Morphologic and Biochemical Changes in Dogs After Portacaval Shunt Plus Bile Fistula or Ileal Bypass: Failure of Bile Fistula or Ileal Bypass to Prevent Hepatocyte Atrophy

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External biliary fistula (BF) or ileal bypass (IB) was performed in dogs at the time of or 2 weeks after portacaval shunt (PCS). The pathologic changes in the dog livers 2 to 4 weeks later were compared to those caused by PCS alone. Histopathologic differences between PCS alone vs. PCS plus BF or IB could not be found. Thus, the experiments did not confirm recent observations by others in rats that BF prevents or reverses the hepatic injury of PCS. As estimated by plasma mevalonic acid determinations, the increase in hepatic cholesterol synthesis that is characteristic after BF or IB was suppressed in animals with PCS. BF and IB reduced but did not eliminate the postprandial elevation in serum bile acid that occurs after PCS. The findings have possible relevance in planning the treatment of patients with familial hypercholesterolemia with the combined use of PCS and IB.

Completely diverting portacaval shunt (PCS), also called Eck's fistula, has been used for its effect upon the metabolism of patients with glycogen storage disease (1), familial hypercholesterolemia (2, 3), and α_1-antitrypsin deficiency (4). The metabolic palliation in these diseases has been ascribed in part to the hepatocyte alterations caused by portal diversion including atrophy, deglycogentation, fat accumulation, increased mitosis, and major ultrastructural disruptions (2, 4–9). These changes are identical in rats, dogs, and humans (5). Thus, research with any of these species should be applicable to all in spite of the fact that humans are unusually resistant to the hepatic encephalopathy that may complicate PCS.

Balabaud et al. (10) and Bioulac et al. (11) have reported that the hepatocyte abnormalities caused by PCS in rats could be prevented or reversed by simultaneous or delayed external bile fistula (BF). They speculated that the liver injury of Eck fistula had been avoided in their animals by preventing the elevation in serum bile acids that is a characteristic finding after PCS (12, 13).

We report here an unsuccessful attempt in dogs to confirm this protective effect of BF. In addition, the bile salt and bile acid depleting procedure of ileal bypass (IB) also failed to prevent the hepatic changes after PCS. The findings of this study have potential significance in planning how to manage patients treated for familial hypercholesterolemia with portal diversion.

MATERIALS AND METHODS

OPERATIVE PROCEDURES

After endotracheal intubation, the operations and sacrifice procedures were performed under anesthesia with pentobarbital sodium supplemented with xylazine (Rompun®) and succinylcholine chloride (Anectine®). The

technique for PCS consisted of side-to-side anastomosis of the portal or superior mesenteric vein to the inferior vena cava after excision of an ellipse from both vessels. After completion of the anastomosis, the portal vein was ligated at its bifurcation in the liver hilum, taking pains to eliminate any incoming venous tributaries to the portal branches above the site of ligation.

IB was performed as described by Buchwald et al. (14). The small intestine from the ligament of Treitz to the ileocecal junction was measured in the units of wound length, and it was divided at the junction of the proximal two-thirds and distal one-third. The end of the proximal segment was anastomosed in two layers to the side of the cecum 6 cm distal to the ileocecal junction. The distal segment of bypassed bowel was closed in two layers and sutured to the cecum proximal to the ileocolostomy to prevent intussusception.

For complete external biliary diversion, a silastic catheter was inserted 1 to 2 cm into the transected common duct and tied in place. The catheter was brought through a right flank stab wound and led subcutaneously to the back of the neck where it was allowed to drain freely. The gallbladder was removed.

ANIMAL GROUPS

Fifty-five adult mongrel dogs, with a starting weight of 10 to 34 kg, contributed to the final data. The dogs were preconditioned in the laboratory facility for several weeks and received standard dog chow containing approximately 25% protein and 8% fat. The same diet was continued postoperatively. An additional 100 experiments could not be completed because of a variety of complications including intussusception, infection, and lethal encephalopathy.

