Hepatic Organic Anion Transport Kinetics and Bile Flow during Short-Term Total Parenteral Nutrition in the Rabbit (43147)

JOHN B. DAS,[1] SANTIBRATA GHOSH,* CATHERINE M. COSENTINO, AND G. GHAUS ANSARI

Division of Pediatric Surgery, The Children's Memorial Hospital, Chicago, Illinois 60614 and the Department of Cell, Molecular and Structural Biology, Northwestern University Medical School, Chicago, Illinois*

Abstract. **Plasma disappearance of sulfobromophthalein (BSP) after an intravenous bolus (5 mg/kg) was determined in six lab chow-fed (LCF) rabbits and in six rabbits maintained on total parenteral nutrition (TPN) for 5 days. A common bile duct cannula enabled measurements of bile flow and biliary BSP excretion. Compartmental analysis of the biexponential plasma disappearance curve yielded three fractional transfer rates, plasma to liver (hepatic uptake), liver to plasma (reflux), and liver to bile (canalicular excretion). The transfer rates for hepatic uptake were 0.253 ± 0.061/min for LCF and 0.147 ± 0.040/min for TPN ($P < 0.01$) and for the canalicular excretion of BSP were 0.038 ± 0.019/min for LCF and 0.019 ± 0.002/min for TPN ($P < 0.05$). Model-computed rates for BSP excretion in bile over 60 min were lower with TPN (61%) than with LCF (80%); the measured excretory rates were 53% for TPN rabbits and 75% of injected dose for LCF animals. Basal biliary flow was reduced by 50% in the TPN group. With a two-compartmental model, assuming two pools and three transfer rates, we have demonstrated for the first time significant decreases in hepatic uptake and canalicular excretion of the organic anion BSP during TPN. A decrease in hepatic blood flow due to the enteral fast of TPN could have contributed in part to the decreased hepatic uptake. But, because the second exponent of the biexponential curve is independent of hepatic blood flow, the decrease in liver to bile transfer rate is a true approximation of a diminished canalicular excretory capacity during TPN. It is concluded that the movement of organic anions along the hepatic BSP/bilirubin transport system is impaired early during TPN.** [P.S.E.B.M. 1990, Vol 195]

The impact of total parenteral nutrition (TPN) on hepatobiliary function has not been fully explored. The marked diminution in the volumes of pancreatic and biliary secretions during the "enteral fast" of TPN has been recognized and used to advantage in the management of high enterocutaneous fistulae (1). But quantitative information on the transhepatic traffic of organic anions during TPN and its associated fast is lacking.

Kinetic analysis of the plasma disappearance of sulfobromophthalein (BSP) provides quantitative estimates of uptake, storage, and excretion across the classic hepatic organic anion (BSP/bilirubin) transport system. BSP, a triphenylmethane dye, binds to serum albumin and is treated by the liver in a manner similar to bilirubin. The two organic anions share a common transport pathway (2). Richards *et al.* (3) proposed mathematical relationships to predict, from the plasma disappearance of the dye after a bolus injection, the rates of hepatic BSP uptake and biliary secretion. This approach has been applied to studies in humans in health (4) and in disease (5). The specific aims of this study were (i) to determine the rates of transfer of BSP from plasma to bile in rabbits maintained on parenteral nutrition, with laboratory chow-fed rabbits serving as controls; and (ii) to ascertain whether these rates calculated by computer simulation from the plasma BSP disappearance curve alone would be indicative of variations in hepatobiliary function.

Materials and Methods

Female NZW rabbits (body wt 1.9–2.5 kg) were allotted to two groups: Group 1 consumed laboratory

[1] To whom requests for reprints should be addressed at Pediatric Surgical Research, Box 114, The Children's Memorial Hospital, 2300 Children's Plaza, Chicago, IL 60614.

Received February 6, 1990. [P.S.E.B.M. 1990, Vol 195]
Accepted June 21, 1990.

0037-9727/90/1952-274$2.00/0

chow *ad libitum* (LCF; $n = 6$) and Group 2 was fed by TPN for 5 days ($n = 6$). All animals were allowed water *ad libitum*. They were housed in individual ages with wire mesh floors under controlled conditions of alternate 12-hr light and dark cycles (room temperature, 22°C).

