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Endotoxin Detoxification by Guinea Pig Tissue Homogenates and Possible Significance of This Reaction *in vivo*. (29802)

W. EDMUND FARRAR, JR. (Introduced by S. B. Formal)
Department of Applied Immunology, Walter Reed Army Institute of Research, Washington, D.C.

Homogenates of various animal tissues are capable of enzymatically detoxifying bacterial endotoxins *in vitro*. Trapani *et al*(1) found endotoxin-detoxifying activity in rabbit liver, adrenal, kidney and lymph node but not in brain, heart or spleen. Keene(2), on the other hand, detected activity in rabbit spleen and heart, as well as in liver and kidney. Smith *et al*(3) found dog spleen to be more active than liver, whereas, kidney, heart and skeletal muscle were inactive. Corwin and Farrar(4) investigated the mechanism by which endotoxin is inactivated by guinea pig liver mitochondria, and concluded that inactivation is accomplished by a process of activation and subsequent oxidation of the fatty acid portion of the endotoxin molecule.

None of these studies provides information about the possible physiological significance of enzymatic detoxification by tissues in the response of normal animals to endotoxin administration. Formal *et al*(5) found that guinea pigs with hepatic necrosis due to carbon tetrachloride (CCl_4) were abnormally susceptible to the lethal action of endotoxin. Farrar and Magnani(6) showed that the degree of susceptibility parallels the severity of the liver damage, and that the response of the animal returns toward normal as the hepatic lesion heals. Furthermore, damaged liver tissue showed almost no ability to detoxify endotoxin *in vitro*, whereas normal liver was quite active. Liver tissue removed after regeneration had taken place showed near normal activity. They suggested that CCl_4 may induce abnormal susceptibility to endotoxin by interfering with the ability of the liver to detoxify this toxic bacterial product.

Although the liver appears to be the principal site of removal of endotoxin from the circulation(7), the spleen also participates under certain circumstances. Wiznitzer *et al* (8) have shown that the dog spleen is capable of both removing and detoxifying endotoxin *in vivo*, whereas the kidney is not. While other organs may possess the necessary enzymes for inactivating endotoxin, only the liver and spleen appear to have access to sufficient amounts of injected endotoxin to function as important sites of endotoxin degradation.

This report describes the *in vitro* inactivation of endotoxin by various tissues of the guinea pig, and attempts to assess the physiological role of the spleen in the response of normal animals and those with liver damage to administration of endotoxin.

Materials and methods. Female Hartley strain guinea pigs weighing 300 to 400 g were used. CCl_4 treatment consisted of subcutaneous injection of 0.15 ml, 48 hours before endotoxin challenge. Susceptibility to endotoxin was determined as the proportion of animals dead 24 hours after intravenous challenge. Endotoxins of *Serratia marcescens* and *Escherichia coli* 026:B6 were obtained from Difco Laboratories.

Tissue homogenates were prepared by a simplification of the method used previously (6). Animals were killed by a blow on the head, and the organs to be tested were quickly removed, weighed, minced with scissors, and placed in 9 volumes of cold 0.01 M phosphate-buffered saline (pH 6.8) using aseptic precautions. Organs were ground with a TenBroeck grinder to produce a smooth suspension. This was centrifuged at 2°C for 15 min at 600 × *g*, and the sediment discarded. Endotoxin-detoxifying activity of the supernatant "homogenate" was determined by incubating either full-strength homogenate or a 1:10 dilution with 20 μg of *S. marcescens* endotoxin in a water bath at 37°C for 1 hour, diluting with buffer (pH 7.4), and assaying for residual toxicity by intravenous inoculation into 11-day-old chick embryos, exactly as described previously(6) except that the eggs were kept at 37°C instead of 39.5°C.

Carbon clearances were measured as described previously(6) except that blood samples were obtained from the orbital plexus by means of calibrated capillary pipettes(9).

Splenectomy was performed through a midline incision under ether anesthesia. Sham operation consisted of laparotomy and ligation and amputation of a small segment of omentum. Animals were challenged with *E. coli* endotoxin between 7 and 14 days after operation, and survivors were autopsied to exclude the possibility of splenic regeneration.

Results. Experiments were performed to determine the ability of various tissues of the guinea pig to detoxify *S. marcescens* endotoxin *in vitro*. As shown in Table I, kidney, liver and spleen were the most active tissues tested, and showed approximately equal activity within the limits of accuracy of the assay system used. Heart possessed an intermediate degree of activity, while skeletal muscle, blood and lung were much less active.

TABLE I. Effect of Incubation with Guinea Pig Tissue Homogenates on Toxicity of Endotoxin.*

	10% Homogenate		1% Homogenate	
Tissue tested	Mortality†	% Protection‡	Mortality†	% 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 hr at 37°C.

† Deaths/total eggs.

‡ % Protection = $\frac{\%\text{ mortality saline} - \%\text{ mortality sample}}{\%\text{ mortality saline}} \times 100.$

Subsequent studies were designed to elucidate the physiological role of the spleen in the response of guinea pigs to administration of endotoxin. It was assumed that the liver is the major site of endotoxin sequestration and degradation in normal animals. This is suggested by the finding of Braude *et al*(7), using radioactive endotoxin in the rabbit, that a major portion of injected endotoxin accumulates in the liver. The data shown in Fig. 1 indicate that removal of the spleen does not significantly alter the rate of clearance of carbon particles from the circulation in the guinea pig. Another possibility to be considered was that the spleen might represent an important resource in animals in which the liver was severely damaged (*i.e.*, by CCl_4). CCl_4 administration impairs the ability of liver tissue to inactivate endotoxin *in vitro*(6), but it has no such effect on splenic tissue (Table II).

