The Clearance of Mannitol and Erythritol In Rat Bile.* (32380)

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Recent studies in the cholecystectomized guinea pig(1), have shown that dehydrocholate enhances the biliary clearance of mannitol and erythritol in direct proportion to its choleretic effect. In contrast to the effect of dehydrocholate, secretin did not change the biliary excretion of these solutes even though bile flow was doubled. The divergent effects of these two choleretics led to the conclusion that primary canalicular bile containing the test solutes could be modified by secretion or reabsorption of fluid in the interlobular ducts. Although interlobular ducts in the guinea pig appear pre-minently responsive to secretin, the possibility that distal fluid transfer also occurs during spontaneous flow and bile salt choleresis has not been excluded(1, 2). In this paper we wish to report briefly the results of similar experiments in the rat. Schanker and Hogben(3) have reported a bile:plasma concentration ratio of 1.2 for mannitol in the rat. Since this is 4 times greater than the value found for the guinea pig and specifically suggests that the rat may perform distal fluid reabsorption, we decided to make a more direct comparison of the rat with the recent results obtained in the guinea pig.

Method. The design and procedural details of these experiments as performed in the guinea pig have appeared in detail elsewhere (4). The present studies differ only with regard to the preparation of the biliary fistula. Anesthetized Sprague-Dawley rats (250-300 g body wt) were nephrectomized and provided with a tracheostomy. Small polyethylene catheters were tied into the common duct at the liver hilus and into the pancreatic duct where it traversed the wall of the duodenum. This arrangement allowed collection of bile and pancreatic juice separately. When care was taken to replace fluid losses and preserve normal body temperature, bile flow was stable throughout the experiments. Pancreatic flow, though continuous, was somewhat erratic.

Each animal received a single intravenous injection of erythritol-C-14 (2.5 μc) and mannitol-H-3 (12.5 μc). Control clearances were measured in the steady-state using bile collected during the last 15 minutes of a 2-hour equilibration period and 75 μl of carotid arterial blood taken at the midpoint of the clearance interval. Maximum choleresis was subsequently maintained for 30 minutes with an intravenous infusion of sodium dehydrocholate (100 mg) or Boots§ secretin (5 units). A second measurement of clearance was made from bile and plasma collected at the end of this choleretic period. The plasma equivalent tissue spaces of mannitol and erythritol were calculated from measurements of tissue H_2O (by drying to constant weight) and the radioactivity in the supernatant fluid from liver and skeletal muscle homogenized with an equal volume of 10% trichloroacetic acid. The water content of liver and skeletal muscle was 72% and 75%, respectively. Recovery of the isotopes from liver and muscle was complete within the limits imposed by volumetric and counting errors which did not exceed 7%. Radioactivity was determined in a 3-channel liquid scintillation spectrometer in which the separation of H-3 from C-14 was greater than 90%. Small corrections required for incomplete discrimination and for variations in quenching were determined by internal standardization.

Results. The changes in bile:plasma con-

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§ Prepared according to the method of Crick, Harper, and Raper by Boots Pure Drug Co. Ltd., Nottingham, England.

centration ratios observed following dehydrocholate appear in Fig. 1. Values previously determined in the guinea pig are presented for comparison.* In Fig. 2 the same data

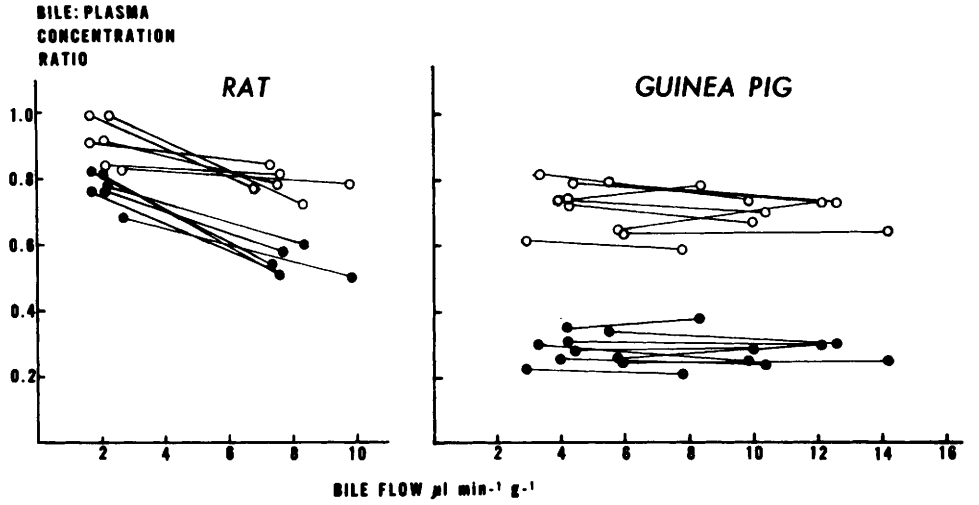

FIG 1. Bile: plasma concentration ratios during dehydrocholate choleresis; O———O, erythritol, ●———●, mannitol.

