Effect of Gram-Negative Endotoxin on Nephrotoxic Serum Nephrosis in Rats.* (23498)

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Experimental renal disease produced in rats by intravenous administration of heterologous nephrotoxic antiserum has been studied by numerous investigators(1-7). The pathologic and clinical manifestations of the disease are variable, and depend upon such factors as manner of preparation and potency of the nephrotoxic serum(8), dose of serum given (3,9), time sequence of injections(7), and age and sex of the animals used(10). Recent efforts to enhance the nephrotoxicity of the serum with streptococcal culture filtrates were largely unsuccessful(11). Piel *et al.*(4) described the occurrence of bilateral renal cortical necrosis in rats given large amounts of nephrotoxic serum. This lesion was associated with deposits of hyaline fibrinoid material in the glomerular capillaries and was strikingly similar to the renal lesion of the generalized Shwartzman phenomenon in rabbits. Since the Shwartzman reaction had not been produced previously in rats, the lesion was considered to be non-specific and was not investigated further by these workers. Recently Gronvall and Brunson(12) reported that the generalized Shwartzman phenomenon could be produced regularly in rats by administration of Gram-negative endotoxin in conjunction with the high molecular weight acidic polymer, Liquoid (sodium polyanethol-sulfonate).

These observations suggested that Gram-negative endotoxin might potentiate the renal disease produced by administration of nephrotoxic serum and led us to investigate the effects produced in rats by intravenous injection of endotoxin in combination with nephrotoxic serum.

Material and Methods. Nephrotoxic serum was produced in rabbits by repeated intraperitoneal injection of rat kidney homogenate prepared according to the method described by Heymann and Lund(3). Lots of nephrotoxic serum from several rabbits were pooled and the potency of the material standardized. Sprague-Dawley rats of both sexes weighing 250-350 g were utilized as a source of renal tissue for preparation of kidney homogenate. The potency of the nephrotoxic serum was established by noting the dosage required to produce a severe nephrotic syndrome in rats. 158 female rats of the Sprague-Dawley strain, weighing 100 to 175 g were used in the study. These were fed Purina Fox Chow and had free access to water. All injectable solutions were made up to a volume of 1 cc. Animals given 2 substances thus received 2 cc of solution. All injections were given intravenously by tail vein. The numbers of animals used in the various experiments are tabulated. Animals dying during the experiment were autopsied and blocks of heart, lungs, spleen, liver and kidney were placed in 10% neutral formalin and sectioned in the usual manner for microscopic examination. *E. coli* endotoxin (lipopolysaccharide Rx B35527) was generously supplied by Mr. Aaron Lane of the Difco Laboratories, Detroit, Mich. Twenty-four hour urine specimens were collected in metabolic cages from rats which had free access to water only. Urinary proteins were measured early in the disease and again at the end of a week or 10 days using the Biuret method with a Bausch and Lomb Spectronic 20 colorimeter. All surviving rats were killed approximately 10 days after injection and autopsied as indicated above.

Results. Table I shows the survival rate and mean urinary protein excretion of rats

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TABLE I. Effect of Nephrotoxic Serum on Survival of Rats Given Gram-Negative Bacterial Endotoxin.

Group	No. of rats	No. surviving	Protein excretion, mg/hr*
Normal rats	79	79	1.32
Normal rabbit serum (1 cc)	5	5	1.44
Endotoxin (250 γ)†	5	5	1.09
" (10 γ)	5	5	1.05
Normal rabbit serum (1 cc) + endotoxin (10 γ)	5	5	1.04
Normal rabbit serum (1 cc) + endotoxin (250 γ)	5	5	1.43
N.T.S.‡ (1 cc)	13	11	8.90
" (2 cc)	5	5	8.95
" (1 cc) + endotoxin (300 γ)	5	0	
" " + " (250)	8	3	13.5
Idem (100)	3	0	
" (50)	8	0	
" (10)	8	0	
" (5)	5	0	
" (1.0)	8	2	12.8
" (.5)	5	2	
" (.1)	5	5	9.47

