THE EFFECT OF CORTISONE ON THE SHWARTZMAN REACTION*

THE PRODUCTION OF LESIONS RESEMBLING THE DERMAL AND GENERALIZED SHWARTZMAN REACTIONS BY A SINGLE INJECTION OF BACTERIAL TOXIN IN CORTISONE-TREATED RABBITS

BY LEWIS THOMAS, M.D., AND ROBERT A. GOOD,‡ M.D.

WITH THE TECHNICAL ASSISTANCE OF JAY N. LITTLE

(From the Heart Hospital Research Laboratories, Department of Pediatrics, University of Minnesota Medical School, Minneapolis)

PLATES 42 TO 44

(Received for publication, January 25, 1952)

The phenomenon of local tissue reactivity in the rabbit, described in 1928 by Shwartzman (1), is a two-stage reaction in which the first event is a local inflammatory response in skin tissue brought about by culture filtrates from certain gram-negative microorganisms; the second event is initiated by an intravenous injection of the same or other culture filtrates, 24 hours later, and consists of hemorrhagic necrosis of the prepared skin area. The presence of an inflammatory exudate in the skin appears to be a necessary feature of preparation for the phenomenon, and it has been suggested that an excessive production of lactic acid in the prepared area is involved in the reaction (2). Evidence which indicates that polymorphonuclear leukocytes may play a role has been presented by Stetson and Good (3), and Stetson (4) has recently proposed that leukocyte-platelet thrombi within small blood vessels may be the cause of necrosis and hemorrhage in the tissues.

It has been reported by Shwartzman *et al.* (5) that this phenomenon is prevented by the administration of cortisone. The inhibitory effect was demonstrable only when large doses of cortisone were administered within a period of 2 to 4 hours prior to the intravenous injection of toxin; when cortisone was given at the time of the intradermal injection of toxin no inhibition was demonstrable.

In the normal rabbit, an intradermal injection of bacterial endotoxin which is capable of preparing for the Shwartzman reaction is followed by the development of edema and erythema at the injected site, with infiltration of the tissue by many inflammatory cells. No hemorrhage or necrosis is demonstrable in such tissue until after the intravenous or "provoking" injection of toxin is given. Thomas and Mogabgab (6) studied the effect of cortisone on the response of skin to meningococcal toxin, in rabbits. Instead of vigorous local inflammatory response, the treated animals exhibited numerous petechial hemorrhages and areas of necrosis throughout the in-

* This work was aided by grants from the United States Public Health Service, the American Heart Association, The Helen Hay Whitney Foundation, and the Minnesota Heart Association.

‡ Markle Scholar in Medical Sciences.

iected skin area. In subsequent experiments, it was observed that a single intravenous injection of meningococcal or *S. marcescens* toxin in cortisone-treated rabbits resulted in bilateral cortical necrosis of the kidneys (7). The nephropathy resembled that of the "generalized Shwartzman reaction," described by Apitz (8) and others (9, 10), which is produced in rabbits when *two* successive intravenous injections of toxin are given 24 hours apart.

The present paper is concerned with an extension of the foregoing studies, and will deal with the following matters: (1) The effect of cortisone on the dermal Shwartzman reaction and, particularly, the question as to its inhibitory effect, (2) the nature of the "primary" hemorrhagic and necrotizing lesions caused by toxin in the skin and kidney tissues of cortisone-treated rabbits, and (3) the relation of the skin and kidney lesions to those encountered in, respectively, the local and generalized Shwartzman reactions. The data to be presented indicate that cortisone does not inhibit the Shwartzman phenomenon; on the contrary, it creates a state of affairs which is equivalent to a stage in the development of the phenomenon. It will be shown that the skin and kidney lesions produced by toxin in cortisone-treated rabbits are similar in several important respects to the local and generalized Shwartzman reactions.

Material and Methods

Rabbits.—Hybrid male and female white rabbits, weighing approximately 1 to 1.5 kilos, were obtained from a single breeder throughout the course of the experiments. They were maintained on a diet of purina rabbit pellets.

Bacterial Toxins.—Meningococcal endotoxin derived from agar washings and prepared by the method of Shwartzman (10) was obtained through the kindness of Dr. Gregory Shwartzman, of the Mount Sinai Hospital, New York. A highly purified polysaccharide toxin from cultures of *Serratia marcescens* (11) was generously supplied by Dr. Murray Shear of the National Cancer Institute.

Cortisone was contributed by Merck and Co., Inc., for all the studies to be reported. The crystalline material was dissolved in ether, dried, and suspended in physiological salt solution in a concentration of 25 mg. per cc. Repeated cultures of the suspended material revealed no viable microorganisms.

EXPERIMENTAL

I. The Effect of Cortisone on the Local Skin Reaction to Bacterial Toxin

Ten rabbits were given a daily intramuscular injection of 25 mg. of cortisone for 4 days. On the 3rd day each rabbit received an intradermal injection of 0.2 mg. of *S. marcescens* polysaccharide toxin in 0.5 cc. physiological saline solution, in the shaven abdominal skin. On the following day, 4 hours after the injection of cortisone, each rabbit was given an intravenous injection of 0.2 mg. of toxin. At the same time, 10 untreated rabbits were similarly prepared and challenged. The results are shown in Table I.

Nine of the 10 rabbits in each group developed typical Shwartzman reactions within 3 hours following the intravenous injection of toxin, and no significant differences in the extent of hemorrhagic necrosis were discernible between the treated and untreated animals.

The effect of a larger dose of cortisone was studied in the Shwartzman reaction with meningococcal toxin, by a method similar to that described by Shwartzman *et al.* (5).

Twelve rabbits were prepared by an intradermal injection of 0.25 cc. of meningococcal agar washing filtrate, in a dilution of 1–2, and challenged 22 hours later by an intravenous injection of 2 cc. of a 1–10 dilution of the same material. Six of these animals were given an intramuscular injection of 60 mg. of cortisone 2 hours prior to the intravenous administration of toxin. All 12 animals developed the Shwartzman reaction (Table I).

It was concluded that cortisone, in the relatively large doses employed, does not inhibit the Shwartzman reaction produced by *S. marcescens* or meningococcal toxin.

TABLE I

The Effect of Cortisone on the Dermal Shwartzman Reaction

Cortisone	Toxin		No. of rabbits	No. with positive reactions
	Skin preparation*	Provocation‡		
25 mg. daily for 4 days	*S. marcescens* on 3rd day	*S. marcescens* on 4th day	10	9
None	*S. marcescens*	*S. marcescens*	10	9
60 mg. 2 hrs. before provocation	Meningococcal	Meningococcal	6	6
None	Meningococcal	Meningococcal	6	6

* Dosage for preparation: *S. marcescens*—0.2 mg., meningococcal—0.25 cc. of 1–2 dilution.
‡ Dosage for provocation: *S. marcescens*—0.2 mg., meningococcal—2 cc. of 1–10 dilution.

The Primary Reaction to Intradermal Toxin in Cortisone-Treated Rabbits.—In rabbits which were pretreated for 3 days with 25 mg. of cortisone daily, no visible reaction at the injected skin site could be demonstrated during the first 12 hours after an intradermal injection of *S. marcescens* or meningococcal toxin. In contrast, local edema and erythema became conspicuous in untreated rabbits during this period. Approximately 18 hours after injection of either toxin, a circular area of pallor was visible at the site of injection in the cortisone-treated rabbits. During the next 8 hours, if the customary intravenous injection of toxin was withheld, small petechiae appeared throughout this area; in some animals these hemorrhages became coalescent, assuming the appearance of mild Shwartzman reactions. As is shown in Table II, this lesion occurred in the majority of cortisone-treated rabbits but not in any of 40 untreated control animals. The gross appearance of typical primary skin lesions is illustrated in Fig. 1.