The design of the completed experiments is shown in Table 1. The duration of most studies (Groups 4 to 7) was 4 weeks; controls were the animals with PCS alone (Group 3). However, the animals tolerated the simultaneous combination of BF and PCS so poorly that an endpoint of 3 weeks was accepted (Group 2), necessitating a separate control series of dogs studied for 3 weeks after PCS alone (Group 1).

PATHOLOGIC STUDIES

Biopsy of one of the left lobes of the liver was performed at each surgical procedure and at sacrifice. The hepatic tissues were fixed in 10% normal buffered Formalin (aqueous solution of formaldehyde), and additional smaller samples were initially fixed in gluteraldehyde solution and postfixed in osmic acid and embedded in Epon (synthetic embedding medium). Two hours prior to sacrifice, 2 mCi per kg body weight tritiated thymidine (specific activity 47 mCi) were injected intravenously. In addition to the final liver biopsy, biopsy of the right kidney was performed. The patency of the vascular anastomosis and the absence of venous tributaries superior to the portal vein ligation were verified. The length of the intestinal bypass, position of the common duct catheter, and the presence of any intraabdominal abnormalities were also determined.

Before weighings, the autopsy livers were cleaned of extraneous tissue, the extrahepatic biliary tract, and other hilar structures. The liver weight (including the final biopsy) was converted to the percentage of both the preoperative and final body weights. Only the latter values will be reported.

For histopathologic studies, frozen sections of the liver were cut and stained with Sudan IV for fat. Paraffin sections were stained with hematoxylin and eosin, Gordon and Sweet's silver impregnation method for reticulin fibers, Pearl's Prussian blue method for iron, trichrome for collagen and fibrin, periodic acid-Schiff reaction for glycogen, and Pearce's method for ceroid and lipofuscin. Half-micron and ultrathin sections were cut from the Epon-embedded material. The former were stained with Azur II for examination by light microscopy, while the latter were stained with lead citrate and examined under a Phillips 300 electron microscope. The size of the midzonal hepatocytes before and after PCS were determined by tracing large numbers of liver cells on standard thickness paper, cutting out the silhouettes, and weighing them (9). Midzonal hepatocytes identified in half-micron Epon sections were also used for measuring on electron micrographs the area of rough and smooth endoplasmic reticulum within these cells and the dimensions of mi-

TABLE 1. DESIGN OF COMPLETED EXPERIMENTS, WEIGHT CHANGES, AND LIVER/BODY WEIGHTS (MEAN ± S.D.)

Group	No. of experiments	Description	Duration of experiment (weeks)	Dog weight loss (% of original)	p[a]	Liver/body weight (%)	p[a]
1	6	PCS (control)	3	18.5 ± 4.8	—	1.8 ± 0.2	—
2	10	PCS + BF	3	14.4 ± 8.8	NS[b]	2.5 ± 0.3	<0.01
3	10	PCS (control)	4	14.4 ± 6.5	—	1.7 ± 0.4	—
4	5	PCS + BF (staged)	4 (2 + 2)	21.4 ± 6.9	NS	2.4 ± 0.3	<0.05
5	5	IB	4	14.4 ± 6.5	NS	2.5 ± 0.3	<0.05
6	10	PCS + IB	4	17.9 ± 10.1	NS	1.7 ± 0.2	NS
7	10	PCS + IB (staged)	4 (2 + 2)	25.2 ± 8.3	<0.05	2.52 ± 0.3	<0.01

[a] Statistical evaluations (Student's t test) of the percentage changes were Group 2 vs. Group 1 (control) and Groups 4 to 7 vs. Group 3 (control).

[b] NS, not significant.

tochondria, lysosomes, lipid inclusions, and clumps of glycogen by the morphometric methods of Loud (15). Autoradiography was performed on dewaxed paraffin sections of liver using Ilford K2 nuclear emulsion. Exposure was for 3 to 12 weeks until counts of the labeled nuclei stopped increasing. All histopathologic observations were recorded without knowledge of the experimental groups from which the specimens came and subsequently decoded.