In the TPN groups, a standard TPN solution (amino acids, dextrose, and soybean oil/egg phospholipid emulsion; 110 kcal/kg/day) was delivered by metered pump via an external jugular vein, utilizing a swivel assembly. The surgical procedures (cannulations and laparotomy) were done under general anesthesia and sterile conditions. Anesthesia was induced with ketamine (40 mg/kg im) and xylazine (4 mg/kg im) and maintained with fractionated doses of both agents, every 60 to 90 min, for the duration of the experiment. Core temperature was maintained at 37–38°C with a heating pad. Implantation of the venous cannula was via short incisions (8- to 10-mm long) in the neck, with minimal surgical dissection. Initiation of TPN was delayed for 24–36 hr after venous cannulation, to allow for recovery from surgery. All animals, LCF and TPN, underwent a laparotomy the morning after their dietary regimens. A carotid artery was cannulated for blood sampling. The cystic duct was occluded with a hemoclip, and the common bile duct was cannulated proximal to the duodenum with polyethylene tubing (i.d., 0.76 mm; length, 40 cm). Consecutive 10-min collections of hepatic bile were made by gravity drainage in preweighed plastic vials. The volume of bile secreted was estimated gravimetrically. After a baseline period of 40 min to allow stabilization of bile flow, a bolus of BSP in 5% dextrose (dose, 5 mg/kg iv) was given. Serial blood samples, at 3- to 10-min intervals, and bile samples, at 10-min intervals, were collected over the next 120 min. The animal was then sacrificed with an overdose of pentobarbital sodium (100 mg/kg iv).

Chemical Analysis. Serum and bile BSP concentrations were determined colorimetrically (6). In our laboratory, the recoveries of BSP in porcine bile were 97–104%, over a BSP concentration range of 5–100 mg/dl of bile. In baseline samples, total bile acids in serum and bile were measured enzymatically with the 3α-hydroxysteroid dehydrogenase assay (test kits by Nycomed AS, Oslo, Norway). Total cholesterol, serum and biliary, was measured by an enzymatic technique (17).

Kinetic Analyses and Calculations. For each individual animal, the plasma BSP concentration data were first fitted to a biexponential equation, against time, by a least squares curve-fitting technique (8). With the parameters obtained as the initial estimates, a direct nonlinear least squares fit of the data was derived by a Simplex algorithm (9). In all cases, the goodness of fit, as judged by the values of the coefficient of determination, was better after this direct fit. The plasma disappearance curve for BSP yielded two slopes with the exponents $K1$ and $K2$ with A and B their respective zero-time intercepts. Such a biexponential fit is consistent with a two-compartmental model for BSP transport (3) (Fig. 1, inset).

The three fractional transfer rates were calculated for movement of dye from pool to pool and to bile: (i) the plasma to liver transfer rate (a), (ii) the liver to bile transfer rate (h), and (iii) the liver to plasma transfer (reflux) rate (b), each fractional rate being constant at all times (3, 10). The transfer rates (per min) were calculated as follows (3, 11, 12).

$$a = (AK1 + BK2)/(A + B)$$

$$h = (K1 \times K2)/a$$

$$b = (K1 + K2) - (a + h)$$

The initial volume of distribution (V_d) in ml was calculated as $V_d = \text{dose}/(A + B)$, a volume equivalent to the plasma volume measured by the albumin-space method (11).

At time = t_{ss}, the liver content of BSP is at its peak and the plasma to liver transfer equals the liver to bile loss.

$$t_{ss} = (\log \cdot eK1 - \log \cdot eK2)/(K1 - K2)$$

The plasma clearance at this point (CL_{ss}) is calculated as

$$CL_{ss} = \{(a \times h)/(b + h)\} \times V_d$$

This value is identical to hepatic clearance derived from the area under the curve for plasma decay of BSP (12).

Basal (resting) bile flow/min was calculated as the average of the last two 10-min collections in the 40-min baseline period.

