catecholamines from adrenergic neural endings in the rat uterus, an organ with a high sensitivity for noradrenaline (Borda et al 1981).

This indirect action should be taken into account when using these drugs as a tool in the pharmacological characterization of the receptors involved in the response to histamine in the isolated uterus of the rat.

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Pharmacokinetics of theophylline in rats with biliary stasis

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Cholestasis in man can be caused by gallstones, tumours of the pancreas or biliary tract, and various drugs (Brooks 1974). The backflow of bile into the liver may produce histological and biochemical abnormalities within the hepatocyte manifested clinically by jaundice and abnormal liver function tests (Brooks 1974). In quantitative electron micoscopy studies, cholestatic rats have demonstrated decreased amounts of hepatic smooth endoplasmic reticulum, the major organelle involved in drug metabolism (Jones et al 1976). Likewise, these same animals were found to have decreased levels of certain drug metabolizing enzymes (Mackinnon & Simon 1975; Drew & Priestly 1976).

Many patients with biliary stasis have concurrent illnesses requiring therapy with several drugs some of which depend primarily on the liver for their metabolic inactivation. Theophylline is such a drug (Piafsky & Ogilvie 1975). In patients with liver dysfunction caused by chronic passive congestion (Piafsky et al 1977) and cirrhosis (Mangione et al 1978), the elimination half-life and clearance of theophylline are significantly prolonged. No information is currently available on the pharmacokinetics of theophylline in patients with abnormal liver function secondary to cholestasis. The purpose of this present study is to investigate the pharmacokinetic parameters of theophylline in rats with extrahepatic cholestasis as a model for the human condition.

Materials and methods

Male Sprague-Dawley rats (220–270 g) were housed one to a cage over corn cob bedding in a well ventilated room (24 ° ± 0·5 °C) with alternate 12 h periods of light and dark. The animals were divided into three groups. The first group consisted of six bile duct-ligated animals fed water and Purina Rodent Chow *ad libitum*. The second group was composed of six sham-operated rats which were pair-fed daily to the bile duct-ligated animals. The third group consisted of three non-operated controls fed *ad libitum*. Double ligation of the common bile duct was performed under sodium pentobarbitone anaesthesia (50 mg kg^{-1}) through a midline excision. Sham animals were anaesthetized similarly but only had their bile ducts exposed before being closed. *Ad lib*-fed control animals were not anaesthetized.

Theophylline kinetics were performed 72 h after the operation under 1·7 g kg^{-1} urethane, an anaesthetic that does not inhibit certain drug metabolic reactions

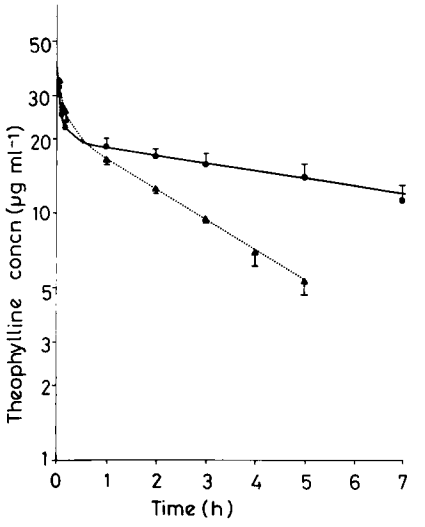

FIG. 1. Plasma concentration of theophylline after 15 mg kg^{-1} i.v. in bile duct ligated rats (●——●) and pair-fed shams (▲ · · · · ▲). Values are mean ± s.e. with n = 6 at each point.

* Correspondence.

Table 1. Biochemical data on experimental animals obtained from the pooled serum of two rats and analysed by a commercial automated serum multiple analyser. Values are mean (with s.d.).

