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THE ESSENTIAL ROLE OF THE LIVER IN DETOXIFICATION OF ENDOTOXIN

W. Edmund Farrar, Jr.,* and Laurence M. Corwin
Department of Applied Immunology and Division of Biochemistry, Walter Reed Army Institute of Research, Washington, D. C.

Bacterial endotoxins are thought by many to be responsible for at least some of the clinical manifestations of human diseases caused by Gram-negative bacteria.[1-4] There has been much interest, therefore, in the ways in which endotoxin which gains access to the mammalian circulation may be rendered nontoxic. There is evidence that a major proportion of endotoxin injected into experimental animals localizes within tissues of the reticuloendothelial system,[5-7] and experimental procedures which alter the function of the reticuloendothelial system may have profound effects on the response of the animal to endotoxin.[8]

During the past decade, a number of investigators have observed that serum,[9,10] and more especially plasma,[11] of various animal species is capable of detoxifying endotoxin *in vitro* by what appears to be an enzymatic reaction.[12] Since this reaction is inhibited by concentrations of calcium present in serum,[13] it appeared to be of questionable physiological significance. However, in 1960, Waravdekar and coworkers announced the detection of endotoxin-detoxifying activity in cell-free homogenates of rabbit liver.[14] Two years later, Trapani and others demonstrated that this reaction was not inhibited by calcium.[15] Endotoxin-detoxifying activity was not limited to tissues rich in phagocytic elements, since spleen, which is rich in RES cells, displayed no activity, whereas kidney, which is not, was quite active. A somewhat similar type of activity was found by Smith *et al.* in dog spleen.[16]

We have been interested in the possible physiological significance of this type of endotoxin inactivation by tissues for the intact animal exposed to endotoxin. Most of our studies to date have been done in the guinea pig. The methods used have been described in detail in previous publications.[17-19] Briefly stated, the endotoxin-detoxifying activity of tissues was determined by grinding the freshly removed organ in cold buffered saline, centrifuging to remove nuclei and cell debris, and incubating the resulting supernatant homogenate in various dilutions with a known amount of endotoxin. The reaction mixture was then diluted with cold buffered saline, and residual endotoxin activity assayed by intravenous injection into 11-day-old chick embryos. Previous experiments by us and by Finkelstein[20] had shown that the lethality of endotoxin for chick embryos was proportional to dosage within the range employed.

*Present address: Department of Preventive Medicine and Community Health, Emory University School of Medicine, 69 Butler Street, S.E., Atlanta, Georgia.

Distribution of Endotoxin-Detoxifying Activity in Guinea Pig Tissues

The relative endotoxin-detoxifying activity of various tissues of the guinea pig studied by this method is shown in TABLE 1. Kidney, liver, and spleen were the most active tissues studied, and showed approximately equal activity. Heart showed an intermediate degree of activity, whereas skeletal muscle, blood, and lung were much less active.[19] This distribution of activity in organs of the guinea pig is similar to that reported by Keene[21] in the rabbit.

TABLE 1
EFFECT OF INCUBATION WITH GUINEA PIG TISSUE HOMOGENATES ON TOXICITY OF ENDOTOXIN*

Tissue tested	Ten per cent homogenate		One per cent homogenate	
	Mortality (deaths/total eggs)	Per cent protection†	Mortality (deaths/total eggs)	Per cent protection†
Kidney	1/20	94	8/20	50
Liver	2/20	88	9/20	44
Spleen	2/20	88	9/20	44
Heart	8/20	50	11/20	31
Skeletal muscle	11/20	31	11/20	31
Blood	11/20	31	12/20	25
Lung	11/20	31	15/20	6
Saline control	24/30	--	24/30	-

*Incubation medium contained 20 μg of endotoxin and either 180 mg (10% homogenate) or 18 mg (1% homogenate) of tissue homogenate (supernatant fraction after 600 × g centrifugation), except in the case of blood, which was used whole. Incubation was for 1 hour at 37°C.

†$\text{Per cent protection} = \frac{\%\text{ mortality saline} - \%\text{ mortality sample}}{\%\text{ mortality saline}} \times 100.$

Since others had shown that most injected endotoxin is removed from the circulation by the liver and spleen,[5-7] and not by the kidney,[22] our attention was directed toward these two organs. An attempt was made to determine the role which the spleen might play in the response of animals to endotoxin. When animals were subjected to splenectomy and then challenged with endotoxin, they were found to be no more susceptible to the lethal effect of endotoxin than normal controls (TABLE 2). It was therefore concluded that any role which the spleen plays in the response of the guinea pig to endotoxin cannot be a major one.