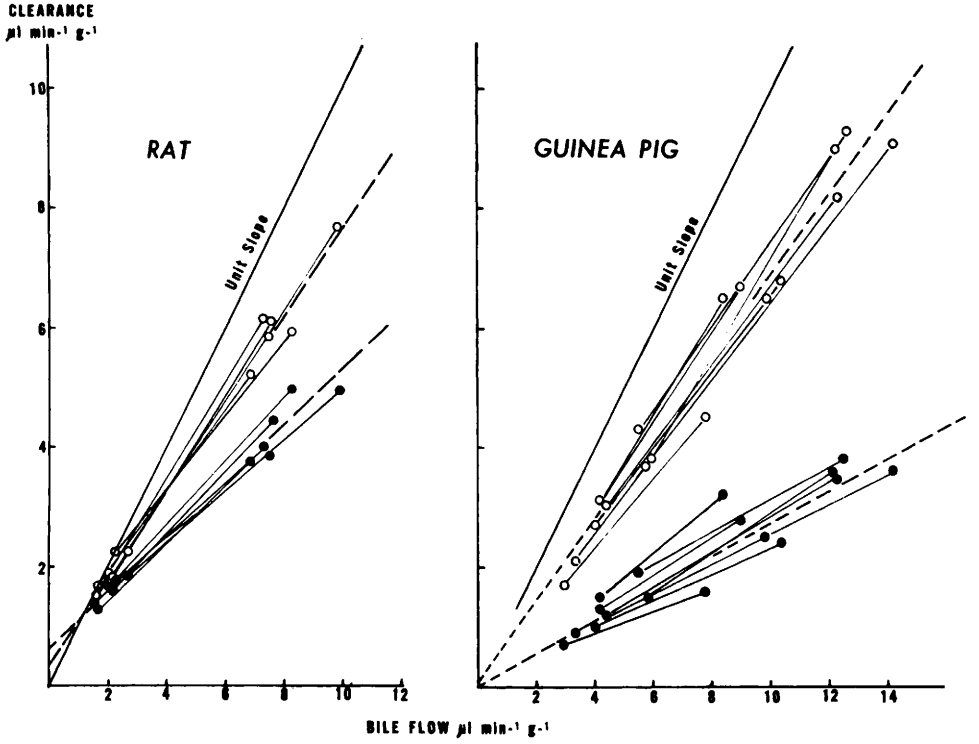

FIG. 2. Changes in clearance during dehydrocholate chloresis; O———O, erythritol. ●———●, mannitol. Equations for mean results (broken lines): RAT, y = 0.73(±.02) × + 0.36(±.29), y = 0.47(±.05)× + 0.61(±.12); GUINEA PIG, y = 0.68(±.06)× + 0.11(±.37), y = 0.27(±.05)× + 0.02(±.19). (±) = 95% confidence interval.

* Parts of Figures 1, 2, and 3 are reproduced with permission from J. Clin. Invest.(4).