A comparison of the histological appearance of the injected skin area in cortisone-treated and untreated rabbits was made in tissues removed 24

hours after the injection of *S. marcescens* toxin. In the untreated animals, the skin showed the characteristic changes of tissue prepared for the Shwartzman reaction, consisting of dense, diffuse infiltrations by inflammatory cells, chiefly polymorphonuclear leukocytes. The small blood vessels showed no evidence of damage, and hemorrhage was not present (Fig. 2). In the cortisone-treated rabbits, the skin showed a milder degree of inflammatory cell infiltration, but many of the small vessels were filled by thrombi apparently composed of leukocytes and platelets. These vascular lesions (Fig. 3) were strikingly similar to the changes observed by Stetson (4) in rabbit skin tissue at the time of occurrence of the Shwartzman reaction. In addition, as in the Shwartzman reaction, many areas of hemorrhage and necrosis of cellular elements were encountered.

TABLE II

Incidence of Primary Reactions to Bacterial Toxin in Skin of Cortisone-Treated Rabbits

Cortisone	Toxin*	No. of rabbits	Hemorrhagic skin reactions‡
25 mg. daily for 4 days	*S. marcescens*, 0.2 mg.	4	4
	S. marcescens, 0.4 mg.	10	9
	Meningococcal§	10	8
None	*S. marcescens*, 0.2 mg.	20	0
	S. marcescens, 0.4 mg.	10	0
	Meningococcal§	10	0

* Toxin injected intradermally on 3rd day of cortisone treatment.

‡ Figures refer to number of rabbits with skin lesions 24 hours after injection of toxin. See text for description of positive reactions.

§ Dosage of meningococcal toxin: 0.25 cc. of 1–2 dilution.

The primary hemorrhagic reaction to toxin in cortisone-treated rabbits, once established, did not interfere with the further development of typical confluent Shwartzman reactions when these animals were subsequently challenged with intravenous injections of bacterial toxin.

As an example, 6 rabbits were given 25 mg. of cortisone for 4 days and injected intradermally with 0.2 mg. of *S. marcescens* toxin on the 3rd day. Twenty hours later discrete petechial hemorrhages were visible in the prepared skin of each rabbit, and at this time the same amount of toxin was injected intravenously. Typical deep blue Shwartzman reactions developed at each prepared site within the next 2 hours.

The capacity of cortisone to bring about enhancement of infection by a variety of bacteria and viruses has been well established (12). The possibility that the primary hemorrhagic skin reactions to toxin might be due to provocation of the Shwartzman reaction by a cortisone-induced blood stream infection was therefore considered. Blood cultures taken during the course of

these experiments in approximately 30 rabbits, on the 3rd and 4th days of treatment with cortisone by the dosage schedules described above, were negative in all instances. It appeared unlikely, therefore, that superimposed bacterial infection was a cause of the observed skin lesions.

Properties of Prepared Skin Tissue in Cortisone-Treated Rabbits.—It was previously observed that skin tissue which is prepared for the Shwartzman reaction by a local injection of toxin is less permeable to intravenously injected dyes than normal skin (13). In this respect the prepared skin differs from other types of local inflammatory reaction in which, according to Menkin (14), the permeability to dye is increased. Experiments were designed to determine whether any change in this property of prepared skin was brought about by cortisone.

Six rabbits were given 25 mg. of cortisone intramuscularly each day for 4 days, and on the 3rd day an intradermal injection of *S. marcescens* toxin was given in the abdominal skin. At the same time, 6 untreated rabbits were similarly prepared with toxin. Twelve hours later each of the rabbits received an intravenous injection of 5 cc. of 1 per cent trypan blue. In the control animals, the dye caused deep blue staining in all skin areas except for the zone of erythema and edema at the site of injection. In the cortisone-treated rabbits, although no gross change was visible at the injected site prior to the administration of trypan blue, staining failed to occur in an area of approximately the same size as that observed in the untreated rabbits.

This observation indicated that the relative impermeability to circulating dye which characterizes the prepared skin in normal rabbits is also present in cortisone-treated animals despite the absence of erythema or edema.

Earlier investigations (2, 3) showed that a characteristic feature of prepared skin tissue is a significant increase in the local concentration of lactic acid, with a concomitant increase in the aerobic production of lactic acid by skin slices *in vitro*. In order to determine whether cortisone had any effect on this property of prepared skin, the following experiment was carried out.

Six rabbits were given an intradermal injection of 0.4 mg. of *S. marcescens* toxin in one side of the abdomen. Twenty hours later 3 of the animals were injected intramuscularly with 50 mg. of cortisone. Four hours after the injection of cortisone the animals were sacrificed and samples of skin from the prepared areas and from normal abdominal skin were removed. The content of lactic acid in each sample and the degree of aerobic production of lactic acid *in vitro* were studied by the methods previously described (2). The results are summarized in Table III.

Each of the prepared skin samples showed an increased content of lactic acid, compared with normal skin tissue from the same animals, and no significant differences between the cortisone-treated and control animals were demonstrable. Moreover, all the prepared skin samples exhibited an increased rate of aerobic lactic acid formation *in vitro*, and although wide variations in the degree of increase were observed there was no effect attributable to cortisone.

Inhibition by Nitrogen Mustard of the Primary Skin Reaction in Cortisone-Treated Rabbits.—It was shown by Becker (15) and Stetson and Good (3) that the dermal Shwartzman reaction is inhibited by the administration of nitrogen mustard (HN_2), in a dosage of 1.5 mg. per kilo 3 days before preparation of the skin. According to the latter workers, the inhibitory effect was only demonstrable during the period of polymorphonuclear leukopenia caused by HN_2. The direct relationship of inhibition to leukopenia was shown by applying a clamp to the abdominal aorta at the time of injection of HN_2,

TABLE III

Aerobic Lactic Acid Formation in Prepared and Normal Skin Samples from Cortisone-Treated Rabbits

Cortisone	Rabbit No.	Preformed lactic acid*			*In vitro* lactic acid production‡		
		Normal skin	Prepared skin	Increase in lactic acid	Normal skin	Prepared skin	Increase in lactic acid
				per cent			*per cent*
50 mg., 4 hrs. before removal of skin samples	541	0.8	1.6	100	0.4	0.95	138
	542	0.7	1.4	100	0.3	1.0	233
	543	0.9	1.1	22	0.1	1.25	1150
None	538	0.9	1.3	44	0.1	1.5	1400
	539	0.9	1.3	44	0.2	1.15	475
	540	0.7	1.1	57	0.7	1.0	43

* Lactic acid determined by Barker-Summerson method in sample of skin tissue placed in 5 per cent trichloracetic acid immediately after death. Results are expressed as milligrams per gram wet weight of tissue.

‡ Samples of tissue were placed in Krebs-Ringer-phosphate solution containing 0.2 per cent glucose, in Warburg vessels gassed with 100 per cent oxygen, and agitated at 37°C. for 1 hour. Trichloracetic acid added and lactic acid determined as above. Results expressed as difference between amount of preformed lactic acid and amount in tissue after 1 hour incubation, in milligrams per gram wet weight.

thus protecting the femoral marrow and preventing subsequent leukopenia; this procedure eliminated the inhibitory effect of HN_2 on the Shwartzman reaction (3). In view of the gross and microscopic similarity between the Shwartzman reaction and the primary hemorrhagic reaction observed in cortisone-treated rabbits, it was of interest to study the effect of HN_2 on the latter phenomenon.

Cortisone was given to 17 rabbits in a daily dose of 25 mg. for 4 days. On the first day, 11 of the animals received intravenous HN_2, 1.5 mg. per kilo. The femoral marrow was excluded by a clamp on the lower abdominal aorta in 5 of these rabbits at the time of injection. On the 3rd day an intradermal injection of 0.4 mg. of *S. marcescens* toxin was given to all 17 rabbits. At the end of the 4th day the skin at the injected site was examined for hemorrhages.

