Hemodynamics of Endotoxin Shock in the Rat and the Effects of Phenoxybenzamine.* (31316)

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Intravenous injection of Gram-negative bacterial endotoxin produces in dogs characteristic circulatory changes including hepatic venoconstriction, portal hypertension, splanchnic pooling, diminished venous return and a lowered arterial blood pressure(1-3). The resulting impaired mesenteric perfusion, mediated primarily by adrenergic vasoconstrictors, is believed to be an important factor leading to irreversible shock(3-9). The alpha adrenergic blocking agent, phenoxybenzamine, has been reported to reduce mortality and prolong survival in canine endotoxin shock(3,7). Other species have been studied less extensively, and there is little information concerning cardiovascular changes following administration of endotoxin in rats. In this report we have described hemodynamic and histological studies of endotoxin shock in the rat and the effects of pretreatment with phenoxybenzamine.

Methods. Forty-three Sprague-Dawley rats weighing about 400 g each were divided into 3 groups: a) hemodynamic studies, 15 rats, b) histological studies, 12 rats, and c) lethality studies, 16 rats.

Hemodynamic studies. Rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg kg $^{-1}$). The superior mesenteric artery was exposed through a mid-line laparotomy, and a 1 mm size non-cannulating transducer(10) of a gated sine wave type electromagnetic blood flow meter (Medicon, Statham, Puerto Rico) was implanted on the vessel. Mechanical occlusion of the artery distal to the transducer was performed several times during the control period to verify baseline position. Polyethylene catheters connected a femoral artery and a branch of the portal vein to pressure transducers (Statham, Puerto Rico). Mesenteric blood flow and both systemic arterial and portal venous pressures were recorded simultaneously and continuously on a direct-writing polygraph (Grass Instrument Co., Harvard, Mass.). A sample record is shown in Fig. 1. Vascular resistance across the gut was calculated as (systemic arterial pressure — portal venous pressure)/mesenteric arterial blood flow and expressed as P.R.U. (mm Hg ml $^{-1}$ min $^{-1}$). Following a 30-minute control period, an LD_{85} of *E. coli* 0-111 endotoxin, 15 mg kg $^{-1}$ (Difco, Detroit, Mich.), was injected into a femoral vein, and hemodynamic measurements were obtained for 4 hours.

In one-third of these animals administration of endotoxin was preceded by an intravenous injection of phenoxybenzamine, 5 mg kg $^{-1}$ (Dibenzyline, Smith, Kline & French, Philadelphia, Pa.), which completely blocked the constrictor response of the superior mesenteric artery to 1.0 μg intravenous norepinephrine. In another third of these rats successive ligation of the splanchnic arteries and portal vein was performed after which the entire gut, spleen, pancreas and stomach were resected. Endotoxin was then injected, and systemic pressure responses were observed.

Histological studies. Endotoxin was injected into the tail veins of 10 conscious rats. In 5 of these animals phenoxybenzamine was administered intravenously before the endotoxin. The animals were sacrificed 4 hours after injection of endotoxin. If a rat receiving both agents died before the time of sacrifice, a rat administered endotoxin alone was killed at that time. The entire small gut of these 10 rats (and an additional 2 animals serving as controls) was rapidly excised, slit longitudinally and placed in formalin. The fixed bowel was wrapped around a wooden stick and held with pins. It was dehydrated in that position and embedded in paraffin. Thus, sections of the entire gut could be examined on one or two large slides.

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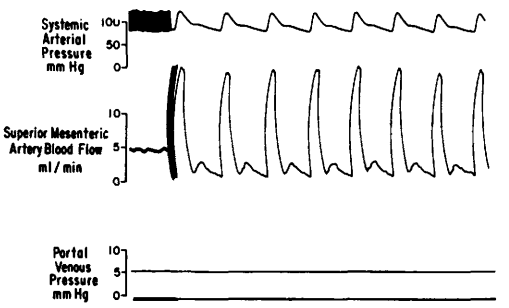
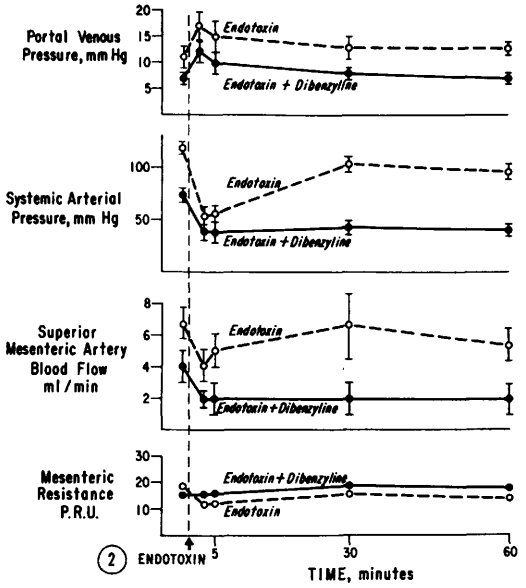


