A MECHANISM OF THE GLYCOGENOLYTIC ACTION OF BACTERIAL ENDOTOXIN*

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Determination of the site of action of bacterial endotoxin has been the object of extensive investigation (1-6).

Hyperglycemia and depletion of liver glycogen are the most constant biochemical changes which have been observed to follow the injection of bacterial endotoxin (1-3). These changes have been assumed to represent a direct hepatotoxic action of endotoxin, implying that bacterial endotoxin is capable of poisoning a specific enzyme system or systems that mediate hepatic glycogenesis. If these assumptions are correct, they constitute an extremely important clue regarding the mechanism of action of endotoxin on an enzymatic level.

However, hyperglycemia and depletion of hepatic glycogen are also well defined consequences of epinephrine release. It is conceivable that the disturbances in carbohydrate metabolism in the liver may not be the result of a direct toxic action on hepatic enzyme systems, but rather the passive consequence of epinephrine release. The hypothesis that an adrenergic mechanism may be involved in the action of endotoxin is supported by a considerable body of indirect evidence, cited by Thomas (7). On the basis of such evidence, he has suggested "it is possible that some, if not all of the effects are closely related to the adrenergic action..."; however, "the mechanism by which endotoxins produced the observed metabolic abnormalities requires much further study" (7).

The following studies were designed to test the hypothesis that the changes in hepatic glycogen content following the administration of bacterial endotoxins are mediated through epinephrine release rather than by a direct toxic action. It is concluded that sublethal quantities of bacterial endotoxin influence hepatic glycogen stores as a consequence of epinephrine release.

Methods and Materials

Animals.—Male rats of the Sprague-Dawley strain, weighing 105 to 115 gm., obtained from Holtzman Farms, Houston, were maintained on a standard antibiotic-free laboratory diet and

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acclimatized at least 1 week at 25°C. before any experimental procedure. The mice used for determination of endotoxin toxicity were females of the Swiss-Webster strain, weighing 18 to 20 gm., obtained from Rockland Farms New City, New York. They were maintained under the same laboratory conditions.

Adrenalectomy.—Bilateral adrenalectomy was performed through a dorsal incision under anesthesia consisting of pentobarbital and ether. Following adrenalectomy, animals were maintained on one of the following regimens: Desoxycorticosterone acetate, 0.5 mg subcutaneously daily (Schering Corporation, Bloomfield, New Jersey lot No. 6T-A5-P58-769, DOCA® in sesame oil, 5 mg. per ml.), or cortisol acetate, 0.1 mg. subcutaneously daily (Upjohn Company, Kalamazoo, Michigan, lot No. KM 010 FE). The time interval between adrenalectomy and study was 5 to 7 days. During this interval the adrenalectomized animals were placed on 0.9 per cent saline solution as drinking water.

Dietary Regimens.—Animals were maintained on a standard antibiotic-free laboratory diet for the first 3 days postadrenalectomy. Both the adrenalectomized and the normal control animals which were to be studied in the well nourished state then were fed by gastric tube twice daily for an additional 3 days to assure comparable food intake in both groups. The animals were either tube-fed a balanced high glucose diet or allowed the standard laboratory diet ad libitum, then tube-fed a supplemental 3 ml. of saturated glucose solution. Both dietary regimens resulted in comparable concentrations of hepatic glycogen.

Animals that were to be studied under conditions of fasting had food withdrawn 96 hours prior to study. During this interval the fasted animals were allowed water ad libitum.

Endotoxin.—Endotoxins from a variety of Gram-negative bacteria were utilized. The characteristics of these endotoxin preparations are tabulated (Table I). The Boivin-type endotoxins were prepared according to the technique of Noyes et al. (8). The quantities of endotoxin administered were sublethal for normal rats and adrenalectomized rats maintained on the large doses of DOCA®. In most studies, the rats received an estimated 2.5 mouse LD₅₀ doses of endotoxin per 100 gm. of body weight.

Glycogen Assay.—On the morning of study, animals were tube-fed. Two hours later adrenalectomized and control animals received either endotoxin or saline by intraperitoneal injection. The animals were sacrificed 60 minutes later by decapitation, the livers were quickly removed and glycogen determinations carried out according to the method of Good, Kramer, and Somogyi (9). Glycogen values are reported as milligrams of glycogen per gram of wet liver.