BIOCHEMICAL STUDIES

After an overnight fast, serum samples were obtained for determination of bilirubin and SGOT. Total serum cholesterol (16) and triglyceride (17) concentrations were determined by enzymatic analysis. In addition, 2-hr postprandial serum samples were obtained for analysis of total serum bile acids by the enzymatic-fluorometric analysis of Brunsgaard et al. (18). Total 3 α-hydroxy bile acids were measured.

Fasting blood samples collected in heparin tubes were separated and the plasma frozen for eventual measurement of plasma mevalonic acid concentrations by the method of Popjak et al. (19), as modified by Parker et al. (20). Attempts to analyse postprandial samples for mevalonic acid proved to be impractical because of the presence in the plasma of substances that interfered with the measurement.

Usually, blood samples for the various analyses were obtained preoperatively about 2 weeks after surgery and at the time of sacrifice.

RESULTS

POSTOPERATIVE BEHAVIOR

Weight loss occurred in all of the animal groups (Table 1), to the most extreme degree in the animals of Group 7 which had PCS and IB in two stages. Hair loss was severe in all of the dogs subjected to PCS, with or without the additional procedures of BF or IB.

Several animals in Groups 1 to 4, 6, and 7 had findings of hepatic encephalopathy including ataxia and convulsions. The only experimental group which was spared

these classical complications of Eck fistula was that with IB alone (Group 5).

PATHOLOGIC STUDIES

Gross Liver Weight. In normal unoperated dogs, the liver weight per body weight percent is 2.43 ± 0.66 (S.D.) per cent (21). Using the reduced final body weight as reference (Table 1), the control animals with PCS alone (Groups 1 and 3) had a diminished liver weight per body weight. The liver weight per body weight per cent was less severely reduced in animals given BF plus PCS (Groups 2 and 4), in animals with IB alone (Group 5), and after PCS plus delayed IB (Group 7).

Histopathologic Observations. The biopsy specimens obtained before the initial operation were normal by light and electron microscopy. Few hepatocytes were seen in mitosis.

After PCS (Groups 1 and 3), the hepatocytes shrank to roughly half their original size (Table 2). They became irregular in shape and lacked glycogen. A small excess of fat accumulated in their cytoplasm. Measured on electron micrographs, the rough endoplasmic reticulum was found to be reduced in amount to less than one-third the quantity found preoperatively, and there were fewer ribosomes on the membranes. What remained of the rough endoplasmic reticulum was dilated and fragmented. The amount of smooth endoplasmic reticulum and the number of free ribosomes were increased. The Golgi apparatus was poorly developed and some of the mitochondria and their cristae disrupted. Lipid droplets were common in the cytoplasm of the hepatocytes. Glycogen granules were greatly reduced in number. The Kupffer cells were increased in number and size, and their cytoplasm contained abundant ceroid, lipfuscin, and lipid. Take up of thymidine by the hepatocyte nuclei increased roughly 9-fold over normal values by 3 and 4 weeks after PCS (Table 2). There was a corresponding rise in the mitotic count. In Groups 4 and 7, all of these changes were established by 2 weeks, at which time BF or IB was performed at a second stage (Table 2).