The results of this experiment, summarized in Table IV, indicated that the primary hemorrhagic reaction to toxin in cortisone-treated rabbits is, like the Shwartzman reaction, completely inhibited by nitrogen mustard. No skin lesions were demonstrable in the 6 animals which received HN_2 without having had the aorta clamped, as contrasted with the occurrence of hemorrhagic reactions in all 6 untreated controls. Two of the 5 animals in which the aorta was clamped at the time of injection of HN_2 exhibited dermal hemorrhages, indicating partial interference with the action of HN_2 by this procedure.

Severe polymorphonuclear leukopenia occurred in each of 6 rabbits on the 3rd day after the injection of HN_2, while the animals in which the aorta was

TABLE IV

The Effect of Nitrogen Mustard on the Primary Skin Reaction to Toxin in Cortisone-Treated Rabbits

Experimental procedure*	No. of PMN's‡ per c. mm. blood (mean)		No. of rabbits	Number with primary skin reaction
	Before experiment	At time of injection of toxin		
Cortisone for 4 days, toxin on 3rd day	2720	3500	6	6
Cortisone for 4 days, HN_2 on 1st day, toxin on 3rd day	1932	440	6	0
Cortisone for 4 days, HN_2 on 1st day with aorta clamped, toxin 3rd day	2075	3302	5	2

* See text for details of procedure.

‡ Numbers refer to the mean polymorphonuclear leukocyte counts in the rabbits of each group at the indicated time.

clamped at the time of injection showed normal leukocyte counts (Table IV). The correlation between leukopenia and protection against skin hemorrhage resembled that observed by Stetson and Good (3) in the Shwartzman reaction.

II. The Effect of Cortisone on the Reaction to an Intravenous Injection of Toxin

The dermal reaction to locally injected bacterial toxin in cortisone-treated rabbits was similar to the local Shwartzman phenomenon in its gross appearance, in the microscopic evidence of thrombosis and hemorrhagic necrosis, and in its inhibition by nitrogen mustard. The systemic counterpart of the dermal Shwartzman reaction is the generalized Shwartzman reaction (8–10), which is characterized by bilateral cortical necrosis of the kidneys. This reaction occurs following two successive intravenous injections of bacterial toxin, spaced 24 hours apart. Experiments were undertaken to determine

whether an analogous renal lesion could be produced in cortisone-treated rabbits by a single intravenous injection of toxin.

Rabbits weighing between 1 and 1.5 kilos were given an intramuscular injection of 25 mg. of cortisone each day for 4 days. On the 3rd day an intravenous injection of 0.4 mg. of Shear's *S. marcescens* toxin was administered. Within the next 24 hours the majority of animals became obviously ill, with extreme weakness and varying degrees of respiratory distress. Approximately 50 per cent died during this period, and the remainder were sacrificed at the end of 30 hours. At autopsy, bilateral cortical necrosis of the kidneys was present in 70 per cent of the rabbits (Table V), with accompanying hemorrhagic lesions of the lungs, spleen, liver, and gastrointestinal tract in most instances. Similar results were obtained in cortisone-treated rabbits with a single intravenous injection of meningococcal toxin, in a dose of 2 cc. of a 1-10 dilution.

In 50 control animals given the same amounts of cortisone without toxin, and in 150 rabbits given *S. marcescens* toxin without cortisone, no gross or microscopic renal lesions were observed (Table V). In order to test the possibility that the generalized Shwartzman reaction may have been provoked by superimposed spontaneous infections in the animals

TABLE V

Occurrence of Bilateral Renal Cortical Necrosis after a Single Injection of S. marcescens Toxin in Cortisone-Treated Rabbits

Experimental procedure*	No. of rabbits	No. with bilateral renal cortical necrosis
25 mg. cortisone daily for 4 days, 0.4 mg. *S. marcescens* toxin i.v. on 3rd day	100	70
25 mg. cortisone daily for 4 days, no toxin	50	0
0.4 mg. *S. marcescens* toxin, no cortisone	150	0

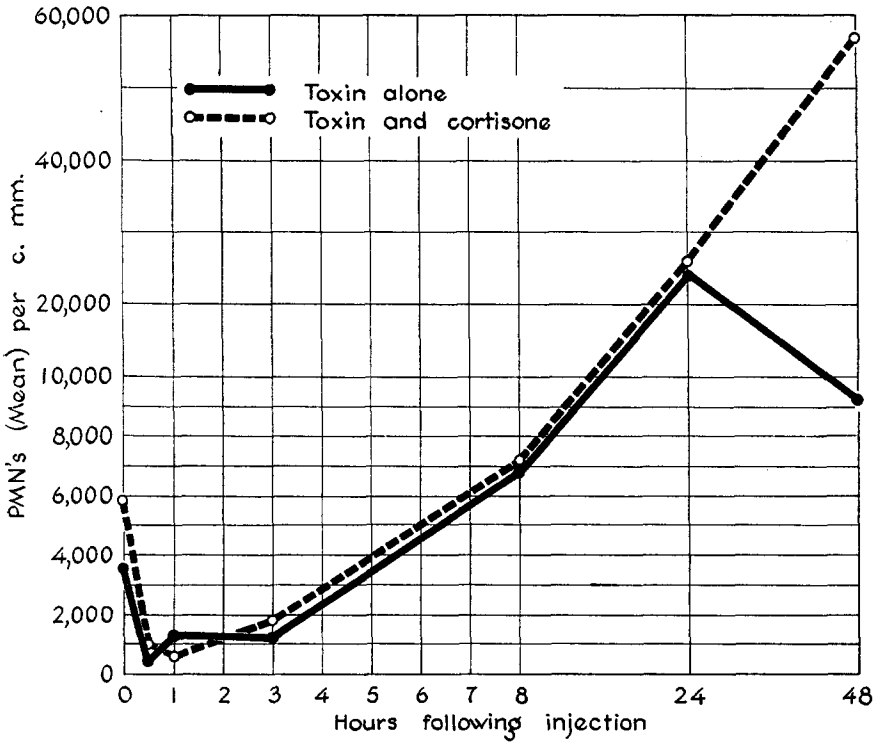
* See text for details of procedure.

receiving both cortisone and toxin, cultures of the blood and kidney tissue were made in 12 rabbits with advanced renal cortical necrosis. No microorganisms were recovered in any instance.

The gross appearance of the kidney lesions resulting from the procedures just described is illustrated in Fig. 4. A striking feature was the apparent limitation of hemorrhage and necrosis to the renal cortex; extension of hemorrhage into the medulla was observed in only a few instances. Microscopically (Fig. 5), the most conspicuous findings were widespread parenchymal hemorrhage and necrosis of glomeruli and tubules within the cortex. All the glomeruli within or adjacent to the affected areas showed accumulations of homogeneous eosinophilic material in glomerular capillaries, which often filled the entire glomerular tuft (Figs. 6 to 7). This material was stained deeply by the Hotchkiss adaptation of the Schiff reaction with periodic acid. Other histochemical studies, to be described elsewhere (16), suggest that it may be a mucopolysaccharide. Neither platelets, leukocytes, nor erythrocytes could be demonstrated in the occluded glomerular capillaries. In some of the kidneys with

far advanced renal necrosis, thrombi composed of leukocytes and platelets were seen in afferent glomerular arterioles; such thrombi were not observed in kidneys with mild or early cortical necrosis.