### Table I

**Characteristics of Bacterial Endotoxin**

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Source</th>
<th>Method of preparation</th>
<th>Toxicity (Mouse LD₅₀, mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aerobacter aerogenes</em></td>
<td>Sanford</td>
<td>Boivin*</td>
<td>1.84</td>
</tr>
<tr>
<td><em>Shigella flexneri 2A</em></td>
<td>Noyes</td>
<td>Boivin*</td>
<td>0.24</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>Difco® No. B25624</td>
<td>Boivin</td>
<td>0.50</td>
</tr>
<tr>
<td><em>Escherichia coli 0-111</em></td>
<td>Sanford</td>
<td>Acetone-killed whole organisms</td>
<td>5.0</td>
</tr>
</tbody>
</table>

* Prepared according to technique published in reference 8.
† Difco Laboratories, Detroit.
Statistical Analysis.—Most experiments were replicated on several occasions with different lots of rats. The replicate experiments were combined and the mean values and standard errors of the means calculated for each procedure. Differences between the means were compared by the t test.

EXPERIMENTAL RESULTS

The effect of bacterial endotoxin on the hepatic glycogen content of well nourished normal and adrenalectomized rats is presented in Table II. The administration of sublethal quantities of Boivin-type endotoxin uniformly decreased the hepatic glycogen content of normal well nourished rats. To ascertain whether the response was confined to endotoxin derived from a single bacterial species, or whether instead endotoxins from different species could elicit the same response, Boivin-type endotoxins were prepared from these bacterial species. In all instances a similar reduction in hepatic glycogen (30 to 38 per cent of control values) was observed. That the method of preparation of endotoxin was not responsible for this effect could be inferred from the observation that killed whole organisms (Escherichia coli O-111) caused a comparable depletion of liver glycogen (48 per cent of control values).

A second group of experiments was performed on adrenalectomized rats (maintained on either cortisone or desoxycorticosterone acetate) in order to

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Bacterial species</th>
<th>Hepatic glycogen content</th>
<th>Normal</th>
<th>Adrenalectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saline</td>
<td>S.E.M. mg./gm.</td>
</tr>
<tr>
<td>1</td>
<td><em>Aerobacter aerogenes</em></td>
<td></td>
<td>Mean ± S.E.M</td>
<td>4.5 ± 1.1</td>
</tr>
<tr>
<td>2</td>
<td><em>Shigella flexneri 2A</em></td>
<td></td>
<td>6.5 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Salmonella typhimurium</em></td>
<td></td>
<td>7 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>E. coli O-111</em></td>
<td></td>
<td>4 ± 1.0</td>
<td></td>
</tr>
</tbody>
</table>

TABLE II

Effect of Various Bacterial Endotoxins on the Hepatic Glycogen Content of Normal and Adrenalectomized, Fed Rats*

*Adrenalectomized rats were maintained on either desoxycorticosterone acetate or cortisone. The quantity of endotoxin administered was a sublethal quantity, approximating 2.5 mouse LD₅₀ per 100 gm. of body weight. Hepatic glycogen was determined 60 minutes after endotoxin administration.

† n, abbreviation for number of animals in a given experimental group.
obviate the effects of release of epinephrine from the adrenal medulla. The liver glycogen content of the cortisone-maintained adrenalectomized rats was the same as those treated with DOCA®; and both of these groups had values for liver glycogen content quite comparable to those values observed in normal rats (normal, 21.4 mg. per gm. wet weight of liver; cortisone-maintained adrenalectomized, 20.6 mg. per gm.; DOCA®-maintained adrenalectomized, 22.0 mg. per gm.). In contrast to the decreases in hepatic glycogen observed in normal rats, no significant decrease in the mean hepatic glycogen content occurred following the administration of either Boivin-type endotoxin or killed whole organisms to adrenalectomized rats. Thus, adrenalectomy followed by

