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In Vivo Quantitation of the Rat Liver's Ability to Eliminate Endotoxin from Portal Vein Blood

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The in vivo uptake of endotoxin by the liver from portal vein blood was assessed during a single passage through the liver. ^{51}Cr labeled and unlabeled endotoxin were infused in different amounts into the femoral vein of three groups of lead-sensitized rats: a nonoperated, a sham-operated, and a surgically created reversed Eck fistula (REF) group. Whereas in the former two the infused endotoxin encounters the lung as the first filter organ, the liver performs this function in the latter experimental model. The mortality rates observed in control and sham-operated, lead-sensitized rats were found to correlate closely and reproducibly to the degree of endotoxemia. This assay was then applied to determine the amount of endotoxin eliminated by the liver by establishing, in the REF rat, the amounts of endotoxin that escaped hepatic clearance. Following infusion of 1 μg of endotoxin/hr into REF rats, approximately 985 ng is found to be taken up by the liver; following 2 μg, 1965 ng is sequestered; following 3 μg, 2810 ng; and after 4 μg, 3175 ng is retained by the liver. Hence, the capacity of the liver to eliminate endotoxin from portal vein blood during a single passage increases as the portal vein endotoxin level rises; it approaches a maximum, suggesting that endotoxin's interaction with the Kupffer cells conforms to classical saturation kinetics. A Lineweaver-Burk plot prepared from these data indicates that the maximal in vivo capacity of the liver to remove endotoxin from portal vein blood approximates 1.5 μg/gm liver/hr. Data obtained with the use of radiolabeled endotoxin corroborate the information obtained with the bioassay technique. Endotoxin eliminated by the Kupffer cells in these quantities is slowly disintegrated; 4 hr after termination of the endotoxin infusion, less than 4% of the radiolabel is found in the urine and none in the bile. These observations indicate that the Kupffer cell's functional capacity to sequester and detoxify endotoxin is extensive and far exceeds the requirements imposed by physiological and most pathological conditions.

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INTRODUCTION

Ever since the studies of Braude et al, the liver has been known to be the principal organ involved in the clearance of endotoxin (lipopolysaccharide, LPS) from the systemic circulation [1]. Beeson [2] indicated that this occurs by phagocytosis, a process mediated by the Kupffer cells. In patients with liver cirrhosis changes occur in liver scans that are consistent with the notion that Kupffer cell clearance is impaired. Hence when Bjorneboe [3] and Triger [4] observed elevated serum E coli antibody titers in patients with liver cirrhosis, it suggested inadequate clearance of E coli or its endotoxin by the diseased liver, thus allowing it to reach antibody-producing sites. The source of the endotoxin remained to be established.

Further evidence for development of endotoxemia during experimentally induced hepatic failure was presented in 1972 [5,9], confirming observations by Wilkinson et al [6] for its presence in patients with acute fulminating hepatic failure and by Wardle for patients with hepatic cirrhosis [7]. Findings suggesting escape of endotoxins from the intestine into the portal vein blood [8,9] were subsequently substantiated in vivo [10] and in vitro [11]. Finally, this fact was also established for patients with liver cirrhosis by Prytz et al [12].

Hepatic clearance of endotoxin, irrespective of whether it reaches the liver via the systemic circulation or by way of the portal vein, takes place by the Kupffer cell; in fact, the role of the hepatocyte in eliminating physiological amounts of endotoxin is negligible [13]. Whereas certain aspects of endotoxin clearance by Kupffer cells remain to be assessed, detailed information concerning endotoxin uptake by monocytes was provided by Bona, who demonstrated the rapid sequestration of labeled endotoxin by these cells using electron microscopy [14]. During the first 15 min he noted that endotoxin is avidly absorbed upon the cell membrane. A similar feature was previously described to occur in vivo [15]. This membrane adhesion is believed to constitute a lipid-lipid interaction [16]. The endotoxin is then incorporated by the cell following membrane invagination. Forty-five min later it is observed on the membrane and in phagocytic vacuoles.

The intracellular fate of endotoxin was studied in vitro by Filkins and in vivo by Mori et al. Filkins demonstrated that sonicates of peritoneal, alveolar, and hepatic macrophages are effective in detoxifying S enteritidis endotoxin, while those of polymorphonuclear cells are not, even at high concentrations [17]. Mori et al [18] used a rat model (reversed Eck fistula rat) in which femoral vein blood is shunted into the portal vein circuit and compared its effects to those noted in animals that encounter the lung as first filter. Using these models they demonstrated that both the liver and lung eliminate small quantities of endotoxin from the passing blood. However, the amount of endotoxin cleared from the portal vein blood during a single passage through the liver far exceeded that eliminated by the lung. The limits of this hepatic function remained to be determined. In the present study we show that endotoxin clearance by the Kupffer cell as determined by two different assay procedures conforms to a saturable membrane receptor system reaching a maximum of approximately 1.5 μg/g liver tissue/hr.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 250–300 g were used in these experiments. The total number of rats studied number 356: 129 controls, 149 sham-operated, and

78 reversed Eck fistula (REF) rats. They were fed Purina chow ad lib, kept in individual cages in an air-conditioned room, and subjected to a 12-hr light/dark cycle.