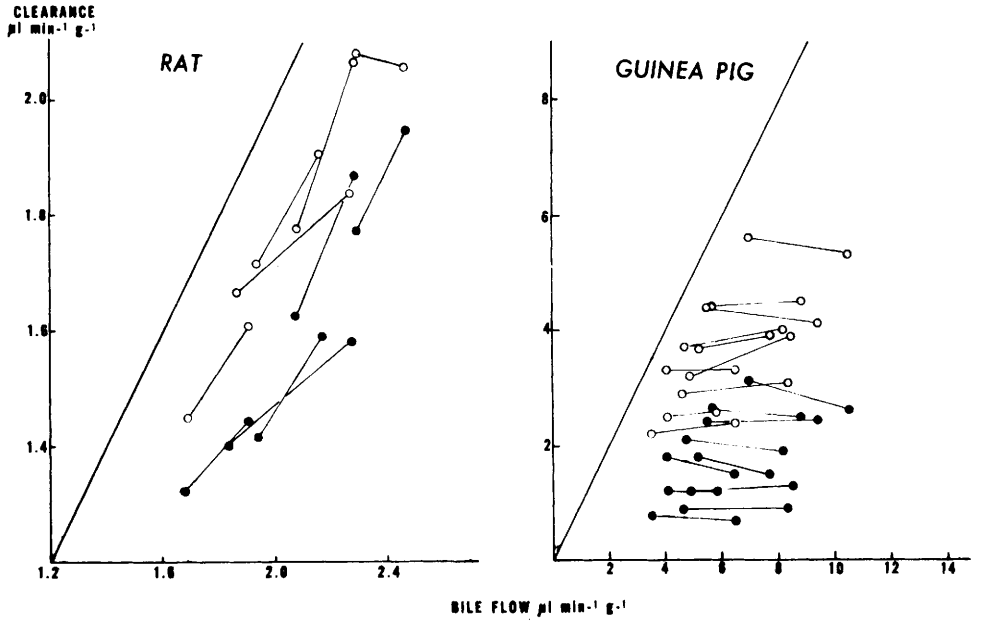

FIG. 3. Changes in clearance during secretin choleresis; O———O, erythritol. ●———● mannitol. Scale for the rat is expanded 10×.

appear as graphs of bile flow *vs* solute clearance (concentration ratio times flow). The clearance data for secretin are presented in Fig. 3. In the rat secretin produced only minor choleresis though as shown in Table I the effect on pancreatic flow was substantial, exceeding by several fold the highest spontaneous flow recorded during the control period. Changes in plasma radioactivity during the 30-minute interval between clearance periods were less than 10% of the initial value.

An earlier study has shown that the radioactivity appearing in guinea pig bile after injection of labeled mannitol or erythritol is homogeneous and chromatographically identical with the material injected(4). These solutes are also reported to resist metabolic transformation in the rat(5). Nevertheless, initial studies disclosed that in both the rat and the guinea pig tritiated mannitol had an unexpectedly large volume of distribution in muscle from the abdominal wall suggesting that H-3 might be present in part as water

TABLE I. Flow Rates.

	Bile				Pancreatic juice	
Animal	Spontaneous flow $\mu l\ min^{-1}g^{-1}$	Spontaneous flow (from Schanker and Hogben) $\mu l\ min^{-1}kg^{-1}$	Dehydrocholate (Δ F) $\mu l\ min^{-1}g^{-1}$	Secretin (Δ F) $\mu l\ min^{-1}g^{-1}$	Spontaneous flow $\mu l\ min^{-1}kg^{-1}$	Secretin (Δ F) $\mu l\ min^{-1}kg^{-1}$
Rat	2.04 ± .32 (n = 11)		5.79 ± .72 (n = 6)	.24 ± .08 (n = 5)	1.1 ± .2 (n = 9)	8.3 ± 4.0 (n = 5)
Rat*	50.2 $\mu l\ min^{-1}kg^{-1}$ (n = 11)	31.1 (n = 18)				
Guinea pig	4.70 ± 1.02 (n = 19)		6.31 ± 1.43 (n = 9)	3.09 ± .71 (n = 10)		

Values are means ± S.D.; g = gram liver wt; kg = kg body wt; Δ F = change in flow; n = No. of animals.

* The units are changed to facilitate comparison of the present results with those obtained by Schanker and Hogben(3).

TABLE II. Plasma Equivalent Tissue Spaces.*

Tissue	Rat			Guinea pig	
	Erythritol-C-14	Mannitol-H-3	Mannitol-C-14	Erythritol-C-14	Mannitol-H-3
Liver	1.08 ± .10 (n=9)	1.00 ± .03 (n=9)		1.06 ± .07 (n=19)	.94 ± .04 (n=19)
Liver		.96 ± .04 (n=5)	.96 ± .03 (n=5)		
Abdominal muscle	.91 ± .08 (n=9)	.32 ± .03 (n=9)		.94 ± .08 (n=10)	.35 ± .06 (n=10)
Abdominal muscle		.33 ± .02 (n=5)	.32 ± .01 (n=5)		
Gastrocnemius		.16 ± .02 (n=5)	.16 ± .01 (n=5)	.81 ± .09 (n=6)	.18 ± .02 (n=6)

* Computed as the ratio of concentration in tissue water to concentration in plasma water. All values have been determined in doubly labeled experiments employing mannitol-H-3 and either erythritol-C-14 or mannitol-C-14. Mean values ± S.D.

or some other intracellular constituent. To resolve this diffculty the content of tritiated mannitol in guinea pig gastrocnemius was examined and several rats were given both mannitol-H-3 and mannitol-C-14 to evaluate possible differences in their distribution. These results appear in Table II along with tissue spaces determined for liver.

