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On the In Vivo Clearance and Detoxification of Endotoxin by Lung and Liver

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BRAUDE and associates by using ^{51}Cr labeled endotoxin, a particulate lipopolysaccharide[20] derived from gram negative organisms, found that after its injection the label gradually disappears from the blood.[5,6] Beeson's studies were the first to indicate that this blood clearance occurs predominantly by phagocytosis.[1,2,3] The rate of disappearance is rapid if small sublethal doses of endotoxin are given. Following administration of larger quantities this process occurs at a much slower pace.[5,6]

The critical endotoxin dose, the absolute amount cleared from blood if all phagocytes come into play, is unknown. From previous observations we have to assume that it is relatively small. After an initial rapid phase, blood clearance for endotoxin proceeds more slowly suggesting that the actual pre-empting of the attachment sites on the phagocyte, incorporation by the cell and intracellular inactivation take place at relatively slow rates.

Rudback and Johnson[21] and Oroszlan[16] and associates demonstrated that the prolonged presence of endotoxin in blood is further enhanced through its association with and reversible inactivation by certain plasma proteins. Thus, under normal conditions, endotoxin in this form is hard to detect in plasma or serum. Special technics for its dissociation are required to release the lipopolysaccharide before it can react in certain identification reactions, *e.g.* the Limulus lysate test as recently described by Levin and associates.[15]

Braude *et al*,[6] found the liver to be the primary organ involved in the clearance of endotoxin. Previously, Waravdekar and associates,[30] Rutenburg *et al*,[22] Trapini and co-workers[28] as well as Corwin and Farrar[7] and Filkins[8] noted, however, that several organs or the homogenates prepared from these organs, including those *without phagocytic activity e.g.* the kidney, inactivate endotoxin *in vitro*. Hence the precise role of the liver in this process remains to be defined.

Recently we observed that gram-negative septicemia occurs in dogs during acute hepatic failure.[10] Subsequently Hume[13] and associates reported similar findings in man during hepatic failure. Bjorneboe *et al*.[4] and Triger and associates[29] noted in patients with hepatic cirrhosis that antibody titers for *E coli* were markedly increased. These various findings suggested that endotoxin may be responsible for some of the clinical manifestations of hepatic failure.

Potential sources of endotoxin are many, but because of their abundance the gram negative organisms of the intestine would seem to constitute a primary source. Previously Ravin *et al*.,[18] Sanford and Noyes[23] and Kocz-sar and associates[14] tried to demonstrate the absorption of endotoxin from the gut, nevertheless, there does not seem to be satisfactory unequivocal evidence for such a process at this time.

Assuming that intestinal absorption of endotoxin would take place under certain circumstances, the

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TABLE 1

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

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

question whether a normal liver is able to clear and detoxify endotoxin from portal vein blood remains to be answered. If this process of clearance exists an approximate assessment of its inactivating capacity has yet to be made. This process was studied in the following manner.

Procedures

A close correlation between endotoxin dose and its lethality has been established in rats in both the presence and absence of lead acetate potentiation, a process first described by Selye and associates[26] and subsequently applied by Filkins.[9] Therefore we selected rats of the Sprague-Dawley strain, weighing 250–275 Gm, for our experiments. Under light ether anesthesia the abdomen was opened through a subcostal incision, and in initial experiments a small branch of the ileal vein was intubated after it was established that no intestinal infarction resulted and that the animals survived ligation of this vein. Infusion of even very small quantities of endotoxin via this route was unsuccessful because infarction of the mesenteric vein invariably occurred. The use of heparin only seemed to aggravate the problem. Hence an alternate approach was taken, as has been previously described by us for dogs.[11] The inferior vena cava was divided below the liver and the distal end anastomosed to the side of the superior mesenteric or portal vein using magnifying glasses and 7–0 sutures. This group of animals was called the reverse Eck fistula group. Intact rats served as controls.

Twenty-four hours after operation the animals were re-anesthetized and the femoral vein was cannulated. After intravenous injection of 5 mg. of lead acetate in distilled water, each animal received endotoxin (lipopolysaccharide W. *E. coli* 0.26; B6 Difco) in isotonic glucose. In some animals it was administered by rapid injection and in others by slow infusion over a period of approximately 1 hour with the aid of a Harvard infusion pump. After injection or infusion the catheter was removed and the cutdown incision closed with interrupted sutures.

TABLE 2. *Effect of Heparin (50 U) on Mortality from Endotoxin in Lead Acetate Pretreated Rats*

No. of Animals	Mode of Injection	Heparin Dose	Mortality
12	Rapid Injection	0	10/12
5	Rapid Injection	50 U	4/5
18	Slow Infusion	0	13/18
6	Slow Infusion	50 U	5/6

Results and Discussion

First we compared the difference in mortality between rapid *injection* and slow *infusion* in lead acetate pretreated, intact rats. Slow infusion was chosen since it appeared to mimic the *in vivo* development of endotoxemia more closely.