Endotoxin

Phenol-extracted lipopolysaccharide E coli 055:B5 and S minnesota (Difco Laboratory, Detroit, MI) were used.

Lead Acetate

Lead acetate, Pb $(C_2H_3O_2)$ $3H_2O$ (Matheson Coleman and Bell Manufactury Chemist, Norwood, OH) was dissolved in pyrogen-free water. Five mg were injected into the dorsal vein of the penis immediately prior to the intravenous infusion of endotoxin.

Heparin

Each animal received 50 units of heparin (Heparin Sodium, Upjohn Company, Kalamazoo, MI) immediately prior to endotoxin infusion.

^{51}Cr-labeled Endotoxin

E coli 055:B5 endotoxin was suspended in pyrogen-free distilled water in a concentration of 5 mg per ml and labeled with $Na^{51}CrO_4$ by the procedure described by Braude et al [1] as follows: 35 mg of the dissolved endotoxin was incubated at 37° C for 24 hr with 0.4 μg of $Na^{51}CrO_4$ and dialyzed for three days against distilled water at room temperature until there was no longer any significant loss of radioactivity in the dialysate. These preparations had relatively low specific activities. In parallel experiments 50 μg E coli endotoxin was labeled with 100 μc of $Na^{51}CrO_4$. The specific activity of the latter preparation shortly before infusion was 750 cpm/μg of endotoxin. The labeled E coli endotoxin preparation was chromatographed on a gel filtration column (30 cm $\times$ 1 cm) of Sephadex G-200 (Pharmacia Fine Chemicals, Piscataway, NJ) and the tubes containing Cr^{51}-LPS were pooled (tubes 5–16; Fig. 1).

Preparation of the REF Rat and Sham-Operated Control

Where in nonoperated and sham-operated rats endotoxin administered via the femoral vein encounters the lung first, in the experimental model with a reversed Eck fistula the liver constitutes the first filter. The liver was shunted into the perfusion circuit by the following surgical procedure. Under ether anesthesia, a midline abdominal incision was made and the inferior vena cava exposed below the liver. This vein was dissected free and then divided close to the liver. The distal end was anastomosed to the side of the portal vein using 7-0 silk running suture (Ethicon, Inc, Somerville, NJ) (Fig. 2). The sham-operated rats underwent a midline abdominal incision with exposure of the inferior vena cava exposed below the liver under general (ether) anesthesia. The portal vein was also exposed. Both vessels were cross-clamped for 15 minutes. Subsequently the abdomen was closed. Twenty-four hr after operation, both REF rats and sham-operated animals were re-anesthesized and the femoral vein cannulated. The cannula (Silastic tube: 0.25 $\times$ 0.47 in, Dow Corning Corporation, Midland, MI) was threaded subcutaneously to the midtail region. Femoral vein perfusions were performed with a Harvard infusion pump in fully awake but restrained rats [19] for approximately 1 hr. During this hour the animals received 1, 2, 3, or 4 μg of endotoxin per 100 g body weight. Following this, the cannula was then removed and the femoral vein ligated; the cut down incision was closed with

