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Gram-Negative Bacterial Infections and Mode of Endotoxin Actions

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On the Escape of Endotoxin from the Intestine

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We became interested in endotoxin as a result of our work on liver failure. We noted during experimentally induced acute hepatic insufficiency in the dog a number of changes (1, 2) which could be attributed to the development of gram-negative sepsis of enteric origin (3). Subsequent reports, notably those by Bjørneboe (4) and by Triger (5), indicate that in patients with liver cirrhosis E. coli antibody titers are markedly increased. This suggests that during hepatic insufficiency in man enteric organisms or their products are also able to escape from the intestine (6).

How is this possible, since as Dr. Brobmann again reiterated yesterday, very few investigators have been able to confirm unequivocally the presence of such an escape mechanism?

To explain the observed changes one can postulate that either enteric organisms and their endotoxins continuously escape from the intestine in considerable quantities, but that these materials are no longer cleared by the liver during hepatic insufficiency, or, that during this condition the efficiency of the mechanisms that normally retain gram-negative organisms and their products within the intestinal lumen, mechanisms which Dr. Cottier summarized for us so admirably day before yesterday (7), is somehow interfered with. For example, it is conceivable that intestinal permeability is increased as a result of portal hypertension and that routes other than the portal vein are followed in the development of sepsis. The fundamental difference between these 2 mechanisms is that in the first instance we are dealing with normal intestinal function and with a primary defect of the liver - while in the second circumstance the primary defect resides in the intestine.

If we scrutinize the first proposition, it is quite clear that it, in its turn, can be broken down further into 2 basic questions.

1. is endotoxin absorbed from the normal intestine into the portal vein in amounts sufficiently large to cause clinical manifestations? and

2. if the material enters the portal vein is the liver able to remove it? The latter question was of some significance to us since we found that the clearance of Cr^{51} labeled endotoxin from blood in hepatectomized dogs occurred at the same rate as in the intact dog, which made us wonder whether the liver was indeed important in this regard (8). I do not wish to go into this question at this time. Suffice to say that we showed that the liver is quite capable of clearing and detoxifying endotoxin and to refer here to a recently published report on this subject from our laboratory (9).

So let us move on then to the next question which is, is endotoxin normally absorbed into the portal vein in quantities sufficiently large to cause problems, if the liver would fail to clear and detoxify it?

To study this problem we made use of rats with a Thiry Vella fistula, which is an isolated, bypassed bile-free ileal segment, prepared as illustrated in Fig. 1. After opening the abdomen in the midline the vascular arcade immediately proximal to the ileocecal valve was divided together with the bowel and the ileum was measured along the mesenteric border in a proximal direction for the required distances. The bowel and mesentery were then divided proximally at the measured point. Intestinal continuity was re-established by approximating the two ends of the ileum with a continuous 7-0 suture, while the two ends of the isolated ileal loop were ex-

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teriorized as proximal and distal ileostomies through separate stab wounds in the left flank.

One week later, after the animals had fully recovered they were lightly anesthetized with ether following which pursestring sutures were placed around both ileostomies. Then a soft rubber catheter was threaded into the loop through the proximal ileostomy through which we injected a mixture of Cr^{51} labeled (1/2 mg) and unlabeled endotoxin (4 1/2 mg) (Difco Laboratories), and an indicator dye, methylene blue (0.1ml-0.25 %). This dye is readily absorbed from the intestine and rapidly excreted in the urine, staining it blue. Both agents were suspended in 3ml of isotonic saline, distilled water or hypertonic (50 %) glucose in water. Then the animals were given lead acetate intravenously (3 1/2 mg/100 g body weight), which has been shown to sensitize the rat to endotoxin more than a thousand fold, presumably through its effect on the reticuloendothelial system (9).

By frequently changing filterpaper placed underneath the wirebottom cages in which these animals were housed were we able to follow the dye excretion in the urine and to collect sequential urine samples for determination of radioactivity excreted in the urine. Also, the dye allowed us to detect possible spillage from either ileostomy. If this occurred to any significant degree the animal was discarded from the study. Data summarized in Table I show how sensitive a Pb acetate treated rat is for intravenous endotoxin; with as little as 1 /ug of endotoxin/ 100 g body weight the mortality rate, irrespective as to whether the endotoxin is administered over a protracted period or as a bolus, ranges from 65 - 80 %.

