Shwartzman-Like Phenomenon of the Rabbit Colon Induced by a Single Injection of Endotoxin.* (27257)

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(Introduced by W. E. Ehrich)
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One of the characteristic responses to endotoxin is the hemorrhagic necrosis of the Shwartzman phenomenon(1). It is produced in skin(2) or colon(3) by 2 injections of endotoxin spaced approximately 24 hours apart. The second dose given intravenously causes hemorrhagic necrosis in the site of the first dose given into the tissue.

This paper is a preliminary report of a new phenomenon produced by endotoxin, namely that a single dose of endotoxin causes a reaction indistinguishable from the Shwartzman phenomenon in the rabbit colon when given into the superior mesenteric artery.

Method. Albino hybrid male rabbits weighing 3 to 5 lb were fed a diet of Purina Rabbit Pellets Complete, and water *ad lib.* No information regarding prior feeding by the breeders was known. Most rabbits remained healthy for varying intervals preceding experimentation, although at least one-half of all animals was infested with coccidia. A few rabbits died without manipulation.

Pyrogen-free saline was used to prepare fresh solutions of endotoxin (*E. coli,* O111: B4, Difco Laboratories). Cultures were taken to verify sterility.

Rabbits were anesthetized with intravenous pentobarbital. Under sterile surgical technic a midline incision was made and the intestinal content retracted to the left, exposing the superior mesenteric artery. A non-traumatic ligature was placed temporarily around the vessel at its base near the aorta or on a peripheral branch depending on the injection site. Care was taken to avoid damage to the neighboring celiac plexus. This ligature served to facilitate the injection and to effect hemostasis.

The dose of endotoxin per injection was 0.05 to 200 μg, in a volume of 0.1 to 2.0 cc given in a tuberculin syringe with a 24-gauge needle. Following the injection, the abdomen was sutured and the wound covered with collodion. Approximately 5 minutes were required for each operation.

Animals were sacrificed at intervals of 15 minutes to 3 months following injection of endotoxin. Autopsies were performed on all animals dying prior to scheduled time of sacrifice. Autopsies included inspection of abdomen, thorax and cranial contents. Tissues for microscopic study were fixed in alcohol-corrosive sublimate. The brain was fixed in 4% formalin. Special stains in addition to H & E included Masson's trichrome, elastic tissue and reticulum. Cultures for bacteriologic study of stomach, bowel, cecum and large bowel were made at random.

The controls consisted of a series of studies throughout the investigation and differed from the above protocol in that saline was substituted for endotoxin. In some animals endotoxin was injected into vessels other than the superior mesenteric artery.

Results. Seventy-four rabbits survived the surgical procedure. Eighteen of them served as controls. Eight rabbits were sacrificed between 15 minutes and 4 hours after operation. Of the remaining 66 rabbits, 8 died within 24 hours. They had received a dose of over 20 μg. Eleven of 19 animals survived more than 72 hours with a dose of 5-20 μg. Fifteen of 21 survived 72 hours or more with a dose of less than one μg.

All but 5 of the 56 animals receiving endotoxin developed gross intestinal lesions. All lesions produced were qualitatively the same but differed quantitatively depending upon the dose. Grossly, there was increased motor activity following the injection and in 15 minutes there was an area of blanching in haustral segments of the cecum that later were the site of ulceration. Hemorrhages were noted in the serosa and erosions of the mucosa were evident one to 4 hours after the injection. In 24 hours extensive ulceration in the cecum

* Supported by grant from Nat. Inst. Health.

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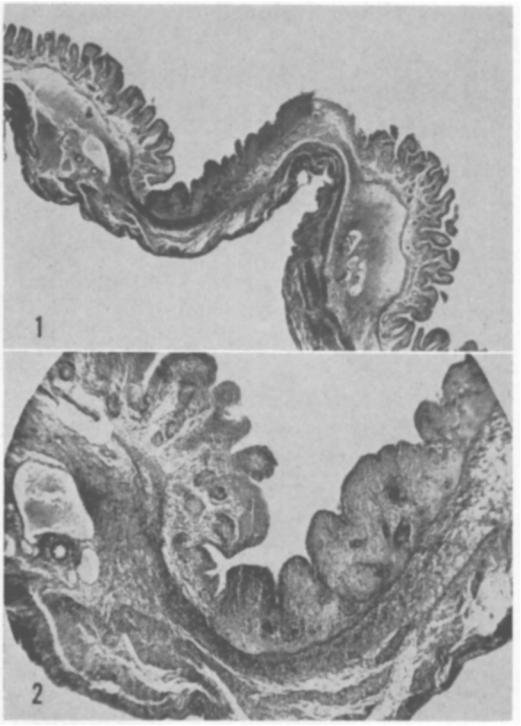

FIG. 1. Survey view of ulcer. On either side the mucosa is well preserved, the submucosa shows edema and dilated vessels.