The mannitol space in rat gastrocnemius is similar to that previously reported for insulin, sucrose, and mannitol(3) regardless of which isotopic label is employed, but the values obtained for abdominal muscle are approximately twice as large. Mannitol-H-3 gives similar values in the guinea pig. Thus, the high content of mannitol in abdominal muscle is not an artifact due to labeling but must be attributed to a property of the tissue itself. The discrepancy is evidently not attributable to localized edema, since the water content of muscle from either site was the same, 75%. The probable explanation is that muscle from the abdominal wall has a much higher proportion of fibrous connective tissue which being relatively acellular has a large tissue space for extracellular markers(6).

Discussion. Mannitol and erythritol are assumed to enter bile by restricted diffusion and osmotic filtration. Two lines of evidence suggest that the site of entry is the intralobular bile canaliculi. First, parenchymal hepatocytes which form the epithelial lining of the canaliculi have been shown to be unusually permeable to large lipid insoluble solutes (3), a finding confirmed in the present studies by the nearly complete equilibration of mannitol in hepatic water. Second, secretion of an osmotically active bile salt into the canaliculi enhances the clearance of the test solutes. In the guinea pig, failure of another choleretic, secretin, to alter solute excretion suggests that there is a second locus distal to the canaliculi capable of important fluid exchange but impermeable to mannitol and erythritol.

The effect of dehydrocholate in the rat, though generally similar to that observed in the guinea pig, is different in detail. Both mannitol and erythritol achieve higher concentrations in rat bile than in guinea pig bile. The differences are more pronounced during spontaneous flow than choleresis and much more striking for the larger solute, mannitol, than for erythritol, indicating an important species difference in relative permeability of the biliary tree to these solutes.

The choleretic response of the rat to secretin is less than 10% of that observed in the guinea pig. The increase in flow of rat bile, though unequivocal, is so small that it may be questioned whether the hepatic effect of secretin is specific. Alterations in bile flow of this magnitude might, for example, simply reflect a change in hepatic blood flow rather than a specific hormonal effect on the biliary epithelium. The brisk increase in pancreatic flow, similar to that observed by Ramirez *et al*(7), would appear to exclude the possi-

bility that the secretin was not potent. Though poorly defined because of the small increments in flow, the changes in biliary clearance which followed secretin in the rat are clearly greater than those obtained in the guinea pig. Final interpretation of this result must await a more refined investigation which may reveal, for example, whether secretin enhances bicarbonate excretion in rat bile as it does in the guinea pig and other animals (8,9).

In the present experiments bile:plasma concentration ratios greater than unity were not observed. Although we are unable to explain the difference of our results from those reported for mannitol by Schanker and Hogben, their experiments differed from ours in procedural details which may have been important. In the absence of fluid replacement and control of body temperature, their animals may have been dehydrated and hypothermic, both factors which could account for the lower flow rates which they observed (Table I). At a lower rate of bile formation we would expect to find a bile:plasma concentration ratio closer to the theoretical equilibrium value of one, but it is not easy to visualize how steady-state dehydration or hypothermia could lead to values higher than unity unless they activated a mechanism for distal fluid reabsorption or unmasked an underlying reabsorptive process by selectively inhibiting distal fluid secretion.

Summary. In the rat, as in the guinea pig, dehydrocholate choleresis leads to increased excretion of mannitol and erythritol, but in the rat the amounts of the solutes appearing in bile are greater. This difference is much more pronounced for the larger solute, mannitol. For mannitol, but not for erythritol, the rate of change of clearance following dehydrocholate is also greater in the rat. Unlike its effect in the guinea pig, secretin produces only a trivial choleresis in the rat. Despite a previous report that mannitol is concentrated in rat bile, bile:plasma concentration ratios greater than unity were not observed in the present studies for either mannitol or erythritol. In both the rat and the guinea pig the extracellular fluid volume of skeletal muscle as estimated from the plasma equivalent tissue space of mannitol is twice as large for muscle from the abdominal wall as for gastrocnemius. The discrepancy is attributed to differences in connective tissue content.

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Cholesterol Precursor Pools of Progesterone in the Bovine Ovary Perfused *in vitro*.* (32381)

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Studies of cholesterol and progesterone synthesis from acetate-1-^{14}C by luteal tissue incubated *in vitro* have consistently demonstrated that the specific activity of newly synthesized cholesterol is lower than that of

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