TABLE III

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Bacterial species</th>
<th>Mean body weight</th>
<th>Hepatic glycogen content</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Original n</td>
<td>Decrease</td>
<td>Saline Mean ± s.e.m.</td>
</tr>
<tr>
<td>1</td>
<td>Aerobacter aerogenes</td>
<td>103</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Aerobacter aerogenes</td>
<td>110</td>
<td>31</td>
<td>7</td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td>17</td>
<td>1.0 ± 0.2</td>
<td>18</td>
</tr>
</tbody>
</table>

* Food was withdrawn 96 hours prior to experiment. Animals were allowed water ad libitum. Approximately 2.5 mouse LD$_{50}$ per 100 gm. body weight of a Boivin-type endotoxin was administered and hepatic glycogen content determined after 60 minutes.

adequate corticosteroid replacement abolished the hepatic glycogenolytic action of sublethal quantities of representative bacterial endotoxins.

The effect of bacterial endotoxin on the hepatic glycogen content of normal rats fasted for 96 hours is presented in Table III. This period of fasting resulted in a 25 to 30 per cent decrease in body weight. Fasting effected a reduction of hepatic glycogen content from a mean of 21.4 mg. per gm. of wet liver to 1.0 mg. per gm. of wet liver. In contrast to the decreases in hepatic glycogen content effected by the administration of bacterial endotoxin in well nourished rats, the injection of the same quantity and lot of endotoxin into fasted rats resulted in a highly significant increase in mean hepatic glycogen content from 1.0 mg. per gm. of wet liver to 3.0 mg. per gm. of wet liver ($P < 0.001$). Thus, liver glycogen content may either rise or fall after endotoxin administration depending upon the antecedent diet.

These data exclude an hepatotoxic action of bacterial endotoxin either
through direct interference with glycogen synthesis or through direct acceleration of glycogen degradation. Our experiments differ in two fundamental aspects from those interpreted by Kun as demonstrating a direct action of endotoxin on glycogen synthesis. First, he did not examine the effects of antecedent diet. His animals received glucose 2 and 3 hours before the administration of endotoxin. Our observations of well nourished animals are in accord with those of Kun and other investigators. The increase in hepatic glycogen content demonstrated in fasted rats following the administration of endotoxin necessitates an alternative explanation. Second, he administered extremely large quantities of endotoxin. To reconcile this difference in experimental design, the effect of lethal doses of bacterial endotoxin was studied in rats receiving 20 mouse LD₅₀ doses of endotoxin per 100 gm. of body weight rather than 2.5 mouse LD₅₀ doses per 100 gm. as in the earlier studies (Table IV). Under these circumstances, adrenalectomy failed to abolish the fall in hepatic glycogen content.

**DISCUSSION**

These experiments confirm the observations that depletion in liver glycogen content is a constant consequence of endotoxin administration to normal well nourished rats. However, reduction in liver glycogen content is not an invariable consequence of endotoxin administration. Indeed, the administration of bacterial endotoxin to normal rats subjected to fasting results in a highly

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**TABLE IV**

*Effect of Lethal Doses of Bacterial Endotoxins on Hepatic Glycogen of Normal and Adrenalectomized, Fed Rats*

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Bacterial species</th>
<th>Hepatic glycogen content</th>
<th>Normal</th>
<th>Adrenalectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saline</td>
<td>Endotoxin</td>
</tr>
<tr>
<td>1</td>
<td><em>E. coli</em> O-26</td>
<td>7 34.6 ± 3.9</td>
<td>4 13.4 ± 3.2</td>
<td>7 34.3 ± 2.5</td>
</tr>
<tr>
<td>2</td>
<td><em>Shigella flexneri</em> 2A</td>
<td>4 25.6 ± 3.4</td>
<td>6 4.2 ± 0.4</td>
<td>4 22.7 ± 2.4</td>
</tr>
<tr>
<td>3</td>
<td><em>Shigella flexneri</em> 2A</td>
<td>1 42.6</td>
<td>2 24.5</td>
<td>1 43.7</td>
</tr>
<tr>
<td>4</td>
<td><em>E. coli</em> O-111</td>
<td>2 32.5</td>
<td>2 11.6</td>
<td>2 38.9</td>
</tr>
</tbody>
</table>

* The quantity of endotoxin administered approximated 20 mouse LD₅₀ per 100 gm. body weight.
significant increase in liver glycogen content. In view of the fact that liver glycogen may either rise or fall after endotoxin administration, depending upon the antecedent diet, to assume poisoning of a specific enzyme system or systems that mediate glycogen synthesis is not reasonable. An alternative mechanism is required.