Statistics. The measured and derived parameters were expressed as means and standard deviations. Between-group comparisons were made by the unpaired Student's t test, with statistical significance set at $P < 0.05$.

Results

Preliminary comparisons of bi- and triexponential fits for the plasma BSP disappearance data failed to show any improvement in the goodness of fit with a triexponential curve analysis. Therefore, a two-compartmental model with an open-ended biliary run-off was considered physiologically realistic for compartmental analysis.

All animals gained weight during the study, averaging 26 g/day. The concentrations of total serum bile acids and cholesterol were higher in the TPN group, indicative of an early onset of cholestasis. Steady-state hepatic clearance of BSP was also significantly diminished in the TPN group (Table I). Plasma volume remained unchanged.

Table I. Serum Bile Acids and Cholesterol and the Model-Independent Parameters in LCF and TPN Rabbits

	Serum bile acids (μM)	Serum cholesterol (mM)	Plasma volume (V_d) (ml/kg)	Extraction coefficient (K_e) (fraction/min)	CL_{ss}[a] ($K_e \cdot V_d$) (ml/kg·min)
LCF (n = 6)	3.65 ± 1.31	1.33 ± 0.28	39.0 ± 7.8	0.189 ± 0.037	7.48 ± 2.58
TPN (n = 6)	8.43 ± 4.64	4.94 ± 0.54	45.2 ± 5.1	0.083 ± 0.039	4.94 ± 0.54
P	<0.05	<0.001	NS[b]	<0.01	<0.05

[a] CL_{ss}, steady-state clearance of the organic anion BSP.
[b] NS, not significant.

Plasma decay of BSP was slower after 5 days of TPN (Fig. 1). The model-dependent transfer rates, a, b, and h, and the derived data for t_{ss} and for the maximal liver content at t_{ss} (as fraction of injected dose) are shown in Table II. In the TPN-fed rabbits, sinusoidal uptake (a) and canalicular excretion (h) were significantly decreased after intravenous feeding. The transfer rate for liver to plasma reflux (b) was the same in both groups. During TPN, the slower sinusoidal uptake of BSP was reflected in a prolonged t_{ss}. In spite of this, the maximal liver content at t_{ss} equaled that in rabbits on laboratory show.

Basal (resting) bile flow was reduced by 50% after 5 days of TPN (Table III). The biliary concentration of total bile acids decreased, with the biliary concentration of cholesterol remaining unchanged. Computer simulation of the distribution of BSP (as fraction of injected dose) between plasma, liver, and bile showed that, in the LCF rabbits at the third minute, the dye was equally distributed between plasma (48%) and liver (49%) pools. But, in the parenterally fed animals, 66% of the dye was still in the plasma pool. Model-computed cumulative BSP excretion in bile (percentage of injected dose) over the first 60 min was lower with TPN (61%) than with LCF (80%). The corresponding cumulative measured rates were 53% for TPN and 75% for LCF.