There is, as can be seen from Table 1, a slight, but not significant difference in the mortality rate between rapid injection and slow infusion of endotoxin. In this experiment the phagocytes lining the pulmonary arteries constituted the first filter encountered by the endotoxin. The findings suggest that the mortality rate is not greatly affected whether these cells are rapidly flooded with endotoxin or gradually exposed to it. This indicates that the rate of endotoxin clearance and detoxification by the cells of this filter may be very slow and allows the spilling over of endotoxin into the rest of the circulation. Histologic studies failed to reveal pulmonary abnormalities, suggesting that the lethal effect was not exerted on the lung itself.

Since slow infusion of endotoxin into freshly operated rats frequently resulted in thrombus formation at the site of the anastomosis, heparin was used to prevent clotting. Thomas *et al.*[27] and Rodriguez-Erdman[19] previously demonstrated that endotoxin activates factor XII. Hageman factor activation not only induces coagulation but as Ratnoff[17] has indicated it is associated with activation of the kinin systems and of C′ 1 esterase activity. Previous observations by Schultz and Becker[24,25] showed that heparin may activate a blood lipase system which could inactivate endotoxin through the enzymatic degradation of the lipid fraction. Hence, the effect of heparin (50 Units per rat) was studied in lead acetate pretreated intact rats.

This small dose of heparin did not cause any significant changes in mortality following endotoxin administration (Table 2) a finding at variance from that pre-

TABLE 3. *Effect of the Type of First Filter Encountered by the Endotoxin* on the Mortality Rate of Rats*

Material Infused via the Femoral Vein	1st Filter	Dose of Endotoxin	No. of Animals	Mortality	Statistical Significance
Endotoxin	Lung	3 mg./100 Gm.	10	9/10	$p < .01$
Lead Acetate (5 mg.) + Endotoxin	Lung	1 ug/100 Gm.	12	8/12	
Endotoxin	Liver	3 mg./100 Gm.	5	1/5	
Lead Acetate (5 mg.) + Endotoxin	Liver	1 ug/100 Gm.	10	0/10	$p < .0001$

* Slow infusion over 1 hour period (with a Harvard pump).

viously observed by Schultz and Becker[24,25] with larger doses of heparin.

Therefore heparin was used in the following experiment designed to study *in vivo* endotoxin clearance and inactivation by the liver. It should be noted that in the reverse Eck fistula animal the liver constitutes the first filter that encounters the highly diluted endotoxin solution while, in the intact animals the lung exerts this function. With the help of the reverse Eck fistula rats, we were able to compare the effect of the clearance and detoxifying activity of the liver, c.q. Kupffer cells, with that of the lung, c.q. pulmonary macrophages, in the group of intact rats (Fig. 1).

In contrast to the limited efficiency of pulmonary macrophages, the Kupffer cells appear to exert a very active and beneficial effect during slow infusion of endotoxin. Under these circumstances they not only seem to eliminate part or all of the minute quantities of endotoxin from blood during the first passage through the

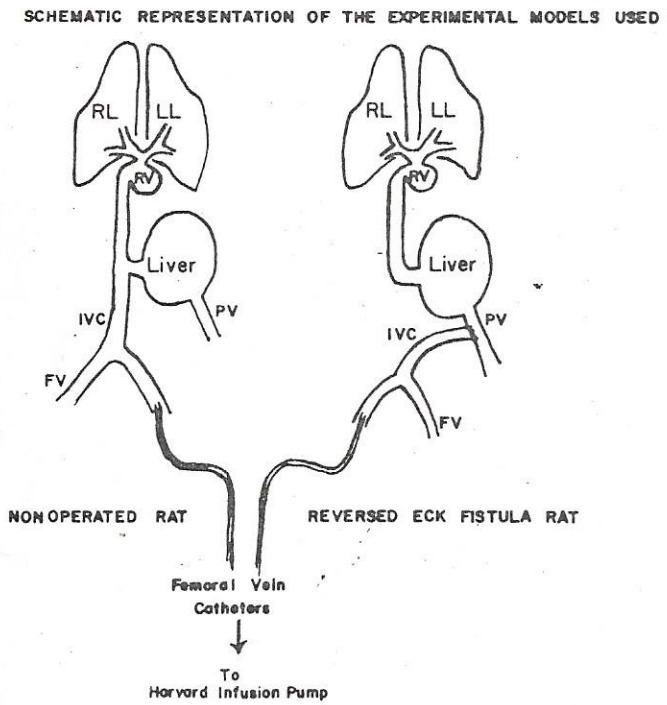

FIG. 1. Study of *in vivo* endotoxin clearance and inactivation by the liver. In the reverse Eck fistula animal (schema on the right) the liver constitutes the first filter that encounters the endotoxin infused into the femoral vein while in the intact animal (schema on the left) the lung exerts this function. With the help of the reverse Eck fistula rats we were able to compare the effect of the clearance and detoxifying activity of the liver, c.q. Kupffer cells, with that of the lung, c.q. pulmonary macrophages in the intact rats.

