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Mechanism of Action of Staphylococcal Toxin in Rabbits.* (27043)

J. ISRAEL,** M. OLDSTONE, S. LEVENSON, E. D. FRANK AND J. FINE
Department of Surgery, Yamins and Kirstein Laboratories for Surgical Research, Beth Israel Hospital and Harvard Medical School, Boston, Mass.

Introduction. Bacterial exotoxins are known to inflict serious damage on specific systems. Thus, the alpha toxin of the Welch bacillus contains a red-cell lecithinase; the diphtheria toxin produces a myocardial lesion; and the exotoxin of the Staphylococcus when given intravenously causes renal necrosis. Bacterial exotoxins, like endotoxins, also can cause peripheral vascular collapse. The endotoxins do so when the reticulo-endothelial system (R.E.S.) loses its capacity to destroy them, so that they remain free in the circulation to inflict vascular damage(1). If exotoxins can injure the R.E. system, the shock and death from exotoxins might be brought about by development of a secondary endotoxemia, the source of which are the endotoxins continuously entering the circulation from the intestine(2).

Some of the experiments to be reported below were done to see whether the death caused by staphylococcal toxin is due to loss of R.E.S. function. Other experiments were done to see whether the renal injury which is a constant feature of the pathology at death from this exotoxin is involved in the death.

Materials & Methods. Adult albino rabbits (wt 2kg) were used throughout. Two separate batches of staphylococcal toxin† were used. The MLD/100 of a 1:10 dilution in saline was determined by administering increasing volumes into the ear veins of rabbits until the amount necessary to kill all rabbits within 24 hours was reached. For the first batch of toxin this MLD/100 was 1.6ml/kg, and for the second batch 2.5ml/kg. Gross postmortem examination was performed, but only the kidney was studied histologically‡.

To exclude contamination of the exotoxin with endotoxin each batch of exotoxin was extracted by the Boivin method(6), and found to contain no endotoxin on assay by the chick embryo technic(7).

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** N.I.H. Fellow in Surgery, School of Med., Univ. of Concepcion, Chile.

† Staphylococcal toxin, #42925-199 (Wood #46) preserved in 0.01% thiomerosal, Lederle Lab., Pearl River, N. Y.

‡ The effect of intravenously administered Staphylococcal toxin on the kidney is well established(3,4,5) and consists of diffuse cortical necrosis and focal medullary hemorrhagic necrosis. Other findings at death in about ½ the animals in a series include scattered petechial hemorrhages among the viscera.

The relationship between the R.E. system and the staphylococcal exotoxin was examined in 2 ways: (a) by testing the blood of the rabbit for endotoxemia following a lethal dose of exotoxin; and (b) by determining any change in toxicity of staphylococcal exotoxin in the rabbit with an injured R.E. system. Injury to the R.E.S. was inflicted by a non-lethal dose of Thorotrast (2.75ml/kg) given 3 hours before or by exposure of the rabbits to 2 hours of hemorrhagic shock(8,9) and assaying for toxicity 20 minutes after a curative transfusion.

The role of the renal lesion in the lethality of staphylococcal exotoxin was determined by noting survival time of rabbits nephrectomized 3 hours before a sublethal dose of exotoxin or 3, 7, or 10 hours after a lethal dose. This survival time was compared to that of rabbits to which no toxin was administered, but from which the kidneys were removed or the arterial circulation totally occluded. In the latter 2 groups of rabbits, survival time was 5 days.

The role of the vegetative nervous system in production of the renal lesion was studied by giving a sublethal dose of the exotoxin to rabbits with one renal pedicle denervated a week in advance. The effect of Dibenamine on the renal lesion was also observed(10). Survivors for examination were killed 48-96 hours later.

Results. Of 7 rabbits given a lethal dose of staphylococcal exotoxin none showed an endotoxemia. Pretreatment with Thorotrast did not alter the lethality of the exotoxin, but 2 hours of hemorrhagic shock increased it considerably (Table 1).

The usual survival time after an MLD/100 of toxin was 24 hours; after excision of both kidneys, 5 days. Removal of both kidneys 3 hours *after* a lethal dose of toxin delayed death until the fifth day in half the animals. Removal of both kidneys 7 or 10 hours *after* a lethal dose of toxin delayed death in all animals until the fifth day (Table 2).

TABLE I. Effect of Injury to Reticulo-Endothelial System on Mortality of Staphylococcal Toxin.

