EFFECT OF PREGNANCY ON THE EXCRETION OF SULPHOBROMOPHTHALEIN IN BILE*

By H. M. Shaw† and T. Heath†

A decreased plasma clearance of sulphobromophthalein (BSP) is often observed during the latter half of normal human pregnancy (Tindall and Beazley 1965). It also occurs during the ingestion of oral contraceptives (Roman and Hecker 1968) and after treatment with oestrogens at doses similar to those produced daily in late pregnancy (Kappas 1968). A reduction in bile flow has been described after oestrogen treatment in rats (Javitt and Harkavy 1969; Forker 1969; Kreek *et al.* 1967), but no attempts appear to have been made to associate changes in bile flow rate with alterations in BSP excretion.

In this study, the effect of variations in the flow of bile on the excretion of BSP was assessed in pregnant and non-pregnant rabbits.

Materials and Methods

Seven 4-month-old New Zealand white rabbits weighing 2·7–3·8 kg were obtained at the time of nesting, which was within 2–3 days of term. Fourteen virgin rabbits of the same age and weight acted as controls. Experiments were conducted alternately with pregnant rabbits and controls and both groups of animals were fasted for at least 18 hr before operation.

Rabbits were anaesthetized with pentobarbitone sodium (i.v. 25 mg/kg body weight). In each rabbit, the cystic duct was ligated and a polyethylene catheter (1·50 mm o.d.), 15 cm in length, was inserted into the common bile duct at the hilus of the liver. Initial rates of bile flow were determined for both pregnant and control rabbits immediately after cannulation. Sulphobromophthalein (Bromthalein, E. Merck, Darmstadt, Germany; 50 mg/ml) was injected into the marginal ear vein at the dose of 0·75, 2·0, and 5·0 mg/kg in non-pregnant rabbits and 2·0 and 5·0 mg/kg in pregnant rabbits, and sequential samples of bile were collected into tared containers until the samples were free from the dye. Each rabbit was used for up to three separate determinations of BSP excretion rate. In order to estimate the excretion rate of BSP at low rates of bile flow in control rabbits, bile was allowed to drain from all control rabbits for a period of 4 hr. After this time, bile flow rate had declined significantly due to interruption of the enterohepatic circulation of bile salts.

The concentrations and excretion rates of BSP were estimated after dilution of 0·07-ml portions of bile with 3 ml of 0·08N NaOH; this achieved concentrations of BSP that could be measured at 585 nm in a Beckman DU 4700 spectrophotometer. Since the excretory rates of BSP into bile increased to a peak about 12 min after injection, then gradually decreased with time, the excretory rate for a particular dose of dye was taken arbitrarily as the product of bile

* Manuscript received 10 January 1972.

† School of Physiology and Pharmacology, University of New South Wales, Kensington, N.S.W. 2033.

flow rate (ml/min) and concentration of dye in bile (mg/ml) at the time when dye concentration had reached half its maximal value.

Student's *t*-tests coupled with analyses of variance were used to estimate whether significant differences existed between means obtained for the different parameters, and regression coefficients and analyses of variance were used to determine the relationships between bile flow rate and BSP excretion.

Results and Discussion

Rate of Flow of Bile.—Pregnant rabbits produced significantly less bile than non-pregnant rabbits ($P < 0{\cdot}025$). Mean $\pm$ S.E.M. for the rates of bile flow during the period immediately after the duct was cannulated were:

	Bile flow rate (ml/kg body wt./min)	(ml/kg liver/min)
Non-pregnant rabbits	$0{\cdot}089 \pm 0{\cdot}009$	$1{\cdot}73 \pm 0{\cdot}18$
Pregnant rabbits	$0{\cdot}044 \pm 0{\cdot}006$	$0{\cdot}88 \pm 0{\cdot}17$

Neither the body nor liver weights of pregnant rabbits were significantly different from the controls.