In our first experiment we made 25 cm T-V loops in 2 groups of rats, the first had undergone end-to-side porta-caval shunt operation, allowing the portal vein blood to bypass the liver, while animals of the second group had undergone only a sham operation consisting of cross-clamping the portal vein and inferior vena cava for 20 minutes, which is the time required to complete a porta-caval shunt in the rat.

In both groups of animals the administration of lead acetate alone was well tolerated, none of the animals died as a result (see Table II). Also, if lead acetate was injected 4 hours after endotoxin was placed in the bypassed ileal segment every animal of either group survived. However, if lead acetate was injected at the same time the endotoxin was placed in the loop 1 out of 10 sham-operated rats and 4 out of 10 porta-caval shunt rats succumbed.

The finding that porta-caval shunt rats tolerated lead acetate alone or lead acetate and endotoxin 4 hours apart would suggest that neither the porta-caval shunt procedure nor the lead acetate alone can be held responsible for the observed increase in mortality in the porta-caval shunt group. Instead, it would suggest that the simultaneous administration of these 2 agents in porta-caval shunt rats resulted in animals that were more susceptable to endotoxin than were the sham-operated rats. Because the porta-caval shunt permits portal vein blood to bypass the liver, the increase in mortality may be due to a diminished clearance by the liver of portal vein blood and the endotoxin it contains. This would suggest that at least part of the endotoxin absorbed from the isolated loop is transported by way of the portal vein. Certainly the amount of endotoxin absorbed is minute, considerably less than 1 /ug/100 g body weight. If it were as much as 1 /ug, we could expect that at least twice the number of porta-caval shunt rats would have died.

In our next experiment we lengthened the intestinal loop from 25 cm to 40 cm. As a result (see Table III) the mortality rose from 10 % to 36 % indicating that the larger the surface area, the more endotoxin is absorbed. It should be pointed out, here, that none of these animals had a porta-caval shunt.

This observation poses an interesting problem. It suggests that, although the amount of endotoxin absorbed is minute, not enough is cleared by the liver to save every animal. We have to conclude, therefore, that some of the endotoxin is able

to bypass the liver. The only other route available for its escape is via the lymphatics.

If part of the endotoxin, indeed, escapes by way of the lymphatics, our first proposition, which states that in hepatic insufficiency, failure of hepatic clearance is responsible for possible endotoxemia of enteric origin, becomes untenable. Since it seems that a considerable part of the endotoxin that escapes from the intestine bypasses the liver, it is obvious that the role of the liver is not a primary but rather a secondary one.

This is an important distinction. It is indeed unfortunate that limitation of time does not allow me to elaborate on this point.

We are left then with the 2nd proposition, namely, that if E. coli sepsis and endotoxemia are frequent complications of liver insufficiency this is probably not so much because of failure of hepatic clearance but rather because the simultaneous involvement of the intestine allows for the escape of more endotoxin.

This proposition remains to be investigated. On reviewing the literature we found that presently only few conditions are known to be associated with increased absorption of intestinal contents. These include total body irradiation (10), graft versus host reaction (11) and possibly treatment with certain chemotherapeutic agents.

The mechanism we choose to induce an increased absorption from the intestine utilized the effect of osmotic shock on the intestine. We resorted to it because of its ready accessibility, the ease with which we were able to control it and the uniformity of results it elicited. We found that the mortality rate rose steeply when hypo- or hyper-osmolar solutions were used to resuspend the endotoxin prior to its installation in a 40 cm T-V loop of a lead acetate sensitized rat (see Table IV). As is shown in Table V this increased mortality was not caused by a nonspecific, dehydrating effect of the hypertonic glucose, rather that it represented a highly specific reaction.

These animals, at autopsy, exhibited a number of very marked changes of which the most unexpected one was the presence of radioactivity in the peritoneal cavity. Hence under these circumstances, labeled endotoxin had escaped from the gut lumen into the free abdominal cavity.