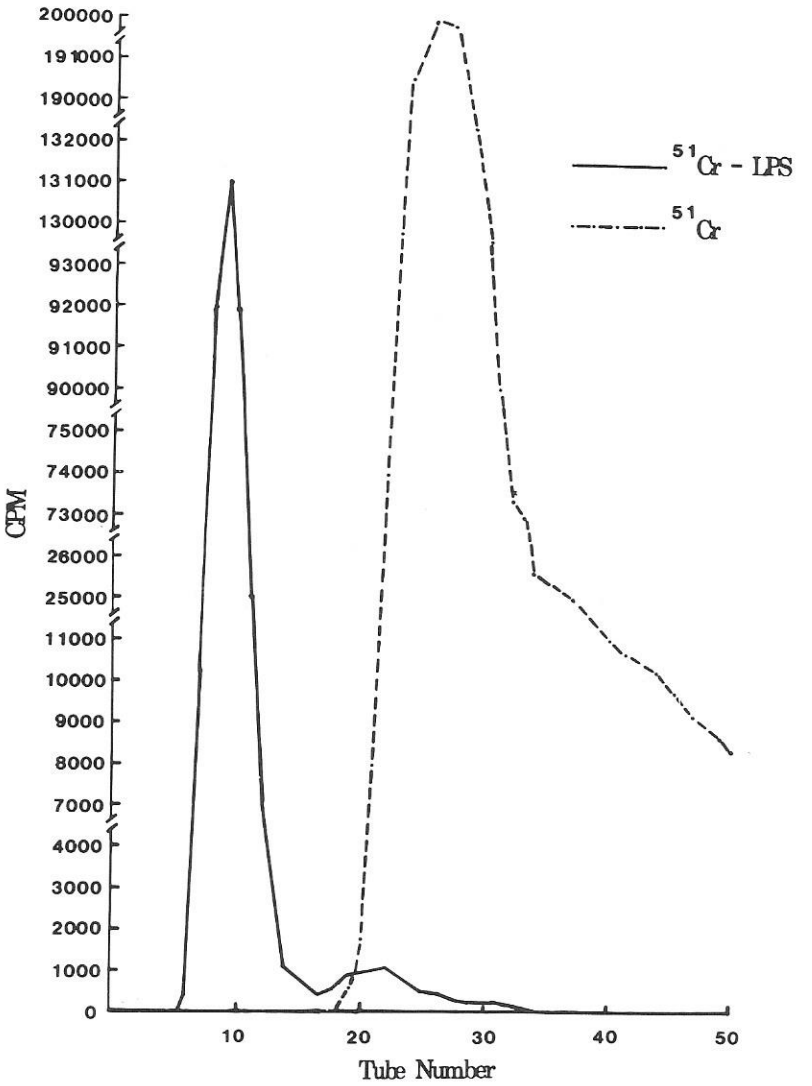

Fig. 1. Chromatography of Cr^{51} labeled E coli LPS on a Sephadex 200 column (30 × 1 cm). One-ml fractions were collected and the radioactivity determined in a gamma counter and expressed in counts/min. Solid line represents Cr^{51} labeled E coli, prepared as outlined in Methods; the dotted line represents free $NaCr^{51}O_4$.

interrupted silk sutures. The animals were returned to their cages where they were allowed full access to water and food.

Mortality Rates

Mortality rates were assessed following operation (sham operation and REF procedure), after administration of lead acetate alone and after injection of lead acetate followed by a 1-hr endotoxin infusion.

Operated rats. Following operation animals were observed for 48 hr, mortality was determined 24 and 48 hr postoperatively.

After Pb administration. Ten animals received 5 mg of lead acetate into the dorsal vein of the penis. The animals were then returned to their cages and observed for 24 hr.

Following lead acetate injection and endotoxin infusion. These animals were observed for 24 hr. No animals were excluded from these mortality studies. Those that died succumbed within 24 hr and were autopsied at that time. Surviving rats were sacrificed 24 hr after endotoxin infusion and autopsied and liver weight was assessed. Liver weight of control and sham-operated rats was 9.5 ± 1.2 g; of REF rats it was 10.0 ± 1.5 g, a slight but not significant increase in liver weight after the operation.

Clearance Studies with ^{51}Cr-labeled Endotoxin

Under ether anesthesia, the femoral vein and artery were cannulated in six control and nine REF rats. The bladder and bile duct were cannulated with a silastic tube via an abdominal incision in three animals. ^{51}Cr-labeled endotoxin was infused through the femoral vein with a Harvard pump over a 1-hr period in anesthesized, minimally restrained animals. Blood samples (0.3 ml) were obtained from the femoral artery catheter during the infusion of labeled endotoxin at 1, 3, 6, 15, 30, and 60 min and 1, 2, and 3 hr after termination of the infusion. Blood samples for counting were added to polyvinyl test tubes containing 2 ml 10% EDTA. Five hr after infusion, the animals were sacrificed; the liver, lung, spleen, and kidney were removed, weighed, washed free of blood, and placed in polyvinyl test tubes for counting. Similarly, urine samples and bladder washings, using pyrogen-free water, were obtained from the urinary catheter during infusion of

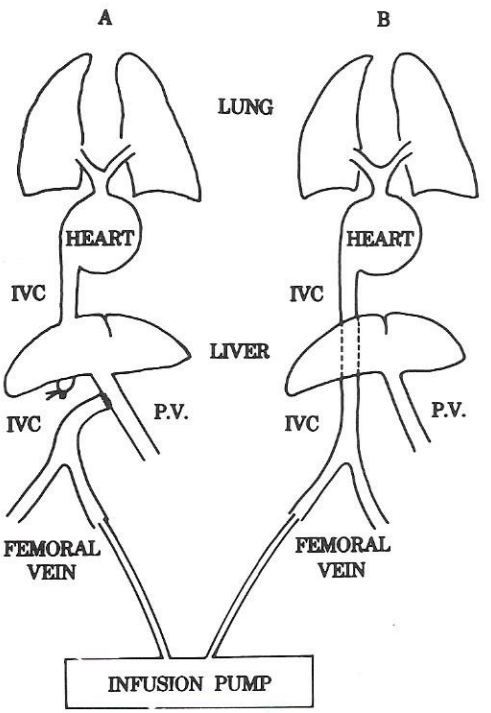

Fig. 2. Schematic representation of the two experimental animal models used. Right, nonoperated and sham-operated rat (B). Left, reversed Eck fistula (A). Note that in the reversed Eck fistula animal, the liver is shunted between the femoral vein and the systemic circulation.

labeled endotoxin at 15, 30, and 60 min. In addition, samples were collected at 30, 60, 90, 120, 150, and 180 min after termination of the infusion and at time of sacrifice. Urine, bile, and blood samples were counted for radioactivity in a well-type scintillator (Tracor Analytic Inc, Elk Grove Village, IL). Radioactivity was expressed in cpm/ml after correction for background. The percent of radioactivity per organ was calculated by dividing total activities per organ by the total activity originally administered.

