The Influence of Acute Stress on the Response of Rabbits to Intravenous Endotoxin

By James M. Anderson, M.D., and Joel G. Brunson, M.D.

Immunated rabbits were subjected to an acute stress period in a modified Noble-Collip drum, alone and in conjunction with an intravenous injection of E. coli endotoxin. The lethal effects and incidence and severity of the morphologic changes were appreciably greater in the animals given endotoxin in association with stress than in those subjected to rotation alone.

The similarity of these lesions to those produced by two properly spaced injections of endotoxin is discussed. The results of the study support the hypothesis that adrenal gland hormones participate in the effects produced by endotoxin.

Various experimental procedures associated with the administration of gram-negative endotoxin have been used to produce cardiovascular fibrinoid lesions in rabbits. Although these studies have provided considerable information concerning the alterations produced by endotoxin, its fundamental mechanisms of action are still unknown.

Certain of its actions, however, resemble those of a profound sympathomimetic reaction. Thomas, and Zweifach, Nagler, and Thomas suggested that endotoxin altered the reactivity of vessels to epinephrine, and these workers produced local necrosis by an intradermal injection of epinephrine or levarterenol in rabbits previously given endotoxin. The production of vascular fibrinoid lesions by an intravenous administration of epinephrine or levarterenol in dogs is also of interest.

These observations strongly suggest the participation of adrenal gland secretions in the changes produced by endotoxin, and led us to investigate the influence of acute stress on the response of rabbits to an intravenous injection of endotoxin. The present paper reports the results of these studies.

Methods

One hundred and thirty-eight hybrid albino rabbits of both sexes weighing approximately 1 Kg. were used in the experiments. They were fed Purina rabbit pellets and had free access to water.

In initial studies to determine the lethal effects of stress, rabbits of approximately 1.5 to 2 Kg. were used. It was found that these animals tolerated the procedure very poorly, and in all subsequent studies smaller rabbits were used. These appeared to withstand the procedure well.

The device used to produce the stress episode consisted essentially of a Noble-Collip type of drum. E. coli endotoxin (lipopolysaccharide 0:111 lot B35527), used in certain of the experiments, was generously supplied by Difeo Laboratories, Detroit, Michigan. This was injected into the marginal ear vein in a concentration of 50 μg. ml., in sterile, isotonic saline solution. Details concerning the numbers of animals used in the experiments are tabulated in the text.

The animals died or were killed within 24 hours after either the injection of endotoxin or stress. Routine postmortem examinations were performed and the tissues were fixed in 10 per cent neutral formalin. Sections from the heart, lungs, liver, kidney, spleen, adrenal glands, small intestine, and skeletal muscle were regularly examined. Hematoxylin and eosin were used routinely. Certain selected sections were stained by the periodic acid-Schiff method and by Mallory's phosphotungstic acid hematoxylin.

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RESULTS

Lethal Effects

Death rarely occurred in animals rotated in the drum for less than 20 min. (table 1). With the standard stress period of 20 min., approximately 10 to 20 per cent of the animals died within 8 hours. Increasing the duration of drum rotation markedly increased the lethality of the procedure and at 25 min. 3 of 4 animals died shortly after rotation. The administration of endotoxin in conjunction with stress resulted in an increased death rate whether the toxin was given 4 or 24 hours prior to stress, or immediately following stress. In the latter group, as shown in table 1, 8 of 11 animals died within 12 hours.

Morphologic Changes

The structural changes following the administration of endotoxin in conjunction with rotational stress are summarized in tables 2 and 3. These changes were similar in each of the groups, but their incidence varied considerably.

Gross changes observed at autopsy included subendocardial, myocardial and valvular hemorrhages. Congestion, hemorrhage, and occasional infarcts were observed in the lungs and spleens. Animals which died within 4 hours after stress were found to have enlarged, friable livers which bled freely on sectioning, while in those which died later, the liver appeared hyperemic, and showed varying degrees of necrosis. This was manifested as opaque, pale yellow areas, most often subcapular, and without predilection for any particular lobe. Congestion, mucosal hemorrhages and full hemorrhage into the lumen of the small intestine were frequently observed. The kidneys were congested and mottled in many of the animals, and in 3 instances bilateral cortical necrosis was present.

Heart. Microscopic changes were noted frequently in all groups. These consisted of myocardial necrosis, cellular reaction, muscle calcification and intravalvular hemorrhages, edema and increased cellularity. The cellular reaction about the areas of muscle necrosis consisted primarily of mononuclears with varying numbers of heterophiles. Occasional aggregates of Anitschkow cells were also observed. The valves displayed marked edema and hemorrhage, and an apparent increase in cellularity (fig. 1). These consisted of Anitschkow cells and other cells with large, deeply basophilic nuclei and indistinct cytoplasmic borders.