These changes lent themselves to a further, semi-quantitative analysis. As can be seen from Table VI, at autopsy none of the animals that received endotoxin in isotonic saline had radioactivity in the abdominal cavity. In contrast, in 9/10 rats that received endotoxin in 50 % glucose in water was free radioactivity found to be present in the peritoneal cavity at the time of death, approximately 5-6 hours after the beginning of the experiment. This group of animals had been sensitized with lead acetate. If no lead acetate was given only 5/10 animals had radioactivity in the peritoneal cavity at the time of sacrifice, 6 hours after the start of the experiment. Also, the relative amount of radioactivity in the first group of rats that died as a result of endotoxemia was much larger than in the latter group that was sacrificed, suggesting that either lead acetate itself or the conditions that preceeded death from endotoxemia, such as shock, were associated with an increased permeability of the gut wall, thus promoting the transfer of endotoxin from the gut lumen to the peritoneal cavity.

Daniele et al. previously showed that if E. coli endotoxin is placed in the peritoneal cavity, part of it remains there while some leaves, predominently via the thoracic ducts (12). This then would indicate that, if intestinal permeability increases, significant quantities of endotoxin (and possibly other intestinal contents) are able to leave the intestine, not by way of the portal vein but by way of the peritoneal cavity, to be taken up by the abdominal lymphatics and transported further via the thoracic ducts, suggesting that also under those conditions not much endotoxin escapes directly into the portal vein circuit.

This study, which is still in progress, has raised a number of interesting problems. There is, for instance, the possibility that lead acetate - as I pointed out - may affect intestinal permeability. Hence we are currently repeating a number of the described studies in adrenalectomized rats. Also, in order to further demonstrate the role of the lymphatics, we intend to repeat some of these studies in rats with a thoracic duct fistula. An important question to be answered relates to the problems that led us to perform these studies in the first place, namely - what is the cause of the sepsis of intestinal origin observed during hepatic insufficiency? We believe that we now have some of the models that will allow us to study this particular problem under controlled conditions in the laboratory.

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Table I.

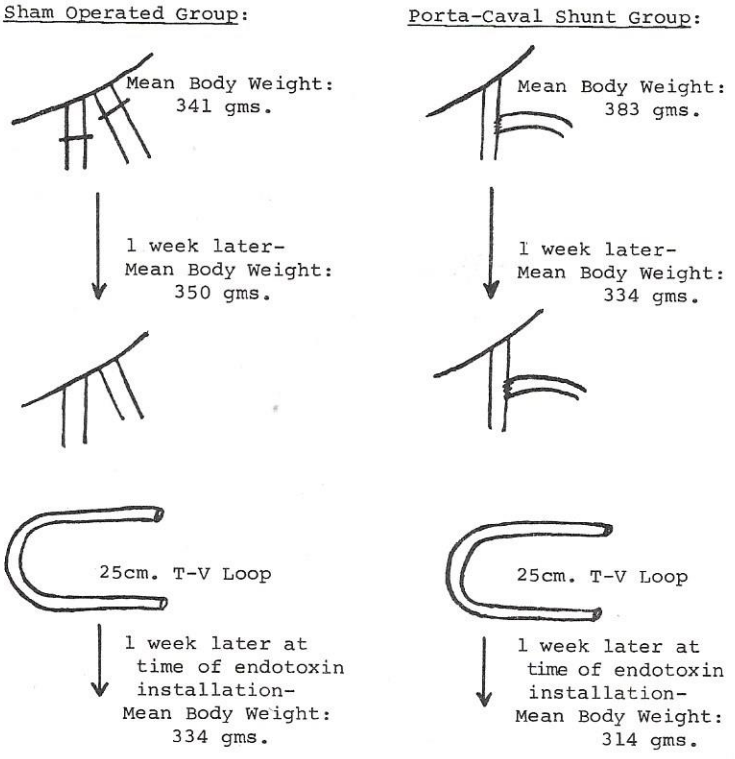
Mode of Injection	Dose of Endotoxin	No. of Animals Studied	Mortality
Rapid Injection			
Endotoxin	1 /ug/100 g	10	0/10
Endotoxin	3 mg/100 g	10	9/10
Lead Acetate (5mg/rat)	0	5	0/5
Lead Acetate (5mg/rat) + Endotoxin	1 /ug/100 g	12	8/12
Slow Infusion*			
Endotoxin	1 /ug/100 g	5	0/5
Endotoxin	3 mg/100 g	5	4/5
Lead Acetate (5 mg/rat)	0	5	0/5
Lead Acetate (5 mg/rat) + Endotoxin	1 /ug/100 g	18	13/18

*Over 1 hour period (with a Harvard pump).