RESULTS

Initially, the mortality observed after preparation of REF was studied. Death occurred postoperatively in one of ten animals within 24 hr. The other animals survived. No death occurred among 20 sham-operated rats. Subsequently, the lethality of lead acetate administration (5 mg/rat) alone was studied. This, if administered alone, was well tolerated in both ten control and ten sham-operated animals. If animals survived the first 24 hr after surgery, the intravenous infusion of endotoxin into the femoral vein of lead-sensitized nonoperated, sham-operated, and REF rats was studied over a 1-hr period using

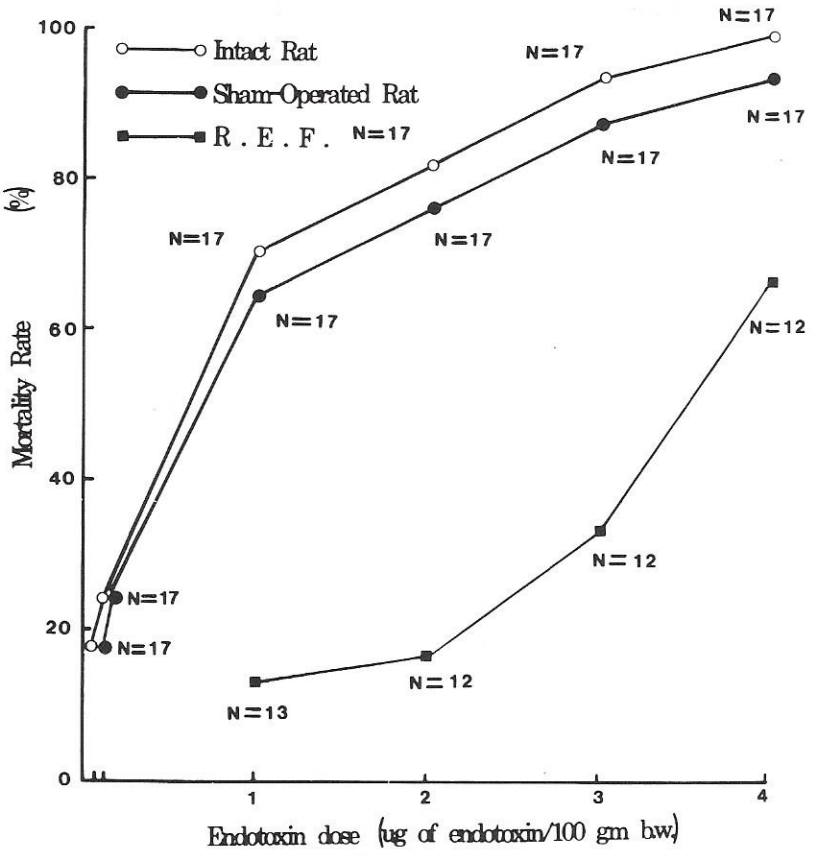

Fig. 3. Relationship between mortality rate and endotoxin dose. The effect of various doses of endotoxin on mortality rate was studied in nonoperated, sham-operated, and reversed Eck fistula, lead-sensitized rats. N = the number of animals in each group.

TABLE 1. Mortality Rates Observed After a 1-hr Infusion of Various Amounts of Endotoxin into Intact, Sham-operated, and REF Rats

Dose of endotoxin 100 gBW/hr	Percent lethality					
	intact rats		sham-operated rats		REF rats	
10 ng	5.8%	(1/17)[1]	5.8%	(1/17)		
50 ng	17.6%	(3/17)	17.6%	(3/17)		
100 ng	25.3%	(4/17)	23.5%	(4/17)		
1 μg	64.7%	(11/17)	70.5%	(12/17)	7.6%	(1/13)
2 μg	76.4%	(13/17)	82.3%	(14/17)	16.6%	(2/12)
3 μg	88.2%	(15/17)	94.1%	(16/17)	33.3%	(4/12)
4 μg	94.1%	(16/17)	100.0%	(17/17)	66.6%	(8/12)

[1]No. died/no. tested.