TABLE 2
EFFECT OF SPLENECTOMY ON MORTALITY IN GUINEA PIGS FOLLOWING INTRAVENOUS INJECTION OF ENDOTOXIN (E. COLI 026:B6)

Endotoxin (mg/kg)	Mortality	
	Splenectomized	Sham
15	5/5	6/6
5	0/5	4/7
1.67	2/5	2/5
.56	0/6	0/5
.19	0/6	0/6

Effect of Experimental Liver Injury on Susceptibility to Endotoxin

On the other hand, when severe acute liver injury was produced with a sublethal dose of carbon tetrachloride, the animals became approximately 150 times more susceptible than normals to the lethal effect of endotoxin.[17,23] As shown in TABLE 3, this susceptibility was maximal 48 hours after carbon tetrachloride administration, and returned toward normal during the next few days. When the animal's susceptibility to endotoxin was compared with the

TABLE 3
LETHALITY OF ENDOTOXIN FOLLOWING CCl_4 ADMINISTRATION

Endotoxin (mcg)	Normal animals	Interval between CCl_4 administration* and intravenous endotoxin challenge (hr.)							
		0	8	12	24	48	72	96	168
2500	17/17†								
500	19/27								
100	0/22	7/23	8/23	13/15	15/15	15/15	15/15	3/15	2/13
20				0/8	10/16	20/21	8/16		
4				0/8	7/16	15/22	0/16		
0.8				0/8	2/8	4/16	1/8		
LD_{50} (mcg)	334			51	6.7	2.2	18.2	> 100	

*0.15 ml subcutaneously.
†Deaths/total.

TABLE 4
COMPARISON BETWEEN ENDOTOXIN SUSCEPTIBILITY AND MORPHOLOGIC CHANGES IN THE LIVER AT VARIOUS INTERVALS AFTER CCl_4 ADMINISTRATION

Morphologic change	Time (hr.)				
	24	48	72	96	120
Necrosis	++	++++	++	+	0
Mitosis	0	+	+++	++++	0
Fatty metamorphosis	++	++	++++	+++	++
Endotoxin LD_{50} (mcg)	6.7	2.2	18.2	> 100	

