The Generalized Shwartzman Phenomenon in Rabbits with Denervated Kidneys[1] (34925)

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It is well accepted that the lesions seen in the generalized Shwartzman phenomenon are due to the accumulation of fibrin as a result of intravascular coagulation (1–3). Although other organs are involved frequently, the kidneys seem to be affected quite regularly and thus glomerular capillary thrombosis and bilateral symmetrical renal cortical necrosis have been considered as the hallmarks of the phenomenon.

It appeared interesting to perform experiments designed to demonstrate the effect of complete denervation achieved by autografting on the production of the generalized Shwartzman phenomenon. The animal of choice was the rabbit since most of the experimental work in this field has been done with it. Such a study seemed also relevant because renal cortical necrosis develops in man and rabbits bearing homografts to which they have been sensitized (4–7).

Materials and Methods. New Zealand white rabbits of either sex, weighing 2 kg were used. Autografts were performed in 16 using microvascular surgical techniques previously described (7). The left kidney was transplanted into the cervical region. The site of the cutaneous ureterostomy was examined several times daily to ensure ureteral patency. The Shwartzman reaction was induced 2 to 36 days after transplantation by infusing, over a 4-hr period, 400 μg of lipopolysaccharide from *E. coli* $O_{26}B_6$ (Difco Laboratories, Detroit, Michigan) dissolved in physiological saline. A group of 10 normal rabbits was also infused in a similar manner. The animals were killed 4 to 24 hr after the infusion.

Antiserums to rabbit fibrinogen, prepared according to the method of Kekwick (8), were raised in guinea pigs. Two potent antiserums were used; only one had to be absorbed with rabbit serum to render it fibrinogen specific as determined by demonstrating a single line in double diffusion gel precipitation and immunoelectrophoresis with rabbit plasma. The antiserums were conjugated with fluorescein isothiocyanate according to the method of Beutner *et al.* (9). The fluorescein:protein ratio was 3, 0 and the conjugates were used at protein concentration of 0.2 mg/ml.

Immunofluorescent staining was carried out on 4-μ sections of tissue which had been quick-frozen in liquid nitrogen. The sections were air-dried for 1 hr, washed briefly in phosphate buffered saline, pH 7.3, incubated with the fluorescein-labeled antiserum to fibrinogen at room temperature for 30 min, then washed again, and mounted in 50% glycerol. For controls, normal tissues were stained with the conjugates; the results of these tests were consistently negative. The activity of the conjugates could also be completely abolished by absorption with fibrinogen.

The serological procedures employed have been previously detailed (10). The double diffusion gel precipitation tests were performed in 1% agarose gel. Plates were incubated at room temperature for several days. The tanned cell inhibition test was performed by treating human blood group O erythrocytes with tannic acid diluted 1:40,000 and coating with fibrinogen at a concentration of 10 μg/ml. The guinea pig antirabbit fibrino-

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TABLE I. Shwartzman Reaction in Rabbits; Effect of Denervation by Autotransplantation.

Rabbit no.	Time (days) between transplantation and endotoxin infusion	Time (hr) between endotoxin infusion and sacrificing	Degree of fibrin deposition in glomerular capillaries[a]	
			Autograft	Kidney left *in situ*
2944	2	24	+	+
3403	3	24	±	±
6045	5	5	±	++++
6126[b]	5	4	—	+++
6044	6	24	—	+
2966	7	20	+++	++++
6111	7	4	+	++++
6008	7	18	+	+++
6112	7	24	+	+
3409	7	20	+++	++++
2956[c]	7	7	++	++
2939[d]	11	24	—	++++
3370[d]	16	24	—	++++
3351	19	24	±	+
3325	34	16	±	+
3327	36	24	±	±

[a] Evaluated on the basis of PAS and/or immunofluorescent staining.

[b] Rabbit 6126 developed acute pyelonephritis in the autograft.

[c] Rabbit 2956 received 200 μg of endotoxin; all others received 400 μg.

[d] Rabbits 2939 and 3370 had no urine flow from the autograft as a result of ureteral obstruction.

gen serum was used at a dilution of 1:500 which corresponded to 5 hemagglutinating units. The inhibiting serum specimens were obtained from blood which had been collected in tubes containing thrombin (Parke, Davis & Co., Detroit, Michigan) at a final concentration of 10 units/ml and epsilon-aminocaproic acid (Amicar, American Cyanamid Corp., Pearl River, New York) at a final concentration of 0.04 *M*. The whole test was performed using a citrate–saline buffer, pH 6.0. The diluent was normal rabbit serum at a dilution of 1:250. The tests were read after 2 hr of incubation at room temperature and again after 18 hr of incubation at 4°.