Stetson (4) has presented evidence indicating that an intravenous injection of meningococcal toxin is followed by a marked decrease in the number of



TEXT-FIG. 1. The effect of a single intravenous injection of *S. marcescens* toxin on the circulating polymorphonuclear leukocytes in normal rabbits, and in rabbits treated for 3 days with 25 mg. cortisone daily. The mean cell count in 3 rabbits is represented by each point on the graph. The two groups showed similar changes during the first 24 hours after the injection of toxin. The cortisone-treated rabbits, which developed renal cortical necrosis, showed increasing polymorphonuclear leukocytosis during the 2nd day.

circulating polymorphonuclear leukocytes, which begins within less than an hour after injection and reaches a maximum at about the time of the development of the dermal Shwartzman reaction. In order to determine whether any alteration in this hematological response occurs in cortisone-treated rabbits, total and differential leukocyte counts were made in 3 normal and 3 cortisone-treated animals before and at varying intervals after a single intravenous injection of toxin. The results are illustrated in Text-fig. 1. Within 30 minutes

after the intravenous injection, both groups showed a sharp drop in the number of polymorphonuclear leukocytes which persisted for 3 hours and was then followed by a rise to a higher level than normal at the end of 24 hours. During this time no significant differences were discernible between the leukocyte response to toxin in the cortisone-treated and normal rabbits. During the second 24 hours after the injection of toxin the leukocyte counts in the untreated animals returned to normal, while the cortisone-treated animals which developed renal cortical necrosis exhibited a progressive increase in the number of polymorphonuclear leukocytes up to the time of death.

Resemblance of Renal Necrosis Following Toxin in Cortisone-Treated Animals to the Generalized Shwartzman Phenomenon.—The generalized Shwartzman reaction was produced in the following manner. Rabbits were given an intravenous injection of 0.4 mg. of Shear's *S. marcescens* toxin, and 24 hours later this was repeated. Within the following 30 hours the majority of animals died, and more than 75 per cent were found to have bilateral cortical necrosis of the kidneys. The gross and microscopic appearance of the kidneys are illustrated in Figs. 8–11. The lesions were indistinguishable from those produced by a single injection of toxin in cortisone-treated rabbits. Hemorrhages were also observed in the lungs, liver, spleen, and gastrointestinal mucosa; the intestinal hemorrhages were usually more extensive than those in the cortisone-treated group. Histologically, the kidney lesions were identical with those observed in the cortisone-treated animals after a single intravenous injection of toxin. The renal necrosis was generally sharply confined to the cortices, and the capillaries of the glomerular tufts were filled with similar homogeneous eosinophilic material which was stained deeply by the Hotchkiss method. Hematological studies revealed no differences in the degree of leukopenia produced by intravenous toxin in the two groups. The animals receiving two successive intravenous injections exhibited a sharp fall in the number of circulating polymorphonuclear leukocytes after each dose of toxin, followed by a subsequent rise to levels higher than normal in the animals which developed renal necrosis.

The Effect of Nitrogen Mustard on Bilateral Renal Cortical Necrosis.—In view of the previous finding that nitrogen mustard prevents the dermal Shwartzman reaction, the effect of this substance on the incidence of bilateral renal cortical necrosis in the generalized Shwartzman reaction was studied.

Twenty-one rabbits were given two successive injections of 0.2 mg. of *S. marcescens* toxin with an intervening interval of 24 hours. Of these 16, or 76 per cent, developed bilateral renal cortical necrosis. At the same time, 10 animals which had received 1.5 mg. of HN_2 3 days earlier were similarly treated. None of the latter rabbits developed renal lesions, indicating complete protection by HN_2 against the generalized Shwartzman reaction. Five rabbits were given the same amount of HN_2 with a clamp applied over the lower abdominal aorta at the time of injection, and toxin was injected intravenously on the 3rd and 4th days respectively. Four of these rabbits developed renal cortical necrosis, indicating that clamp-

ing of the aorta interfered with the protective action of HN_2. Another group of 6 rabbits was similarly treated with HN_2 and a clamp was applied to the left renal artery at the time of injection of mustard; renal necrosis did not occur in either kidney in these rabbits, indicating that exclusion of the kidney from the effect of HN_2 did not provide any interference with the protective action of the latter substance. The results are summarized in Table VI.

TABLE VI

The Effect of Nitrogen Mustard on the Occurrence of Bilateral Renal Cortical Necrosis in the Generalized Shwartzman Reaction

Group	Experimental procedure*	No. of rabbits	No. with bilateral renal cortical necrosis
I	2 i.v. injections of 0.2 mg. *S. marcescens* toxin, 24 hrs. apart	21	16
II	As in (I), with HN_2 3 days before 1st injection of toxin	10	0
III	As in (II), with aorta clamped at time of injection of HN_2	5	4
IV	As in (II), with left renal artery clamped at time of injection of HN_2	6	0

* See text for details of procedure.

TABLE VII

The Effect of Nitrogen Mustard on the Occurrence of Bilateral Renal Cortical Necrosis after a Single Injection of S. marcescens Toxin in Cortisone-Treated Rabbits

Group	Experimental procedure*	No. of rabbits	No. with bilateral renal cortical necrosis
I	25 mg. cortisone daily for 4 days, 0.2 mg. *S. marcescens* toxin i.v. on 3rd day	33	21
II	As in (I), with HN_2 on 1st day	17	0
III	As in (II), with aorta clamped at time of injection of HN_2	36	8
IV	As in (II), with mock operation at time of injection of HN_2	8	0

* See text for details of procedure.

The occurrence of bilateral renal cortical necrosis in cortisone-treated animals, following a single injection of bacterial toxin, was also prevented by the administration of HN_2 in the same fashion as in the rabbits subjected to the generalized Shwartzman reaction. As is shown in Table VII, of 17 cortisone-treated rabbits which were given an injection of HN_2 3 days prior to toxin, none developed renal necrosis. When the abdominal aorta was clamped in 36 rabbits at the time of injection of nitrogen mustard, 8, or 22 per cent of the animals developed renal necrosis. In 8 animals which were subjected to a

mock operation at the time of injection of HN_2, with a clamp placed on the abdominal aorta but released before the HN_2 injection, no interference with the protective action of HN_2 was demonstrable.

In summary, the injection of nitrogen mustard prior to the administration of two successive intravenous injections of bacterial toxin, or prior to a single injection of toxin in cortisone-treated rabbits, prevented the occurrence of bilateral renal cortical necrosis. The protective effect of HN_2 was inhibited by clamping of the aorta at the time of its administration, although the cortisone-treated animals showed less complete inhibition of protection than the group receiving two intravenous injections of toxin. No explanation is available for the difference between the two groups in this respect. It is noteworthy that a similarly incomplete degree of inhibition was observed in the experiments dealing with the primary hemorrhagic reaction of skin to toxin in cortisone-treated rabbits (Table IV).

Failure to Provoke Renal Cortical Necrosis with Non-Bacterial Materials.— Provocation of the local Shwartzman reaction in the skin has been accomplished by the intravenous injection of a variety of non-bacterial materials, including tissue extracts (17), glycogen (18), and kaolin (2), and, in sensitized rabbits, foreign protein antigens (10). These materials were tested for the capacity to produce renal necrosis in cortisone-treated rabbits and in animals which were prepared for the generalized Shwartzman reaction by a preceding intravenous injection of *S. marcescens* toxin.

Rabbits were treated for 4 days with 25 mg. of cortisone daily and, on the 3rd day, groups of 3 each were given intravenous injections of (1) 5 cc. of a 10 per cent suspension of normal rabbit liver in physiological saline, (2) 2 cc. of a 1 per cent suspension of kaolin, and (3) 10 cc. of a 1 per cent suspension of rabbit liver glycogen. Six animals which had been sensitized to egg white 14 days previously were injected intravenously with 1 cc. of a 25 per cent suspension of egg white in saline on the 3rd day of cortisone treatment. Renal lesions did not occur in any of the animals in these groups.

The same materials were tested as provoking agents in animals which had received, 24 hours previously, an intravenous injection of 0.4 mg. of *S. marcescens* toxin and were thus prepared for the generalized Shwartzman reaction. None of the rabbits developed renal lesions or any other gross tissue lesions. In separate experiments it was shown that each of the substances was capable of producing the local Shwartzman reaction in rabbits prepared by intradermal injections of toxin. The results indicated, therefore, that the generalized Shwartzman reaction and the renal necrosis of cortisone-treated animals resemble each other, and differ from the local Shwartzman reaction, in the failure of provocation by these non-bacterial materials.