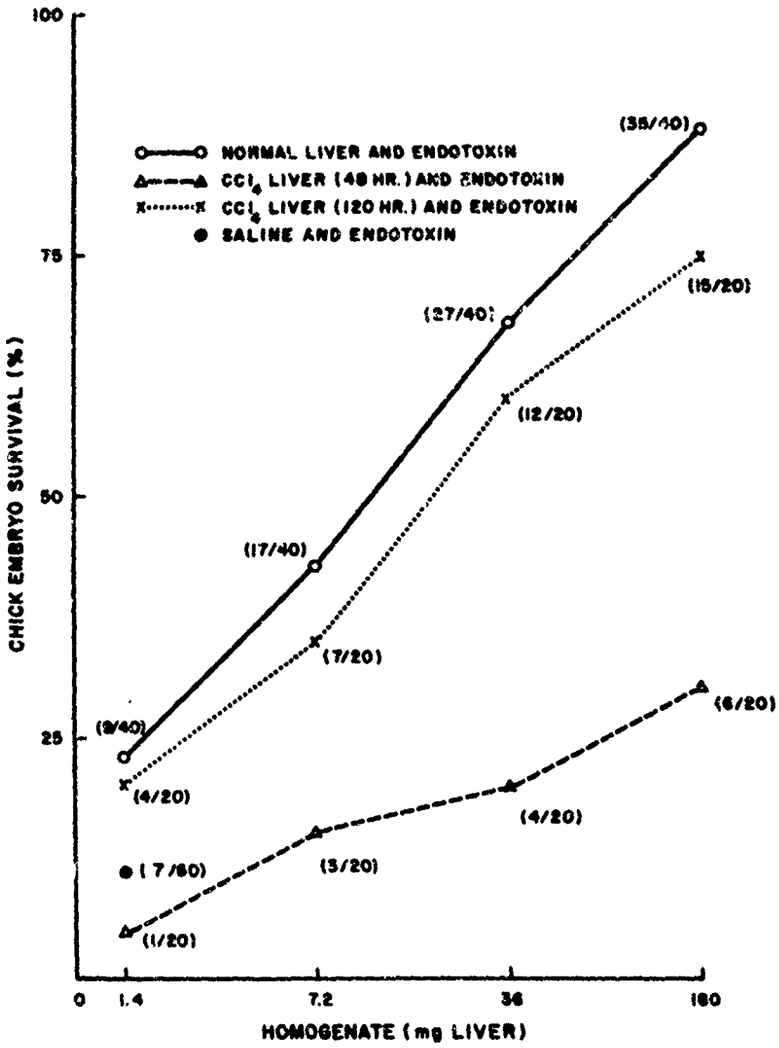

FIGURE 1. Effect of incubation with liver homogenates on toxicity of *S. marcescens* endotoxin for 11-day chick embryo. Pooled data from 4 experiments. The values in parentheses represent surviving embryos/total tested. Each embryo received 0.0125 μg endotoxin intravenously (2 LD_{50}), assuming no inactivation had taken place.

microscopic appearance of the liver, it was found that the susceptibility was maximal when the amount of necrosis seen in the liver was greatest (TABLE 4). As regeneration of the liver took place, the resistance of the animals to endotoxin returned toward normal.

Experiments were next performed to determine the effect of carbon tetrachloride administration on the detoxification of endotoxin by liver homogenates. As shown in FIGURE 1, homogenates prepared from normal liver possessed considerable detoxifying activity. The solid line shows that only 9 milligrams of homogenate from normal liver were required to protect 50 per cent of the chick embryos used in the assay. In contrast, liver homogenate prepared from animals given carbon tetrachloride 48 hours before sacrifice was much less active and only 30 per cent of embryos survived when 180 milligrams of liver were used (dashed line). By extending this line it can be seen that 2300 mg of liver would be required to protect 50 per cent of the embryos, a reduction in activity to 1/250 of normal. The survival rate of control embryos injected with endotoxin incubated in saline was 12 per cent. When the liver was removed 5 days after carbon tetrachloride administration, at a time when the liver had returned to essentially normal microscopic appearance, the ability of the homogenate to detoxify endotoxin approached that of normal liver (dotted line). Thus there appeared to be a relationship between the degree of liver necrosis, the susceptibility of the animal to endotoxin, and the ability of liver tissue homogenates to detoxify endotoxin *in vitro*.

Nature of Endotoxin Inactivation by Guinea Pig Liver

Since the foregoing experiments suggested that the liver might be an important factor in the response of normal guinea pigs to bacterial endotoxin, it seemed important to elucidate if possible the nature of the specific factor, or factors, in liver homogenate which inactivate bacterial endotoxin *in vitro*. Attempts were first made to localize the endotoxin-detoxifying activity within a specific cell fraction, but activity was found in all fractions studied—nuclear, mitochondrial and supernatant. We were also unable to purify the endotoxin-detoxifying factor by means of ammonium sulfate precipitation or by calcium phosphate gel absorption.[18]

When the effect of pH on the system was studied, it was found that there are two distinct pH optima when the whole homogenate is used (FIGURE 2). The first optimum is at pH 6.5 to 7.0, and the second is at pH 8.5 to 9.0. This finding suggested that endotoxin inactivation by whole liver homogenate may involve more than one enzyme. When washed mitochondria were studied it was found that significant activity occurred only at the more acid pH optimum.

In order to ascertain the nature of the enzymatic activity in the mitochondria, an investigation of cofactor requirements was made. Mitochondria were used in these experiments because it is possible by washing to free them, for the most part, from soluble cofactors. As shown in TABLE 5, con-