Hyaline fibrinoid material was noted also within coronary arterial walls, beneath the endocardium, and within the substance of the cardiac valves (figs. 2 and 3). This material

TABLE 1.—Lethal Effects of Stress* Alone and in Conjunction with an Intravenous Injection of Endotoxin†

<table>
<thead>
<tr>
<th>1st procedure</th>
<th>2nd procedure</th>
<th>Interval</th>
<th>No. of animals</th>
<th>No. dying in 12 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>—</td>
<td>11</td>
<td>4</td>
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<tr>
<td>Stress</td>
<td>Endotoxin</td>
<td>Immed.</td>
<td>11</td>
<td>8</td>
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<td></td>
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<td>10</td>
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<td></td>
<td></td>
<td>12 hrs.</td>
<td>9</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>18 hrs.</td>
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<tr>
<td></td>
<td></td>
<td>24 hrs.</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>Stress</td>
<td>Immed.</td>
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<td></td>
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<td>4 hrs.</td>
<td>12</td>
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<td>8 hrs.</td>
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*20 min. period of drum rotation.
†50 μg. E. coli lipopolysaccharide I.V.
was morphologically and tinctorially similar to that described previously.² The incidence of fibrinoid in the control groups (18 per cent) and in those groups given endotoxin prior to stress (16 per cent) was low, as shown in tables 2 and 3. When endotoxin was given after stress, the incidence of fibrinoid lesions was approximately double (38 per cent) that of the other groups. The time interval between endotoxin administration and stress, however, did not seem to be a significant factor in production of the cardiac fibrinoid lesions in those groups of animals.

Lungs. Pulmonary congestion, hemorrhage, and edema, associated with the presence of hyaline thrombi or emboli in the pulmonary arterial branches, were significantly more frequent (50 per cent) in those animals in which endotoxin administration followed stress. In the control group, hemorrhage was the only lesion noted (27 per cent).

Liver. Animals which died within 1 to 2 hours after stress alone, or stress in conjunction with endotoxin, showed engorgement of the larger hepatic vessels, perivascular hemorrhages, and vacuolization of liver cells. In those animals which survived for a longer period of time, marked congestion, hemorrhage, and areas of focal to diffuse necrosis were observed (fig. 4). In the areas of necrosis, calcification of liver cells was noted occasionally (fig. 5). These lesions were observed more frequently in the animals in which endotoxin was given in conjunction with stress (40 to 45 per cent) but were noted in one instance in the group of control animals (9 per cent).

Small Intestine. Striking intestinal lesions were observed both in the control animals and in those given endotoxin, if death occurred shortly after stress. These consisted of marked acute congestion, often accompanied by intraluminal hemorrhage (fig. 6). Animals which survived for a longer period showed extensive thrombosis of intestinal vessels associated with areas of ischemic necrosis in addition to the lesions mentioned above (fig. 7). In 3 instances, congestion and hemorrhage were noted also in the large intestine.

Spleen. The splenic lesion consisted of the presence of material with the morphologic and tinctorial features of fibrinoid, which lay in the splenic sinusoids. This was associated with areas of infarction and hemorrhage. These changes were observed more frequently in those animals in which stress preceded the
administration of endotoxin (83 per cent). Conversely, splenic lesions were noted much less frequently when these procedures were reversed (29 per cent) and in control animals (9 per cent).

Other Organs. Hemorrhage within the thymus gland was observed almost invariably. Congestion was the only consistent alteration noted in the kidney, although deposits of fibrinoid material within the lumina of the glomerular capillaries were noted in 5 instances. Areas of adrenal hemorrhage and necrosis were present in one animal; otherwise the adrenal glands showed no microscopic changes. No alterations were observed in the sections of skeletal muscle.

DISCUSSION

In the present study it has been shown that diffuse vascular lesions may occur in rabbits subjected to acute rotational stress. Cardiac lesions were observed in 73 per cent of the animals, of which approximately 20 per cent were associated with the presence of hyaline fibrinoid material. Lesions of the liver, spleen, and small intestine were noted in approximately 10 per cent, and pulmonary lesions occurred in approximately 30 per cent of the animals. The incidence and severity of these lesions are strikingly similar to those which occur in immature rabbits given a single intravenous injection of endotoxin.1

When the animals were subjected to acute stress in conjunction with an intravenous injection of endotoxin, more diffuse, extensive lesions developed. The incidence of the lesions varied, however, depending on the sequence of procedures and the interval between administration of endotoxin and rotation of the animals in the drum. Only 16 per cent of the animals given endotoxin prior to stress developed fibrinoid-containing cardiac lesions, but this material was observed in 38 per cent of the cardiac lesions in animals in which endotoxin was given after stress. Likewise, a higher percentage of the animals given endotoxin after stress developed pulmonary and splenic lesions than did those of the groups in which toxin was administered before stress.

