Impaired Biliary Excretion of Phenol 3, 6-Dibromphthalein Disulfonate in Neonatal Guinea Pigs[1] (35629)

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Previous studies in this laboratory in which either submaximal loading doses of unconjugated and conjugated BSP (1), or in which doses sufficient to result in maximal rates of excretion of dye into bile (2) were administered to neonatal and adult guinea pigs revealed depressed excretion of dye into bile in neonatal animals when unconjugated BSP was given, but normal excretion when conjugated BSP was injected. Hepatic uptake did not limit hepatic disposition of unconjugated dye (2). With increasing age, injected unconjugated BSP was excreted at progressively more rapid rates. Increased output of unconjugated BSP into bile was the major factor accounting for improvement in dye excretion in neonatal animals infused with unconjugated BSP (2). The studies have been interpreted as indicating that impaired hepatic disposition of injected unconjugated BSP in the neonatal guinea pig is due to a combination of decreased transport of unconjugated dye from liver cells into bile, and decreased conjugation of BSP. The first defect is the major one.

The availability of phenol 3,6-dibromphthalein disulfonate, hereafter, referred to as DiBSP, an analog of BSP differing only by two less bromine atoms and reported not to be or only slightly metabolized prior to excretion into bile (3–5), provides a valuable tool for further exploring the hepatic disposition of organic anions in the neonatal period. In the present studies, hepatic uptake and biliary excretion of DiBSP have been compared in neonatal and adult guinea pigs. The data demonstrate the presence of an excretory defect for the dye in the newborn animal.

Materials and Methods. Phenol 3, 6-dibromphthalein disulfonate (DiBSP) supplied by Hynson, Westcott and Dunning as a powder, was dissolved in sterile saline (0.85%) prior to injection.

Biliary excretion of DiBSP. Adult female guinea pigs, 270–350 g, and neonatal guinea pigs from the 2nd to 16th day of life (Maxfield, Cincinnati) were anesthetized throughout with ethyl ether. Bile was collected through an indwelling polyethylene (PE-50) common bile duct cannula, the cystic duct was ligated and the midline abdominal incision was sutured. Body temperature was monitored via a rectal probe (Tele-Thermometer, Yellow Springs Instrument Co.) and controlled between 37 and 38° by an electric heating pad placed underneath the animal. Dye was administered into a femoral vein by single injection in a volume of 0.5 ml/100 g of body weight, over a 30-sec timed period. The first bile sample, which contained cannula washout and the initial appearance of dye in bile, was collected over a 5-min interval. Subsequent samples were collected during three successive 10-min periods. At the end of each experiment, a blood sample was taken via the aorta into a heparinized syringe.

Bile was collected into previously tared bottles, and volume was considered equivalent to the weight of the bile. Concentration

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of DiBSP in bile was determined as recorded previously (4). Dye excretion in any sample was calculated as the product of dye concentration and bile volume and recorded (μmoles of dye/100 g of body wt/10-min sample).

We discarded specimens from animals experiencing respiratory difficulty or a sharp decline in bile flow during the experiment.

Hepatic uptake of DiBSP. Net hepatic uptake of DiBSP was assessed in both adult and newborn guinea pigs on the second day of life by methods previously described for measurement of hepatic uptake of BSP (6). To minimize excretion of dye into bile during the experiments, animals prepared via a midline abdominal incision under ether anesthesia, were studied either with patent biliary trees (designated nonligated) and their livers were removed at 2 min following injection, or with ligated cystic and common bile ducts (designated ligated) and livers removed at 5.5 and 10.5 min after injection. Ten min after preparation of the animal was completed, 24 μmoles/100 g of body weight of DiBSP was administered intravenously. A timed blood sample from the aorta was taken over a 30-sec interval immediately preceding removal of the liver. Livers were blotted, weighed, and analyzed for DiBSP content by a method described previously for extraction of BSP from the liver (6). In preliminary studies, it was demonstrated that dye is completely recovered by the extraction procedure. Results of hepatic dye content of DiBSP, corrected for trapped plasma content, are expressed both as micromoles per gram of liver and as micromoles per 100 g of body weight. Total plasma volume and hepatic trapped plasma volume used for these calculations were determined in a previous series of experiments. Total plasma volume averaged 4.3 ml/100 g of body weight and 5.8 ml/100 g for adults and neonatal animals, respectively, and trapped hepatic plasma volume averaged 0.13 ml/g of liver wet weight in neonatal guinea pigs (unpublished observations in this laboratory). Trapped hepatic plasma volume averaged 0.10 ml/g of liver in the adult guinea pig (6).

