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Comparison of the effects of Enovid and α-naphthylisothiocyanate on bromosulphophthalein excretion in Syrian golden hamsters

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Bromosulphophthalein (BSP) is an anionic dye which is rapidly removed from plasma by the liver, conjugated, and then excreted into bile. Its excretion rate into bile is saturable, and this upper limit (transport maximum, T_M) has been used as a sensitive measure of the integrity of the excretory capacity of the liver [1].

A very small percentage of women in general, and a greater number of women in certain geographic regions, show recurrent cholestatic jaundice during pregnancy, or while taking oral contraceptives such as Enovid [2–4]. However, in both conditions, the women have a defective biliary excretion demonstrable as reduced BSP-T_M [5, 6]. This was interpreted as indicating a 'subclinical' cholestasis which is manifested as cholestatic jaundice only in certain sensitive women [7].

Anabolic steroids, oral contraceptives and estrogen derivatives are cholestatic agents and reduce BSP-T_M in experimental animals [6, 8, 9]. α-Naphthylisothiocyanate (ANIT) is another cholestatic agent in many species [9], but hamsters proved less susceptible than rats, requiring higher doses to produce cholestasis [10]. We have also noted the apparent resistance of hamsters to the cholestatic effects of Enovid, i.e. an acute pretreatment which reduced bile flow by 50 per cent in rats had no such effect in hamsters.* The present studies, therefore, were undertaken to determine whether acute or chronic Enovid pretreatment might have a more subtle effect on the bile secretory apparatus in hamsters, being evident only as a reduction in maximal transport rate, and thus resembling more closely the human situation. ANIT was included in this study for comparison of any effect of Enovid with a known cholestatic agent in hamsters.

Chemicals. Enovid is a mixture of 96% norethynodrel and 4% mestranol which were purchased separately from the Sigma Chemical Co., St. Louis, MO. ANIT was obtained from Aldrich Chemicals (Milwaukee, WI), BSP from Sigma, and Diabutal (pentobarbital) from Diamond Laboratories (Des Moines, IA).

Animal treatment. Female Syrian golden hamsters (12- to 14- weeks-old) were given a single oral dose of ANIT (300 mg/kg in paraffin oil) 2 days prior to BSP infusion. In the acute Enovid studies, female hamsters were given five daily oral doses of Enovid (50 mg/kg in propylene glycol) prior to experimentation on day 6. Controls received equivalent doses of vehicle only. In the chronic studies, female hamsters were fed 0.004% Enovid in powdered food (Wayne Lablox) from 7 to 27 weeks of age.

BSP studies. Animals were anesthetized with pentobarbital (125 mg/kg), the common bile duct was ligated, and the gall bladder was cannulated for bile collection.* The jugular vein was then cannulated for BSP infusion using a Cheminert metering pump (Laboratory Data Control, Riviera Beach, FL) after the animals had been stabilized for 30 min at 36–38° on heating pads. Temperatures were monitored by rectal thermometers (model 47, Yellow Springs Instrument Co., OH).

BSP-T_M was determined after the method of Klaassen and Plaa [11]. BSP was dissolved in physiological saline (25 or 35 mg/ml) and infused at the rate of 20μl/min/100 g body weight. Bile was collected for five consecutive 15-min periods, after initiation of the infusion, into preweighed vials which were then reweighed. After dilution with 0.1 N

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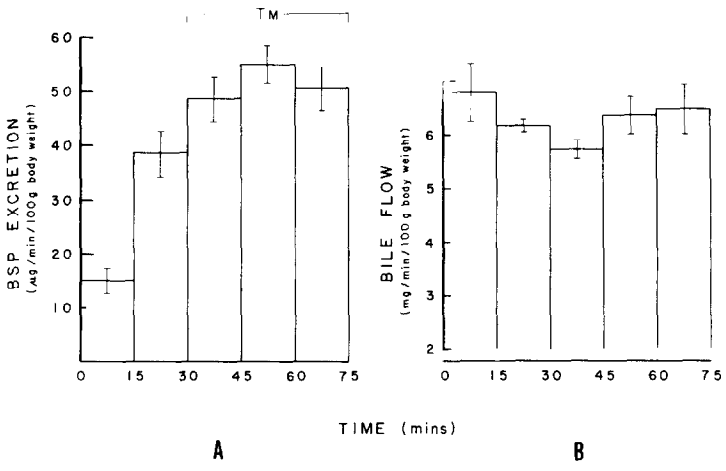

Fig. 1. Panel A: control BSP excretion rate for each 15 min of bile collection, showing the maximal level reached at 30–75 min. T_M was calculated as the average excretion during the third, fourth and fifth periods. Panel B: control bile flow rate during each 15-min period of bile collection.

Table 1. Effects of infusion rate on bile flow, BSP maximal concentration, and T_M in bile

BSP infusion rate (μg/min/100 g body wt)	Bile flow (mg/min 100 g body wt)	BSP maximal concentration (μg/mg bile)	BSP-T_M (μg/min/100 g body wt)
500 (7)*	6.17 ± 0.27†	8.45 ± 0.17	52.1 ± 2.8
700 (4)	5.92 ± 0.38	8.39 ± 0.50	48.0 ± 6.0

* Numbers of animals per group are given in parentheses.
† Values are expressed as means ± S.E.M.

NaOH, the absorbance at 580 nm was measured with a Spectronic 20 colorimeter (Bausch & Lomb) to determine BSP concentration.

