Experimental Chronic Portal Vein Bacteremia.* (28899)

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The liver plays a major role in extracting bacteria and bacterial products from portal blood(1,2). The consequences of this on hepatic structure and function have not been studied. In some animals portal blood contains various microorganisms(3), but in normal man it is sterile(4). In certain patients with chronic ulcerative colitis, portal venous blood contains enteric organisms(5,6,7), which has been incriminated as the cause of pericholangitis, a lesion commonly encountered in colitis patients(8).

The effect of single injections of bacteria into the portal vein in animals has been studied(9,10,11,12), but experimental chronic portal bacterial infusion has not been investigated. The purpose of this preliminary report is to describe some of the alterations in hepatic structure and function consequent to prolonged portal vein bacterial infusion.

Material and methods. Experimental animal. The criteria for a suitable animal were: 1) sterile portal blood; 2) no indigenous hepatic lesions; and 3) sufficient size and temperament to permit obtaining multiple blood and liver samples. The rat has sterile portal blood(13), but prolonged mesenteric vein infusion was impractical in this animal. Portal venous blood in dogs is contaminated with numerous microorganisms(3), and rabbits develop hepatic necrosis due to coccidiosis infestation(14). Calves were employed because they are known to thrive and to remain docile despite long periods of limitation of activity.

Procedure. One- to three-week old animals were permitted to adjust to laboratory environment for several weeks. Just prior to anesthesia blood was obtained from the external jugular vein for determination of serum bilirubin, alkaline phosphatase, 5-nucleotidase, protein electrophoresis, and SGOT by standard methods. Atropine premedication and ether drip provided excellent anesthesia. A right subcostal incision was made under sterile conditions. The liver was biopsied with a Menghini needle. The rumenal vein was identified and isolated, and 2 ligatures placed about the vein in close proximity. The proximal ligature was tied securely while the distal ligature was loosely maintained. The vein was incised obliquely and a sterile PE 280 polyethylene tube was inserted and threaded into the portal vein. Its position in the portal vein was verified by palpation. A culture of portal blood was obtained. The catheter was anchored securely and brought to the outside through a stab wound in the right dorso-lateral abdominal wall, and further anchored with skin sutures. The portal catheter was connected to a constant infusion pump and saline was administered. After operation the animal was placed in a specially constructed stanchion which limited movement to standing or lying down. Within one hour the animal was alert and standing. Food and water were offered 24 hours later. No antibiotics were given.

Control period. During the control period of 3 to 13 days the portal catheter was infused with 600 ml of saline per day. The clinical status was evaluated by rectal temperatures, general vigor and appetite. Frequent portal vein blood samples were collected for quantitative cultures 24 to 72 hours after operation. One or 2 additional external jugular blood samples were collected for the biochemical studies listed above, and a percutaneous needle biopsy of the liver was secured at the end of control period.

Complications. The study was initiated in 13 calves, but was completed in only 6, because of complications in the other 7. Two died with pneumonia within 24 hours after pentothal anesthesia, which was then abandoned. One calf expired on the third post-

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TABLE I. Summary of Blood Chemistries.

	Animal #	SGOT (units)	Alkaline phosphatase (Bodansky units)	5-nucleotidase (units)	Bilirubin (mg/100 ml)
Control*	1–13	80 ± 21	3.3 ± 1.6	.07 ± .10‡	.31 ± .27
Exp†	2	670	5.1	3.7	.25
	5	780	5.0	.24	.30
	6	160	13.5	.61	.40

* Mean and standard deviation of 20 determinations.
† Maximum level achieved.
‡ There was no detectable 5-nucleotidase in the serum on 9 occasions.

operative day because of intestinal obstruction caused by volvulus of ileum about the catheter. Three calves expired 2 to 4 days following operation due to bacteremia caused by *Salmonella B.*, *Staphylococcus aureus* or *E. coli*. The source of the bacteremia was not established. The experiment was abandoned in one calf 24 hours after surgery because of its large size and adverse temperament.

Experimental period. Six calves were given continuous infusions of *E. coli* for 4 to 51 days. The organisms were suspended in saline with 0.1% bovine serum albumin to insure their viability without multiplication (15). The number of bacteria per minute was arrived at arbitrarily and was varied considerably in an effort to find a concentration of bacteria which would produce an hepatic lesion without killing the animal. External jugular vein blood was obtained every one to 7 days for biochemical studies, culture, and bacterial agglutinations. Liver was secured for histologic study by percutaneous biopsy every one to 7 days.