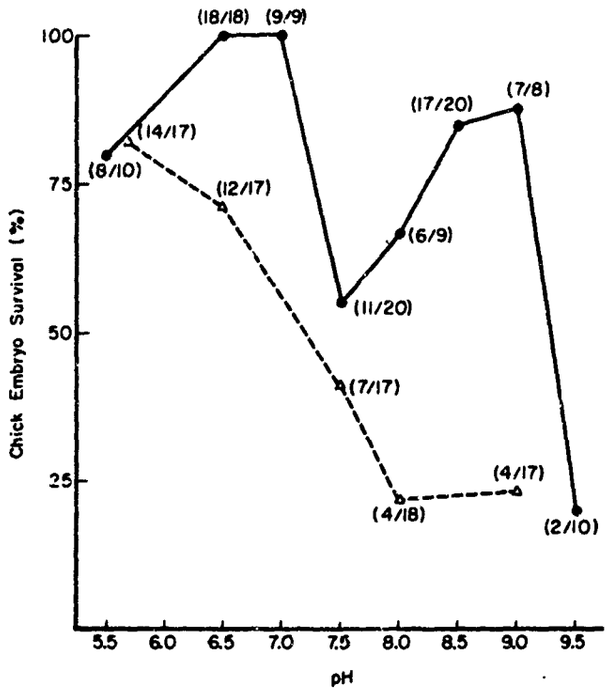

FIGURE 2. Effect of pH on endotoxin inactivation by whole-liver homogenate (•) and by washed mitochondria (△). Homogenate was prepared as a 20 per cent suspension of liver in 0.33 M sucrose after centrifugation at 600 × g. This was diluted 1:1 with 0.01 M phosphate buffer (pH 7.0) and then adjusted to the desired pH with either 0.1 N HCl or 0.1 N NaOH. Reaction mixture contained 1.8 ml of homogenate and 0.2 ml (20 μg) of endotoxin. Mitochondria consisted of a 20 per cent suspension of washed mitochondria in 0.33 M sucrose, diluted 1:1 with 0.1 M buffer of desired pH. Reaction mixture contained 1.5 ml of mitochondria, 1.5 mM ATP, 1.5 mM NAD, 1.5 mM MgCl and 20 μg of endotoxin in a final volume of 2 ml. All eggs received 2 LD_{50} of endotoxin, which killed 90 per cent of control eggs.

TABLE 5
EFFECT OF COFACTORS UPON ACTIVITY OF MITOCHONDRIA

Determination*	Mortality	Per cent Protection†
Mitochondria alone	9/24	45
+ ATP	5/24	69
+ Malate	2/23	87
+ ATP + malate	2/24	88
Saline control	16/24	

*ATP and malate were added at a concentration of 1.5 mM.

†Per cent protection = $\frac{\%\text{ mortality saline} - \%\text{ mortality sample}}{\%\text{ mortality saline}} \times 100.$

TABLE 6
EFFECT OF COFACTORS UPON ACTIVITY OF MITOCHONDRIAL ACETONE POWDER EXTRACT*

Determination	Mortality	Per cent Protection
Extract alone†	20/44	36
+ ATP	8/42	75
+ NAD	12/52	69
+ Malate	21/43	35
+ NAD + malate	14/51	64
+ ATP + NAD + malate	13/50	65
Saline control	40/53	

*The incubation medium contained 1.4 ml of acetone powder extract in 0.01 M phosphate buffer (pH 6.8) and 20 μg of endotoxin. ATP, NAD, and malate were added at a concentration of 1.5 mM.
†Protein concentration = 1.55 mg/ml.

siderable stimulation of endotoxin-detoxifying activity could be effected by the addition of ATP or malate to the reaction mixture. Since it was obvious that mitochondria alone had substantial activity it was suspected that they might contain significant amounts of bound endogenous cofactors. Therefore, an acetone powder was prepared from mitochondria and this powder was extracted with phosphate buffer. The activity of this extract was enhanced by ATP and NAD, but malate was not effective in this system (TABLE 6).

The enhancing effect of the addition of ATP, NAD, and malate was strongly suggestive of fatty acid activation and oxidation. Therefore the ability of guinea pig liver to activate the fatty acids of endotoxin to form the acyl coenzyme A derivatives was tested. As shown in TABLE 7, almost half the fatty acid esters in *Salmonella typhimurium* endotoxin were activated by incubation with liver homogenate.

Since Smith *et al.*[16] had shown that the endotoxin-detoxifying activity present in homogenates of dog spleen was inhibited by tetraethylpyrophosphate (TEPP), a potent esterase inhibitor, the effect of this substance on guinea pig liver homogenate was tested. At a concentration of 0.1 mM TEPP there was no inhibition of endotoxin inactivation at either acid or alkaline pH optimum, despite complete inhibition of liver esterase activity with *p*-nitrophenylbenzoate as substrate. It was therefore concluded that esterase activity is probably not involved in the inactivation of endotoxin by guinea pig liver homogenate.

These findings fit very well into the hypothesis that inactivation of endotoxin by homogenates and mitochondria of guinea pig liver is accomplished by

TABLE 7
ACTIVATION OF FATTY ACID ESTERS BY GUINEA-PIG LIVER HOMOGENATES

Determination	μg of oleic per mg of endotoxin
Fatty acid ester*	123
Fatty acids activated†	59

*Used 2.66 mg of *Salmonella typhimurium* endotoxin; fatty acid ester was determined with oleic acid as a standard.