	Bile duct ligation	Pair-fed shams	Ad-lib fed controls
Creatinine*	0·95 (0·35)	1·15 (0·07)	1·40 (0·14)
BUN*	37 (20)	45 (2)	14 (1)
Albumin**	2·2 (0·1)	2·1 (0·1)	2·9 (0·1)
Total bilirubin*	7·0 (0·3)	0·3 (0·1)	0·6 (0·2)
SGOT†	816 (28)	239 (16)	171 (42)
SGPT†	243 (9)	67 (34)	62 (4)
Alkaline phosphatase†	133 (4)	43 (6)	52 (6)
Cholesterol*	205 (65)	66 (18)	73 (3)

* Units are mg dl^{-1}.
** Units are g dl^{-1}.
† Units are international units $litre^{-1}$.

Table 2. Theophylline pharmacokinetic parameters and weight changes for experimental animals give 15 mg kg^{-1} theophylline i.v. while under urethane anaesthesia. Kinetics were studied 72 h after bile duct ligation or sham operations, and at the time the ad lib-fed controls were approximately equal in weight to the other two groups. Percentage weight changes are over the 72 h between operation and kinetic study. Values are mean (with s.d.).

	Bile duct ligation (n = 6)	Pair-fed shams (n = 6)	Ad lib-fed controls (n = 3)
$t_{1/2}$ (α) (min)	3·5 (2·3)	5·5 (2·9)	—
$t_{1/2}$ (β) (h)	9·3 (4·5)*	2·6 (0·6)	2·2 (0·4)
V_D (litre kg^{-1})	0·74 (0·09)	0·68 (0·09)	0·49 (0·05)*
Cl (ml h^{-1} kg^{-1})	68 (33)**	184 (26)	155 (46)
% weight change	−10·4 (2·3)	−8·1 (1·8)	10·9 (1·3)**

* Statistically different from pair-fed shams, $P < 0.01$.
** Statistically different from pair-fed shams, $P < 0.001$.

(Umeda & Inaba 1978), and which has been reported to maintain hepatic blood flow at a level equal to that of an awake animal (Hiley et al 1978). The right and left jugular veins were surgically exposed and 15 mg kg^{-1} theophylline (18·75 mg kg^{-1} aminophylline) was injected i.v. through the right jugular vein. Blood samples (0·4 ml) were taken at 3, 6, 9 min and 1, 2, 3, 4, 5 h after injection for the pair fed shams and at 1, 2, 3, 5, 7 h for the bile duct-ligated rats. Samples were obtained by needle puncture of the left jugular vein through the pectoralis major muscle with a 1 cc heparinized tuberculin syringe. Plasma theophylline concentrations were determined by a commercially available enzyme immunoassay kit (EMIT) as previously described (Gushaw et al 1977). Liver enzymes and other biochemical parameters were obtained from the pooled serum of two animals by a standard serum multiple analyser.

Pharmacokinetic calculations were performed using the two compartment open model by the successive 'feathering' technique (Gomeni & Latini 1977). All data was compared by the two tailed Student's *t*-test for unpaired samples, using a $P>0.05$ as being statistically insignificant.

Results

Biochemical parameters for all three animal groups are listed in Table 1. Kidney function was judged to be normal in all groups as measured by normal serum creatinines. The cholestatic animals demonstrated the usual laboratory evidence of obstruction, namely elevated levels of transaminases, bilirubin, alkaline phosphatase and cholesterol compared with the two other control groups. Albumin was higher, and BUN was lower in the *ad lib*-fed controls, compared with the other two groups which lost about 10% of their body weight.

As Table 2 demonstrates, animals in the bile duct ligation group had a significant loss in body weight compared with the *ad lib*-fed, non-operated controls, however the weight loss of the pair-fed sham group was similar to that of the bile duct ligation group.