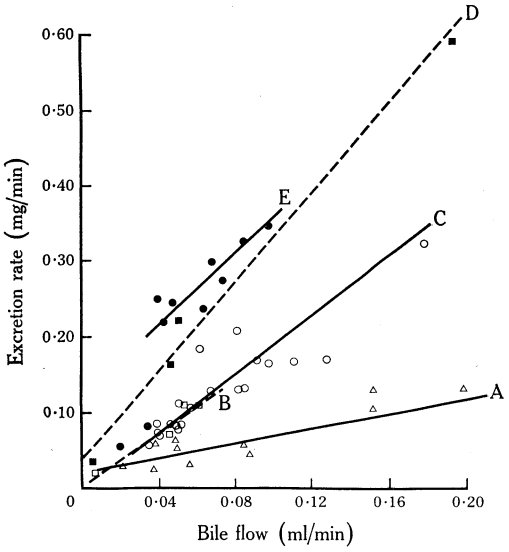

Fig. 1.—Relationship between excretion rate of sulphobromophthalein and bile flow rate in non-pregnant (A, C, and E) and pregnant (B and D) rabbits.

Line	Dose (mg/kg)	Relationship	P
A(△)	0·75	$y = 0{\cdot}6x + 0{\cdot}021$	$< 0{\cdot}001$
C(○)	2·0	$y = 1{\cdot}9x - 0{\cdot}006$	$< 0{\cdot}001$
E(●)	5·0	$y = 2{\cdot}4x + 0{\cdot}120$	$< 0{\cdot}01$
B(□)	2·0	$y = 1{\cdot}8x - 0{\cdot}001$	$< 0{\cdot}05$
D(■)	5·0	$y = 2{\cdot}9x + 0{\cdot}037$	$< 0{\cdot}01$

where x = bile flow (ml/min) and y = excretion rate (mg/min).

Sulphobromophthalein Excretion.—When BSP was given to non-pregnant rabbits at the dose of 0·75 mg/kg, it was excreted into bile at a rate that was related directly to bile flow; each increase in flow of 0·1 ml/min was associated with an increase in excretion rate of 0·06 mg/min. When BSP was injected at 2·0 or 5·0 mg/kg, however, an increase in bile flow of 0·1 ml/min was associated with an alteration in excretory rate of 0·19 and 0·24 mg/min respectively. However, in non-pregnant rabbits which were given a dose of 5·0 mg/kg, this region of proportionality could only be demonstrated for bile flow rates of more than 0·04 ml/min (Fig. 1). Pregnancy did not appear to have any effect either on the relationship between bile flow rate and BSP

excretion (Fig. 1) or on the concentration of BSP in bile (half-maximal value):

	Dose of BSP (mg/kg)	Bile BSP concn. (mg/ml)
Non-pregnant rabbits	2·0	1·9±0·4
	5·0	4·1±1·2
Pregnant rabbits	2·0	2·1±0·7
	5·0	4·8±1·1

The maximum biliary BSP concentration was determined at the end of each experiment by administration of BSP at 75 mg/kg. This dose had been previously determined in trial experiments by constant infusions of BSP that exceeded the transport maximum. After the BSP was administered to both pregnant and control rabbits, the maximum concentration of BSP in the bile of the two groups reached similar values:

Non-pregnant rabbits 12·3±2·3 mg/ml; pregnant rabbits 14·0±1·0 mg/ml.

A choleresis occurred during the excretion of BSP and was particularly marked in the pregnant rabbits. As a result of this, BSP appeared in the bile of pregnant and non-pregnant rabbits at about the same rate—1·9 mg/min.

The depression in the rate of removal of BSP from the circulation of pregnant women has been attributed by Tindall and Beazley (1965) to the effects of high circulating levels of oestrogens. No account was taken in this study, however, of the slowing of bile flow that could occur during pregnancy. Klaassen and Plaa (1967) suggested that biliary excretion of BSP might be the limiting factor in the elimination of BSP from plasma. They showed that in species such as the dog in which bile flow was slow, the maximum excretion rate of dye was much less than in animals such as the rat and rabbit which secrete bile at a rapid rate.