The addition of IB (Group 7) or BF (Group 4) 2 weeks

TABLE 2. HISTOPATHOLOGIC STUDIES

Group	Description	n	Hepatocyte size units (mean ± S.D.)			p[a]	No. of labeled hepatocytes per 100 hepatocytes[b] (mean ± S.D.)	p[a]
			Before	2 weeks	Sacrifice		Sacrifice	
1	PCS	6	0.365 ± 0.048		0.141 ± 0.020	—	13.8 ± 1.6	—
2	PCS + BF	10	0.358 ± 0.070		0.158 ± 0.020	NS[c]	13.9 ± 1.2	NS
3	PCS	10	0.354 ± 0.031		0.154 ± 0.036	—	14.3 ± 1.8	—
4	PCS + BF (staged)	5	0.356 ± 0.063	0.159[d] ± 0.025	0.158 ± 0.009	NS	15.1 ± 2.9	NS
5	IB	5	0.351 ± 0.034		0.328 ± 0.035	<0.01	1.64 ± 0.3	<0.001
6	PCS + IB	10	0.354 ± 0.048		0.138 ± 0.011	NS	13.8 ± 2	NS
7	PCS + IB (staged)	10	0.358 ± 0.054	0.173[d] ± 0.036	0.162 ± 0.030	NS	16.1 ± 1.6	NS

[a] Statistical comparisons were between Group 2 vs. Group 1 (control) and Groups 4 to 7 at sacrifice vs. Group 3 (control).

[b] In normal unoperated dogs, the labeling is 1.6 ± 0.4 (7).

[c] NS, not significant.

[d] The atrophy at 2 weeks was explained by the PCS which was the first stage operation.

after PCS increased the amount of iron pigment in the Kupffer cells. Dogs with BF, whether this was performed at the time of PCS (Group 2) or later (Group 4), also developed cholestasis with bile "thrombi" in the centrilobular canaliculi. The severity of the abnormalities caused by PCS was neither increased nor alleviated by any of the additional procedures. IB by itself caused no structural alterations of the liver and the uptake of thymidine by the hepatocytes remained normal (Table 2).

BIOCHEMICAL STUDIES

Bilirubin and Transaminases. Elevations in serum bilirubin were common only in the animals of Groups 2 which were submitted to simultaneous PCS and BF 3 weeks before sacrifice (Table 3). Five of the dogs in this group had serum bilirubin concentrations >1.2 mgm% at some time postoperatively, whereas only 1 dog of the 46 in the other six groups had this finding. Minor serum transaminase elevations occurred postoperatively in all of the experimental groups (Table 3).

Serum Lipids. Serum cholesterol was lowered in all of the experimental groups, to the least degree in animals with IB alone (Table 4). The decreases seen after PCS alone were not augmented with the addition of BF or IB (Table 4). Significant changes in serum triglycerides were not caused by any of the experimental procedures.

Mevalonic Acid. Plasma mevalonic acid was unchanged (Group 3 and following Stage 1 of Group 7) or depressed

(Group 1) after PCS (Table 4), but was tripled after IB alone (Group 5). Small or no increases in mevalonic acid occurred in the animals of Groups 6 and 7 in which PCS was combined with IB (Table 4). A small increase was seen in the dogs of Group 2 which was submitted to simultaneous PCS and BF (Table 4).

Postprandial Serum Bile Acids. Major increases were caused by PCS alone (Table 5). After IB alone, there was no change from baseline. When either BF or IB was combined with PCS, serum bile acids increased at 1 to 2 weeks and/or before sacrifice, but to significantly lower levels at 2 weeks or at sacrifice than after PCS alone. The clearest results were in the staged procedures of Groups 4 and 7 in which the major increases after 2 weeks reflected PCS alone. BF (Group 4) and IB (Group 7) then caused significant subsequent falls in serum bile acids.

Special Assessment of Group 2. The results of the histopathologic and biochemical studies were separated into those obtained from dogs which did or did not develop jaundice (Table 6). Except for the bilirubin, there were no obvious differences between the two subgroups.