TABLE 2. The Amount of Endotoxin that Escapes Hepatic Sequestration During Endotoxin Infusions into the Lead-Sensitized REF Rats

Dose of endotoxin 100 gBW/hr	Mortality rates		The amount of endotoxin that escapes into the systemic circulation (ng/hr)
1 μg	7.6%	(1/13)[1]	15
2 μg	16.6%	(2/12)	35
3 μg	33.3%	(4/12)	190
4 μg	66.6%	(8/12)	825

[1]No. died/no. tested.

a Harvard infusion pump. Results of these studies are summarized in Figure 3 and Table 1. The difference in the mortality rates observed after infusion of endotoxin into lead acetate-sensitized control and sham-operated rats was slight and insignificant.

Mortality rates observed in REF rats receiving 1 μg of endotoxin/100 g body weight (BW) are approximately the same as those noted in sham-operated rats receiving 10 ng of endotoxin/100 g BW. More precisely, the amount of endotoxin that escaped hepatic clearance and hence spilled over into the systemic circulation was extrapolated from the dose-mortality curve. Thus we found that in the latter, approximately 15 ng of endotoxin spills over into the systemic circulation (Table 2). Likewise the mortality rates following the infusion of 2 μg of endotoxin/100 gm BW were found to be equivalent with a spillover of approximately 25 ng of endotoxin; 3 μg with approximately 85 ng; and 4 μg with approximately 815 ng of endotoxin.

These data allowed us to calculate the amount of endotoxin sequestered by the liver. Plotting these quantities against the amounts of endotoxin administered, they were found to increase as the blood endotoxin level rises (Fig. 4). This function reaches a maximum, thus lending itself to treatment with the Michaelis-Menten equation. Preparing a Lineweaver-Burk plot from the data the following values were obtained: A correlation coefficient of 0.93, a Km equaling 14.5 μg/hr, and Vmax of 1538 ng/gm/hr or approximately 1.5 μg/gm/hr (Fig. 5).

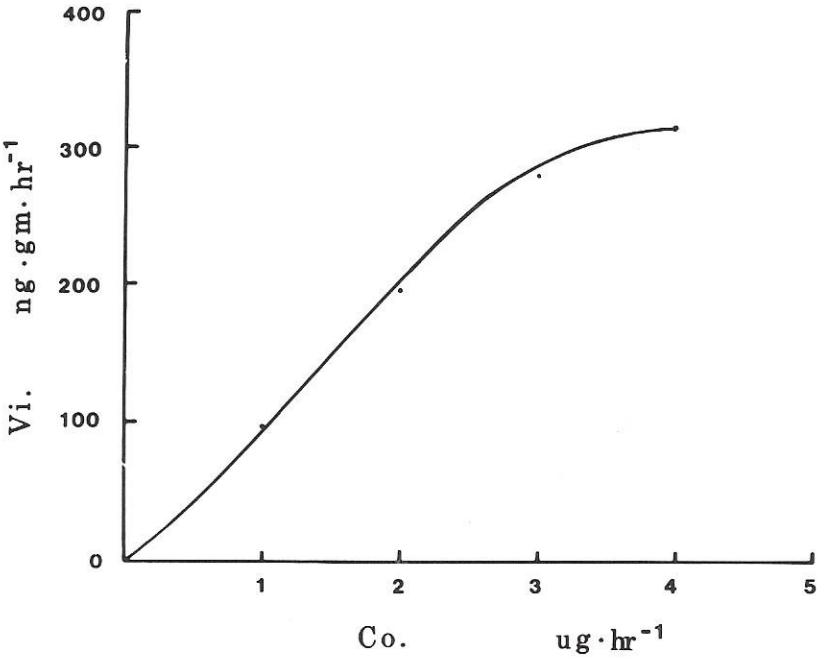

Fig. 4. In vivo capacity of the liver to eliminate endotoxin from portal vein blood as a function of portal vein blood endotoxin level. The quantity of endotoxin eliminated by the liver from the portal vein blood during a 1-hr infusion period, Vi (expressed in ng/gm liver/hr) is plotted against the total amount of endotoxin, Co (expressed in μg/hr) used to perfuse the liver per hr.