A similar divergence in changes in liver glycogen was demonstrated by the Coris to follow the administration of epinephrine to well nourished rats as compared to rats subjected to fasting (10-12). Epinephrine produces hyperglycemia and depletion of liver glycogen when administered to well nourished animals. In contrast, the sequence of events that follow epinephrine administration to a fasted rat is: (a) a fall in muscle glycogen,(b) a rise in blood lactate, and (c) a rise in liver glycogen. Though temporally different, the similarity in changes in liver glycogen content observed following the administration of either epinephrine or endotoxin under different nutritional circumstances suggested that the action of endotoxin on liver glycogen was mediated through the release of epinephrine.

The importance of this mechanism was confirmed by the demonstration that total adrenalectomy followed by corticosteroid replacement abolished the depletion in liver glycogen following the administration of sublethal quantities of bacterial endotoxin to well nourished rats.

While adrenalectomy did not abolish the glycogenolytic effect associated with the administration of extremely large doses of endotoxin such as used by Kun (1, 2), this glycogenolytic effect may have been a secondary manifestation of prolonged anoxia secondary to the hypotension which accompanies the administration of such large doses of endotoxin, the result of stimulation of extra-adrenal medullary sources of catechol amines or of a direct hepatotoxic effect.

The data presented indicate that the derangement of carbohydrate metabolism following sublethal quantities of endotoxin cannot represent a direct interference with glycogen synthesis or direct acceleration of glycogen degradation since the content of liver glycogen may either rise or fall after endotoxin administration, depending upon the antecedent diet. The fact that total adrenalectomy followed by corticosteroid replacement abolished the depletion in liver glycogen following the administration of sublethal quantities of bacterial endotoxin to well nourished rats is interpreted as indicating that the derangement of carbohydrate metabolism following sublethal quantities of endotoxin represents the passive consequence of epinephrine release.

There is a considerable body of evidence, much of which is cited by Thomas, which suggests that bacterial endotoxin exerts a sympathomimetic effect through the release of epinephrine (7, 13-17). Reilly and coworkers first proposed that the sympathetic nervous system was primarily involved in the action of endotoxin (14). Indeed, the physiological and pathological resemblances
between endotoxin and epinephrine shock are striking. The histological evidence that depletion of adrenal chromaffin substance occurs after endotoxin was directly confirmed by Egdahl, who demonstrated a significant release of catechol amines into adrenal venous blood following administration of sublethal quantities of bacterial endotoxin of the Boivin type to dogs (16, 17). Pretreatment of animals with adrenergic and ganglionic blocking agents affords protection against endotoxic shock (18–20). On the other hand, the injection of epinephrine or norepinephrine into the skin of rabbits within 4 hours after an intravenous injection of endotoxin resulted in extensive dermal hemorrhagic necrosis (21). The changes seen within the gastrointestinal tract of animals following lethal doses of epinephrine and norepinephrine are similar to those seen in the intestinal mucosa following shock secondary to bacterial endotoxin (22, 23). An additional similarity in the actions of endotoxin and epinephrine is the interference with the migration of leucocytes noted by DeLaunay and associates and by Miles and Niven (24, 25).

Total sympathectomy does not protect animals from the lethal effects of endotoxin (20). Also, the development of tolerance to levarterenol is not associated with the acquisition of cross-resistance to bacterial endotoxin (26). These observations suggest that while increased amounts of either epinephrine or norepinephrine may be deleterious in the course of endotoxic shock, neither epinephrine nor norepinephrine are of themselves the common denominator in the lethal action of bacterial endotoxin.

CONCLUSIONS

These experiments have demonstrated that liver glycogen may rise or fall after endotoxin administration, depending upon the antecedent diet and that total adrenalectomy followed by corticosteroid replacement abolishes the glycogenolytic effect of sublethal doses of endotoxin.

It is concluded that the derangements of carbohydrate metabolism observed following the administration of sublethal quantities of bacterial endotoxin represent, not a direct hepatotoxic effect of endotoxin, but rather the passive consequence of epinephrine release.

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