†Used 5 mg of endotoxin and 0.2 ml of 20% liver homogenate, centrifuged at 2,000 × g and incubated with tris (hydroxymethyl) aminomethane buffer (pH 7.4), 10 mM ATP, 5 mM $MgCl_2$, 0.5 M hydroxylamine (pH 7.4), and 3 mM CoA. The pyrophosphate formed was measured and converted to oleic acid equivalents. Incubation was for 30 min. at 37°C.

the activation and subsequent oxidation of fatty acid residues in the endotoxin molecule. The enhancing activity of ATP in this reaction is difficult to explain on any other basis. Although ATP can activate amino acids, purified endotoxins of very high toxicity can be prepared which contain virtually no amino acid or protein nitrogen. ATP, as a cofactor in the hexokinase reaction, can phosphorylate a variety of monosaccharides. But the carbohydrate moiety of endotoxin is a polysaccharide. Furthermore, incubation of endotoxin with hexokinase, ATP, and magnesium ion did not affect toxicity. In general, no enzymes hydrolyzing glycosidic linkages require ATP for their action, nor are they found in guinea pig liver mitochondria, which are rich in endotoxin-detoxifying activity. The enzyme in guinea pig liver which activates fatty acids was discovered by Kornberg and Pricer in 1953,[24] and was found by them to be present in both mitochondrial and supernatant fractions. This is very similar to our experience in being unable to localize the endotoxin-inactivating properties within a single fraction. They were also unable to purify their enzyme by means of ammonium sulfate precipitation. The actions of NAD and malate are readily explained by this hypothesis. NAD could exert its effect through its role in the oxidation of activated fatty acids. Malate, in common with other Krebs cycle acids, could spark fatty acid oxidation by the formation of ATP for activation of the fatty acids, and as well might remove the acetyl coenzyme A molecules to form citrate and continue the cycle. The lack of effect of malate in the acetone powder extracts of mitochondria can be explained by the fact that oxidative phosphorylation, which is necessary for the production of ATP, does not occur in these preparations.

An important corollary of these results is the implication that the lipid moiety of endotoxin is at least one of the factors required for the expression of endotoxin toxicity. Although we believe that the lipid component is a necessary ingredient of the complete endotoxin molecule, it should not be inferred

that the lipid fractions themselves will necessarily be found to be toxic when extracted from the integrated structure of which they are only one part.

Mitochondrial Oxidation and Susceptibility to Endotoxin

If these hepatic enzymes which are involved in the activation and oxidation of the fatty acid component of endotoxin participate in the detoxification of endotoxin *in vivo*, it should be possible to demonstrate in various types of experimental liver injury a correlation between hepatic enzyme activity *in vitro* and the susceptibility of the animals to the effect of endotoxin. In order to investigate this question, acute liver injury was produced in guinea pigs with two additional hepatotoxins, allyl alcohol and DL-ethionine, which presumably have very different mechanisms of action from that of carbon tetrachloride.[25-30] The morphological effects of these various hepatotoxins on the liver are shown in FIGURES 3-6. FIGURE 3 shows a section of normal guinea pig liver. FIGURE 4 shows a section made from a liver removed 48 hours after the administration of carbon tetrachloride. The prominent features are the severe necrosis involving the central areas and the adjacent zone of fatty meta-

FIGURE 3. Normal guinea pig liver. Hematoxylin and eosin stain × 180.