Chromatograms. Preliminary studies were performed with bile from animals injected with DiBSP. Aliquots were applied to Whatman No. 1 filter paper and subjected to descending chromatography as previously described (4). A single band chromatographically indistinguishable from that of injected DiBSP, was observed on exposure of the dried chromatograms to ammonia vapor. This did not react with ninhydrin and was considered to be unaltered DiBSP, a finding similar to that noted in the rat (3, 4, 5). Thus detailed analysis of bile by chromatography was not performed in the rest of the study.

Statistical Analysis. Statistical evaluation of the results of the above experiments was performed utilizing the Mann-Whitney U Test for nonparametric analytical procedures (7).

Results. Biliary excretion of DiBSP. a. Adult animals. Maximal rates of excretion were achieved 5 to 15 min after administration of DiBSP as a single injection to adult guinea pigs. The rate of dye excretion during this first 10-min collection period reached maximal values at a DiBSP dose of 24 μmoles/100 g of body weight, and was not raised further by progressively increasing the dose up to 60 μmoles/100 g of body weight (Fig. 1). With doses of 42 μmoles/100 g of

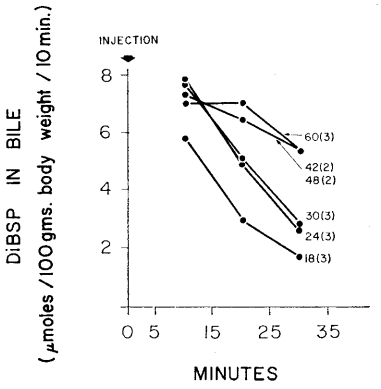

FIG. 1. Biliary excretion of DiBSP in adult guinea pigs: Single injections of DiBSP were administered at zero time. The doses of DiBSP given (μmoles/100 g of body wt) are listed on the right; and the number of animals receiving each dose are given in parentheses. Maximal rates of dye excretion were achieved in the 5- to 15-min collection period with doses of DiBSP of 24 to 60 μmoles/100 g of body weight.

TABLE I. Biliary Excretion of Dye in Guinea Pigs Given Single Intravenous Injections of DiBSP.[a]

Age (day of life)	No. of animals	Body wt[a] (g)	Liver wt/body wt[a] (%)	Dose (μmoles/100 g)	Bile flow[a] (ml/100 g/10 min)	Dye in bile: Conc[a] (μmoles/100 ml)	Dye in bile: Maximal rate of excretion[a] (μmoles/100 g/10 min)
2	15	92 ± 9.8	3.9 ± 0.6 ($p > 0.1$)[b]	18 (5)[c] 24 (5) 30 (5)	0.19 ± 0.04 ($p < 0.01$)[b]	2244 ± 426 ($p < 0.001$)[b]	3.78 ± 1.40 ($p < 0.001$)[b]
6	14	97 ± 21.2	3.1 ± 0.3 ($p < 0.001$)	24 (9) 30 (5)	0.24 ± 0.05 ($p > 0.1$)	2211 ± 451 ($p < 0.001$)	5.34 ± 1.54 ($p = 0.001$)
9	9	113 ± 14.2	3.2 ± 0.3 ($p < 0.001$)	24 (5) 30 (4)	0.25 ± 0.04 ($p = 0.1$)	2478 ± 482 ($p < 0.01$)	6.36 ± 1.86 ($p > 0.1$)
16	6	153 ± 16.4	3.6 ± 0.3 ($p < 0.025$)	24 (3) 30 (3)	0.25 ± 0.04 ($p > 0.1$)	2905 ± 477 ($p > 0.1$)	7.13 ± 1.49 ($p > 0.1$)
Adults	13	296 ± 21.7	4.4 ± 0.8	24 (3) 30 (3) 42 (2) 48 (2) 60 (3)	0.24 ± 0.07	3199 ± 574	7.54 ± 1.06

[a] Results are expressed as mean ± 1 standard deviation.

[b] p values for each vertical column are expressed by comparison with the results obtained in adult animals.

[c] Number in parentheses denotes the number of animals injected with that dose of DiBSP.

body weight, and higher, the rate of excretion during the second and third 10-min collection periods fell less sharply however, and, at 60 μmoles/100 g of body weight, average excretion maintained a plateau over two successive 10-min collection periods. For tabulation in Table I, the maximal rate of DiBSP excretion into bile for each individual animal receiving 24 to 60 μmoles/100 g of body weight was considered to be the highest value achieved during any bile collection period or the mean of this value and values obtained during consecutive collection periods that were within 10% of the highest value. Thus the maximal rate of DiBSP excretion in 13 adult animals averaged 7.54 ± SD 1.06 μmoles/100 g of body weight/10 min (Table I). This value is a little higher than, but statistically not significantly different from, the average value of 6.45 ± SD 0.53 μmoles/100 g/10 min ($0.05 < p < 0.1$), obtained in unpublished studies from this laboratory in five animals given DiBSP as a prime of 7.4 μmoles/100 g of body weight followed by a constant infusion of 0.74 μmoles/100 g/min. Since studies in neonatal guinea pigs were performed with a single injection technique, the value of 7.54 μmoles/100 g/10 min calculated in adults by this technque was used for comparison with neonatal animals.