III. The Absorption of Toxin from the Skin in Cortisone-Treated Rabbits

One possible explanation for the hemorrhagic skin and renal lesions occurring after a single injection of toxin in cortisone-treated rabbits is that these ani-

mals may have a reduced capacity to localize toxin or remove it from the blood, with the result that their tissues are exposed to recirculating toxin for a longer period than normal animals. If this were the case, an intradermal injection of toxin capable of bringing about local preparation of the skin might be followed by the absorption of a sufficient amount of toxin to take the place of a challenging injection, with local dermal hemorrhage and necrosis as the result. Similarly, intravenously injected toxin would not only act as the preparing stimulus for the generalized Shwartzman reaction but might also, on recirculation, provoke the reaction.

Little is known concerning the mechanisms in the normal animal which are responsible for the removal of bacterial toxin from the blood. It is possible that cells of the reticuloendothelial system may be mainly involved, in view of the observation by Beeson (19) that blockade of this system by thorotrast results in an increased susceptibility to the Shwartzman reaction and a decrease in acquired resistance to the pyrogenic effect of bacterial toxins. The susceptibility of lymphoid tissues to hemorrhagic necrosis in the local and generalized Shwartzman reaction (10) suggests that localization of toxin may occur in such tissues. It is known that cortisone causes dissolution and atrophy of lymphoid tissue (20), and conceivably this might result either in a delayed removal of circulating toxin or a subsequent discharge of absorbed toxin from lymphoid tissues undergoing destruction.

No direct approach to this problem in the present study appeared feasible, in the absence of satisfactory methods for determining the amounts of toxin in blood or tissues. As an indirect method, the effect of cortisone on the retention of toxin in the skin was studied. In normal rabbits there is evidence that the bacterial toxins employed for provocation of the Shwartzman reaction are absorbed only to a small extent, if at all, when injected intradermally (10). Thus, when an intradermal injection of highly concentrated meningococcal or *S. marcescens* toxin is given 24 hours after skin preparation, the Shwartzman reaction does not occur, although much smaller amounts of toxin provoke the reaction when injected intravenously. Moreover, rabbits exhibit no evidence of systemic intoxication following intradermal injections of quantities of toxin which would be rapidly lethal if given intravenously.

The optimal amount of intravenously injected *S. marcescens* toxin required to produce renal necrosis in cortisone-treated rabbits was found to be 0.4 mg. per rabbit. Doses of 0.2 mg. or less failed to produce the lesion in more than a small percentage of rabbits. Considerably smaller doses, in untreated rabbits, produced the generalized Shwartzman reaction when two intravenous injections were given 24 hours apart; renal necrosis occurred consistently following two successive injections of 0.05 mg. each. With this information a method was available for comparing the degree of absorption of intradermally injected toxin in cortisone-treated and untreated rabbits, and the following experiment was carried out.

Eight rabbits were given 25 mg of cortisone daily for 3 days, and on the 3rd day each received an intradermal injection of 0.4 mg. of *S. marcescens* toxin. Eight untreated rabbits were prepared for the generalized Shwartzman reaction by an intravenous injection of 0.4 mg. of toxin; 24 hours later 4 were given 0.4 mg. intravenously, and 4 received the same dose intradermally. Four additional untreated rabbits received two intradermal injections of 0.4 mg. of toxin 24 hours apart.

The outcome is indicated in Table VIII. Seven of the cortisone-treated rabbits developed renal necrosis within 30 hours after the intradermal injection of toxin. Extensive renal lesions occurred in each of 4 untreated rabbits when this amount of toxin was given twice by the intravenous route, but no lesions were produced when the first dose was given intravenously and the second intradermally, or when both were given intradermally.

The results indicate that absorption of toxin from the skin occurred in the cortisone-treated rabbits, to the extent that intradermally injected toxin was

TABLE VIII

Bilateral Renal Cortical Necrosis after a Single Intradermal Injection of S. marcescens Toxin in Cortisone-Treated Rabbits

Group	Experimental procedure*	No. of rabbits	No. with bilateral renal cortical necrosis
I	25 mg. cortisone daily for 4 days, 0.4 mg. *S. marcescens* toxin intradermally on 3rd day	8	7
II	2 i.v. injections of 0.4 mg. *S. marcescens* toxin, 24 hrs. apart. No cortisone	4	4
III	0.4 mg. *S. marcescens* toxin i.v., followed 24 hrs. later by 0.4 mg. intradermally. No cortisone	4	0
IV	2 intradermal injections of *S. marcescens* toxin, 24 hrs. apart. No cortisone	4	0

* See text for details of procedure.

capable of producing the same degree of renal damage as a similar amount of toxin given by vein. Since the dose of toxin employed (0.4 mg.) was not in excess of the amount previously shown to be required for the consistent production of renal necrosis in cortisone-treated rabbits when given intravenously, it can be assumed that a considerable proportion of toxin was absorbed.

In contrast, the animals which did not receive cortisone showed no evidence of absorption of toxin from the skin. Although the generalized Shwartzman reaction was regularly produced when the second injection of toxin was given intravenously, no renal lesion occurred when it was injected into the skin. It is of interest that the amount of toxin employed for intradermal injection in these animals was at least eight times greater than the dose known to be effective in provocation of the generalized Shwartzman reaction when given intravenously.

On the basis of these observations, it is suggested that cortisone may interfere with the capacity of skin tissue to localize or retain injected toxin. The bearing of this on the mechanism of dermal and renal necrosis will be discussed in the section which follows.

DISCUSSION

These studies were the outcome of experiments originally designed to determine whether the dermal Shwartzman reaction could be prevented by cortisone. The amounts of bacterial toxin employed were sufficiently large so that positive reactions were obtained in approximately 90 per cent of the untreated controls. The doses of cortisone were also large; it is reasonable to assume that 25 mg. daily in rabbits weighing between 1 and 1.5 kilos, with a pretreatment period of 3 days before testing, constitutes a maximum therapeutic regime when compared with the amounts of cortisone required for other biological effects in animals. Under these conditions, no inhibition of the Shwartzman reaction by cortisone could be demonstrated.

Instead of causing inhibition of the Shwartzman reaction, cortisone treatment produced hemorrhagic skin lesions at the site of injection of bacterial toxin without the agency of the intravenous or "provocative" injection of toxin. These lesions bore some resemblance to the dermal Shwartzman reaction in their gross appearance, although the hemorrhage was less extensive and confluent. Histologically, numerous thrombi composed of platelets and leukocytes were seen in the small vessels of the skin, similar to the vascular lesions described by Stetson (4) as an early stage in the development of the Shwartzman reaction. The primary skin reaction in cortisone-treated animals was, like the Shwartzman reaction, completely inhibited by nitrogen mustard. Also, as in the Shwartzman reaction, the inhibitory effect of nitrogen mustard was partly reversed when the femoral marrow was protected against the leukopenic effect of mustard.

The systemic counterpart of the dermal Shwartzman reaction is produced by two intravenous injections of bacterial toxin, spaced 24 hours apart. According to Apitz (8), Gerber (9), and others the cardinal pathological feature of the generalized Shwartzman reaction is bilateral cortical necrosis of the kidneys. The only circumstance in which this lesion has been described in rabbits after a single injection of such toxin is during pregnancy (21, 10).