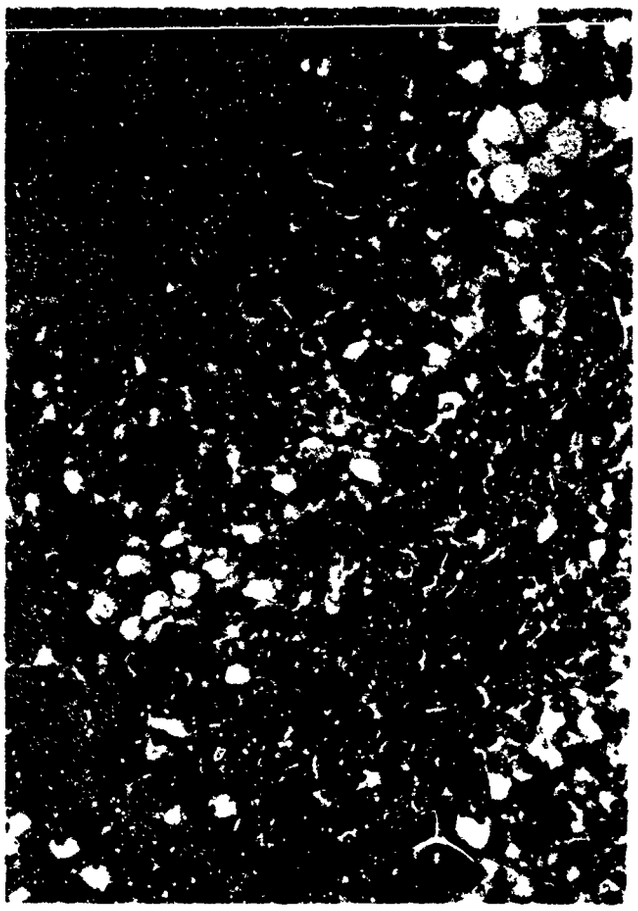

FIGURE 4. Guinea pig liver 48 hours after CCl_4, 0.15 ml subcutaneously. There is a zone of necrosis connecting two central areas at the bottom. Fatty metamorphosis is present adjacent to the necrotic areas. A portal area is at the top. Hematoxylin and eosin stain × 180.

morphosis. FIGURE 5 shows a section made 48 hours after administration of allyl alcohol. The central vein area appears uninvolved, whereas there is severe necrosis and extravasation of blood from the capillaries surrounding the portal area. FIGURE 6 shows the effect of ethionine on the liver. The characteristic feature is extensive panlobular fatty metamorphosis with little or no necrosis. In the case of each of the last two hepatotoxins, as in the studies with carbon tetrachloride, a dose was used which was just below that which would produce a significant mortality among the guinea pigs. Like carbon tetrachloride, each of the agents produced a maximal histological change 48 hours after its administration. The effect of these hepatotoxins on the susceptibility of animals to endotoxin was quite different from that of carbon tetrachloride. As shown in TABLE 8, allyl alcohol increased the susceptibility of guinea pigs to endotoxin only threefold, in contrast to the 150 to 250-fold increase in susceptibility which followed the administration of carbon tetrachloride. Ethionine exerted an effect intermediate between that of carbon

FIGURE 5. Guinea pig liver 48 hours after allyl alcohol, 0.042 ml/kg intragastrically. Large areas of necrosis and hemorrhage surround portal areas. The parenchyma about the central vein (lower left) appears unaltered. Hematoxylin and eosin stain × 75.

TABLE 8
SUSCEPTIBILITY OF GUINEA PIGS TO LETHAL EFFECT OF E. COLI ENDOTOXIN FOLLOWING ADMINISTRATION OF ALLYL ALCOHOL

Endotoxin (mg/kg)	Normal animals	Interval between allyl alcohol administration* and i.v. endotoxin challenge (hr.)				
		6	12	24	48	144
15	12/13†	6/6	6/6	6/6	5/6	4/4
5	3/13	2/6	3/6	7/10	5/10	4/4
1.67	2/14	0/6	2/6	3/10	4/10	0/4
0.56			0/6	2/10	2/10	0/4
0.19				1/10	2/12	
0.063					1/12	
LD_{50} (mg/kg)	6.9	6.7	3.6	2.3	2.2	2.9

*0.042 ml/kg by gastric tube.
†Deaths/total.