FIG. 2. Higher magnification of lesion. Several thrombi are visible in the acutely necrotic mucosa.

was noted. The ulcers measured 3 to 4 cm in greatest diameter and were located along the antimesenteric border of the cecum. Ulcers tended to be confluent. The surrounding mucosa appeared normal. Free perforations did not occur. Healing occurred with slight scar formation. There was no tendency to chronicity of the ulcers or pseudopolyposis, fistula formation, stricture or neoplasm.

Diarrhea occurred in the first few hours and lasted several days. The feces were invariably liquid and yellowish brown in color.

Injection of India ink into the superior mesenteric artery of several living animals demonstrated that it supplied the bowel from the distal duodenum to the sigmoid colon. However, endotoxin injection into the vessel produced lesions confined to the cecum and to a lesser extent to the proximal colon. In 2 rabbits there were associated mucosal hemorrhages of the stomach.

Microscopically, lesions were noted in the 15-minute specimens. The mucosa was denuded in many areas which were relatively bloodless. Some vessels in these areas showed what appeared to be platelet thrombi. Other bloodless areas did not show thrombi. Segmented neutrophils coated the endothelium of vessels in the lamina propria. The lesions at one hour showed marked vasodilation and thrombosis of veins in the submucosa. India ink injection into the mesenteric artery of rabbits sacrificed 4 hours after injection of endotoxin revealed vessels empty of ink in the region of the denuded mucosa. Thrombi of fibrin and leukocytes in the mucosa were noted. Plasma cells were numerous in these lesions. In the fully developed ulcers at 24 hours, necrosis of all the layers of gut was noted. The most severe damage was in the muscular layers and was present in some places with relatively normal overlying mucosa. Intense edema and polymorphonuclear infiltration accompanied these lesions. The walls of venules and arterioles showed marked fibrinoid degeneration and remnants of white blood cells. The presence of hemorrhage was still detectable in the mucosa and large venous thrombi were recognizable in spite of the almost total loss of architectural features. Coccidia were noted in the submucosa in many areas, occasionally associated with plasma cells, but often with no cellular response. Coccidia were not observed, however, in the areas of hemorrhagic necrosis or intense inflammation. Lesions similar to "crypt abscesses" were seen in several specimens. In older lesions neutrophils were replaced by plasma cells, histiocytes and other mononuclear cells. Fibrous elements appeared early, indicating onset of healing with slight scar tissue formation. Attempts at reepithelialization were noted.

Sixteen of the control rabbits which received commercially prepared pyrogen-free saline solution into the superior mesenteric artery did not develop intestinal lesions. Two such controls did develop ulcers grossly indistinguishable from the endotoxin-induced ulcers.

Four rabbits received a single dose of endotoxin into the carotid artery and none developed intestinal lesions.

Random cultures of the stomach and small bowel were sterile. *E. coli* and *B. subtilis*

were recovered from cecum and colon.

Discussion. The colon lesion produced by a single injection of endotoxin into the superior mesenteric artery of the rabbit is a Shwartzman phenomenon as judged by its pathologic features. Thrombosis of small vessels, fibrinoid degeneration of the vessel and walls and necrosis characterize this lesion. The omission of a preparatory dose of endotoxin makes this observation of interest. The classical lesion occurs only after tissues are first prepared by a local dose of endotoxin. The second or provocative dose given intravenously elicits the reaction. Since the single dose of endotoxin under the conditions described produces the Shwartzman reaction, it seems logical to assume that the gut is already in some way prepared. This may come about by the presence in the gut of naturally occurring endotoxin from the normal flora of Gram negative bacteria.

The mechanisms operating in production of this lesion have been studied(4). Repeated injections of epinephrine into the peritoneal cavity of the experimental animal produced colon lesions grossly resembling the lesions in this study(5). Ileal lesions were noted by that technic but not in the present study.

Another interesting feature regarding the mechanism of action of endotoxin is that 2 of the rabbits that received endotoxin into the superior mesenteric artery had lesions in the stomach as well as in the colon. This combination of gastric and intestinal lesions is suggestive of the Reilly phenomenon which is produced by mechanical stimulation of the splanchnic nerves or injection of endotoxin in the region of these nerves(6). It is unlikely, however, that the Reilly phenomenon explains the other observations reported here because the control animals did not show this combination of lesions. Furthermore, the gastric lesions in the 2 rabbits in question consisted exclusively of hemorrhage and were confined to the mucosa.

It remains to be demonstrated that the phenomenon occurring in the colon in response to a single dose of endotoxin meets the characteristics of the classic Shwartzman phenomenon other than pathologic criteria(1). For example, the classic Shwartzman can be prevented by inducing leukopenia with cytotoxic agents, by inhibiting thrombosis with anticoagulants, by use of cortisone or by blocking the R. E. system according to Ehrich(7). Such studies are in progress.

Summary. The effects of endotoxin given as a single dose into the superior mesenteric artery of the rabbit are presented. An ulcerative lesion develops in the colon which has the microscopic features of the classic Shwartzman phenomenon.

The authors thank Drs. William Antopol and Chandler A. Stetson for evaluating the microscopic material and especially Dr. H. L. Bockus without whose constant advice and encouragement this work would have been impossible.

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Received September 28, 1961. P.S.E.B.M., 1962, v109.