b. Neonatal animals. Guinea pigs, on the second day of life, achieved their highest rate of excretion in the second collection period (Fig. 2) with comparable results obtained at doses of DiBSP of 18 to 30 μmoles/100 g of body weight. Older animals achieved maximal rates of excretion that were comparable when given 24 and 30 μmoles/100 g of body weight. Doses of 42 μmoles/100 g, and above, were poorly tolerated by the neonatal guinea pigs, resulting in a sharp fall in bile flow and subsequently death of the animal. For tabulation in Table I, maximal rates of dye excretion were calculated in a manner similar to that for adult guinea pigs.

On the second day of life, the maximal rate of dye excretion was 3.78 ± SD 1.40 μmoles/100 g/10 min, a value 51% of that found in adult animals. Thereafter, dye excretion increased progressively and by the 16th day reached 95% of the adult value. On the second day of life, the rate of bile flow during maximal dye excretion was 72% of the adult rate (0.24 ± SD 0.07 ml/100 g/10 min). Bile flow reached adult values by the 6th day of life. The average concentration of DiBSP in bile on the second day of life was 66% of the adult value of 3199 ± SD 574 μmoles/100 ml. The rise in dye concentration in bile roughly paralleled the increase in maximal dye excretion into bile with increasing age. Plasma values of DiBSP at the end of the experiment exceeded 12 μmoles/100 ml in both newborn and adult animals. Because of the wide range of dosages used in adult animals no comparison between plasma values in adult and neonatal animals was attempted.

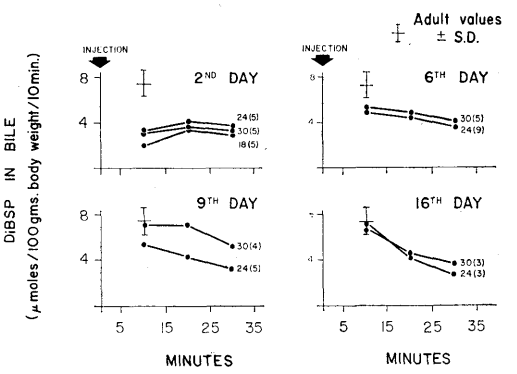

FIG. 2. Biliary excretion of DiBSP in neonatal guinea pigs: Rates of DiBSP excretion into bile in neonatal animals on the 2nd, 6th, 9th, and 16th days of life are shown for different doses of administered dye. The number of animals receiving each dose are listed in parentheses on the right of each section. The mean maximal rate of excretion ± 1 SD for adult animals is indicated in the upper left of each section by a crossed vertical bar.

Hepatic uptake of DiBSP. Studies involving neonatal animals were limited to the second day of life since the defect in hepatic disposition of DiBSP was maximal at this age. The initial rate of accumulation of DiBSP in liver was virtually the same in both adult and newborn guinea pigs at all time periods studied (Fig. 3). The average value for hepatic content of dye (μmoles/g of liver wet wt) was a little higher in newborn animals than in adults. Since liver size per unit of body weight is smaller at this age, when hepatic content is expressed as micro-

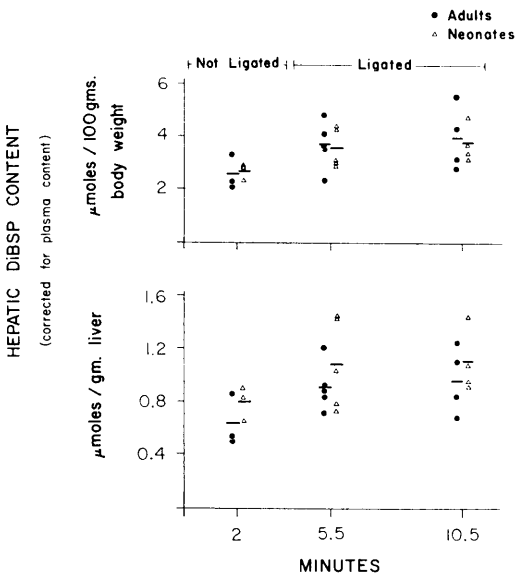

FIG. 3. Hepatic content of DiBSP in adult and neonatal guinea pigs: The values listed were corrected for plasma content of dye. Livers removed at 2 min after injection of dye were obtained from animals with patent biliary tracts and are designated nonligated. Common bile ducts were ligated in the animals whose livers were removed 5.5 and 10.5 min after administration of DiBSP.

moles per 100 g of body weight, the average values are almost identical. With either method of comparison there was no significant difference in hepatic dye content between adult and neonatal guinea pigs.

