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

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Endotoxin Detoxification by Guinea Pig Tissue Homogenates and Possible Significance of This Reaction in vivo. (25802)

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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 (CCL4) 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 CCL4 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. Witznitzer 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. CCl₄ 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. Endotoxin *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 activity 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.

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 CCl₄). CCl₄ administration impairs the ability of liver tissue to inactivate endotoxin in vitro (6), but it has no such effect on splenic tissue (Table I).

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

**Table I. Effect of Incubation with Guinea Pig Tissue Homogenate on Toxicity of Endotoxin.**

<table>
<thead>
<tr>
<th>Tissue tested</th>
<th>10% Homogenate Mortality</th>
<th>% Protection</th>
<th>1% Homogenate Mortality</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>1/20 94</td>
<td>8/20 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>2/20 88</td>
<td>9/20 44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>2/20 88</td>
<td>9/20 44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>8/20 80</td>
<td>11/20 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal</td>
<td>11/20 31</td>
<td>11/20 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>11/20 31</td>
<td>12/20 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>11/20 31</td>
<td>15/20 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline control</td>
<td>24/30 100</td>
<td>24/30 100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 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 =  

\[
\frac{\text{% mortality saline} - \text{% mortality sample}}{\text{% mortality saline}} \times 100. 
\]
ENDOTOXIN DETOXIFICATION BY TISSUES

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 CCI4-treated Guinea Pigs

<table>
<thead>
<tr>
<th>Endotoxin (mg/kg)</th>
<th>Normal CCI4-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Splenectomized Sham</td>
</tr>
<tr>
<td>15</td>
<td>5/5+</td>
</tr>
<tr>
<td>5</td>
<td>0/5</td>
</tr>
<tr>
<td>1.67</td>
<td>2/5</td>
</tr>
<tr>
<td>.56</td>
<td>0/6</td>
</tr>
<tr>
<td>.19</td>
<td>0/6</td>
</tr>
<tr>
<td>.063</td>
<td>—</td>
</tr>
<tr>
<td>.021</td>
<td>—</td>
</tr>
<tr>
<td>.007</td>
<td>—</td>
</tr>
</tbody>
</table>

* .15 ml subcutaneously 48 hr before endotoxin administration.
† Death/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 CCI4, 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 CCI4 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 CCl₄ 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 CCl₄, 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.

I am indebted to S. B. Formal for many valuable suggestions, and to J. G. Watson, S. Austin and A. L. Fisher, Jr., for excellent technical assistance.


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