Fig. 1. Recording of systemic arterial pressure, superior mesenteric artery blood flow and portal venous pressure in an anesthetized rat. Mean arterial pressure was approximately 100 mm Hg, mesenteric blood flow 5 ml/min and portal pressure 5 mm Hg.

Lethality studies. Seven conscious rats were injected with endotoxin alone, 4 rats received phenoxybenzamine alone, and 5 rats were administered both agents. The animals were observed for 24 hours.

Results. Hemodynamic studies. Prior to the injection of endotoxin, values for portal pressure, systemic arterial pressure and mesenteric blood flow were 11 ± 4 (S.D.) mm Hg, 120 ± 8 mm Hg and 7 ± 1 ml/min respectively. Calculated mesenteric vascular resistance from these measurements was 15.6 P.R.U. (Fig. 2).

The injection of endotoxin prompted a rapid increase in portal venous pressure and a decline in systemic arterial pressure and mesenteric blood flow. These changes began within 2 minutes after endotoxin was administered and were maximal within 5 minutes. In 4 of 5 animals the onset of portal hypertension preceded the development of arterial hypotension. Portal pressure increased 54%, systemic arterial pressure decreased 57%, and mesenteric blood flow declined 39% from control values during the first few minutes of endotoxin shock (Fig. 2). All 3 measured parameters returned toward control values in the next 30 minutes, but declined thereafter until the death of the animals. Calculated mesenteric vascular resistance showed an initial fall after administration of endotoxin, followed by a near recovery to the control value at 30 minutes. After several hours of endotoxemia there was usually a rise in resistance.



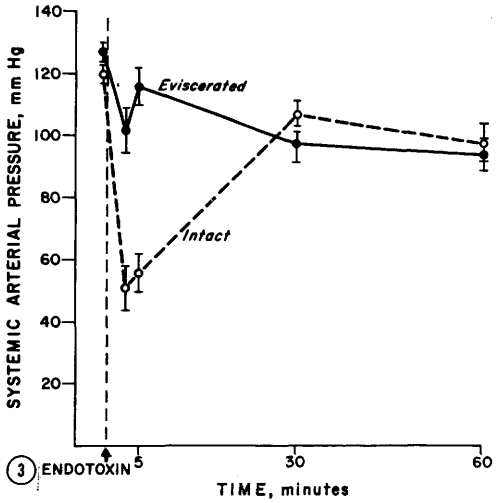


FIG. 2. Hemodynamic responses of rats in the first hour of endotoxin shock. Open circles connected by broken lines represent measurements (mean ± S.E.) of portal and arterial pressure, mesenteric blood flow and calculated resistance in 5 rats administered endotoxin alone; filled circles connected by solid lines indicate comparable measurements in rats administered phenoxybenzamine before endotoxin.

FIG. 3. Comparison of arterial pressure responses to endotoxin in 2 groups of rats: intact and eviscerated.

