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The enterohepatic circulation of ^{3}H-phenolphthalein in the rat

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Many drugs are excreted in bile and, consequently, may undergo an enterohepatic circulation (EHC) (see Smith & Millburn, 1975). In the rat, phenolphthalein is conjugated with glucuronic acid and then extensively excreted in bile (Millburn, Smith & Williams, 1967). This paper reports on the EHC of phenolphthalein.

In bile-duct-cannulated rats injected with [^{3}H]-phenolphthalein (25 mg/kg i.p.), 89 ± 1.6% (n = 3) of the ^{3}H was excreted in bile in 3 h, whereas in intact rats four days are required for the elimination of 86 ± 3.9% (n = 3) in faeces. This delayed faecal excretion appears to be due to EHC.

Following intraduodenal infusion of [^{3}H]-phenolphthalein into bile-duct-cannulated rats, there is a rapid biliary excretion of radioactivity (Figure 1a). By contrast, on infusion of bile containing [^{3}H]-phenolphthalein glucuronide obtained from rats injected with [^{3}H]-phenolphthalein, there is a lag period of some 4 h before a comparable rate of excretion occurs (Figure 1a). This suggests that the glucuronide may require hydrolysis to the aglycone before significant absorption occurs, as is the case for stiboestrol glucuronide (Fischer, Millburn, Smith & Williams, 1966).

Rats were treated daily for 3 days with antibiotics to suppress the intestinal microflora. Figure 1b shows that this treatment did not inhibit the absorption of free phenolphthalein. However, on the intraduodenal infusion of bile containing [^{3}H]-phenolphthalein glucuronide the biliary excretion of ^{3}H (Figure 1b) was much lower than in untreated animals (Figure 1a). This indicates that hydrolysis of phenolphthalein glucuronide by bacterial β-glucuronidase is an important step in the EHC of phenolphthalein.

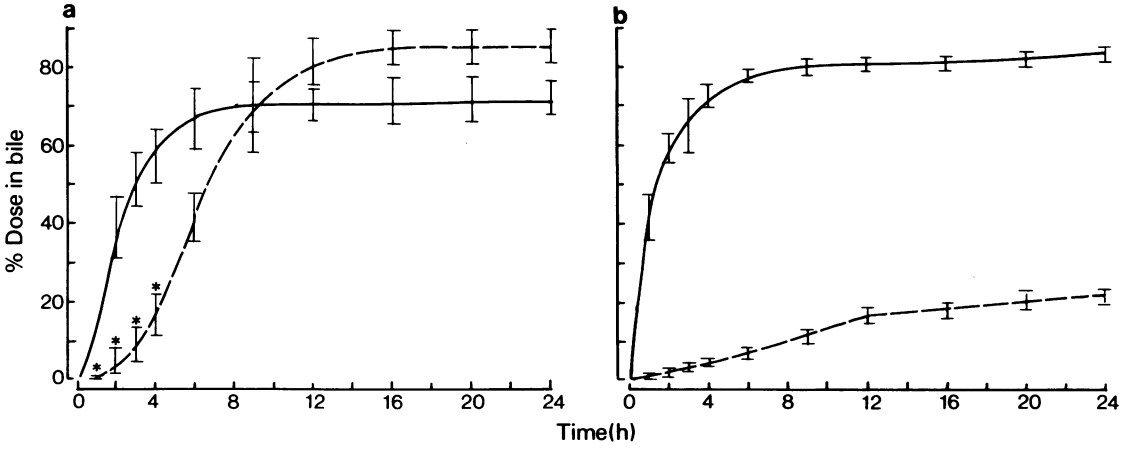

Figure 1 Biliary excretion of radioactivity after intraduodenal infusion of ^{3}H-phenolphthalein (——) or ^{3}H-phenolphthalein glucuronide (— — —) into bile-duct-cannulated female Wistar albino rats (200-250 g body weight). The dose was 79 μmol/kg. The vertical bars represent s.e. mean (n = 3).
a: without antibiotic treatment. *These values are significantly ($P < 0.05$) lower than the corresponding values for phenolphthalein.
b: after 3 days treatment with neomycin (100 mg kg^{-1} day^{-1}), tetracycline (50 mg kg^{-1} day^{-1}) and bacitracin (50 mg kg^{-1} day^{-1}).

Carotid arterial blood level measurements suggest that phenolphthalein may be systemically bioavailable from the EHC. Thus, in intact but not in bile-duct-cannulated rats there is a secondary plasma peak of radioactivity 5-6 h after the intravenous administration of [^{3}H]-phenolphthalein. This peak coincides with the absorption of the aglycone from the intestine following the bacterial hydrolysis of phenolphthalein glucuronide (see Figure 1a).

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The effects of cyproheptadine pretreatment on insulin release from isolated pancreatic islets

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Cyproheptadine, an antiserotonin-antihistaminic agent with a chemical structure similar to the tricyclic antidepressants (Stone, Wenger, Ludden, Stavorski & Ross, 1961) inhibits glucose-mediated insulin release by an immediate and direct effect on the rat pancreatic islet of Langerhans (Richardson, McDaniel & Lacy, 1975).

The present studies describe the effects of different secretogogues on insulin release from cyproheptadine-pretreated rat islets. Approximately 200 islets were isolated from two 200-300 g ten week old male albino rats (OFA Sandoz SPF strain) by the collagenase technique (Lacy & Kostianovsky, 1967). An equal number of islets were placed in each of two perfusion chambers and perifused at 37° C and pH 7.40 with Krebs Ringer bicarbonate containing 5.6 mM D-glucose at a rate of 1 ml/min as described previously (Lacy, Walker & Fink, 1972). After 45 min, the test islets were exposed to 100 μM cyproheptadine hydrochloride monohydrate for five minutes. Subsequently both chambers were stimulated with an insulin secretogogue for a further 60 min as indicated in the Table. The perfusate was collected at 1- or 5-min intervals throughout the study. The insulin content was determined by radioimmunoassay (Wright, Makulu, Vichick & Sussman, 1971) and expressed as (μU/islet)/minute. All data was subjected to complete statistical analysis.

Cyproheptadine pretreatment completely abolished tolbutamide- or glucose-evoked insulin release. Conversely the responsiveness of islets to

Table 1 The effects of cyproheptadine pretreatment on insulin release from perifused islets

Mean rate of secretion with 5.6 mM D-glucose (μU/islet)/min ± s.e.		*Insulin secretogogue added*	*n*	*Mean rate of secretion after addition of secretogogue $\mu\mu$/islet per min ± s.e. mean*		**P*	*% Inhibition*
Control	*Test*			*Control*	*Test*		
0.59 ± 0.04	0.54 ± 0.09	1.1 mM tolbutamide	3	1.37 ± 0.08	0.48 ± 0.09	< 0.001	100.0
0.47 ± 0.21	0.78 ± 0.29	11.1 mM D-glucose	3	2.67 ± 0.65	0.72 ± 0.22	< 0.01	100.0
0.73 ± 0.15	0.67 ± 0.08	11.1 mM D-glucose + 6.0 mEq/1. Ca^{++}	3	3.17 ± 0.37	0.96 ± 0.15	< 0.001	88.5
0.53 ± 0.35	0.49 ± 0.24	5.0 mM theophylline	4	1.19 ± 0.44	1.17 ± 0.33	NS	0.0

* Control versus test values after addition of secretogogue
NS = not significant