had ratios of 1.58 ± 0.23 and 1.53 ± 0.32, respectively, and showed no significant differences compared to controls. However, pretreatment with I resulted in significant increases in the final ratios, 1.95 ± 0.29. Nevertheless, this 24% increase may not be justification to assume that active transport was enhanced. Effects such as a decrease in serosal to mucosal flux in pretreated animals may result in misleading effects.

For this reason, experiments were performed with cannulated hanging sacs from segments of pretreated animals to compare transfer rates. In contrast to the ratio studies, the serosal solutions were free of the sugar, so transfer rates reflect both active and passive transfer. Table IV summarizes the rates observed after oral and intraperitoneal dosing of the inducing agents and demonstrate no significant differences between control and treated animals. As in the case of previous experiments with this technique, steady state was maintained throughout the 2-hr experiment. Comparing the total amounts of sugar transferred for each treatment showed no difference from control values.

These data appear to contradict the previous studies in that no increase in mucosal to serosal transport occurred with I treatment. It was apparent that further experiments would be necessary to separate the active component to examine pretreatment effects. To interpret the previous data better, investigations were conducted with ligated everted sacs containing buffer free of the sugar. Information obtained from short-time tissue uptake studies and transfer of 3-*O*-methylglucose at a low concentration (0.1 m*M*) would be more reflective of the active component.

Table V contains the millimoles of the sugar present in the sac tissue and in the serosal fluid after 10 min of incubation. Intraperitoneal pretreatment with II enhanced labeled sugar transfer; however, the amount in the tissue, although somewhat higher, was not significantly different than control values. Both oral and intraperitoneal administration of I resulted in enhanced 3-*O*-methylglucose transfer, but the tissue levels were increased significantly only for intraperitoneal administration. Phenobarbital pretreatment had no observed effect on either quantitative measurement compared to control experiments.

It is difficult to interpret why the effects of II were not noted in the two previous experiments performed at high sugar concentrations, and this

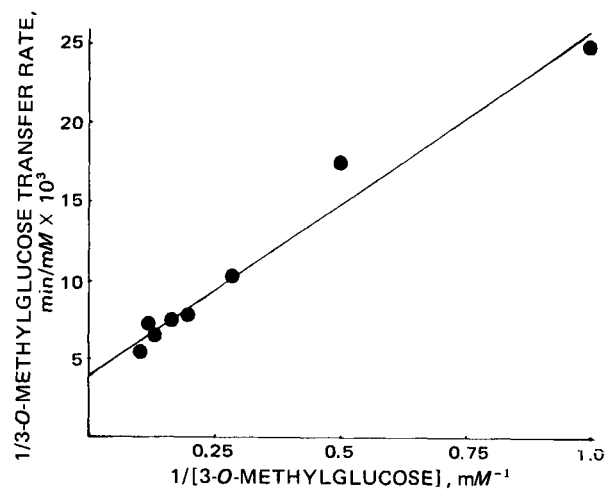

Figure 2—*Plot of the reciprocal of the 3-O-methylglucose transfer rate* versus *the reciprocal of the sugar concentration.*

Table V—Effect of Pretreatment on Transfer and Tissue Uptake of ^{14}C-3-*O*-Methylglucose[a] across the Everted Rat Small Intestine

Treatment	*n*	Amount Transferred to Serosal Fluid in 10 min, m*M* × 10^{-5}	10-min Tissue Uptake, m*M* × 10^{-5}
Control	28	0.38 ± 0.06	1.90 ± 0.32
Phenobarbital, oral	12	0.35 ± 0.06	2.09 ± 0.39
Phenobarbital, intraperitoneal	12	0.39 ± 0.08	1.89 ± 0.32
II, oral	12	0.36 ± 0.10	1.97 ± 0.21
II, intraperitoneal	12	0.58 ± 0.14[c]	2.22 ± 0.37[b]
I, oral	12	0.50 ± 0.09[c]	2.16 ± 0.50
I, intraperitoneal	12	0.60 ± 0.11[c]	2.64 ± 0.28[c]
Phlorizin	4	0.143 ± 0.004[c]	0.52 ± 0.13[c]

[a] Initial 3-*O*-methylglucose concentration = 0.1 m*M*. [b] $p < 0.1$. [c] $p < 0.001$.

result deserves further study. These experiments again demonstrate that everted intestinal sacs from I-treated animals appear to enhance abilities to transfer and uptake sugar. The fact that serosal accumulation of 3-*O*-methylglucose is not a complicating factor in these experiments points to a possible explanation of opposing effects of I on active and passive transport. This would explain the apparent lack of effect of I pretreatment in the cannulated everted intestinal sac experiments.

The present investigations demonstrated that pretreating rats with I, an enzyme-inducing agent, results in alteration of 3-*O*-methylglucose transfer across everted rat small intestinal sacs. Comparisons of results from several studies suggest that pretreatment with I may produce changes in both active and passive transfer. Alterations in the *in vitro permeability of red blood cells* by I were documented (23) and were believed to result from stability changes in the biological membrane, which were found to depend on the drug concentration in the suspension.

With regard to the active transport enhancement, the literature (10, 11) describes such changes with pretreatment of known liver microsomal-inducing agents. However, consideration must be given to visual observations made on the appearance and condition of the intestine removed from animals treated with I. As reported by Levy and Hayton (24), this drug has an inhibitory effect on GI motility. Their studies were performed at a lower dose (40 mg/kg), and their observations were made shortly afterward (40 *min*) compared to the present pretreatment. However, food material and bile were consistently noted in the small intestine of the treated animals in the present study.

Bile salts alone inhibit or depress active absorption of various substrates through intestinal tissue (25). On the other hand, bile salts are capable of altering the permeability of the intestinal membrane to drugs (26). Measurements of sac tissue weight showed an approximate 10–13% increase in the wet tissue weight of the sac prepared from treated rats. Twenty-four-hour fasting was reported to result in a loss in the intestinal tissue weight (27); however, the effects on active absorption were inconclusive after this fast period. The complexity of the situation existing in treated animals requires additional studies before a single conclusion can be reached. Further investigations are currently underway.

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