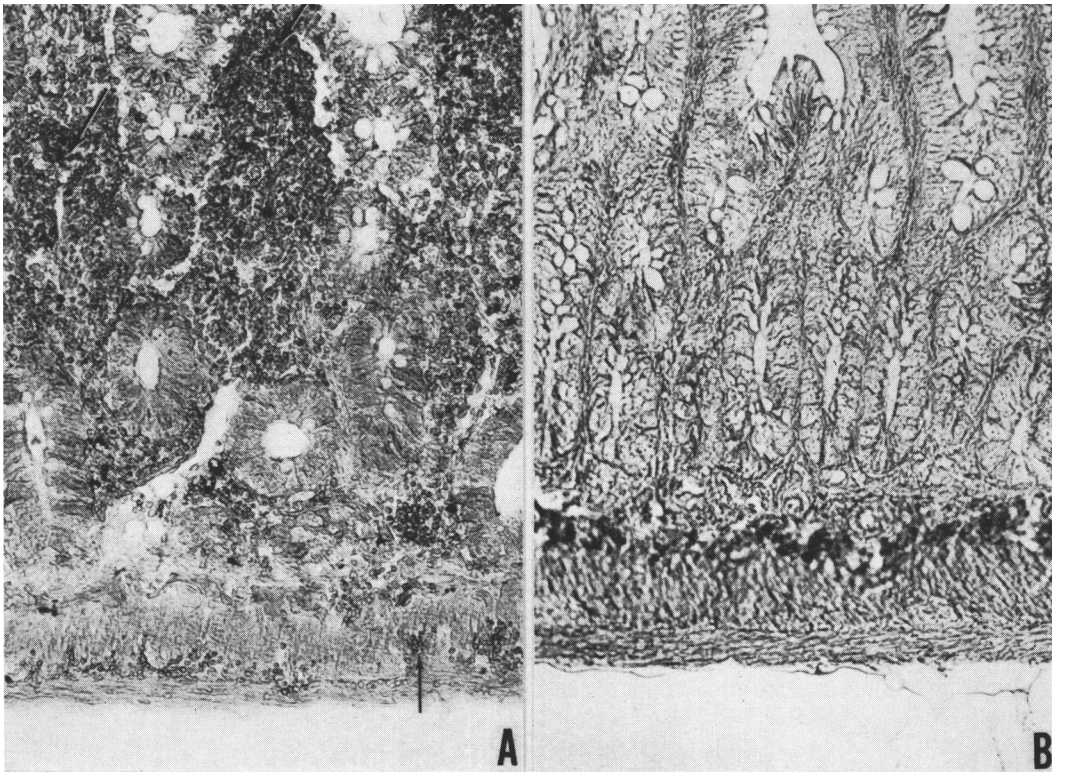


FIG. 4. Rat small intestine. A. Gut shows severe hemorrhage in the lamina propria (upper arrows); there is also evidence of hemorrhage in the muscularis (lower arrow). Tissue obtained from a rat receiving endotoxin. B. Control gut. Okajama's alizarin stain for hemoglobin. Magnification × 216.

Hemodynamic responses to endotoxin in 5 rats pretreated with phenoxybenzamine are also shown in Fig. 2. The alpha adrenergic blocking agent induced a lower portal pressure (7 ± 1 mm Hg), arterial pressure (70 ± 12 mm Hg) and mesenteric blood flow (4 ± 1 ml/min) before endotoxin was injected. The usual early hemodynamic responses to endotoxin were also observed in these animals; portal pressure increased 57%, while systemic arterial pressure and mesenteric blood flow decreased 43 and 50%, respectively, within a few minutes after injection of endotoxin. However, the transient recovery preiod seen in non-blockaded rats was impaired, and both arterial pressure and mesenteric blood flow remained at low levels till the death of the phenoxybenzamine-treated rats, which usually occurred within 2 hours after injection of endotoxin. Calculated mesenteric vascular resistance showed no increase from control values in this group during the first hour of endotoxin shock.

In eviscerated rats systemic arterial pressure fell far less abruptly than in intact rats (Fig. 3).

Pathological studies. Macroscopic. Grossly, the gut of rats administered endotoxin appeared diffusely reddened, particularly along the mucosal surface. Intestines from 4 of 5 animals receiving phenoxybenzamine before endotoxin were indistinguishable from controls. The fifth appeared similar to the intestines of the endotoxin group.

Microscopic. At the time of examination, slides were identified only by number. Two pathologists graded them independently and labeled them "severe" when extensive tracts of bowel were markedly congested and hemorrhagic (Fig. 4A), "moderate" when focal areas of marked congestion and hemorrhage involving only a few contiguous villi were

occasionally seen and "slight" when only focal areas of congestion were noted. The lesions were located mainly in the lamina propria, but in slides graded "severe" or "moderate," lesions were also found in the muscular and serosal layers.

Four out of 5 animals receiving endotoxin alone and one of those receiving endotoxin and phenoxybenzamine had "severe" lesions. One animal receiving endotoxin alone had "moderate" lesions, and 2 of the rats pretreated with phenoxybenzamine had "slight" lesions. The other 2 (endotoxin and phenoxybenzamine) were indistinguishable from controls (Fig. 4B).

Lethality studies. The injection of endotoxin (15 mg kg $^{-1}$) into the tail vein was lethal to 6 of 7 conscious rats within 24 hours, but all animals survived at least 4 hours. Phenoxybenzamine alone (5 mg kg $^{-1}$) was not lethal to any of 4 rats over a 24-hour period. All 5 rats receiving both agents were dead within 4½ hours after administration of endotoxin.