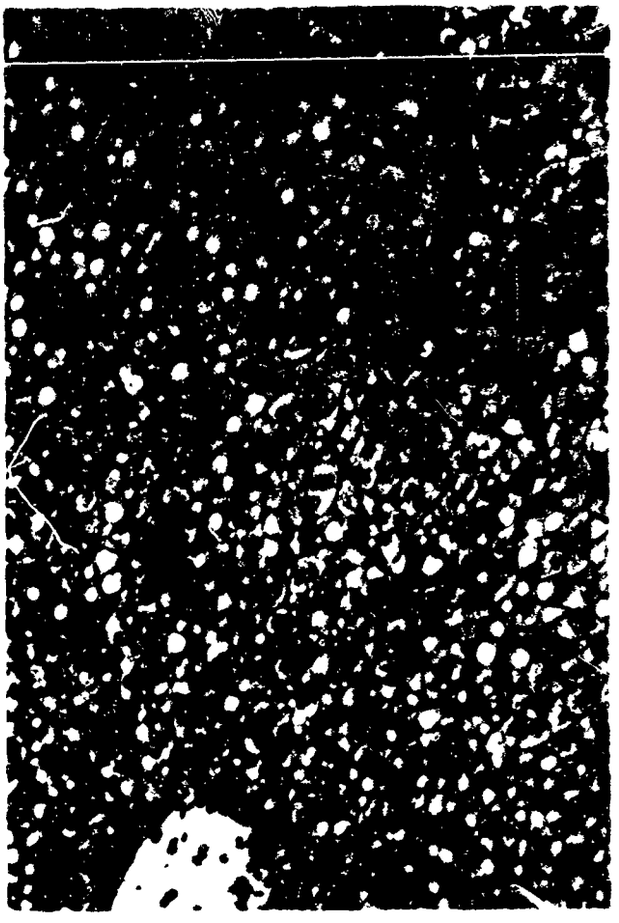

FIGURE 6. Guinea pig liver 48 hours after ethionine, 100 mg intraperitoneally. Diffuse fatty metamorphosis is present throughout the lobule. A central vein is at the bottom and portal area at the top. Hematoxylin and eosin stain × 180.

tetrachloride and allyl alcohol, increasing the sensitivity of animals approximately 12-fold, as compared with normal animals which were fasted for the same length of time as the ethionine-treated animals (TABLE 9). Neither allyl alcohol nor ethionine could be shown to have any effect on the endotoxin-inactivating ability of liver, spleen, or kidney tissue, using the chick embryo bioassay system.

In TABLE 10 are shown the effects of various hepatotoxic agents on substrate oxidation by liver mitochondria. It is evident that the most striking reduction in oxidation with octanoate as substrate was produced by carbon tetrachloride. Allyl alcohol showed very little effect on oxidation, and ethionine exhibited an effect intermediate between these two agents. These effects cannot be attributed to the anorexia which some of the animals given hepatotoxins manifested, since starvation throughout this whole period had much less effect on oxidation of octanoate, particularly during the first 30 minutes. Effects very similar to those shown for octanoate were obtained when malate

TABLE 9
SUSCEPTIBILITY OF GUINEA PIGS TO LETHAL EFFECT OF E. COLI ENDOTOXIN FOLLOWING ADMINISTRATION OF DL-ETHIONINE

Endotoxin (mg/kg)	Normal animals	Fasted animals*	Interval between ethionine administration and i. v. endotoxin challenge (hr.)				
			6	12	24	48	144
15	6/10	9/10	8/8	6/8	10/10	10/10	6/6
5	2/10	1/10	2/8	4/8	6/10	12/14	4/6
1.67	0/10	0/10	0/8	0/8	1/10	8/14	0/6
0.56					0/10	6/14	
0.19						4/14	
0.063						2/12	
LD_{50} (mg/kg)	12.7	8.7	7.2	6.4	3.8	0.74	3.8

*Animals fasted for 60 hrs. before endotoxin challenge.
†100 mg intraperitoneally.

or β-hydroxybutyrate was used as substrate. In contrast, none of the hepatotoxins studied decreased the oxidation of succinate. The increased oxidation of succinate observed after the administration of all three hepatotoxins may reflect an effect of the agents on the mitochondrial membrane, with an increase in permeability of the membrane to the substrate.

In the preparation of the homogenates and mitochondrial preparations described in these studies no attempt was made to separate hepatic parenchymal cells from reticuloendothelial cells of the liver. The possibility might be raised therefore that some of the enzymatic activities demonstrated might represent functions of reticuloendothelial cells rather than hepatic cells. Certainly the overwhelming majority of the cells from which these materials are derived are hepatic parenchymal cells, and preparations of this type are routinely used in biochemical studies of hepatic cell functions. Preliminary studies of the effect of the hepatotoxins used in these experiments on the function of the reticuloendothelial system have failed to yield evidence that these agents consistently alter the rate of clearance of colloidal carbon from the circulation.

Recent publications have described the reversible inactivation of endotoxin by plasma,[31] tissue extracts,[32] and papain.[33] This type of reversible inactivation was shown to be due to the complexing of the anionic endotoxin with cationic proteins. A similar type of reversible inactivation could be accomplished with synthetic cationic polyglucose derivatives.[32] Most of the previous studies of endotoxin inactivation have involved plasma[11 13,31] or tissue ex-