Fig. 1 illustrates the the average plasma disappearance curves for theophylline in the sham-operated and bile duct-ligated animals, and Table 2 lists the theophylline pharmacokinetic parameters calculated for these two experimental groups and the *ad lib*-fed controls. Compared with pair-fed shams, the cholestatic rats had unchanged distribution half-lives and volumes of distribution for theophylline. The theophylline elimination half-life was prolonged 350% in the cholestatic rats compared with shams, with a corresponding reduction in total clearance to approximately one-third of that in the shams. There was no statistical difference in elimination half-life or total clearance between the *ad lib*-fed controls and pair-fed shams, however the volume of distribution was statistically lower in the *ad lib*-fed controls than in the other two groups.

Discussion

Extrahepatic biliary obstruction caused a substantial reduction in theophylline clearance and a prolongation in its elimination half-life without significantly affecting its distribution half-life or volume of distribution. Conformation of biliary stasis was obtained by changes in the various biochemical parameters illustrated in Table 1. Because theophylline metabolism can be affected by changes in diet and nutrition (Alvares et al 1976), the animals in our study were compared with carefully pair-fed, sham-operated controls. Kinetic parameters were also measured in three non-operated *ad lib*-fed rats to serve as a comparison to the pair-fed, sham controls. The volume of distribution was significantly lower in the *ad lib*-fed animals compared with the pair-fed shams, a result which could possibly be explained by increased theophylline protein binding due to a higher serum albumin (Table 1) in the *ad lib*-fed rats.

Theophylline is metabolized in the liver to a number of inactive metabolites by means of the microsomal enzyme system and xanthine oxidase (Grygiel & Birkett 1980). Its ultimate disposition differs markedly in the rat

compared with man in that 46% of the drug is excreted unchanged in the rat (Williams et al 1979) compared with less than 10% in man (Jenne et al 1976). Biliary excretion amounts to less than 5% of the administered dose in the rat (Williams et al 1979). These facts suggest that the decreased theophylline clearance present in our bile duct-ligated animals is most likely due to an inhibition of theophylline metabolism, since cutting off the biliary route of excretion would only slightly decrease total clearance, and renal clearance should be normal as represented by the normal serum creatinines in Table 1.

The effects of cholestasis on drug metabolism and kinetics has been previously studied in both man and rats with somewhat inconsistent results. Seventy-two hours after bile duct ligation, rats were found to have decreased activities of cytochrome P-450 and other drug metabolizing enzymes (Drew & Priestly 1976; Mackinnon & Simon 1975), with corresponding reductions in the total clearances of pethidine and pentobarbitone (Knodell et al 1980). Schaffner & Popper (1969) have postulated that these changes are probably due to a bile salt-mediated alteration in hepatocyte function producing what they called a 'hypoactive hypertrophic smooth endoplasmic reticulum' with detrimental effects on the various enzyme systems. However, liver biopsy specimens from patients with cholestatic jaundice have been associated with a small but statistically insignificant depression in cytochrome P-450 and cytochrome b_5 content (Ahmad & Black 1977) and a statistically significant depression of hexobarbitone metabolizing enzymes (Auranen 1972). Pharmacokinetic studies in similar patients demonstrated normal elimination half-lives of antipyrine (Hepner & Vesell 1975), paracetamol (Elfström & Lindgren 1974), and hexobarbitone (Richter et al 1980), but prolonged half-lives of meprobamate and pentobarbitone in certain sub-groups of cholestasis (Carulli et al 1975). Thus is appears that the effect of biliary stasis on drug disposition in man is variable and cannot accurately be predicted without doing the actual pharmacokinetic study.

Our results in the rat seem to indicate that theophylline metabolism is particularly sensitive to cholestasis, since its elimination half-life was prolonged about 350% even though only about 50% of the drug is actually being metabolized. Because theophylline is a drug with a narrow therapeutic range, small decreases in its clearance leading to elevated serum levels can potentiate toxicity, such as seizures and cardiac arrhythmias (Jacobs et al 1976). Therefore cautious monitoring of theophylline in cholestatic patients is recommended till definitive human pharmacokinetic studies are done.

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