Discussion. Our results indicate that the rat and the dog exhibit similar hemodynamic and histological responses to the injection of a lethal dose of *E. coli* endotoxin. The early phase of endotoxin shock in the dog(2), characterized by the abrupt onset of portal hypertension, systemic arterial hypotension and decreased mesenteric vascular perfusion, is well-developed in the rat. In both species these changes give way to partial recovery, which is maximal around 30 minutes after injection of endotoxin, after which there is a gradual decline in arterial pressure and mesenteric blood flow until death. The histological lesions in the gut of the dog in endotoxin shock—diffuse hemorrhages and venous congestion—were also noted in the rat. In both the rat and the dog the early hemodynamic changes of endotoxin shock can be attenuated by splanchnic evisceration. These observations suggest a fundamental role for the splanchnic circulation in the early hemodynamic responses to endotoxin in both species.

Ross(11) described arterial pressure responses of rats to the injection of lethal doses of endotoxin. He observed no decline in mean arterial pressure till death many hours after administration of endotoxin. However, experimental conditions in his study differed from our own: his rats were conscious one hour after anesthetization with ether and injection of endotoxin, control pressure was ⅓ lower than in our rats, and a different endotoxin (*S. newport*) was employed.

There are conflicting reports(3,7,12,13) regarding the protective effects of phenoxybenzamine in dogs in endotoxin shock. In the rat phenoxybenzamine conferred no protection, although it blocked the mesenteric vasoconstrictor response to norepinephrine and reduced the severity of the bowel lesions. Moreover, the similarity of hemodynamic reactions to endotoxin in rats with and without phenoxybenzamine pre-treatment suggests that alpha adrenergic stimulation is not an important splanchnic mechanism in the early phase of endotoxin shock. It is possible, however, that some alternate dose of phenoxybenzamine might have protected rats against endotoxin.

The currently dominant explanation for the pathophysiology of endotoxin shock maintains that a prolonged diminution of mesenteric perfusion is critical in causing a breakdown of the intestinal barrier to endogenous endotoxin and a breakdown of abdominal reticuloendothelial protective functions(3,5). If this concept is correct, the adverse effects of phenoxybenzamine in the rat could be due to: 1) reduced mesenteric perfusion before endotoxin was injected, 2) failure to abolish the initial effects of endotoxin on mesenteric perfusion, and 3) interference with the usual mesenteric vascular recovery from effects of endotoxin.

Examination of suggested therapies for endotoxin shock over the past decade reveals considerable controversy. There are champions of adrenergic pressor agents(13-15) and of adrenergic blocking agents(3,4,7-9). The advocacy of phenoxybenzamine in human endotoxemia has been based largely upon canine studies. If this is a valid basis for therapy, our results provide no support for the use of phenoxybenzamine in human endotoxin shock. The failure of this drug to protect rats against endotoxin and its apparent

acceleration of death indicates caution in the use of this drug in the treatment of clinical endotoxin shock.

Summary. We have investigated the hemodynamics of endotoxin shock in the rat and the influence of phenoxybenzamine. Within a few minutes after injecting endotoxin there was a marked rise in portal venous pressure and a fall in both arterial pressure and mesenteric blood flow. Considerable recovery of these values toward control occurred within 30 minutes after administration of endotoxin, following which there was a gradual decline in measured parameters till death. Animals pretreated with phenoxybenzamine had lower portal and arterial pressures and mesenteric blood flow before injection of endotoxin. The magnitude and direction of the early vascular responses to endotoxin were the same as in rats not administered the adrenergic blocking agent; however, arterial pressure and mesenteric perfusion failed to recover from the early effects of endotoxin in rats receiving phenoxybenzamine. Furthermore, prior treatment with phenoxybenzamine conferred no protection against the lethality of endotoxin although it reduced the severity of the intestinal lesions.

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ACTH Stimulation and Dexamethasone Inhibition in Newcomers to High Altitude.* (31317)

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Previous work from this laboratory has shown that sea level residents exposed to high altitude increase their cortisol secretion rate; however, the response of the adrenal cortex was not maximal(1). This may represent: 1) a not intense enough stimulation or 2) adrenal inability of a greater response. The present investigation was undertaken in an effort to answer this question, and also to study whether dexamethasone was able to prevent the transitory hyperactivity of the adrenals when sea level residents are exposed to high altitude.

Material and methods. We have studied 3 groups of young adult males, native residents of sea level. They have been observed in Lima, at sea level, and then taken by train to Cerro de Pasco, Perú (14,000 feet). The departure was at 8:00 a.m. Two hours later the train travels at altitudes over 10,000 feet.

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