A single intravenous injection of either meningococcal or *S. marcescens* toxin produced bilateral renal cortical necrosis in rabbits which were treated with cortisone for 3 days prior to the injection. The kidney lesion was in the gross indistinguishable from that observed in rabbits subjected to the generalized Shwartzman reaction by two intravenous injections of toxin. Histologically, both showed hemorrhage and necrosis of all cellular elements in irregular zones within the cortex. In both the glomerular capillaries appeared

to be filled with homogeneous, eosinophilic material which stained deeply in Schiff periodic acid preparations. Hemorrhages and areas of necrosis were also observed in the lungs, spleen, liver, and gastrointestinal tract in the cortisone-treated rabbits; similar lesions were encountered in rabbits in which the generalized Shwartzman reaction had been produced by two intravenous injections of toxin.

The resemblance between the two phenomena was further substantiated by the finding that nitrogen mustard provided complete protection against both. In each case the protective effect of mustard was associated with the polymorphonuclear leukopenia caused by this substance, and when leukopenia was prevented by clamping the aorta at the time of administration, protection against renal necrosis did not occur.

From the above observations it seems reasonable to conclude that there is a close similarity, if not identity, between the skin and kidney lesions produced by a single injection of toxin in cortisone-treated rabbits and the lesions of the local and generalized Shwartzman phenomena. In order to account for this effect of cortisone, the following possibilities must be considered.

1. Cortisone treatment may have induced a systemic bacterial or virus infection which was capable of provoking the Shwartzman reaction. There is ample experimental evidence for the enhancing effect of cortisone on infection by a variety of agents, and spontaneous septicemia is known to occur during cortisone treatment (12). Although cultures of the blood and kidney tissue of animals with renal necrosis were negative, the possibility of a transient, undetected bacteremia during cortisone treatment cannot be ruled out. It seems unlikely, however, that this event occurred with sufficient regularity to bring about the generalized Shwartzman reaction in over 70 per cent of animals.

2. Cortisone treatment may have increased the susceptibility of skin and kidney tissue to direct, primary damage by the toxins employed. There is evidence that cortisone causes impairment of the inflammatory response of tissues to various types of irritants (22), and it is conceivable that inflammation may have a protective effect against bacterial toxin. If this were the case, it might be expected that similar lesions would occur following a single injection of toxin in animals treated with nitrogen mustard prior to the injection of the toxin, since here also the inflammatory response at the site of injection is much diminished (3), but such lesions have not been observed.

3. Cortisone treatment may have reduced the capacity of tissue cells, especially those of the lymphoid system, to remove toxin from the circulating blood or to retain removed toxin. It is known that continuing dissolution of lymphoid cells occurs during cortisone treatment with doses as large as were employed in these experiments (20). It is possible that such cells may be involved in the removal and binding of endotoxins from Gram-negative organisms; this is suggested by the observation of Beeson (19) that large amounts of injected thorotrast will increase the susceptibility of rabbits to such toxins.

The amount of *S. marcescens* toxin which is required to produce renal cortical necrosis in cortisone-treated animals is more than four times greater than the amount which will consistently cause this lesion in untreated rabbits when given in two doses, 24 hours apart. It is possible that either a failure to remove toxin from the blood or a subsequent release of toxin into the blood might accomplish the same end-result in cortisone-treated animals as is accomplished by two separate injections of toxin in rabbits developing the generalized Shwartzman reaction.

An observation which may be of significance in this regard was made in cortisone-treated rabbits given a relatively large quantity (0.4 mg.) of *S. marcescens* toxin in the skin. In untreated animals this amount of toxin produces local edema and erythema but does not cause systemic evidences of intoxication, nor does it provoke the local or generalized Shwartzman reaction in appropriately prepared animals. Following 3 days of treatment with cortisone, a single intradermal injection of 0.4 mg. of toxin resulted in bilateral renal cortical necrosis in the majority of rabbits tested. Since the same amount of toxin is required to produce renal necrosis consistently in treated rabbits when given intravenously, the following interpretation is suggested:—

The cortisone-treated animal is lacking in the capacity to retain toxin within the injected skin area, and intradermally injected toxin causes the same systemic effects as when given intravenously. A comparable mechanism, involving internal tissues normally concerned with the removal or fixation of toxin in the blood, may allow the toxin to recirculate for a sufficient length of time so that both preparation and provocation of the generalized Shwartzman reaction can be brought about by a single injection of toxin.

This explanation would also account for the occurrence of the primary hemorrhagic skin reaction in cortisone-treated rabbits at the site of intradermal injection of toxin. At the same time that the skin is undergoing preparation for the local Shwartzman reaction, sufficient toxin may be absorbed into the blood as to act as the provoking stimulus.

The relation between the local and generalized Shwartzman reactions is not entirely clear. Two points of difference became apparent in the present study. The intravenous injection of non-bacterial materials which are capable of provoking the dermal Shwartzman reaction, such as tissue suspensions, glycogen, kaolin, and protein antigen in sensitized rabbits, failed to produce renal lesions in animals prepared by an intravenous injection of toxin 24 hours previously, as well as in cortisone-treated rabbits. Second, the glomerular lesion which occurs early in the course of renal cortical necrosis consists of capillary occlusion by eosinophilic material in which neither platelets nor leukocytes are demonstrable, unlike the plugging of small vessels by formed elements, which, according to Stetson (4) characterizes the beginning of the dermal Shwartzman reaction. The possibility that platelets in the glomerular

capillaries may have disintegrated before the time of removal of the kidneys has been entertained, and further studies on the point are in progress. The ultimate identification of the material in the glomeruli is considered to be a possible key to the mechanism of the generalized Shwartzman reaction.

SUMMARY

1. Cortisone, in a dose of 25 mg. daily and with a pretreatment period of 3 days, in rabbits weighing 1 to 1.5 kilos, did not inhibit the dermal Shwartzman reaction produced by meningococcal or *S. marcescens* toxin.

2. In cortisone-treated rabbits, a single intradermal injection of toxin produced a primary reaction of hemorrhage and necrosis in the skin at the injected site. This lesion resembled the Shwartzman reaction in its gross and histological appearance.

3. Like the Shwartzman reaction, the primary hemorrhagic reaction in cortisone-treated rabbits was prevented by nitrogen mustard, and the preventive effect of nitrogen mustard was partly eliminated when the femoral marrow was protected against the latter agent.

4. A single intravenous injection of meningococcal or *S. marcescens* toxin, in cortisone-treated rabbits, was followed by bilateral cortical necrosis of the kidneys in the majority of instances. The renal lesions, as well as hemorrhages in the lungs, spleen, liver, and gastrointestinal tract, resembled the lesions of the generalized Shwartzman reaction. Histologically, the glomerular capillaries in both types appeared to be occluded by homogeneous, eosinophilic material which showed a strongly positive Schiff reaction.

5. The renal lesion following a single injection of toxin in cortisone-treated animals, and that following two intravenous injections in the generalized Shwartzman reaction, were both completely prevented by nitrogen mustard. This effect of nitrogen mustard was inhibited when the femoral marrow was protected against the latter agent.

6. The injection of *S. marcescens* toxin into the skin of normal rabbits did not cause systemic symptoms, nor was it possible to provoke the generalized Shwartzman reaction by this route. In cortisone-treated rabbits, a similar intradermal injection was regularly followed by the development of bilateral cortical necrosis of the kidneys, indicating that absorption of toxin from the skin occurred in these animals.

7. Possible mechanisms to account for the observations are discussed.

The authors are obliged to Professor James R. Dawson for many helpful suggestions during the course of this investigation.

BIBLIOGRAPHY

1. Shwartzman, G., *Proc. Soc. Exp. Biol. and Med.*, 1928, **25,** 560.
2. Thomas, L., and Stetson, C. A., *J. Exp. Med.*, 1949, **89,** 461.
3. Stetson, C. A., and Good, R. A., *J. Exp. Med.*, 1951, **93,** 49.