* Mean mg/hr. † Lipopolysaccharide endotoxin from *E. coli*. ‡ Nephrotoxic serum.

given normal or nephrotoxic rabbit serum and *E. coli* endotoxin separately or in combination. All animals that were given normal rabbit serum or lipopolysaccharide endotoxin alone or in combination survived. Rats given nephrotoxic serum in doses (1-2 cc per rat) sufficient to produce proteinuria regularly survived 10 days. In contrast, when 1 cc of nephrotoxic serum was given together with endotoxin, a majority of the animals died in periods of 3 to 24 hours following injection. For example, in the groups given amounts of endotoxin varying from 300 γ to 5 γ, only 3 survived longer than 24 hours. In each instance death was preceded by prostration, lethargy, ruffling of the fur, bloody nasal discharge and diarrhea. The survival rate increased when endotoxin was given in doses of less than 1 γ. At 0.5 γ 2 of 5 rats survived, and all animals given 1 cc of nephrotoxic serum in combination with 0.1 γ *E. coli* endotoxin survived.

Urinary protein excretion of rats given nephrotoxic serum alone in the dosages used was about 7 times that of normal rats. The protein excretion of animals injected with normal rabbit serum or endotoxin alone and in combination varied little from the normal controls. Proteinuria among the few animals which survived injections of nephrotoxic serum and endotoxin was of approximately the same magnitude as in rats given nephrotoxic serum alone.

Morphologic changes. Gross changes in the animals which died following combined nephrotoxic serum and *E. coli* endotoxin injection included ascites, pulmonary hemorrhages and enlarged bluish-red kidneys with tense capsules. On cross section the kidney lesions were suggestive of early bilateral cortical necrosis and if the animals had survived longer it is probably that the characteristic changes of the generalized Shwartzman phenomenon would have been more apparent.

Microscopic examination of the kidneys from rats dying after simultaneous injection of endotoxin (all dosages) plus 1 cc of nephrotoxic serum revealed deposition of hyaline fibrinoid material in the glomerular capillaries together with tubular necrosis, eosinophilic staining tubular casts and proliferation of glomerular capillary endothelial cells (Fig. 1).

Kidneys from rats given nephrotoxic serum alone showed only a proliferation of endothelial cells similar to that which occurs in acute glomerulonephritis in man (Fig. 2).

Discussion. The relative resistance of rats to the lethal action of large doses of lipopolysaccharide endotoxin observed by others(13) is supported by our investigations. Intravenous injection of as much as 250 γ failed to kill

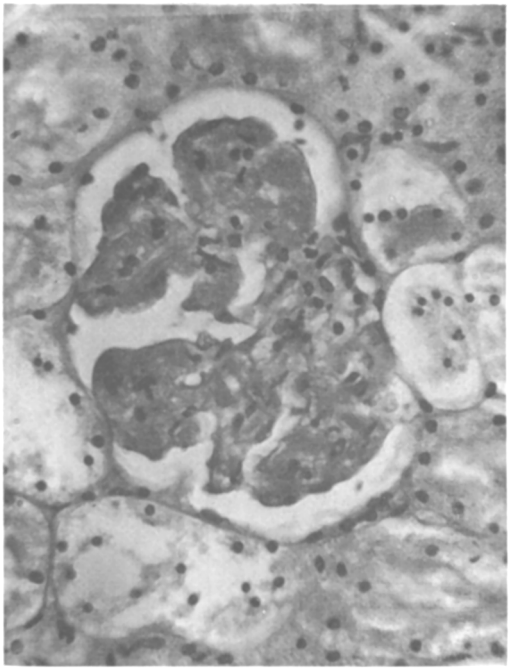

FIG. 1. Periodic acid-Schiff stain of a glomerulus from a rat given 1 cc of nephrotoxic serum simultaneously with 150 γ of *E. coli* endotoxin. Death occurred within 24 hours. Note moderate proliferation of capillary endothelial cells, glomerular capillary occlusion with Schiff-positive fibrinoid material, and early tubular necrosis. × 360.

