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Behavior of Microorganisms and Endotoxin Perfused Through the Isolated Rat Liver.* (28875)

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The mechanisms by which enteric organisms gain access to the bile is not known. Three routes are theoretically reasonable; hematogenous, lymphatic and direct ascending infection. This problem has been investigated with conflicting results since the early part of this century. Experiments utilizing common duct fistulae in dogs and rabbits have demonstrated the appearance of a variety of enteric organisms and of the *Staphylococcus aureus* in the bile within 5 to 10 minutes following an intravenous or endoportal inoculation. On the basis of these observations, it was concluded that the passage of bacteria from the blood to the bile was almost instantaneous(1). A number of other investigators, however, using similar organisms in the rabbit and guinea pig, observed no such rapid transit of bacilli from blood to bile. Indeed, positive bile cultures after intravenous inoculation were obtained only inconstantly, and then after a latent period varying from 3 hours to a week(2,3).

It occurred to us that perfusion of microorganisms through the isolated rat liver might help clarify the issue of whether or not bacteria are rapidly filtered from the blood into the bile. The main advantages of this approach would be: (1) the system is isolated from the predominant lymphatic flow along deep lymphatic vessels that follow the course of the portal veins and bile ducts draining toward the hilum of the liver, (2) organisms could be constantly perfused at a known rate directly into the portal vein and (3) hemodynamic effects could be directly observed without the influence of other tissues and organs.

Materials and methods. The isolated perfused rat liver preparation essentially involves rapidly removing the liver from an animal and placing it in a system where it is perfused by oxygenated, heparinized donor blood. The liver maintains its histologic and metabolic integrity for a period of some 5 to 6 hours and bile is produced at a rate comparable to that observed in the intact animal. The procedure used for removal and perfusion of the liver was that described by Miller *et al*(4). Male Sprague-Dawley rats were

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TABLE I. Bacteriology of Bile in Typical Perfusions Employing Various Microorganisms.

Hr of infusion	Colonies per ml in bile		
	S. marcescens	*E. coli*	*S. aureus*
0	0	0	0
1	0	0	0
1½	40	0	0
2	40	5	0
2½	40	180	0
3	750	TNTC*	0
3½	930	TNTC	0
4	—	—	0

* TNTC: Too numerous to count.

used as blood and liver donors and weighed from 400 to 550 g. They were maintained on Purina Lab Chow and water *ad libitum* until just prior to sacrifice.

Because of the difficulty in maintaining absolute sterile conditions in the system, *Serratia marcescens*, the E. C. Y.-9 strain of *Escherichia coli* and the Giorgio strain of *Staphylococcus aureus* were used for ease of identification. These particular strains of *E. coli* and *S. aureus* have been described(5,6). Large numbers of organisms were employed in order to test the capacity of the livers' defenses to their limits. For all bacterial experiments, inoculums of 10^8 organisms/ml were prepared from overnight cultures. An initial injection of 2×10^8 organisms was given after a control period of ½ hour and then 2×10^8 organisms/hr were constantly infused by the endoportal route. Sterile collections of bile were made every ½ hour and pour plates prepared from an aliquot. Purified endotoxin administered as lipopolysaccharide from *E. coli* 0127:B8 was obtained from Difco Laboratories, Detroit, Mich., diluted in Ringer's solution to a concentration of 1 mg/ml just prior to infusion. During and at the end of each perfusion, specimens of liver were removed and studied histologically.

Results. Table I depicts total colonies/ml of bile collected during a representative perfusion for each of the 3 microorganisms used. In every experiment, *S. marcescens* and *E. coli* began to appear in the bile from 1½ to 2 hours after the start of infusion and continued to come out in increasing numbers during the rest of the perfusion. The staphylococci, on the other hand, failed to appear in the bile during 5 of 6 separate perfusion experiments. In no case could bacteria be recovered from bile prior to 1½ hours after the start of infusion.

Fig. 1 shows the mean hepatic blood flow correlated with duration of perfusion for a variety of materials. It is apparent that there are 2 main classes of responses. The normal blood flow is illustrated during the constant infusion of Ringer's solution and shows an initial mean value of about 14 ml/min which rose during the first hour to an average of 19 ml/min, a rate which was sustained for the next 2 to 3 hours, then gradually decreased toward the fifth hour. While mean hepatic blood flow remained unaltered with all *S. aureus* infusions, administration of *S. marcescens* and *E. coli* produced a consistent and reproducible slowing effect in all experiments beginning at about one hour after inoculation. In view of the fact that the presence of endotoxin might have accounted for this difference in behavior between gram-positive and gram-negative organisms, 4 perfusion experiments were performed with purified *E. coli* endotoxin alone. Doses ranged from 200 μg/hr to 2 mg/hr and total doses of 8 to 10 mg were employed in some experiments with

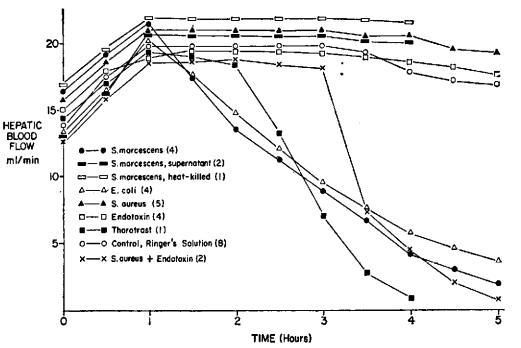

FIG. 1. Mean hepatic blood flow correlated with time perfusions of various microorganisms, endotoxin and Thorotrast. Figures in parentheses represent number of perfusion experiments.

no effect on blood flow. In all cases, endotoxin in doses exceeding 3 mg killed intact animals of comparable weight to the donor rats. Washed, heat-killed *S. marcescens* and sterile *S. marcescens* supernatant also failed to slow hepatic blood flow. A constant infusion of Thorotrast (26%) produced almost identical slowing effects on blood flow as that observed with the gram-negative bacilli. Particularly noteworthy was the observation that a combined infusion of staphylococci and endotoxin, neither of which alone showed any hemodynamic effect, slowed blood flow dramatically.