Results. Experimental preparation. The calf proved to be a good experimental animal. It tolerated prolonged periods of limited activity. Samples of blood and liver were obtained with minimal difficulty. The stanchion permitted the animal to stand, lie down, and feed, but generally limited movements which would disrupt the catheter or infusion equipment. A total of 58 percutaneous liver biopsies were obtained, with one complication. A liver biopsy 4 days after starting the *E. coli* infusion in one calf caused a fatal hemorrhage. Most of the complications have been eliminated by experience.

Control observations. During the control period 37 portal vein blood cultures were obtained in the 13 calves. Thirty-three were sterile, and one showed a few diphtheroids, probably contaminants. The 3 animals that died of bacteremia 2 to 4 days after operation had positive portal vein cultures. Twenty-seven portal vein blood cultures were obtained from the 6 calves that were eventually given the bacterial infusion and all were sterile. The control biochemical determinations are summarized in Table I. Gamma globulin was present in all animals.

Response to bacterial infusion. Three of the 6 calves became febrile and weak within 24 hours following *E. coli* infusion and died after 4 to 15 days. Their average age was about 4 weeks and average weight about 100 lb. The initial dose of bacteria varied from 5.4×10^5 to 3.8×10^7 organisms per minute. In all 3 the liver showed varying degrees of patchy necrosis of hepatocytes, edema and inflammation in portal triads, and Kupffer cell hyperplasia (Fig. 1). These changes were present as early as 24 hours after the start of bacterial infusion. In one animal the bacterial infusion was discontinued after 6 days because of fever and lethargy. Reinstitution of a smaller concentration of bacteria 4 days later resulted in increased hepatic inflammation and necrosis. The concentration of bacteria in the other 2 animals was not significantly altered during the experiment. In all 3 animals the lesion was accompanied by an alteration in one or more serum enzyme levels (Table I).

The other 3 calves were older and weighed more. The initial dose of bacteria was somewhat lower than in those above, and varied from 50 organisms to 8×10^5 organisms per minute. These animals did not become ill, develop serum enzyme alterations, nor de-

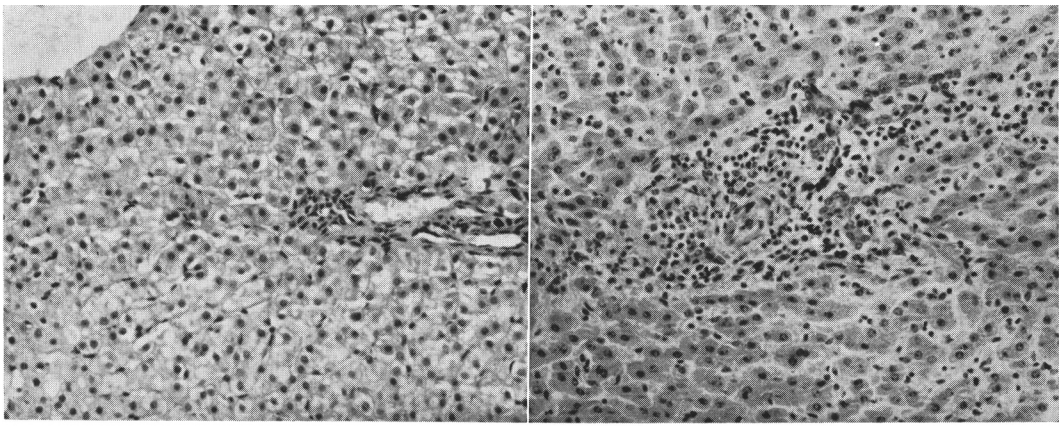

FIG. 1. A. H + E × 156. Photomicrograph of representative portion of liver obtained by biopsy from control period. Note small, compact, normal portal zone, normal parenchymal cells and central vein. B. H + E × 156. Photomicrograph from Calf #6 after 11 days of bacterial infusion showing edematous portal zone, heavily infiltrated with lymphocytes, and moderate ductular proliferation. The surrounding parenchyma is not disturbed, although sinusoids contain increased numbers of Kupffer cells.

velop hepatic histologic abnormalities, even when the dose of bacteria was increased to extremely high levels, and the infusion continued for as long as 51 days.