Similarly, Boyer, Scheig, and Klatskin (1970) have shown that in the isolated, perfused rat liver, the two most important factors responsible for determining the rate of removal of BSP from the blood are the rate of bile production, and the bile BSP concentration. These authors' results were confirmed in whole animal preparations by O'Maille, Richards, and Short (1966) and Ritt and Combes (1967) in the dog and Gronwall and Cornelius (1969) in the sheep. In each study, the maximum excretion rate of BSP was increased after infusion of bile salts. Boyer, Scheig, and Klatskin (1970) considered that the bile salts enhanced transport of the BSP by acting as a choleretic and not by altering membrane permeability or by stimulating a transport system or by influencing BSP–glutathione conjugation. It was the bile flow rate that was the important determinant of BSP excretion.

Gronwall and Cornelius (1969) found that the dose of BSP administered was a critical factor in the rate of elimination of the dye in bile. If the dose of BSP was high, the concentration of BSP in the bile reached a maximum of about 15 mg/ml, and the rate of excretion varied directly with bile flow. If the dose of BSP was less than that necessary to produce a maximum biliary concentration, the rate of excretion was proportional to the rate of infusion, and was independent of changes in bile flow rate that were produced by infusing taurocholic acid at different rates (Gronwall and Cornelius 1969).

In the present study, the excretion rates of single doses of 0·75, 2·0, and 5·0 mg/kg of dye were dependent on bile flow rate. All these doses were much lower than those necessary to produce maximum biliary concentrations of BSP (approximately 50 mg/kg). Excretion rates of single doses of dye by pregnant and non-pregnant rabbits were similar at each rate of bile flow. Thus it seems possible that delay in excretion of the dye during pregnancy may be attributable, at least in part, to the decrease in bile flow encountered during pregnancy.

References

BOYER, J. L., SCHEIG, R. L., and KLATSKIN, G. (1970).—The effect of sodium taurocholate on the hepatic metabolism of sulfobromophthalein sodium (BSP). The role of bile flow. *J. clin. Invest.* **49**, 206–15.

FORKER, E. L. (1969).—The effect of estrogen on bile formation in the rat. *J. clin. Invest.* **48**, 654–63.

GRONWALL, R., and CORNELIUS, C. E. (1969).—Maximal biliary excretion of sulfobromophthalein sodium in sheep. *Am. J. dig. Dis.* **15**, 37–47.

JAVITT, N. B., and HARKAVY, M. (1969).—Ethinyl estradiol-induced cholestasis in female Wistar rats. *Gastroenterology* **56**, 400.

KAPPAS, A. (1968).—Studies in endocrine physiology; biologic actions of some natural steroids on the liver. *New Engl. J. Med.* **278**, 378–84.

KLAASSEN, C. D., and PLAA, G. L. (1967).—Species variation in metabolism, storage and excretion of sulfobromophthalein. *Am. J. Physiol.* **213**, 1322–6.

KREEK, M. J., PETERSON, R. E., SLEISENGER, M. H., and JEFFRIES, G. H. (1967).—Influence of ethinyl estradiol-induced cholestasis on bile flow and biliary excretion of estradiol and bromsulfophthalein by the rat. *J. clin. Invest.* **46**, 1080.

O'MAILLE, E. R. L., RICHARDS, T. G., and SHORT, A. H. (1966).—Factors determining the maximal rate of organic anion secretion by the liver and further evidence on the hepatic site of action of the hormone secretin. *J. Physiol., Lond.* **186**, 424–38.

RITT, D. J., and COMBES, B. (1967).—Enhancement of apparent excretory maximum of sulfobromophthalein sodium (BSP) by taurocholate and dehydrocholate. *J. clin. Invest.* **46**, 1108.

ROMAN, W., and HECKER, R. (1968).—The liver toxicity of oral contraceptives. A critical review of the literature. *Med. J. Aust.* **2**, 682–8.

TINDALL, V. R., and BEAZLEY, J. M. (1965).—An assessment of changes in liver function during normal pregnancy using a modified sulfobromophthalein test. *J. Obstet. Gynaec., Brit. Emp.* **72**, 717–37.