Results. A prolonged infusion of endotoxin instead of two spaced injections as in the usual procedures was employed since this was found to give more reproducible results. The lesions seen in the various organs of normal rabbits in which the reaction was produced were quite similar to those previously described (1). In the early lesions there was noted some PAS staining material lining the capillaries of the kidneys and this material showed immunofluorescent staining for rabbit fibrinogen. In the more severe lesions the loops appeared to be occluded with similar material and occasionally the arterioles were also involved. In the animals killed 8 to 24 hr after infusion, renal cortical necrosis could be observed. Notable, however, was the sparing of individual nephrons and groups of nephrons even in the otherwise necrotic outer cortex. Another feature was the well-known fact that both kidneys tended to be involved to a similar degree.

The rabbits with autografts had similar lesions in the grafted and in the left-*in-situ* kidneys (Table I). However, in most instances the lesions in the grafted kidneys were less extensive and in some autografts there was only focal and local involvement (Fig. 1). In two autografts which had ureteral obstruction and in one graft which developed very severe acute pyelonephritis,

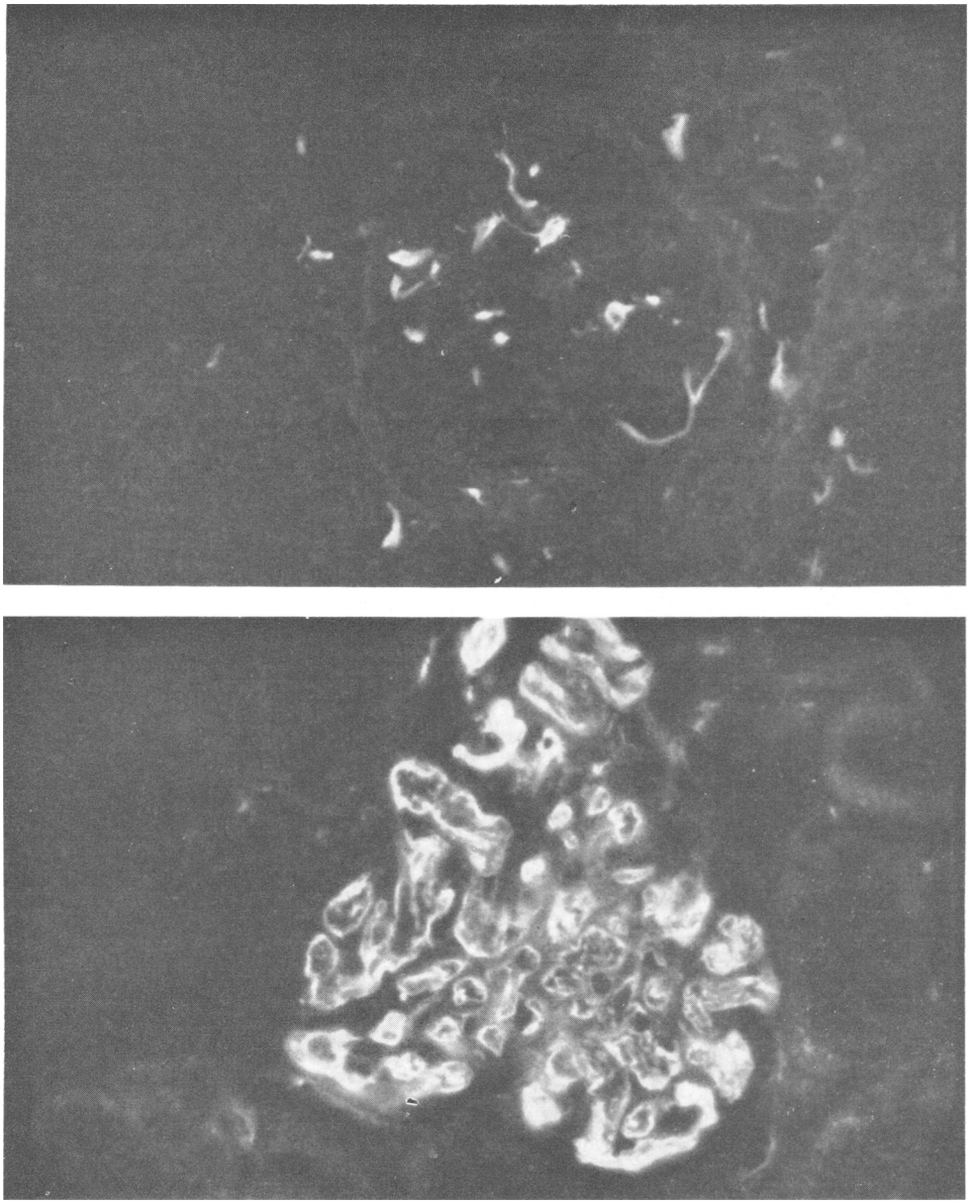

FIG. 1. Effect of denervation of kidney on Shwartzman phenomenon assessed by staining of kidney sections from rabbit 6111 by fluorescein-conjugated antibodies to rabbit fibrinogen: (upper) renal autograft; (lower) kidney left *in situ*.

there was no glomerular deposition of fibrin in spite of the fact that the same animals had very pronounced depositions of fibrin in the kidneys left *in situ*.