DISCUSSION

PCS deprives the liver of first-pass exposure to hormones (especially insulin) and other putative hepatotrophic factors from the splanchnic viscera with consequent morphologic changes in the rough endoplasmic reticulum that explain reductions in many biosynthetic

TABLE 3. EFFECT OF EXPERIMENTS ON SERUM BILIRUBIN AND SGOT AT SACRIFICE

Group	Description	Bilirubin (mg% sacrifice)	p[a]	No. of dogs with bilirubin ≥1.2 mg/dl	SGOT (mU/ml sacrifice)	p[a]
1	PCS	1.1 ± 0.2		0/6	30 ± 6	—
2	PCS + BF	2.8 ± 1.8	NS[b]	5/10	45 ± 31	NS
3	PCS	1.2 ± 0.7	NS	1/10	36 ± 30.6	—
4	PCS + BF (staged)	<1	NS	0/5	49.2 ± 24	NS
5	IB	1.1 ± 0.2	NS	0/5	19.4 ± 17.4	NS
6	PCS + IB	<1	NS	0/10	11.9 ± 8	<0.05
7	PCS + IB (staged)	1.08 ± 0.2	NS	0/10	27.6 ± 14.5	NS

[a] Statistical evaluations were: Group 2 vs. Group 1 (control) and Groups 4 to 7 vs. 3 (control). Bilirubin normal, <1.2 mg/dl; SGOT normal, ≤50 mU/ml.

[b] NS, not significant.

TABLE 4. EFFECT OF EXPERIMENTS ON SERUM CHOLESTEROL AND PLASMA MEVALONIC ACID

Group	Description	Cholesterol (mg%)			% Decrease		p[a]		Mevalonic acid (nm/liter)			p[a]
		Before	2 weeks	Sacrifice	2 weeks	Sacrifice	2 weeks	Sacrifice	Before	2 weeks	±% Variation	
1	PCS	157 ± 38	99 ± 33	92 ± 14	41	42	—	—	59 ± 26	26 ± 4	−56	—
2	PCS + BF	196 ± 100	120 ± 30	126 ± 22	38	36	NS[b]	NS	79 ± 56	116 ± 65	+46	<0.01
3	PCS	168 ± 36	127 ± 29	126 ± 40	24	25	—	—	58 ± 21	57 ± 11	−1.8	—
4	PCS + BF (staged)	139 ± 37	126 ± 19	94 ± 25	9.3	33	NS	NS	—	—	− −	—
5	IB	186 ± 127	184 ± 93	154 ± 51	1.0	17	<0.05	NS	72 ± 49	229 ± 88	+218	<0.01
6	PCS + IB	185 ± 45	158 ± 31	161 ± 36	19	13	NS	NS	63 ± 15	87 ± 40	+38	NS
7	PCS + IB (staged)	155 ± 35	121 ± 28	120 ± 39	22	33	NS	NS	59 ± 16.1	53.5 ± 26[c]	−10.1	NS

[a] The per cent changes of Group 2 were compared with those in Group 1 (control) and those in Groups 4 to 7 were compared to those in Group 3 (control).
[b] NS, not significant.
[c] The results were before the Stage 2 operation and thus reflected PCS alone.

TABLE 5. EFFECT OF EXPERIMENTS ON SERUM BILE ACIDS

Group	Description	Bile acids (µM/liter)			p^a	
		Before	2 weeks	Sacrifice	2 weeks	Sacrifice
1	PCS	2.67 ± 1.38	134 ± 104	75.59 ± 55.3	—	—
2	PCS + BF	5.50 ± 4.2	43 ± 40.9	61.25 ± 60.0	<0.05	NS^b
3	PCS	5.26 ± 5.79	208 ± 71.6	190.8 ± 144.4	—	—
4	PCS + BF (staged)	2.46 ± 1.01	93 ± 67.9^c	16.18 ± 11.3	<0.05	<0.05
5	IB	3.80 ± 2.79	4.9 ± 3.3	4.43 ± 2.1	<0.001	<0.001
6	PCS + IB	2.66 ± 2.65	41.1 ± 25.3	91.14 ± 46.6	<0.001	NS
7	PCS + IB (staged)	5.60 ± 6.3	170.1 ± 74.4^c	29.26 ± 25.7	NS	<0.05

^{a} Statistical comparisons (Student's t test) were of Group 2 vs. Group 1 (control) and Groups 4 to 7 vs. Group 3 (control).
^{b} NS, not significant.
^{c} The results at 2 weeks reflected PCS alone.