Sections of livers obtained during and at the end of the perfusion experiments revealed a similar histologic picture for all 3 organisms. While the integrity of the parenchymal cells was maintained, many of the Kupffer cells were swollen and packed with microorganisms.

Discussion. In the isolated perfused rat liver, no microorganisms appeared in the bile before a latent period of at least 1½ hours after endoportal inoculation. These observations would suggest that the immediate passage of bacilli from the blood to bile noted in previous investigations was mediated by a factor not operative in our isolated system, perhaps the lymphatic vessels. It is of interest that the gram-negative organisms appeared with consistency in the bile, while staphylococci in the same concentration failed to do so. Similar results have been obtained in the intact guinea pig where, following intravenous administration, *E. coli* could be uniformly recovered from the bile, while *S. aureus* could not(3).

The hemodynamic changes in the isolated rat liver produced by infusion of gram-negative bacilli is most striking. Why this marked decrease in blood flow always occurred 1 to 1½ hours after continuous infusion of *S. marcescens* or *E. coli*, but never following a similar inoculum of *S. aureus* is not clear. A vasomotor effect of endotoxin alone does not seem to be the explanation because neither endotoxin nor the supernatant from an overnight culture of *S. marcescens* caused decrease in blood flow. On the other hand, a combined infusion of endotoxin and *S. aureus* resulted in a decrease in mean hepatic flow which was similar to that produced by gram-negative organisms. Infusion of Thorotrast produced an almost identical decrease in flow suggesting that particulate blockade of the reticuloendothelial system (R.E.S.) might be a causative factor, by virtue of swelling of the Kupffer cells, sinusoidal compression and resultant increased resistance to flow. While this explanation remains a distinct possibility, the observations that washed, heat-killed *S. marcescens* had no hemodynamic effect, and that the degree of R.E.S. involvement observed by light microscopy was similar for all organisms, makes this theory less likely. Further studies are in progress to elucidate the mechanisms responsible.

The influence of bacterial endotoxin on hepatic blood flow has been studied previously in the intact animal by indirect clearance and dye dilution techniques(7) and by perfusion of the liver *in situ*(8). The results of these studies in a variety of species, including the rat, have been controversial. Some investigators have suggested that changes in blood flow correlate with alterations in the systemic circulation while others conclude that endotoxin has a direct vascular effect on the liver(9,10). The results of the present studies suggest that endotoxin does not have a direct vascular action in the isolated rat liver and supports, by inference, recent studies demonstrating that decreased hepatic blood flow in intact rabbits was due to a diminished cardiac output induced by endotoxin(7).

Elaboration of histamine, epinephrine, and serotonin have been considered to be possible causative agents accounting for the hepatovascular phenomena induced by endotoxin (10-13). If endotoxin liberated hormonal substances such as histamine from hepatic mast cells, then an increase in blood flow might be expected since this finding has been observed in the isolated perfused rat liver.§ Moreover, if endotoxin increased the levels of epinephrine or serotonin in the isolated

§ R. A. Levine, unpublished observations.

liver, a prompt vasomotor effect should have been present as previously demonstrated by these amines in this isolated system(14). Since endotoxin had no hemodynamic effects on the isolated rat liver, our studies would suggest that if endotoxin liberates humoral agents, it probably does so on an extrahepatic basis.

Summary. Utilizing the isolated perfused rat liver as a model to study the behavior of bacteria and bacterial products in that organ, 3 observations have been made: (1) A latent period of at least 1½ hours was present before organisms which had been injected endoportally appeared in the bile. (2) Gram-negative bacilli appeared in the bile with regularity while staphylococcal organisms failed to do so during the period of perfusion. (3) A decrease in hepatic blood flow was produced by administration of gram-negative bacilli. Neither staphylococci alone, nor endotoxin alone induced this effect, but the 2 in combination slowed blood flow. Endotoxin, even in massive doses, had no direct hemodynamic effect on the isolated perfused rat liver.

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Accelerated Immune Response Induced by D-L Penicillamine.* (28876)

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Mercaptane compounds have been demonstrated to dissociate macroglobulins by cleavage of disulfide bonds *in vitro*(1). Administration of penicillamine, a sulfhydryl amino-acid derivative (β,β-dimethylcysteine), to patients with various disease states characterized by elevated macroglobulins appeared to show that depolymerization can also be effected *in vivo*(2,3,4). However, the late onset of the fall in titer of serum rheumatoid factor in rheumatoid arthritis patients given penicillamine and the persistence of the reduced titer for many weeks after the drug had been withdrawn suggested that effects other than intravascular depolymerization alone may have occurred(5). Since this may indicate that penicillamine exerts a more profound influence on the immune apparatus, the present investigation was undertaken to examine the possible effect of D-L penicillamine on the primary immune response.

Materials and methods. White New Zealand rabbits of both sexes averaging 2 kg were used. The animals were given commercial rabbit feed (Rockland Rabbit Ration) and drinking water *ad libitum*. D-L penicillamine (Aldrich) was dissolved in a 0.15 M phosphate buffer pH 7.2 and injected into

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