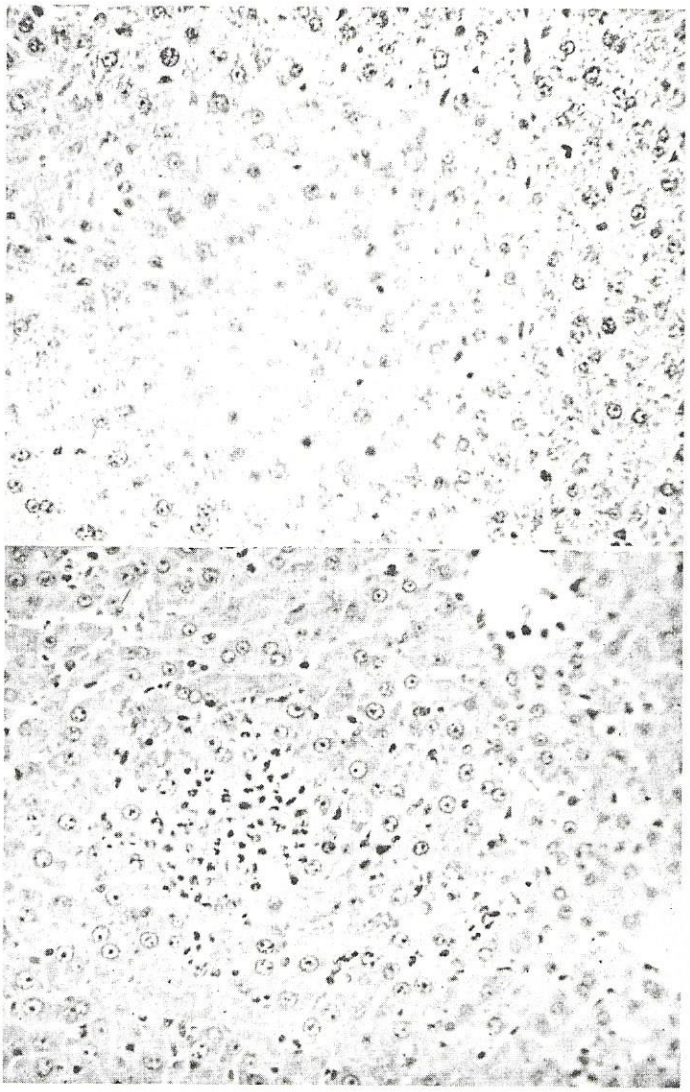

FIG. 2. Effect of endotoxin infusion on the liver was negligible in the reversed Eck fistula rats (top). In contrast focal areas of centrilobular necrosis were observed in livers of non-operated rats that succumbed to systemic infusion of endotoxin (bottom).

liver, they also are able to render a lethal dose harmless, as demonstrated by the fact that all the animals survived the 1 hour infusion of endotoxin (Table 3).

The effect of the endotoxin infusion on the liver was found to be negligible. This finding is in marked contrast to the focal areas of centrilobular necrosis observed in livers of non-operated rats that succumbed to systemic injection or infusion of endotoxin (Fig. 2). These hepatic lesions are therefore probably not endotoxin induced but are rather the result of the shock that precedes the death of these animals.

The result of this experiment would suggest that *in vivo* shunting of the liver into the perfusion circuit can protect the rat against the lethal effects of endotoxin and that Kupffer cells are much more effective in this regard than pulmonary phagocytes suggesting a specific functional difference. Whether this is due to a difference in the number of cells or to functional differentiation is presently not known. Results of studies by Howard and associates[12] would suggest that Kupffer cells are augmented and replaced by recruitment of hematogenous cells, which are probably identical with the precursors of macrophages in other areas. In view of their possible common origin, therefore it would seem that the observed difference in activity is probably not the result of morphologic or functional differentiation. An alternative explanation would be the development of a localized form of tolerance acquired by the Kupffer cells as a result of small but repeated exposures to endotoxin. This would suggest the occasional escape of endotoxin into the portal circulation, a feature for which there is presently no definite proof.

Summary

Little is known about the possible role of endotoxin in hepatic failure. It is a pertinent problem since the liver, because of its strategic location between the intestine and the systemic circulation, might well serve as a filter for endotoxin, if endotoxin is able to escape from the bowel.

If this were the case, interference with endotoxin clearance from the portal vein blood during hepatic failure, would be expected to result in development of a chronic, low grade endotoxemia.

In regard to this problem two questions arise. Is endotoxin absorbed from the gut? This is a point that remains to be settled. If endotoxin escapes, is the liver able to clear and detoxify it? The present studies carried out in rats provide evidence that the liver, in contrast to the lung, can eliminate endotoxin from blood and render it harmless.

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