any animals. The relatively high properdin values(14) found in this species might partially explain the rat's resistance to the lethal and necrotizing effects of endotoxin. One might also postulate a more rapid clearance of endotoxin by the reticuloendothelial tissues (15) in rats than in other animals. Whatever its basis, this resistance to endotoxin is readily overcome by a simultaneous injection of nephrotoxic serum. When given together with nephrotoxic serum, even minute amounts of endotoxin (0.5 γ) produced early death and the morphological picture of the generalized Shwartzman reaction. One hundred per cent survival of rats given 0.1 γ of endotoxin in combination with nephrotoxic serum indicates that a minimum amount of endotoxin necessary for the lethal effect has been reasonably well defined.

The mechanism by which nephrotoxic serum and Gram-negative endotoxin act together to produce this pronounced lethal effect in rats is unknown. However, the observations presented here are similar to those made previously in these laboratories(16-18) demonstrating enhancement of lethal and necrotizing effects of endotoxin in rabbits by prior treatment with cortisone and prior or simultaneous injection of thorotrast, trypan blue, colloidal iron or colloidal carbon. Thus, nephrotoxic serum might interfere with the capacity of the R. E. system to clear endotoxin from the blood. At present, we have no evidence to support this concept but experiments to test the possibility seem indicated.

Gronvall and Brunson(12) produced the generalized Shwartzman reaction and death in rats by administering endotoxin together with or prior to injection of sodium polyanethol sulfonate (Liquoid). It seems unlikely from the timing and requisite sequence of the injections in their studies that the polysaccharide polymer exerts its effect by producing "reticuloendothelial blockade." Investigations by Thomas, *et al.*(19) have indicated that other mechanisms are involved in the apparent synergism of large molecular weight acidic polymers and endotoxin. Experiments to test the possibility of these mechanisms being the basis for the synergistic action of nephrotoxic serum and Gram-negative endotoxin are in progress. These include variations in the sequence and time relationships of injections and attempts to prevent the renal lesions with heparin and nitrogen mustard(20,21). Preliminary studies(22) have shown a depletion of the heparin-precipitable protein nor-

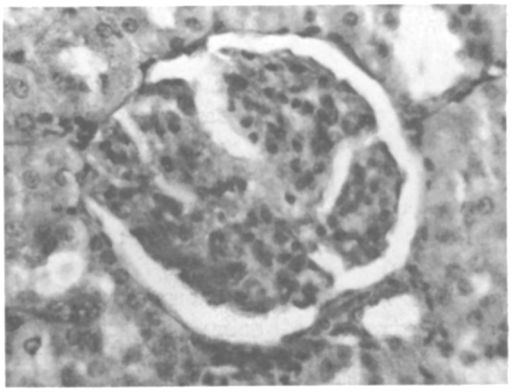

FIG. 2. Periodic acid-Schiff stain of a glomerulus from a rat given 1 cc of nephrotoxic serum alone. Killed 10 days after injection. Note proliferation of glomerular capillary endothelial cells and absence of Schiff-positive material. × 275.

mally present in rat plasma following injection of nephrotoxic serum alone or in combination with endotoxin. This observation suggests that nephrotoxic serum may have a similar action to the large molecular weight acidic polymers.

Our pathological studies indicate that still a third mechanism might be found basic to the synergistic action of nephrotoxic serum and Gram-negative endotoxin. Nephrotoxic serum produces proliferation and swelling of the endothelium of glomerular capillaries which might render these blood vessels more susceptible than normal to the necrotizing action of endotoxin. Similar changes produced in the general vascular system, even though not recognizable by light microscopy, might account for the lethal effect and the general vascular susceptibility to the necrotizing process.