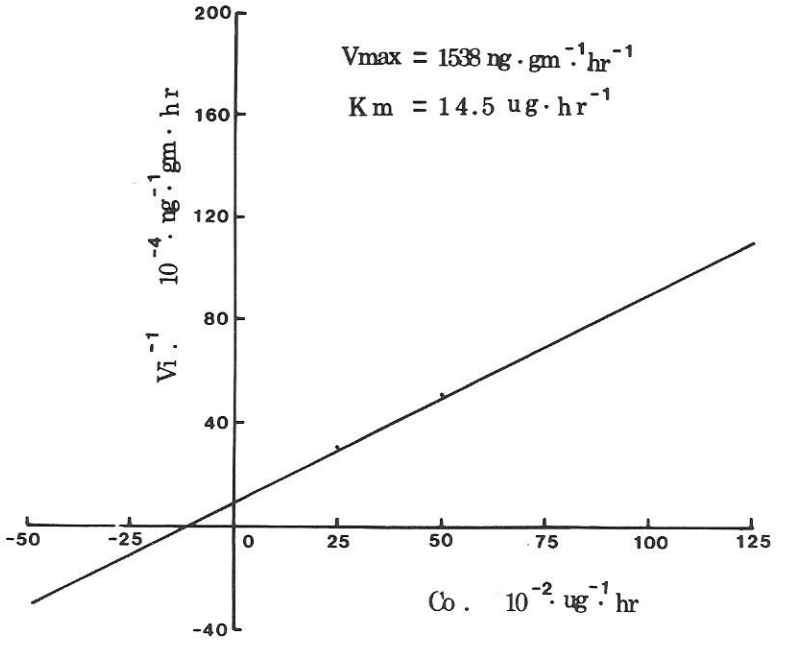

Fig. 5. Lineweaver-Burk plot. Endotoxin concentration sequestered by the liver/hr (Vi) is plotted against the portal vein endotoxin concentration, Co.

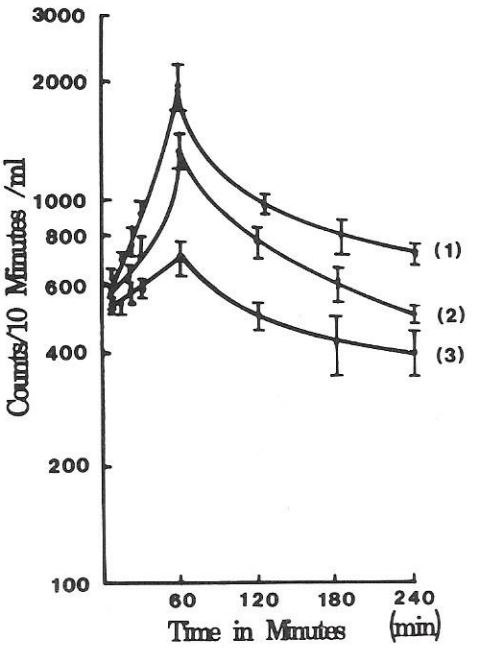

Fig. 6. Levels of blood radioactivity observed in rats after infusion of various amounts of radiolabeled endotoxin. 1) Blood radioactivity expressed cpm/ml after infusion of 1 μg of endotoxin/100 g BW/hr into 3 nonoperated rats. 2) Idem after infusion of 4 μg of endotoxin/100 g BW/hr into 3 reversed Eck fistula rats. 3) Idem after infusion of 1 μg of endotoxin/100 g BW/hr into 3 reversed Eck fistula rats. (Mean ± S.D.)

To evaluate the validity of the bioassay procedure used in obtaining these data, these studies were repeated using radiolabeled endotoxin. ^{51}Cr-labeled endotoxin was infused during a 1-hr period via the femoral vein into lead acetate sensitized REF, sham-operated, and control rats. Infusion of 1 μg of labeled endotoxin into three nonoperated, lead sensitized rats was associated with sharp increase in blood radioactivity. Subsequently, it declined exponentially in a similar fashion to that previously observed by others [15,20]. In contrast, infusion of the same amount of labeled endotoxin into three lead acetate sensitized REF rats resulted in only a slight increase in blood radioactivity, suggesting again a minimal spillover of endotoxin into the systemic circulation (Fig. 6, curve 3). This finding corroborates the previous findings obtained with the bioassay.

During infusion of 4 μg of labeled endotoxin into three REF lead-sensitized rats, peripheral blood radioactivity increased; it was less than that observed following infusion of 1 μg of LPS into three nonoperated lead-sensitized rats (Fig. 6, curve 2); in fact, the activity was about half the activity observed after infusion of 1 μg of LPS, indicating again minimal spillover of endotoxin.

During the radiolabeling experiments urine samples and bladder washings were also obtained. The average total amount of free ^{51}Cr found to be excreted into the urine during the 5-hr observation period was 3.8% of the administered dose. None appeared in the bile collected from these animals.

Tissue distribution of radioactivity was assessed 4 hr after termination of the infusion. Liver uptake in lead acetate-treated controls amounted to 62.7% of the total radioactivity infused; in the REF rat it was 76.2%. In animals receiving no lead acetate

TABLE 3. Organ Uptake of Radioactivity After Intravenous Infusion of 1 μg of Labeled Endotoxin, Determined 3 hr After Termination of the Infusion

	Lead acetate sensitization (5 mg/rat)		No lead acetate sensitization	
Organs	control n = 3	REF Rat n = 3	control n = 3	REF Rat n = 3
Liver	62.7%	76.2%	78.9%	87.5%
Spleen	4.7%	3.4%	3.9%	3.4%
Lung	1.3%	1.4%	1.6%	1.8%
Kidneys	0.6%	0.7%	0.9%	0.6%
Blood	27.0%	14.3%	11.3%	4.1%
Recovery	96.3%	96.0%	96.6%	97.4%