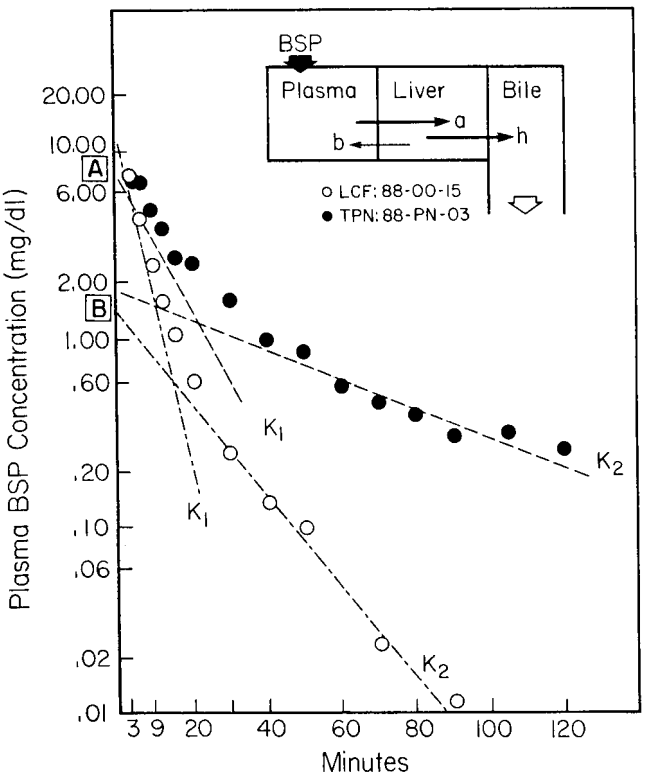

Figure 1. Plasma concentrations of BSP after a bolus injection in a LCF and in a TPN rabbit. The two exponential components (slopes $K1$ and $K2$) and their y-intercepts (A and B, respectively) are shown. Inset, the two-compartmental model with an open-ended biliary outflow. The transfer rates are: a, plasma to liver; b, liver to plasma; and h, liver to bile.

Calculated values for the BSP fractions in plasma, liver, and bile are shown in Figure 2, along with the observed values in plasma and bile for a representative animal from each group. The differences between the observed and calculated values for BSP in bile varied from animal to animal.

Discussion

The conventional graphic method of biexponential fit of data, using semilogarithmic plots (or logarithmic regressions), is arbitrary and not always accurate over the entire anion concentration range. The availability of algorithms for nonlinear least square fits, such as the Simplex method used in this work, and the use of computers have improved the accuracy and power of this approach.

Based on the plasma data alone, it was possible to predict the time and magnitude of hepatic uptake and the biliary excretory rate for BSP. The correlation between the observed and calculated values for plasma BSP was good (r = 0.99). However, in both LCF and TPN animals, the curve for the measured cumulative excretion of BSP in bile was shifted to the right of the curve for the computer-predicted values. The calculated values represent a fraction of the injected dye projected at the biliary canalicular level, whereas all measured values were from bile collected from a more distal common bile duct. No corrections were made for the biliary dead space and the volume of the biliary cannula, both of which would have contributed to delays in the appearance of dye in the collection vial. These collection delays alone could have shifted the curve for the measured excretion to the right.

Under the conditions of our experiments, after 5

Table II. Plasma Clearance of BSP in LCF and TPN Rabbits: Anion Transfer Rates and Model-Dependent Parameters

	Transfer rates/min			t_{ss}[a] (min)	Liver uptake$_{max}$[b] (fraction of dose)
	Plasma to liver (*a*)	Liver to plasma (*b*)	Liver to bile (*h*)		
LCF (n = 6)	0.253 ± 0.061	0.011 ± 0.005	0.038 ± 0.019	9.28 ± 2.00	0.70 ± 0.11
TPN (n = 6)	0.147 ± 0.040	0.009 ± 0.002	0.019 ± 0.002	15.50 ± 3.80	0.67 ± 0.08
P	<0.01	NS[c]	<0.05	<0.01	NS

[a] t_{ss}, time to reach maximum liver uptake of BSP.
[b] Liver uptake$_{max}$, highest fraction of dose at time t_{ss}.
[c] NS, not significant.

Table III. Bile Flow and Biliary Excretions of Bile Acids, Cholesterol, and BSP in LCF and TPN Rabbits

	Basal bile flow[a] (μl/kg·min)	Bile acids (m*M*)	Cholesterol (m*M*)	Cumulative bile BSP excretion (fraction of dose at 60 min)	
				Measured	Calculated
LCF (n = 6)	60.7 ± 19.7	15.0 ± 2.23	0.23 ± 0.05	0.755 ± 0.170	0.797 ± 0.182
TPN (n = 6)	27.2 ± 8.7	7.3 ± 4.8	0.20 ± 0.13	0.533 ± 0.165	0.607 ± 0.071
P	<0.01	<0.01	NS[b]	<0.01	<0.05

[a] Basal bile flow, mean of a 20-min collection from common bile duct before bolus injection of BSP.
[b] NS, not significant.