Even in the short duration of these studies, it is likely that some dye taken up in liver passes back into the blood. Thus hepatic content is not synonymous with hepatic uptake. Nevertheless, a minimal estimate of the initial rate of hepatic uptake of DiBSP can be calculated from the data for hepatic content of dye 2 min after intravenous administration. This value is approximately 1.3 μmoles/100 g of body weight/min in adult and neonatal animals, and is in excess of the maximal rate of excretion into bile of 0.75 μmoles/100 g of body weight/min in the adult and 0.38 in the 2-day-old neonatal guinea pig.

Discussion. As might be expected from their chemical similarity, DiBSP and BSP share processes involved in the movement of these organic anions from blood to bile [(4) unpublished observations by the authors]. It has not yet been established whether these include hepatic uptake or the excretory process or both. Nevertheless, the capacity of the system for each of the compounds as indicated by maximal rates of excretion into bile, differs as demonstrated by our findings in the adult guinea pig. Thus the average value for DiBSP T_m in the present study was 7.54 ± SD 1.06 μmoles/100 g/10 min. This contrasts sharply with maximal excretory rates of 1.62 ± SD 0.35 μmoles/100 g of body weight/10 min when conjugated BSP was injected, and of 0.85 ± SD 0.24 μmoles/100 g of body weight/10 min when unconjugated BSP was administered (6). In the latter instance, both unconjugated and conjugated BSP compounds were excreted into bile. The current findings contrast with those of Klaassen and Plaa (5), who found that the T_m for DiBSP and BSP were similar in rats, rabbits, and dogs. Thus there exists a species difference in the relative order of magnitude of maximal excretory rates for these compounds.

Despite the differences in absolute values for maximal rates of excretion into bile in guinea pigs, previous studies with BSP compounds and the present study with DiBSP demonstrate the presence of a similar major defect in dye disposition by the liver in neonatal guinea pigs. When unconjugated BSP was administered in doses sufficient to saturate the overall transport system, impaired delivery of dye into bile was observed in the neonatal animal (2). Impairment was most marked early in the neonatal period and gradually improved, with adult values of excretion being reached by the 16th day of life. Two important defects were detected: (i) impaired delivery of unconjugated BSP from liver cells to bile; and (ii) impaired conjugation of BSP. Hepatic uptake of BSP was not impaired and did not limit dye transport. The first defect appeared to be the most important quantitatively, and focused attention on the series of processes considered collectively as the excretory step in dye excretion.

The present studies concerned with an assessment of the hepatic disposition of 3, 6-dibromphthalein disulfonate, an analog of

BSP (DiBSP = BSP — 2 Br) which does not undergo metabolic transformation in liver (3–5) also demonstrate a depression of the excretory step for this dye in the neonatal guinea pig. Thus the maximal rate of biliary excretion of DiBSP was depressed in young neonatal animals below values for adults. By contrast, a minimal estimate of the rate of initial hepatic uptake of DiBSP was comparable in neonatal and adult guinea pigs and exceeded values for rates of excretion into bile. Clearly, uptake did not limit hepatic disposition of DiBSP. Since the dye is not metabolized in the liver of the guinea pig, it is logical that impairment in movement of DiBSP from within liver cells to bile is the factor accounting for delayed excretion into bile in the neonatal guinea pig.

The excretory process is composed of a series of steps including (a) intracellular movement of dye from the site of uptake to the bile canaliculus; (b) carrier-mediated active transport into the lumen of the bile canaliculus; and (c) probable back diffusion from the biliary tract along large concentration gradients generated by active secretion of dye into bile. Relatively little is known about these processes. Whether impaired excretion in the neonate is the result of defects in one or some combination of these steps must be established by further study.

Summary. Hepatic disposition of DiBSP was studied in adult and newborn guinea pigs from days 2 through 16 of life after intravenous administration of DiBSP in doses sufficient to achieve maximal rates of dye excretion into bile. Neonatal guinea pigs showed a significant reduction in the maximal rate of dye excretion into bile when compared to adult animals. A minimal estimate of hepatic uptake of DiBSP showed that uptake was similar in neonatal and adult animals and was significantly greater than the maximal rates of excretion into bile. Hence hepatic uptake did not limit hepatic disposition of DiBSP. With aging, injected DiBSP was excreted at progressively more rapid rates reaching adult levels of excretion at the beginning of the second week of life. This study demonstrates the presence of a defect in the excretory process by which DiBSP is transported from liver cells into bile in the neonatal guinea pig.

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