4. Stetson, C. A., *J. Exp. Med.*, 1951, **93,** 489.
5. Shwartzman, G., Schneierson, S. S., and Soffer, L. J., *Proc. Soc. Exp. Biol. and Med.*, 1950, **75,** 175.
6. Thomas, L., and Mogabgab, W. J., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74,** 829.
7. Thomas, L., and Good, R. A., *Proc. Soc. Exp. Biol. and Med.*, 1951, **76,** 604.
8. Apitz, K., *Virchows Arch. path. Anat.*, 1934, **293,** 1.
9. Gerber, I. E., *Arch. Path.*, 1936, **21,** 776.
10. Shwartzman, G., Phenomenon of Local Tissue Reactivity, New York, Paul Hoeber, Inc., 1937.
11. Perrault, A., and Shear, M. J., *Cancer Research,* 1949, **9,** 626.
12. Thomas, L., *Ann. Rev. Int. Med.*, 1952, **3,** in press.
13. Thomas, L., and Stetson, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1948, **69,** 409.
14. Menkin, V., Dynamics of Inflammation, New York, The Macmillan Co., 1940.
15. Becker, R. M., *Proc. Soc. Exp. Biol. and Med.*, 1948, **69,** 247.
16. Kane, D., Good, R. A., and Thomas, L., data to be published.
17. Antopol, W., and Glick, D., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38,** 346.
18. Bier, O. G., and do Amaral, J. P., *Men. Inst. Butantan*, 1945, **18,** 33.
19. Beeson, P. B., *Proc. Soc. Exp. Biol. and Med.*, 1947, **64,** 146.
20. White, A., *Harvey Lectures*, 1947–48, **43,** 43.
21. Apitz, K., *J. Immunol.*, 1935, **29,** 255.
22. Michael, M., Jr., and Whorton, C. M., *Proc. Soc. Exp. Biol. and Med.*, 1951, **76,** 754.

EXPLANATION OF PLATES

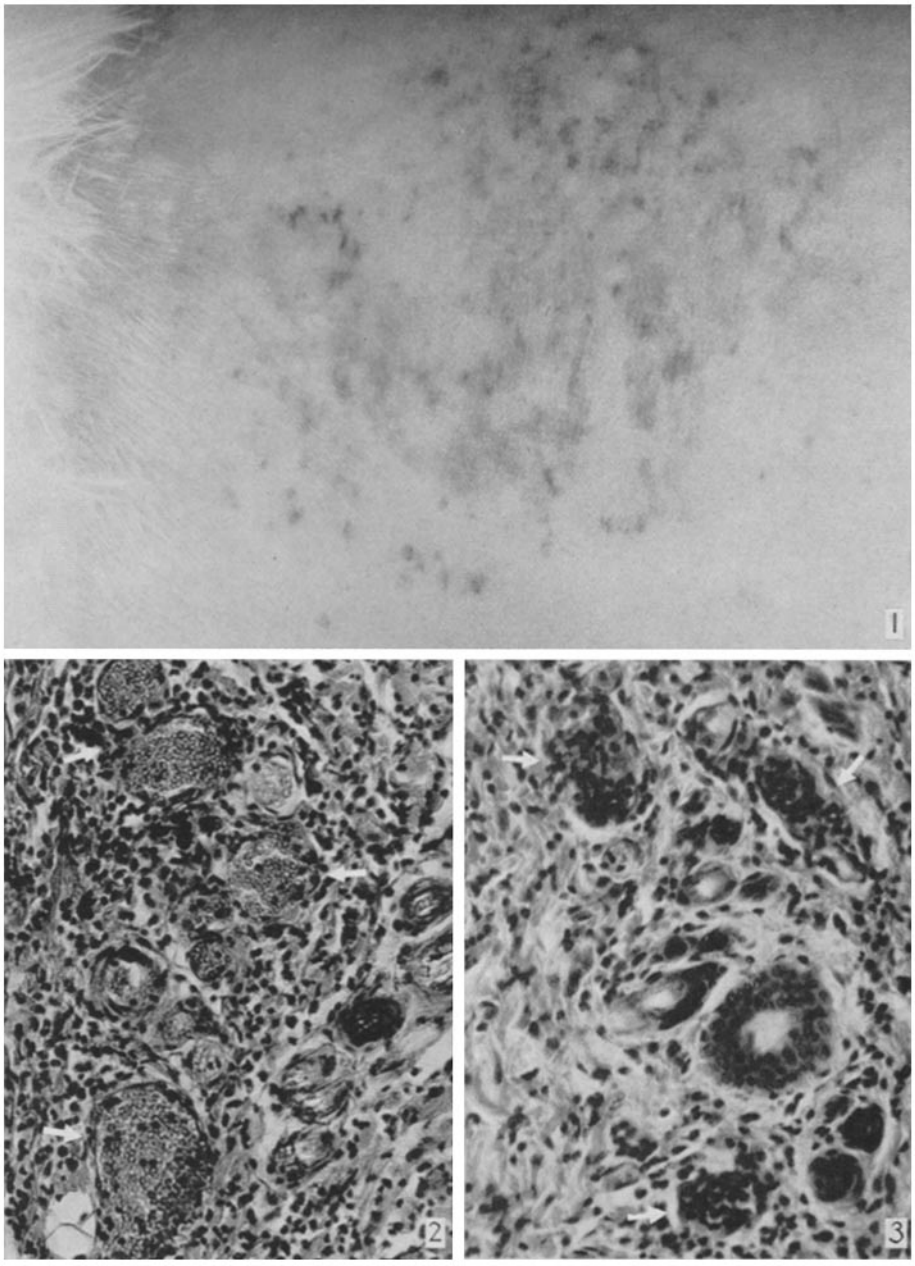
PLATE 42

All the sections shown, except those of Figs. 7 and 11, were stained with hematoxylin and eosin.

FIG. 1. The primary reaction in the abdominal skin to *S. marcescens* toxin in a cortisone-treated rabbit. This animal received 25 mg. cortisone daily for 4 days, and an intradermal injection of 0.4 mg. toxin on the 3rd day. Petechial hemorrhages appeared at the injected site 24 hours after the injection of toxin. ×1.7.

FIG. 2. The histologic appearance of skin prepared for the Shwartzman phenomenon in a normal rabbit. *S. marcescens* toxin was injected intradermally 24 hours previously. The interstitial tissue shows dense infiltration by polymorphonuclear leukocytes. The blood vessels (indicated by arrows) appear to be patent. ×290.

FIG. 3. The histologic appearance of the primary skin reaction to *S. marcescens* toxin in a cortisone-treated rabbit (gross appearance shown in Fig. 1). There is mild cellular infiltration in the interstitial tissue. Small blood vessels (indicated by arrows) appear to be occluded by masses of leukocytes and platelets. ×290.



(Thomas and Good: Cortisone and the Shwartzman reaction)