TABLE 10
EFFECTS OF HEPATOTOXIC AGENTS ON SUBSTRATE OXIDATION BY LIVER MITOCHONDRIA

Animal	Octanoate		Succinate		Malate		β-hydroxybutyrate	
	0' - 30'	30' - 60'	0' - 30'	30' - 60'	0' - 30'	30' - 60'	0' - 30'	30' - 60'
Normal (8*)	9.3 ± .7†	7.8 ± 1.0	6.2 ± .5	5.1 ± .5	2.0 ± .1	0.8 ± .1	1.7 ± .2	1.4 ± .2
CCl_4 (4)	1.6 ± .2	0.4 ± .1	8.5 ± .8	8.0 ± 1.2	0.2 ± .2	0.2 ± .1	0.6 ± .2	0.4 ± .1
Allyl alcohol (8)	7.0 ± 1.1	5.2 ± 1.0	7.8 ± 1.0	6.7 ± .8	2.1 ± .3	0.7 ± .1	1.5 ± .2	1.2 ± .1
Ethionine (8)	4.9 ± .4	1.7 ± .2	10.4 ± 1.8	7.6 ± 1.3	1.4 ± .2	1.0 ± .1	1.4 ± .2	0.9 ± .1
Starved (8)	8.7 ± 1.3	3.0 ± .6	8.1 ± .7	5.7 ± .6	2.2 ± .3	0.6 ± .1	2.1 ± .3	0.9 ± .2

Medium consists of 40 μ moles sodium phosphate buffer (pH 7.4), 300 μ moles NaCl, 12 μ moles KCl and 4 μ moles $MgSO_4 \cdot 7H_2O$. The individual substrate concentrations were 60 μ moles for succinate, 30 μ moles for β-hydroxybutyrate or malate, and 5 μ moles for octanoate. NAD was added at 3 μ moles except when succinate as substrate. When octanoate was substrate, 3 μ moles of ATP, 3 μ moles of malate, and 0.1 ml of 1% cytochrome were added. The value for octanoate oxidation was calculated by subtracting the oxidation when octanoate was absent. The final volume was 3 ml. The oxidation was followed for 1 hour at 37° C.

*Number of animals studied.

†μ atoms O/10 mg protein ± S. E. M.

tracts which did not contain mitochondria[14,15,21,32] and have shown maximal activity at an alkaline pH. In these situations, reversible interaction between endotoxin and tissue or plasma proteins may represent the principal mechanism of endotoxin inactivation.

Although we have not attempted to reactivate endotoxin detoxified by mitochondrial preparations, the participation of such a nonspecific mechanism in this system appears to be extremely unlikely for the following reasons:

1. The presence of two distinct pH optima when homogenates containing mitochondria are used is difficult to reconcile with an electrostatic mechanism of inactivation.

2. Enhancement of the activity of mitochondrial preparations by ATP, NAD and malate strongly suggests that the inactivation is enzymatic in nature.

3. The inactivating ability of various tissues is not strictly proportional to the protein content of the homogenate, which should be the case if a nonspecific mechanism were involved.

4. Homogenates of carbon tetrachloride-damaged livers, which showed little endotoxin-inactivating ability, actually had a higher protein content than homogenates prepared from normal liver.

Summary

In summary, it has been shown that cell-free homogenates of several tissues of the guinea pig are capable of inactivating endotoxin *in vitro*. High degrees of activity were found in the kidney, which is not thought to possess much reticuloendothelial tissue, as well as in the spleen and liver, which are rich in phagocytic cells. Animals which had undergone splenectomy were no more susceptible to the lethal effect of endotoxin than sham-operated controls, indicating that the spleen probably plays no more than a minor role in the response of the guinea pig to endotoxin. Damage to the hepatic parenchyma with carbon tetrachloride increased susceptibility of the animals approximately 200-fold. Maximal susceptibility was correlated with the presence of a maximal amount of necrosis in the liver, and liver tissue removed at this stage of intoxication showed almost no ability to detoxify endotoxin *in vitro*. Healing of the lesion in the liver was associated with a return toward normal resistance to endotoxin by the animal and with a reappearance of endotoxin-inactivating capacity of liver tissue homogenates. Inactivation of endotoxin by guinea pig liver homogenates and mitochondria appeared to be due to activation and oxidation of the fatty acid portion of the endotoxin molecule, a finding which implies that the lipid component of endotoxin is necessary for its toxicity. When the effects of carbon tetrachloride, allyl alcohol and ethionine were compared, a close correlation was found between the effect of the hepatotoxin on NAD-linked substrate oxidation by liver mitochondria and its effect of the susceptibility of the guinea pig to endotoxin. Allyl alcohol and ethionine, which had much less effect than carbon tetra-

chloride on both fatty acid oxidation by liver mitochondria and on susceptibility of the guinea pig to endotoxin, could not be shown to reduce the endotoxin-inactivating capacity of guinea pig tissues.

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