Figure 1 shows the rates of biliary BSP excretion and bile flow in control hamsters. The BSP excretion rate reached a maximum by the third collection period and maintained this level for an additional 30 min (Fig. 1A), as did BSP concentration. The BSP-T_M and maximal biliary concentration for each animal was therefore calculated as the mean of the excretion rates in the third, fourth and fifth periods. Bile flow remained relatively constant throughout the experiment (Fig. 1B), and values quoted hereafter were derived from an average of the bile flow rate in the third, fourth and fifth periods. Increasing the infusion rate from 500 to 700 μg BSP/min/100 g body weight had no effect on biliary BSP-T_M, maximal concentration, or bile flow rate (Table 1). This ensures that, at the former rate, BSP excretory mechanisms were saturated.

Neither acute nor chronic Enovid treatment of hamsters had any effect on bile flow, BSP maximal concentration, or BSP-TM (Table 2). Seven animals received 300 mg/kg ANIT; in three bile flow ceased completely, whereas the other four animals showed normal bile flow rates (Table 2). Although bile flow was normal, there was an almost 50 per cent reduction in biliary BSP-T_M and hence maximal concentration in bile (Table 2).

The use of BSP-T_M to measure biliary transport capacity has been criticized on the basis that, during infusion of high levels of BSP, biliary BSP excretion was directly proportional to bile flow, and both decreased steadily with time [12, 13]. Dhumeaux *et al.* [12] suggested that, at least in the particular rat strain used, maximal BSP concentration in bile might be a more appropriate measure of hepatic excretory capacity than BSP-T_M. However, these criticisms do not apply to the present studies because bile flow remained relatively constant for the 75 min of infusion, but the biliary BSP excretion rate increased to a maximum by 30–45 min and maintained that level for a further 30 min (Fig. 1). Results following ANIT intoxication also indicate that BSP-T_M may vary independently of bile flow (Table 2).

Another problem which has been reported is that the decreased bilirubin T_M observed in rats pretreated with some steroids may be due solely to the induced hypothermia [14]. Therefore, in the present study, hamsters were maintained at a constant temperature throughout the experiment. To ensure that BSP excretory mechanisms were saturated at the infusion rate to be used, some hamsters were infused at higher rates, and indeed did not show an increased BSP-T_M.

In the present studies, neither chronic ingestion of Enovid at doses comparable to those used in women, nor 5 days of pretreatment with high doses of Enovid caused any detectable alterations in hepatic excretion capacity. Therefore, the hamster has proven highly resistant, in contrast to rats and humans, to the cholestatic effects of this oral contraceptive. Hamsters have also been reported to be more resistant to the cholestatic effects of ANIT as measured by hyperbilirubinemia and decreased bile flow [10, 15], and BSP plasma disappearance [15]. In the present studies using three times the dose of ANIT required to produce severe cholestasis in rats [16], three of seven treated hamsters showed complete bile flow stoppage, while the remaining animals showed normal bile flow. Similar results were reported by Indocochea-Redmond and Plaa [10]. However, in animals with normal bile flow, BSP-T_M was reduced by 50 per cent. ANIT is known to have many effects on the rat liver [17] including bile duct necrosis, cholangitis, bile canalicular membrane damage, and consequently impaired transport of substances into bile [9, 16]. The absence of bile flow in some animals may indicate that bile ducts are sufficiently blocked by necrotic debris to prevent the flow of bile from the bile duct cannula, or this may be an artifact of cannulation in ANIT-treated animals. However, in other hamsters, bile flow was not obstructed at all, but a 'subclinical' defect in the biliary transport of

Table 2. Effects of acute ANIT, Enovid and chronic Enovid treatment on bile flow, BSP maximal concentration, and T_M

Animal treatment		Bile flow (mg/min/100 g body wt)	BSP maximal concentration (μg/mg bile)	BSP-T_M (μg/min/100 g body wt)
Acute				
Control	(7)*	6.17 ± 0.27†	8.45 ± 0.17	52.1 ± 2.8
Enovid	(4)	6.55 ± 0.22	8.31 ± 0.18	54.3 ± 1.0
ANIT	(4)	6.53 ± 0.33	4.37 ± 0.70‡	27.8 ± 3.6‡
Chronic				
Control	(4)	5.52 ± 0.24	9.07 ± 0.45	49.1 ± 3.5
Enovid	(4)	5.95 ± 0.32	9.25 ± 0.30	54.0 ± 3.6

* Numbers of animals per group are given in parentheses.
† Values are expressed as means ± S.E.M.
‡Denotes values significantly different from respective controls ($P < 0.001$).

hepatocytes was detectable as a reduced BSP-T_M (Table 2).

These studies have emphasized the resistance of the Syrian golden hamster, in contast to rats and humans, to cholestasis as measured by bile flow and BSP-T_M, following acute or chronic oral contraceptive pretreatment. Cholestasis was induced in hamsters with high doses of ANIT, which appeared to have an all-or-none effect on bile flow; however, BSP-T_M was reduced in those animals where bile flow was normal.

Eppley Institute for Research in Cancer,
University of Nebraska Medical Center,
Omaha, NE 68105, *U.S.A.*

PAUL J. HERTZOG*
VERNA ILACQUA
RALPH GINGELL

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* Present address: Cancer Research Unit, University of York, Heslington, York YO1 5DD, U.K.