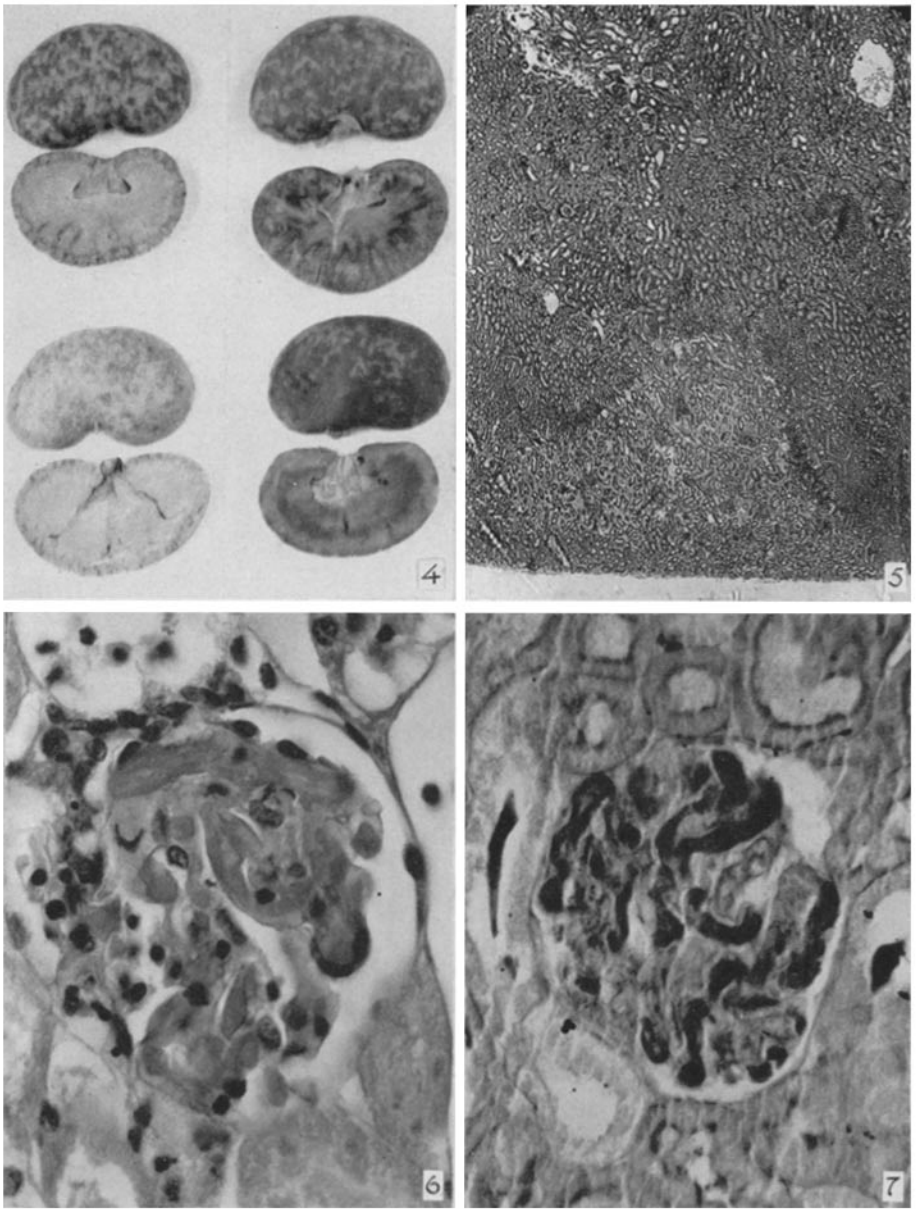
PLATE 43

FIG. 4. Bilateral cortical necrosis of the kidneys in cortisone-treated rabbits, following a single intravenous injection of *S. marcescens* toxin. The animals received 25 mg. cortisone daily for 4 days, and 0.4 mg. of toxin intravenously on the 3rd day. The surface and cut edge of kidneys from four rabbits are shown. ×0.8.

FIG. 5. The histologic appearance of the renal cortex in a cortisone-treated animal with bilateral cortical necrosis following a single injection of *S. marcescens* toxin. Low power magnification shows a sharply demarcated zone of cortical necrosis in the lower part of the section. ×29.

FIG. 6. The histologic appearance of a glomerulus in the kidney of a cortisone-treated rabbit with bilateral cortical necrosis following a single injection of *S. marcescens* toxin. The glomerular capillary tuft is filled with homogeneous acellular material. ×370.

FIG. 7. A glomerulus from the kidney shown in Fig. 6, stained by the Hotchkiss modification of the Schiff reaction with periodic acid. ×370.



(Thomas and Good: Cortisone and the Shwartzman reaction)

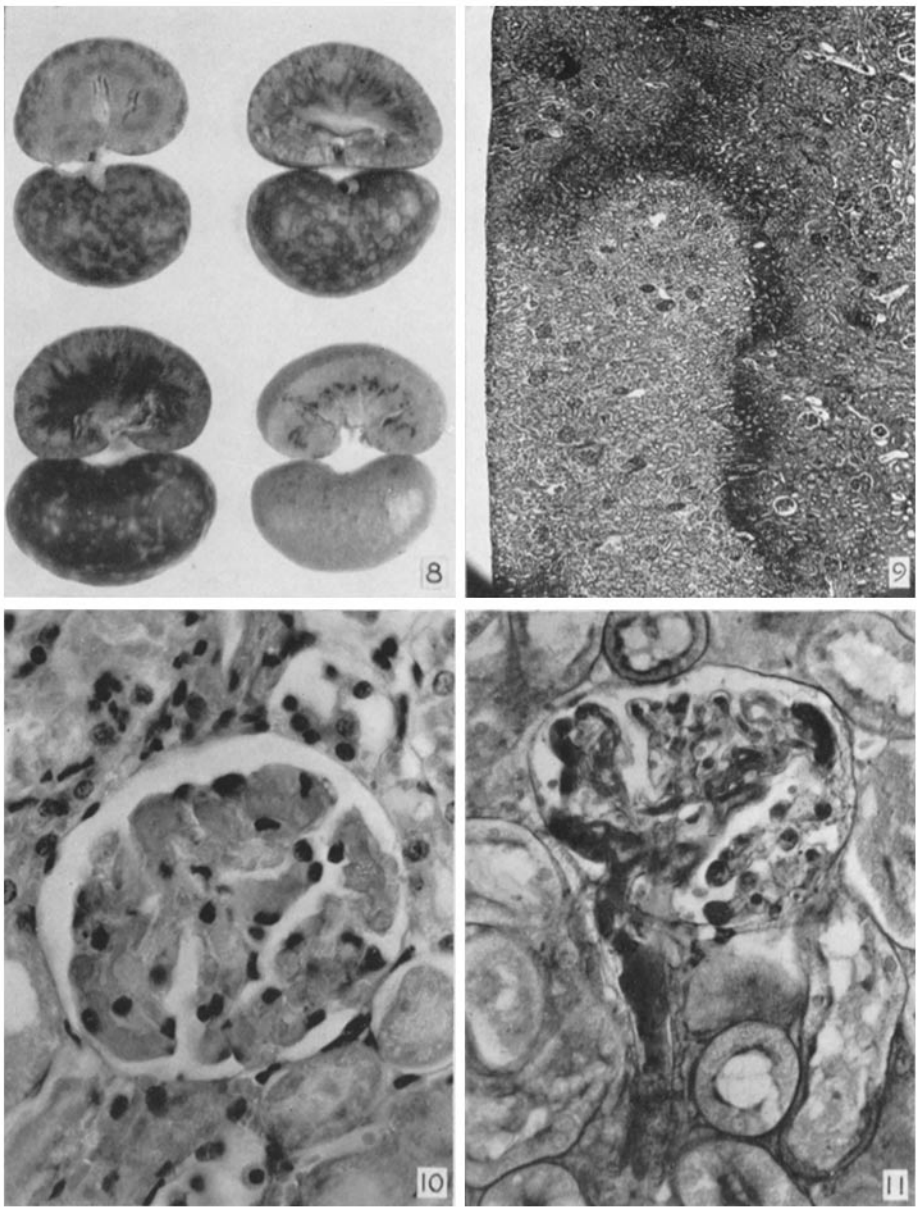
PLATE 44

FIG. 8. Bilateral cortical necrosis of the kidneys in the generalized Shwartzman reaction, produced by two successive intravenous injections of *S. marcescens* toxin, spaced 24 hours apart. The surface and cut edge of kidneys from 4 rabbits are shown. ×0.8.

FIG. 9. A zone of cortical necrosis in the kidney of a rabbit with the generalized Shwartzman reaction. ×29.

FIG. 10. A glomerulus in the kidney of a rabbit with the generalized Shwartzman reaction, showing homogeneous acellular material filling the glomerular capillary tufts. ×370.

FIG. 11. A glomerulus from the kidney shown in Fig. 10, stained by the Hotchkiss modification of the Schiff reaction with periodic acid. ×370.



(Thomas and Good: Cortisone and the Shwartzman reaction)