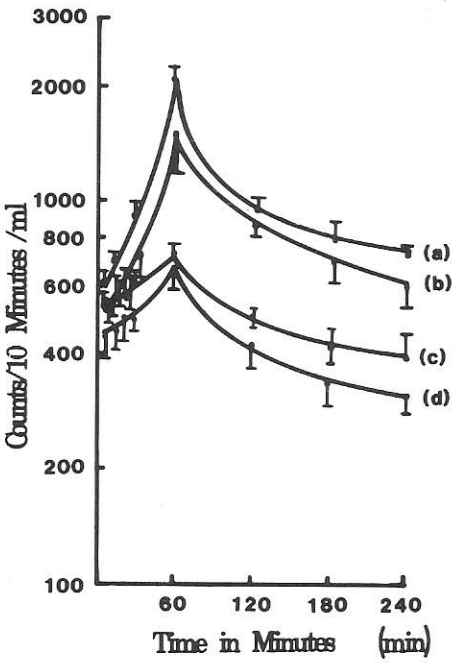

Fig. 7. Levels of blood radioactivity observed in rats after infusion of 1 μg of radiolabeled endotoxin/100 g BW/hr. a) Blood radioactivity expressed cpm/ml after infusion of 1 μg of endotoxin/100 g BW/hr into 3 lead-sensitized, nonoperated rats. b) Idem after infusion of 1 μg of endotoxin/100 g BW/hr into 3 nonlead-sensitized, nonoperated rats. c) Idem after infusion of 1 μg of endotoxin/100 g BW/hr into 3 lead-sensitized, reversed Eck fistula rats. d) Idem after infusion of 1 μg of endotoxin/100 g BW/hr into 3 nonlead-sensitized, reversed Eck fistula rats. (Mean ± S.D.)

(three in each group) these values were 78.9% (controls) and 87.5% (experimental animals) respectively (Table 3).[1] The residual blood radioactivity in lead acetate-treated controls amounted to 27% of the total, infused radioactivity while that observed in REF rats was 14.3%. These values were 11.3% and 4.1%,[1] respectively, in nonlead sensitized rats, suggesting that the uptake of radiolabeled endotoxin by the liver exceeds that of

[1]The noted differences in the observed values represent merely a trend; the limited number of studies performed in this experiment preclude a statistical evaluation.

unlabeled endotoxin in the lead acetate-treated animal, which is expected in view of the fact that salts of heavy metals interfere with phagocytosis [26].

In two REF rats the spillover during a 1-hr infusion of labeled E coli LPS (1 μg) into the systemic circulation was studied as such and, after a preceding injection of 1 mg S minnesota endotoxin, administered as a bolus 10 min prior to starting the infusion. In the latter the spillover of radioactivity into the systemic circulation greatly exceeded that observed in the former, suggesting that both endotoxin preparations probably compete for the same binding site on the macrophage membrane.

DISCUSSION

Endotoxin is sequestered by the liver irrespective of whether it arrives via hepatic artery and portal vein [1] or via the portal vein alone [18]. The question we asked ourselves was, if endotoxin escapes across the intestinal wall, as was shown previously, what quantities can the liver clear from the portal circulation during a single passage? This assumes *first,* that because the amounts of endotoxin involved in this process are minute (approximately 1–5 ng/ml of blood), methods capable of detecting these quantities in the circulation are available. *Second,* it is exceedingly difficult to sample the portal vein blood; hence, to become detectable, endotoxin would have to reach the systemic circulation before it can be demonstrated. Using the REF rat as experimental model, the LPS not cleared by the liver is expected to spill over into the peripheral blood where it becomes potentially demonstrable. *Third,* because we intended to assess Kupffer cell function alone, without participation of the hepatocyte, the amounts of endotoxin to be used had to be less than those known to injure the cells lining the hepatic sinusoids. Where the amounts of endotoxin used in the various experiments reported here approximate those found in vivo in the rat which are very small indeed, they pose difficulties in detection. Three procedures are presently available to determine endotoxins in biological fluids: The radioimmunoassay, the limulus lysate technique, and a bioassay developed by Selye et al [22]. The radioimmunoassay is only specific for detecting 0 antigens, not for lipid A, the active component of LPS. Also, it is unable to detect nanogram quantities of LPS, features severely limiting its usefulness for our study [21]. We felt that the limulus amebocyte lysate technique was unsuitable for our purposes, because it requires the use of an inlying catheter to obtain the required blood samples which poses serious problems in controlling contamination. Hence we used the third technique, a bioassay procedure previously developed in Selye's laboratory which quantitates systemic endotoxin levels [22] and which is reliable while its reproducibility and relative ease of application have been previously noted by others [10,18,23]. This assay depends upon a several thousandfold enhanced sensitivity to endotoxin induced by lead acetate. Where normal rats tolerate milligram amounts of endotoxin per 100 g BW with impunity, lead sensitization results in reproducible mortality rates using nanograms of endotoxin. Our results indicate that this bioassay is not only very sensitive and reproducible but also quite reliable. The latter fact was borne out in the present studies where data obtained with radiolabeled endotoxins were found to correlate closely to those obtained with the lead acetate bioassay.