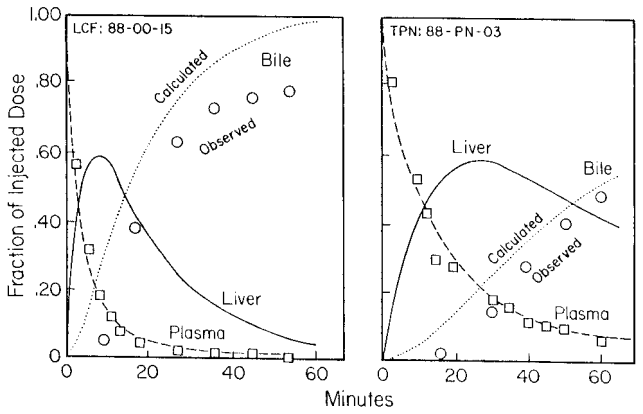

Figure 2. Distribution of BSP, as fraction of dose, between plasma, liver, and bile in a LCF and in a TPN rabbit. ——, Predicted values; □ and ○, measured values.

days of TPN in the rabbit, resting bile flow and the biliary excretion of bile acids and cholesterol were lower than in LCF, whereas serum concentrations of bile acids and cholesterol were elevated, indicative of a "cholestatic state." During TPN, biliary secretion of the exogenous organic anion (BSP) was markedly reduced. Unlike in estrogen-induced cholestasis (an experimental and clinical model for reversible cholestasis), where biliary canalicular secretion of BSP is depressed (13) with little change in hepatic uptake (14), the results of our study showed decreases in the transfer rates for both hepatic uptake and canalicular secretion of BSP during TPN-associated cholestasis. Hepatic uptake, however, exceeded canalicular secretion, demonstrating a substantial capacity for hepatic storage of dye during short-term TPN. The slower hepatic uptake during TPN merely delayed the time (t_{ss}) to reach maximal storage (Table II).

Hepatic uptake is determined by hepatic blood flow and the actual transfer of solute ("intrinsic hepatocyte uptake") across the sinusoidal membrane (15). The enteral fast enforced during TPN would reduce splanchnic blood flow and might contribute in part to the impaired hepatic uptake of BSP. The absence of an increased reflux of dye from liver to plasma during TPN (the transfer rate *b* being similar in LCF and TPN) negates the possibility of biliary regurgitation having contributed to the delay in plasma clearance and hepatic uptake of BSP.

The second component (*K2*) of the plasma disappearance curve is virtually independent of changes in hepatic blood flow and is, therefore, a true approximation of the excretory capacity of the bile canaliculus (16). With canalicular excretion being the rate-limiting step in biliary transport of organic anions (17), this decrease in biliary excretory capacity is probably the earliest and most critical event in the hepatobiliary dysfunction of TPN. Elevated levels of plasma γ-glutamyl transpeptidase and 5′-nucleotidase seen early during TPN in infants have also pointed to damage to the canalicular membrane as a primary event during TPN-induced cholestasis (18). It has been postulated that in the cholestatic syndrome of TPN the liver cell membrane may be damaged by an excess of Na^+-dependent amino acids (19) and relative lack of serine

(a methyl donor that improves membrane fluidity) in the infusate (20). The physicochemical changes in the liver cell membrane and their relation to hepatobiliary function during TPN have yet to be explored.

Finally, this study confirms and extends previous observations that TPN reduces basal bile flow in the experimental animal (21, 22). With a model assuming only two pools and three transfer rates, we have demonstrated for the first time measurable decreases in the transfer rates for hepatic uptake and canalicular excretion of an organic anion, with little change in the storage capacity of the hepatocyte during short term TPN in the rabbit model.

This work was supported by the Children's Surgical Foundation, Inc., Chicago, IL and was presented in part at the XXXI International Congress of Physiological Sciences, Helsinki, Finland, July 1989.

We appreciate the help of Ms. Jean Atchison in the preparation of this manuscript.