Discussion. Beeson *et al* demonstrated in patients with bacterial endocarditis that the liver removes bacteria from the blood(1). A similar capacity of the liver to inactivate bacterial endotoxin has been demonstrated by Greene *et al*(2). Hektoen described inflammation, bile duct proliferation and fibrosis in the portal triads of the liver in guinea pigs following single intraperitoneal or subcutaneous injections of bacteria of the pseudodiphtheria group(10). Weaver similarly injected a strain of *E. coli* into guinea pigs and produced portal inflammation, bile duct proliferation, focal necrosis, and an increase in perilobular connective tissue(9). MacMahon and Mallory produced an acute inflammatory lesion in the portal triads, bile duct proliferation, and focal necrosis in rabbits after a single injection of hemolytic streptococci into a mesenteric vein(11). Wachstein *et al* observed that intraperitoneal injection of *Salmonella typhimurium* resulted in transient focal degenerative changes and Kupffer cell proliferation(12). Chronic portal vein bacterial infusion has not been previously studied.

Our results indicate that portal blood of healthy calves is sterile. Chronic portal vein infusion of *E. coli* in 3 of 6 calves resulted in an hepatic lesion characterized by portal inflammation and edema, hyperplasia of Kupffer cells, focal necrosis of hepatocytes, and beginning proliferation of bile ductules. This was accompanied by alterations in SGOT, serum alkaline phosphatase, and/or serum 5-nucleotidase. The histologic lesion and the biochemical abnormalities resemble pericholangitis, a frequent complication of chronic ulcerative colitis(8).

Three calves did not become ill or develop an hepatic lesion consequent to bacterial infusion. The failure to produce a lesion might be due to the size of the initial dose of bacteria. The 3 calves which developed an hepatic lesion were given a higher dose of *E. coli* during the first 24 hours. It is possible that a larger initial dose might overwhelm the animal's resistance and permit the lesion to develop.

Summary. Chronic portal bacteremia has been experimentally produced in calves. Some of the changes in hepatic structure and function have been studied. Preliminary results suggest that: 1) the experimental preparation is satisfactory for studying the chronic infusion of bacteria, toxins, etc. into the portal vein; and 2) chronic portal bacteremia can cause a lesion resembling pericholangitis. More comprehensive experiments have been undertaken to study the effect of bacterial

perfusion on the liver and biliary tract.

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Effect of Fat on Plasma and Tissue Cholesterol Concentration of Hyper-Eu- and Hypothyroid Rats.* (28900)

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Numerous workers have observed that both metabolic activity and dietary fat have an effect on plasma cholesterol level. Hegsted *et al*(1) showed that in rats with normal metabolic activity, dietary unsaturated fat lowers plasma cholesterol when compared with animals fed saturated fat. Gerson *et al* (2) suggested that the action of unsaturated fats on the level of plasma cholesterol is due to the increased deposition of cholesterol in various organs and an increased fecal excretion. Hill *et al*(3) reported the effect of thyroid hormone and thyroid analogs on cholesterol metabolism in thyroidectomized rats and showed that the compound used lowers serum cholesterol levels when given in a non-toxic dosage. Ruegamer and Silverman(4), Duncan and Best(5) reported that administration of thyroid analogs to rats fed atherogenic diets prevented the elevation of cholesterol level in plasma and liver.

Although a great deal of work has been done with various fats and thyroid hormone and analogs on cholesterol metabolism, no report has shown the effect of the type of fat in the diet on cholesterol metabolism at the same time that the effect of metabolic activity is evaluated. It is obvious from the literature that both the type of fat and metabolic activity affect cholesterol metabolism by altering plasma cholesterol level. The present report shows the effect of different dietary fats at various metabolic activities.

Materials and methods. Rats were fed a purified diet which contained 25% (by weight) casein, 30% sucrose, and 20% fat either as cottonseed oil (Diet A) or as butter (Diet B). These diets also contained 4% cholesterol, 2% vitamin supplements (Fillios and Mann(6)), 3% salt mixture (Hegsted *et al*

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