The manner of lead sensitization has never been clearly established. Recent observations from our laboratory suggest that lead salts may inhibit the activity of endotoxin-inactivating enzymes in serum [24].

The REF rat model was previously applied to study the in vivo clearance of endotoxin and its subsequent detoxification by the liver [18]. Those initial studies established that

the capacity of the liver of lead sensitized rats to clear endotoxin in one single passage from the portal vein blood exceeded 1 μg of endotoxin/100 g BW/hr. The present study made use of this same experimental model to obtain a more precise quantitative assessment of endotoxin sequestration by the liver, a function which in the endotoxin concentration range used here is thought to be nearly exclusively performed by the Kupffer cells. Hence, ultimately we determined these cells' maximum capacity to clear endotoxin from portal vein blood during a single passage through the liver.

First, the mortality rates for intact and sham-operated rats was determined after a 1-hr intravenous infusion of various doses of endotoxin into the femoral vein. The dose/mortality ratios for these two experimental models were virtually identical. The mortality rates observed in the present studies are slightly less than those previously reported by Selye et al [22], Filkins [23], and Mori et al [10,18], presumably because in their studies endotoxin was injected as a bolus, while in the present study it was infused over a 1-hr period. Also, Selye et al found that the simultaneous administration of lead acetate and endotoxin results in somewhat higher mortalities than when these two agents are given an hour apart [22], as was the case in the present study.

Comparing the mortality rates observed in control or sham-operated animals to those found in REF rats allowed us to determine the amount of endotoxin that escapes clearance by the liver and thus spilled over into the systemic circulation. Mortality rates observed after infusing 1 and 2 μg of endotoxin into the REF rat indicated their almost complete clearance from the portal vein blood during a single passage through the liver. However, administration of larger quantities was associated with escape of significant amounts of endotoxin into the systemic circulation (Table 2). At the same time, however, increasing amounts of endotoxin were sequestered by the liver. Plotting endotoxin uptake by the liver as a function of the portal vein endotoxin concentration revealed that the former approaches a maximum (Fig. 4). Since in the concentration range used in the present studies the hepatocyte is presumably not involved in endotoxin clearance [13], it suggests that the LPS associated with and incorporated by the Kupffer cell involves a process that takes place via a saturable membrane receptor system. The actual in vivo capacity of the system that eliminates endotoxin from portal vein blood approaches 1.5 μg/g liver/hr (Fig. 5).

A similar concentration-dependent association of endotoxin for isolated rabbit polymorphonuclear leukocytes has also been previously demonstrated [25]. This process, studied in vitro, occurred independent of temperature or metabolic inhibitors, suggesting that it is a passive one. In addition, it was found that neither complement nor other plasma factors are required for in vivo sequestration of endotoxin [15]. Findings obtained in the present study suggest that elimination of one species of endotoxin from the circulation tends to interfere with the disappearance of a second, different preparation. These various findings suggest that the macrophage receptor for LPS requires neither complement nor antibody for their action; also, they are probably nonspecific in that different lipopolysaccharides can interact with the same binding site, a conclusion consistent with the concept previously advanced by Shands that endotoxin's association with the cell membrane represents a "lipid-lipid" interaction [16]. Eventually the endotoxin thus cleared is incorporated by the Kupffer cell and disintegrated.

This breakdown appears to take place very slowly. Four hr after terminating the infusion less than 4% of the injected radioactivity was recovered in the urine, while none appeared in the bile. Hence, the amount of free ^{51}Cr or labeled endotoxin breakdown products sufficiently small to be able to escape into the urine during this period appears

to be negligible. This finding clearly confirms Bona's electron microscopic findings that intact endotoxin can persist inside these cells for periods exceeding 24 hours [14], a feature that is in marked contrast to the fate of denatured proteins which undergo rapid degradation after their ingestion by Kupffer cells [26–29].

Thus, it appears that the maximal amount of endotoxin cleared by the liver from portal vein blood during a single passage is considerable. It greatly exceeds the quantity that escapes across the rat gut wall. Those are truly minute [10,11]. Where some have expressed the belief that intestinal permeability for enteric endotoxins and other gut-derived antigens may well be increased during liver disease [30,31], others have recently expressed doubts concerning this issue [31]. If it escapes, however, and the preponderance of evidence favors the escape of macromolecules across the gut wall in an unaltered form [10,11,32], a Kupffer cell deficiency as occurs during liver cirrhosis would account for some of the previously reported spillovers of gut-derived antigens in these patients. The repercussions of this process remain to be assessed.

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