Dose of Toxin* ml/kg	No Injury	Thorotrast	Reversible Shock
.5	0/4	0/4	0/6
.8	0/4	1/4	2/4
1.0	0/6	0/6	4/6
1.2	3/13	5/13	2/2
1.4	4/5	3/5	—
1.6	9/10	4/4	—

* Toxin in 1/10 dilution.

Removal of both kidneys 3 hours *before* a sublethal dose of toxin reduced survival time from 24 hours to 2 hours in 1/2 the rabbits, and a uniformly non-lethal dose in normal rabbits reduced survival rate from 100% to 50% (Table 2).

Sympathectomy of a renal pedicle did not prevent renal necrosis. Indeed the injury was clearly worse on the denervated side. Dibenamine did not change survival time or renal pathology.

Discussion. These data indicate that the staphylococcal exotoxin does not affect the function of the R.E.S. or evoke an endotoxemia. Nor does the R.E.S. affect the mechanism of action of this toxin, since Thorotrast does not alter the lethal dose. On the other hand a brief exposure to hemorrhagic shock increases sensitivity to the exotoxin. This may be attributable to an adverse effect of shock on excretion of the toxin or on a detoxifying mechanism outside the R.E.S. Renal denerva-

TABLE II. Mortality of Staphylococcal Toxin in Nephrectomized Rabbits.

	Bilateral Nephrectomy No Toxin	Bilateral Renal Artery Ligation No Toxin	Bilateral Nephrectomy MLD/100 Toxin*		Bilateral Nephrectomy Sublethal Dose Toxin†	MSD Toxin‡
			3 Hr Before	7-10 Hr Before	3 Hr After	
No. Exp.	10	5	8	10	10	6
Mortality 24 hr	0/10	0/5	4/8	0/10	10/10	3/6
5 days	10/10	5/5	8/8	10/10	—	6/6

* Minimum Lethal Dose (MLD/100) = 2.5ml/kg (1/10 dilution).
† Sublethal Dose = 2.0 — 1.8ml/kg (1/10 dilution).
‡ Maximum Survival Dose (MSD) = 1.4ml/kg (1/10 dilution).

tion, which blocks the effect of endotoxin on the renal cortex in the Shwartzman reaction(11), does not prevent the renal lesion caused by exotoxin. Thus it appears that the kidneys remove a considerable amount of staphylococcal toxin from the circulation, for bilateral nephrectomy performed 7 to 10 hours after the toxin is given prevents death. The same conclusion follows from the observation that death from toxin is quicker if the kidneys are removed before the toxin is given. Hence it appears that in the intact animal, extraction of toxin by the kidneys delays but does not prevent death. One may conclude further that while the toxin is held by the kidneys, it is not acting elsewhere. But sometime afterward the toxin or a product of the toxin interacting with the kidney is released and that this released material kills. Since ischemic necrosis of both kidneys does not kill earlier than bilateral nephrectomy, the necrosis produced by toxin is not the cause of death. Presumably, then, the toxin or a modification of it acts on an unidentified site elsewhere to cause death. Vasospasm and other effects of the catechol amines appear not to be involved. The mechanism of death following administration of staphylococcal exotoxin remains unknown.

Summary. The R.E.S., which is the main defense against endotoxin, plays no part in the defense against staphylococcal toxin. The kidneys remove staphylococcal toxin from the circulating blood. The toxin or a modification of it is subsequently released, then exerts a lethal action elsewhere. For some hours after it is taken up by the kidney the toxin cannot act. Thus, the kidney is protective for some hours. Indeed nephrectomy prevents death from exotoxin. The renal necrosis produced by the toxin in itself is not the lethal lesion, because renal necrosis produced by arterial occlusion does not cause such rapid death as occurs in renal necrosis produced by the toxin.

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Renin and Angiotensinase Content of the Kidney of Normal and Renal Hypertensive Rats.*‡ (27044)

PEDRO BLAQUIER§†, DAVID F. BOHR, ALBERTO C. TAQUINI, JR.† AND SIBLEY W. HOOBLER

Departments of Physiology and Internal Medicine, University of Michigan, Ann Arbor

The possible role of renin as a causative agent in experimental renal hypertension has been extensively investigated. Direct determination of the concentration of renin in the blood has been indecisive. It is to be expected that if renin, through its pressor effect, is the direct cause of experimental renal hypertension, it would be present in the blood of hyper-

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§ Research Fellow of Am. Heart Assn. and Univ. of Michigan Inst. of Science and Technology.

† Present address: Centro De Investigaciones Cardiologicas, Med. School, Univ. of Buenos Aires, Argentina.