Table II.

Mortality Following the Installation of Endotoxin

in 25cm. Long Rat T-V Fistulae



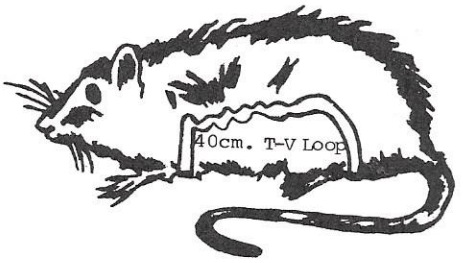
	Mortality Rate	
Pb acetate i.v. alone (3.5mg/100gm body weight)	0/10	0/10
Pb acetate i.v. + Endotoxin (in loop) 4 hours apart	0/10	0/10
Pb acetate i.v. + Endotoxin (in loop) simultaneously	1/10	4/10

Table III.

Effect of the Length of the Bypassed Ileal Segment on the Amount of Endotoxin Absorbed (as Expressed in the Mortality Rate of Lead Sensitized Rats).

	25 cm T-V loop	40 cm T-V loop
Mortality	1/10 (10 %)	5/14 (36 %)

Table IV.



Effect of the Suspension Medium Used to Reconstitute the Endotoxin (after its Installation into 40cm. T-V Loops) on the Mortality Rate of Lead Acetate Sensitized Rats

Endotoxin suspended in	Absolute Mortality	Mortality Rate
isotonic solution	5/14	36%
hypotonic solution	12/19	64%
hypertonic solution	13/14	93%

Table V.

Presence or Absence of Radioactivity in Abdominal Cavity at Time of Sacrifice or Death of the Rat

	Presence of Cr^{51}	Relative Activity
isotonic solution	0/10	
hypertonic solution E + E * and Pb acetate	9/10	++++
hypertonic solution E + E * (no Pb acetate)	5/10	++

Table VI.

Effect of 50 % Dextrose (in the Presence or Absence of Endotoxin and Lead Acetate) on the Mortality Rate of Rats with a 40 cm T-V Loop

Pb acetate i. v.	endotoxin in T-V loop	absolute mortality	mortality rate
-	+	1/20	5 %
+	+	19/20	95 %
+	-	9/10	90 %

Construction of a Thiry-Vella Fistula

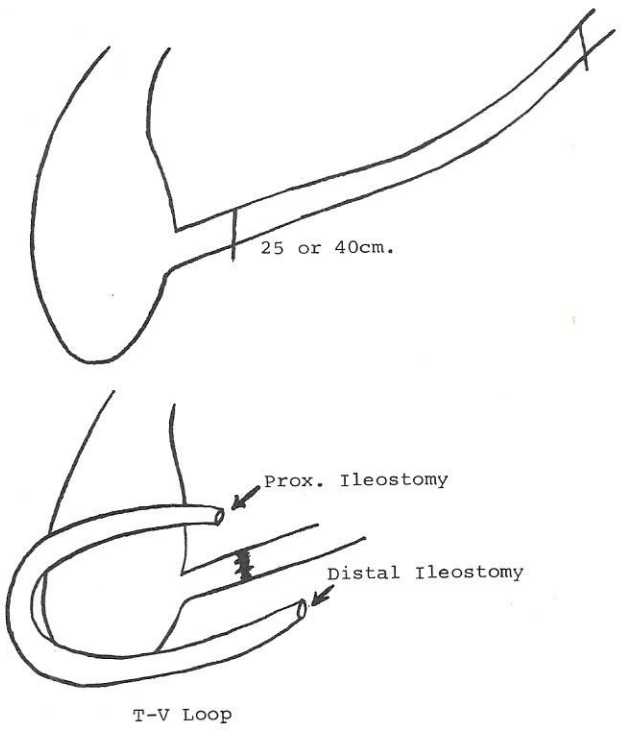


Fig. 1.