TABLE 6. DOGS IN GROUP C WITH BILIRUBIN > OR ≤ 1.2 MG

	No. of dogs		Bilirubin (mgm%)	SGOT (mU)	Mevalonic acid (nm/liter)		Cholesterol (mgm%)		Bile acids (µM/liter)		Hepatocyte size units		No. of labeled hepatocytes/1,000
					Preoperatively	2 weeks	Preoperatively	Sacrifice	Preoperatively	Sacrifice	Before	Sacrifice	Hepatocyte Sacrifice
≥1.2	5	X̄	4.5	54.2	75.5	133	234.8	136	7.4	96.8	0.38	0.16	13.8
	±	S.D.	1	27.6	48.0	71	135.4	15.6	5.8	56.7	0.09	0.03	0.8
<1.2	5	X̄	1.1	26.8	84.3	87.8	156.8	115.2	3.9	45.6	0.34	0.16	14
	±	S.D.	0.2	10.5	88.9	68.6	18.9	24.2	2	56.9	0.05	0.03	1.5
		p	0.001	NS^a	NS	NS	NS	NS	NS	NS	NS	NS	NS

^{a} NS, not significant.

processes (5–9). It was long thought that the hepatic histopathologic and functional changes caused by Eck fistula were a unique feature in dogs. It is now established that these alterations are similar if not identical in all species so far studied including rats, dogs, swine, subhuman primates, and humans (2, 5, 6) even though humans and rats are unusually resistant to the complications of hepatic encephalopathy (3, 5).

The present study was undertaken in dogs in an attempt to verify observations by Balabaud et al. (10) and Bioulac et al. (11) in rats that BF reverses the hepatic changes caused by PCS, and to see if the same effect could be achieved with IB. PCS (1, 6), BF (22), and IB (14) all cause reductions in serum cholesterol levels. The changes in cholesterol and bile acid metabolism resulting from each of the three procedures have been thoroughly studied.

PCS causes a reduction in cholesterol synthesis (6, 13, 23–30) and bile acid synthesis (13, 24, 30, 31). Interruption of the venous enterohepatic circulation has been one presumed cause of the elevation in postprandial serum bile acids caused by PCS (12, 13).

Many of the metabolic effects of either BF or IB alone are opposite to those of PCS and reflect a response to increased bile salt and cholesterol losses. After BF, hepatic cholesterol synthesis (22, 32–35) and bile acid synthesis (22, 35, 36–40) are increased many times. After IB, the same increases are seen in cholesterol (14, 41–43) and bile acid (43, 44) synthesis.

Bioulac et al.'s hypothesis (11) was that BF in rats reversed or prevented the liver injury of PCS by reducing the exposure of the liver to the high levels of serum bile acids which are caused by PCS or, alternatively by stim-

ulating bile acid synthesis. Detailed discussion of such possible mechanisms would be superfluous since our canine experiments did not confirm a protective effect.

In dogs, the failure of either BF or IB to alter the morphologic changes caused by PCS was nearly absolute. Although the animals with BF or IB plus PCS tended to have higher liver weight per body weight ratios at the time of sacrifice than dogs with PCS alone, part of this effect may have been due to a greater average body weight loss during the 2 to 4 weeks after the multiple procedures. The possibility that the hepatic atrophy of PCS had been reduced by BF or IB was not supported by quantitative histopathologic analyses of hepatocyte size. Furthermore, the addition of BF and IB did not alter the increased rate of cell renewal and the organelle disruptions that are characteristic after PCS (5–7). Although some of the dogs with BF in Group 2 had imperfect bile drainage and consequent jaundice, the results in these animals were no different than in others in Group 2 which did not have rises in serum bilirubin, nor did the factor of jaundice alter the results of the lipid, mevalonic acid, and bile acid studies.