These investigations may provide an explanation for the recent observations of Baxter and Goodman(7) who found that nephrotoxic serum incubated with rat intestine resulted in a product which killed all rats when injected intravenously. In contradistinction, nephrotoxic serum incubated with other organs and tissues had no such effect. It is likely that the lethal effect they observed was a function of a synergistic action of nephrotoxic serum and minute amounts of lipopolysaccharide endotoxin of bacterial origin derived from intestinal tissues. Likewise, the occasional instance of bilateral cortical necrosis observed by Piel *et al.*(4) in animals given nephrotoxic serum alone may have been due to a synergistic action of nephrotoxic serum and minute quantities of bacterial endotoxin absorbed from the bowel or endotoxin-like polysaccharides derived from other tissues.

More important are the implications of these experiments that a complicating factor otherwise of so little consequence to the rats can result in death and widespread fibrinoid necrosis when the kidneys have been damaged by nephrotoxic serum. Several diffuse renal diseases in man have a common pathological lesion in the end stage. One might speculate that the underlying disease process induces an extreme vulnerability of the vessels to the necrotizing actions of certain lipopolysaccharides. Thus, a "complicating factor" actually might induce the progressive renal disease and could be primarily responsible for the morphological characteristics of the end state. Stetson (23) and Schwab *et al.*(24) found that group A streptococci contain an endotoxin similar to Gram-negative bacterial endotoxins. Landy (25) discovered polysaccharides with endotoxin-like action to be widely distributed in nature, including mammalian tissues. These observations permit postulation that toxins capable of producing the pathological lesions described here might be derived from sources other than Gram-negative bacteria. Further studies to evaluate this hypothesis are in progress.

Summary. 1) Intravenous injection of small amounts of nephrotoxic serum produced a syndrome in rats whose characteristics include proteinuria and proliferation of glomerular endothelial cells. This dosage of nephrotoxic serum was well tolerated by the rats. 2) Rats were shown to tolerate *E. coli* lipopolysaccharide endotoxin over a wide range of dosages. 3) Simultaneous injection of nephrotoxic serum and minute amounts of Gram-negative bacterial endotoxin resulted in acute death in a high percentage of rats and renal lesions resembling those of the generalized Shwartzman reaction. 4) The possible implications of these observations on susceptibility to necrotizing effects of endotoxin-like substances and on the pathogenesis of renal disease are discussed.

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Blood Pressure of the Normal Rhesus Monkey. (23499)

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This laboratory has been interested in developing procedures for evoking hypertension in subhuman primates such as the rhesus monkey(1). There being essentially no definitive data on variations in blood pressure of the normal rhesus monkey, determination of the limits of such variations was a necessary preliminary to studies on hypertension. This report includes observations on blood pressure variations in normal and splenectomized monkeys acquired with 2 indirect methods as well as comparisons of these indirect readings with measurements of the arterial pressure obtained directly with a strain gauge.

Materials and methods. Animals. Young male and female rhesus monkeys weighing from 3 to 12 kilos were used. Details of the care and feeding of animals in this colony have been described by Schmidt and Genther (2). Three groups of monkeys were employed in these studies. The first group comprised 14 normal monkeys on which blood pressure measurements were made repeatedly over a 2-month period using the 2 indirect blood pressure methods described below. The second group consisted of 27 splenectomized animals on which control blood pressures were measured for 2 months in the same fashion as the first group. These monkeys had been infected with malaria, their infections cured by various chemotherapeutic agents, and this fact confirmed by splenectomy. The blood pressure studies were begun 2 or more months after splenectomy. After the control period the majority of these animals were used in studies on experimental hypertension. However, 6 were observed as controls for an additional 12 months, observations on these animals providing data on stability of simian blood pressure levels over an extended period. The third group consisting of 46 anesthetized monkeys was used in comparing the direct and indirect blood pressure methods. Of these, 43 had no known cardiovascular or renal disease while the remaining 3 had moderate experimental hypertension. The procedure used in handling the monkeys was an important factor in securing reproducible blood pressure measurements. The monkeys were housed in large cages, each containing 6 to 10 animals. The cages opened individually onto a narrow corridor to which animals were admitted one at a time. The monkey was caught on its first run, then allowed to rest