When otherwise normal guinea pigs were subjected to splenectomy and later challenged with endotoxin, they proved to be no more susceptible to its lethal action than sham-operated controls (Table III). When animals

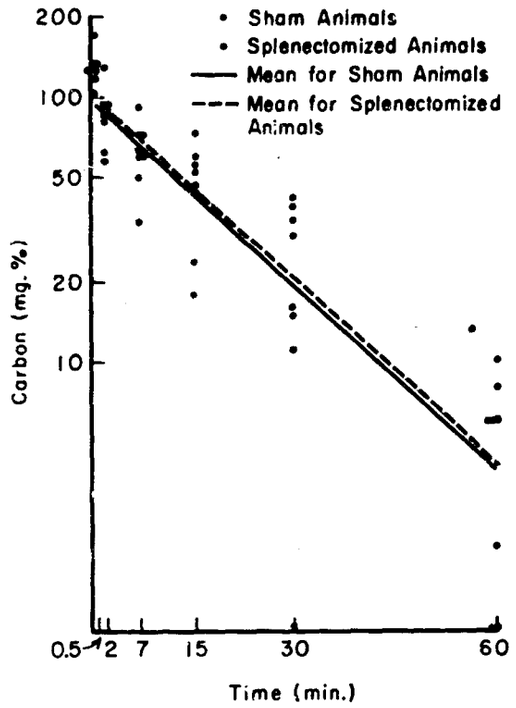

FIG. 1. Rate of disappearance of injected carbon from the circulation in sham-operated and splenectomized guinea pigs. Each animal received 8 mg carbon intravenously per 100 g body weight.

were pre-treated with CCl_4 48 hours before endotoxin challenge, a procedure previously shown to result in severe, non-fatal hepatic damage, they were rendered more than 100 times as sensitive as normal animals to endotoxin. However, absence of the spleen in these animals was not associated with any further increase in susceptibility (Table III).

TABLE II. Effect of CCl_4 Administration* on Inactivation of Endotoxin by Guinea Pig Liver and Spleen Homogenates.†

Tissue used	10% Homogenate Mortality	10% Homogenate % Protection	1% Homogenate Mortality	1% Homogenate % Protection
Normal liver	10/30	65	17/30	40
CCl_4 "	21/30	26	26/30	8
Normal spleen	12/30	58	25/30	13
CCl_4 "	10/30	65	20/30	28
Saline control	19/20	—	19/20	—

* .15 ml subcutaneously 48 hr before sacrifice of animal.

† Incubation medium contained 20 μg of endotoxin and either 180 mg (10% homogenate) or 18 mg (1% homogenate) of tissue homogenate. Incubation was for 1 hr at 37°C.

Discussion. When bacterial endotoxin is injected into experimental animals, it is rapidly removed from the circulation, principally by the liver and spleen(7,10,11). Its ultimate fate is unknown, but the data reported here indicate that several tissues of the guinea pig, notably the liver, spleen and kidney, are capable of detoxifying endotoxin *in vitro*. Corwin and Farrar(4) have previously shown that the enzymatic process by which guinea pig liver tissue inactivates endotoxin appears to involve activation and subsequent oxidation of the fatty acid portion of the molecule.

TABLE III. Effect of Splenectomy on Mortality Following Intravenous Endotoxin (*E. coli* 026:B6) Injection in Normal and CCl_4-treated* Guinea Pigs.

Endotoxin (mg/kg)	Mortality: Normal, Splenectomized	Mortality: Normal, Sham	Mortality: CCl_4-treated, Splenectomized	Mortality: CCl_4-treated, 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	5/5	5/5
.063	—	—	5/5	5/5
.021	—	—	2/5	2/5
.007	—	—	1/5	1/5

* .15 ml subcutaneously 48 hr before endotoxin administration.

† Deaths/total.

The question of the physiological significance of the tissue enzymes which are capable of detoxifying endotoxin *in vitro* constitutes a difficult problem. In experiments previously reported(6) it was found that administration of CCl_4, which produces severe but non-fatal liver injury, rendered guinea pigs extremely susceptible to the lethal action of endotoxin and also markedly inhibited the ability of liver tissue to detoxify endotoxin *in vitro*. One possible explanation of these findings is that CCl_4 induces abnormal susceptibility to endotoxin by disrupting a specific endotoxin-inactivating function of the liver.

Since the spleen is capable of removing endotoxin from the blood(8,11) and contains enzymes capable of detoxifying it, a possible role for this organ in the response of animals to endotoxin injection was sought. Indirect evidence that the spleen can play no more

than a minor role was provided by the finding that CCl_4 administration does not depress the endotoxin-inactivating ability of the spleen. Thus, the presence of a normal spleen does not constitute adequate protection against even small doses of endotoxin if liver function is severely impaired.

Removal of the spleen did not detectably enhance the susceptibility of normal animals to bacterial endotoxin. More significantly, even when the liver was severely damaged by CCl_4, so that the animals were more than 100 times as sensitive as normal animals to endotoxin, splenectomy did not result in a further increase in susceptibility.

The experiments reported herein fail to provide evidence that the spleen participates in any important way in the response of either normal guinea pigs or those with severe liver damage to the injection of bacterial endotoxin.

Summary. Homogenates of guinea pig liver, spleen, kidney and (to a lesser degree) heart are capable of detoxifying bacterial endotoxin *in vitro*. Since only the liver and spleen appear to remove significant amounts of endotoxin from the circulation, these two organs would seem to be the ones in which the inactivation process might be of some physiological significance *in vivo*. Previous studies suggested that the liver may have an important function in the inactivation of endotoxin which gains access to the body. The experiments reported herein are consistent with this hypothesis, but provide no evidence that the spleen plays any significant role in the response of either normal guinea pigs or those with liver damage to administration of endotoxin.

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