Figure 2 shows that the guinea pig antiserum to rabbit fibrinogen gave a line of precipitation with the serums obtained after the infusion of endotoxin. Of 6 postinfusion serums tested, 5 gave such lines indicating the presence of fibrin breakdown products. In some instances two lines were seen corresponding in all likelihood to antigens D and E of fibrinogen (11). The only negative serum was obtained immediately after infu-

TABLE II. Tanned Cell Hemagglutination Inhibition Test.

Dilution of inhibiting serum, 1 to	Normal rabbit		2966		Cohn fraction I 1000 μg/ml
	Serum	Plasma	Preinfusion	Postinfusion	
10	+	—	+	—	—
20	+	—	+	—	—
40	+	—	++	—	—
80	+	—	++	—	++
160	+	±	++	—	+
320	+	+	++	+	+
640	+	++	++	++	+
1280	++	++	++	++	+

sion. Normal rabbit serums including preinfusion serums from experimental animals gave consistently negative results.

Table II shows the results of examining the same serums in the tanned cell inhibition test. Again, the serums before the infusion were negative while those obtained after infusion were positive indicating the presence of fibrin breakdown products. Of 6 postinfusion serums tested, all 6 had fibrin breakdown products, while none could be detected in the preinfusion serums or in normal rabbit serums.

Discussion. It is generally assumed that fibrin accumulates in the glomeruli rather than forms there. McKay *et al.* (12) concluded that glomerular capillary dilation was an important factor. Gourzis *et al.* (13) and Müller-Berghaus and McKay (14) utilizing the adrenergic blocking agents dibenzamine and phenoxybenzamine demonstrated that peripheral adrenergic mechanisms are intimately involved in the effects of *E. coli* endotoxin. Palmerio *et al.* (15) demonstrated the effectiveness of unilateral sympathectomy in ameliorating the Shwartzman reaction in the denervated kidney. It has been contended, however, that denervation may not be complete unless the vessels are severed and therefore performing an autograft was recommended (16, 17).

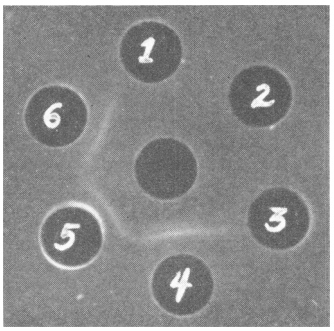

FIG. 2. Double diffusion gel precipitation test: (center well) guinea pig antirabbit fibrinogen serum; (peripheral wells) (1) 2939 serum, before infusion; (2) 2939 serum, at the very end of infusion; (3) 2966 serum, before infusion; (4) 2966 serum, 20 hr after the infusion; (5) 3370 serum, 24 hr after infusion; (6) 6008 serum, 18 hr after infusion.

The present data indicate that although both the renal autograft and the kidney left *in situ* suffered from the Shwartzman reaction, the former appears to be less vulnerable to the noxious effects of endotoxin infusion. This is in agreement with the above reports that implicated the importance of peripheral adrenergic mechanisms. Not unexpectedly, ureteral obstruction also prevented the lesion, presumably by stopping glomerular filtration. Acute pyelonephritis also appeared to protect the kidney by a mechanism which remains to be elucidated.

Several investigators have been unable to demonstrate fibrinolysis occurring in the generalized Shwartzman phenomenon in the rabbit but the methods used were quite insensitive (18–21). The clearance of fibrin from glomeruli after one dose of endotoxin had been noted but it was attributed to the activity of the reticuloendothelial system (18). However, it has been shown that the generalized Shwartzman reaction can be induced with only one dose of endotoxin instead of two (17) or with an infusion of thrombin (18, 22) provided that fibrinolysis is concom-

itantly inhibited by epsilon-aminocaproic acid or by protease inhibitors. Conversely, the reaction can also be prevented by streptokinase which activates the fibrinolytic system of the rabbit (23, 24). These experiments then suggest that the rabbit has a potentially effective fibrinolytic system. In our experiments it was possible to show, using sensitive serologic tests, that all 6 rabbits tested had circulating fibrin degradation products after endotoxin infusion. Consistently negative results were obtained with serum from normal rabbits including the preinfusion specimens of the experimental animals.

It is of interest that Graeff *et al.* (25) have demonstrated disappearance of plasminogen activator from the outer medulla in those rabbits which had extensive glomerular deposition of fibrin. This suggests activation of their fibrinolytic system, the ineffectiveness of which, however, could be explained by the inhibition of fibrinolysis. Rabbits undergoing the generalized Shwartzman phenomenon regularly develop elevated levels of serum lipids (26) which are known to affect the blood coagulation mechanism including the inhibition of fibrinolysis (27). It is conceivable then that the inhibition of fibrinolysis by the lipemia may be of pathogenetic significance in the deposition of fibrin in this phenomenon.

Conclusion. Complete denervation of the rabbit kidney by performing an autograft appears to ameliorate the noxious effect of an endotoxin infusion. Fibrin degradation products appear in the circulation when the generalized Shwartzman phenomenon is induced in rabbits by endotoxin infusion, suggesting activation of its fibrinolytic system.

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