The distribution and morphology of the le-
ACUTE STRESS BY ENDOTOXIN

Fig. 4 Left. Section of liver from rabbit given endotoxin 8 hours before rotational stress. Extensive ischemic necrosis with surrounding inflammatory reaction. Animal was killed 24 hours after rotation. Hematoxylin and eosin. X 85.

Fig. 5 Right. Subcapsular area of hepatic necrosis from another animal given endotoxin 12 hours before stress. Necrotic liver cells heavily calcified. Rabbit was killed 24 hours after stress. Hematoxylin and eosin. X 110.

Lesions in these animals are similar to those produced by 2 intravenous injections of endotoxin, a fact which suggests that an acute stress reaction may act in a manner similar to an intravenous injection of endotoxin.

Although the method by which endotoxin produces necrotizing vascular lesions is unknown, several lines of evidence support the concept that its actions are mediated, at least in part, through hormones from the adrenal gland. The observation that an acute stress reaction, accompanied by an increased output of adrenal hormones, is associated with an increased incidence of systemic lesions in animals given endotoxin, supports this hypothesis. Studies now in progress indicate that there may also be an increased susceptibility to a local injection of endotoxin.

Preliminary results indicate that an intradermal injection of endotoxin in association with acute rotational stress results in an extensive area of dermal necrosis which makes its appearance within 4 hours and progresses to eschar formation and sloughing in 48 to 72 hours. Comparable results have been obtained by an intradermal injection of epinephrine. These lesions are similar to those described by Thomas in rabbits given an intradermal injection of a mixture of epinephrine and endotoxin.

It is possible that the excessive quantities of circulating epinephrine, induced by the acute stress procedure, could explain the necrosis produced by an intradermal injection of endotoxin. It is also possible, as suggested by Fine et al., that endotoxin, or similar lipopolysaccharides derived from tissues, are absorbed from the intestinal tract as a consequence of the shock state induced by rotation. The presence of such materials in the circulation might then explain the necrosis produced by an intradermal injection of epinephrine.

It thus appears, from these and other observations, that fundamentally similar mechanisms may operate in the production of death.
and morphologic changes in animals given 2 intravenous injections of endotoxin or a single injection of endotoxin in association with acute rotational stress. The possibility that adrenal hormones play an important role in the changes produced by endotoxin is supported by these observations.

**Summary**

Groups of hybrid albino rabbits of both sexes, weighing approximately 1 Kg., were subjected to a standard 20 min. period of drum rotational shock, alone and in conjunction with an intravenous injection of *E. coli* endotoxin given simultaneously with or at varied intervals prior to or after rotation. The lethal effects, incidence and severity of the structural changes were appreciably greater in the groups given endotoxin than in those subjected to rotation alone.

The salient morphologic changes in those rabbits given endotoxin in conjunction with rotational stress consisted of areas of myocardial hemorrhage, necrosis, and calcification, cardiac valve hemorrhages, focal to diffuse hepatic necrosis, pulmonary thrombi or emboli, and congestion, hemorrhage, and necrosis of the small bowel. In association with these lesions hyaline fibrinoid material was present in the walls and lumina of the vessels and the splenic sinusoids.

The similarity of these lesions to those occurring in the generalized Shwartzman phenomenon is discussed. These observations, and preliminary data obtained by intradermal injections of endotoxin or epinephrine in conjunction with acute rotational stress, support the hypothesis that adrenal gland hormones participate in the effects produced by endotoxin.

**Summario in Interlingua**

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ACUTE STRESS BY ENDOTOXIN

43

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seva administrate simultaneamente con le ro-
tation o a varie intervallos ante o post illo. Le
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seva subjicite solmente al rotation.

Le plus notable alterationes morphologic in
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tion con le stress del rotation esseva areas de
hemorrhagia myocardial, de necrosis, e de cal-
cification, hemorrhagia de valvula cardiac,
foecal o diffuse necrosis hepatic, thrombos o
embolos pulmonar, e congestion, hemorrhagia,
e necrosis del intestino tenue. In association con
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seva presente in le parietes e le passages del
vasos e del sinusoides splenic.

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currente in le generalisate phenomeno de
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jectiones intradermal de endotoxina o epi-
nephrin e in conjunction con le application de
stress acute per rotation—supporta le hypo-
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