1. Dudrick SJ, Wilmore DW, Steiger E, Mackie JA, Fitts WT Jr. Spontaneous closure of traumatic pancreatic duodenal fistulas with total intravenous nutrition. J Trauma **10**:542–553, 1970.
2. Berk PD, Stremmel W. Hepatocellular uptake of organic anions. Prog Liver Dis **8**:125–144, 1986.
3. Richards TG, Tindell VR, Young A. A modification of the bromsulphthalein liver function test to predict the dye content of the liver and bile. Clin Sci **18**:499–511, 1959.
4. Barber-Riley G, Goetzee AE, Richards TG, Thomson JY. The transfer of bromsulphthalein from the plasma to the bile in man. Clin Sci **20**:149–159, 1961.
5. Quarfordt SH, Hilderman HL, Valle D, Waddell E. Compartmental analysis of sulfobromophthalein transport in normal patients and patients with hepatic dysfunction. Gastroenterology **60**:246–255, 1971.
6. Gaebler OH. Determination of bromsulphthalein in normal, turbid, hemolyzed or icteric serums. Am J Clin Pathol **15**:452–455, 1945.
7. Bolton CH, Nicholls JS, Heaton KW. Estimation of cholesterol in bile: assessment of an enzymatic method. Clin Chim Acta **105**:225–230, 1980.
8. Riggs DS. The Mathematical Approach to Physiological Problems. Baltimore: Williams & Wilkins, pp146–151, 1963.
9. Noggle J. Physical Chemistry on a Microcomputer. Boston: Little, Brown & Co., pp148–163, 1985.
10. Berk PD, Blaschke TF, Waggoner JG. Defective bromosulfophthalein clearance in patients with constitutional hepatic dysfunction (Gilbert's syndrome). Gastroenterology **63**:472–481, 1972.
11. Berk PD, Isola LM, Jones EA. Specific defects in hepatic storage and clearance of bilirubin. In: Ostrow JD,Ed. Bile Pigments and Jaundice: Molecular, Metabolic and Medical Aspects. New York: Marcel Dekker Inc., pp279–316, 1986.
12. Clarkson MJ, Richards TG. Steady-state plasma clearance of bromsulphthalein and indocyanine green measured by single injection. Res Vet Sci **8**:454–462, 1967.
13. Heikel TA, Lathe GH. The effect of oral contraceptive steroids on bile secretion and bilirubin Tm in rats. Br J Pharmacol **38**:593–601, 1970.
14. Mueller MN, Kappas A. Estrogen pharmacology. I. The influence of estradiol and estriol on hepatic disposal of sulfobromophthalein (BSP) in man. J Clin Invest **43**:1905–1914, 1964.
15. Vatner SF, Patrick TA, Higgins CB, Franklin D. Regional circulatory adjustments to eating and digestion in conscious, unrestrained primates. J Appl Physiol **36**:524–529, 1974.
16. Hacki W, Bircher J, Preisig R. A new look at the plasma disappearance of sulfobromophthalein (BSP): Correlation to BSP transport maximum and the hepatic plasma flow in man. J Lab Clin Med **88**:1019–1031, 1976.
17. Wheeler HO, Meltzer JI, Bradley SE. Biliary transport and hepatic storage of sulfobromphthalein sodium in the unanesthesized dog, in normal man and in patients with hepatic disease. J Clin Invest **39**:1131–1144, 1960.
18. Black DD, Suttle A, Whitington PF, Whitington GL, Korones SD. The effect of short-term total parenteral nutrition on hepatic function in the human neonate: A prospective, randomized study demonstrating alterations of hepatic canalicular function. J Pediatr **99**:445–449, 1981.
19. Preisig R, Rennert O. Biliary transport and cholestatic effects of aminoacids [Abstract]. Gastroenterology **73**:1240, 1977.
20. Belli DC, Fournier L-A, Lepage G, Yousef I, Weber AM, Tuchweber B, Roy CC. Total parenteral nutrition-associated cholestasis in rats: Comparison of different amino acid mixtures. J Parent Ent Nutr **11**:67–73, 1987.
21. Zahavi I, Shaffer EA, Kelly J. Reduced bile output with chronic enteral and parenteral infusion of amino acids, glucose and fat emulsion in rabbits. J Pediatr Gastroenterol Nutr **4**:622–627, 1985.
22. Truskett PG, Shi ECP, Rose M, Sharp PA, Ham JM. Model of TPN-associated hepatobiliary dysfunction in the young pig. Br J Surg **74**:639–642, 1987.