It is possible that the data from the canine and rat studies were not as discordant as their divergent interpretations. Major weight loss occurred in our dogs after PCS and in the rats studied by Bioulac et al. (11). In both species, the liver weight per body weight ratios at the time of sacrifice were higher with the addition of BF, but the extent of hepatocyte atrophy was profound in both species when this was assessed by sophisticated histopathologic measurement of the size of hepatocytes. In the experiments of Bioulac et al. (11), the hepatocytes after PCS shrank by 41% compared to results after sham

PCS; after PCS plus BF at a second stage, the shrinkage was still 31% compared to that in control rats after sham PCS and BF. Thus, it would be incorrect to say that atrophy had been prevented by BF. The main difference between the rat and dog experiments was the seeming reversal by BF in the rat of many of the hepatocyte organelle changes caused by PCS including disorganization of the rough endoplasmic reticulum. In the dog, there was no evidence at all of this ameliorating effect with BF or with the bile salt depleting procedure of IB.

In the present study, an attempt was made with biochemical analyses of serum or plasma to assess the interaction of PCS with the mechanistically opposing procedures of BF and IB. These were in no sense controlled metabolic investigations since all of the animals were in a catabolic state with weight losses that ranged from 14 to 25% during the 3 to 4 weeks of study. Nevertheless, observations were possible which might have clinical relevance since PCS and IB have been used together with an apparently cumulative effect to lower the serum cholesterol of at least three patients with familial hypercholesterolemia (45, 46). Guzman et al. (47) have seen similar cumulative effects in dogs after portal diversion (by means of portacaval transposition) plus ileal resection. However, it is theoretically possible that procedures such as IB, BF, and ileal resection, which increase the enteric losses as well as the hepatic synthesis of cholesterol and bile acids, could subvert the antilipidemic mechanisms of PCS which are thought to derive mainly from depressed hepatic cholesterol synthesis (3, 6, 13, 23–30).

The possibility of a cancelling effect of combined procedures was demonstrated from our animal studies. During the 3 or 4 weeks of observation, the anticholesterolemic effect of PCS was approximately the same with or without BF or IB, and thus there was not an obvious advantage in combining the procedures. After IB alone, estimation of cholesterol synthesis by plasma mevalonic acid determination (20) indicated a 3-fold increase as expected. When IB (or BF) was combined with PCS, the mevalonic acid rise was not eliminated. However, it was greatly reduced, reflecting the inability of the Eck fistula liver to fully respond to the stimulus of IB. These findings do not preclude the probity of further clinical trials using PCS and IB together in selected patients with familial hypercholesterolemia inspite of a further precautionary note by Rucker et al. (48). They reported 3-year follow-ups on the dogs described by Guzman et al. (47) on which portal diversion and ileal resection were initially complementary. This additive effect eventually was lost.

In the past, the extraordinary increases in postprandial plasma bile acids seen after PCS have been ascribed in part to disconnection of the enterohepatic bile acid cycle (12, 13). Observations in our dogs emphasized that this is not the only factor since elimination of the hepatic source of enteric bile acids did not prevent but only reduced rises in plasma bile acids after a meal. The 30- to 40-fold increases following PCS were less if BF and IB were also performed, but even with PCS and total external bile diversion (Groups 2 and 4) the postprandial

rises were still 7 to 13 times above the baseline. In contrast, animals with IB as the sole procedure had no increase at all. Bioulac et al. (11) have speculated that the Eck fistula liver can respond to BF with an attempt at heightened bile acid synthesis, but even if this were true, the response would be predicted to be subnormal (13, 24, 30, 31); bile acid synthesis was not measured in our experiments. It seems most likely that the liver injury of Eck fistula renders the hepatic clearance of bile acids less efficient, since elevations of serum bile acids have been described in a wide range of liver diseases (49, 50). In their original canine studies, Horak et al. (12) drew attention to the probable role of depressed hepatic clearance of bile acids, and Poupon et al. (51) came to the same conclusion from studies in patients before and after portacaval shunting.

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