The strain used to initiate the present study was one of 14, each of a different species, which were employed in attempts to enhance the resistance of bacteria to 4 new antibiotics other than penicillin and streptomycin. In no other instance was a dependent variant recognized although each strain had been transferred 40 times in media containing graded concentrations of the respective antibiotics and from the antibiotic-containing media also to antibiotic-free media. It cannot be stated with any assurance that such dependent variants had not developed in other strains or to other antibiotics since the method used was not suitable for their recognition unless they replaced the sensitive and resistant variants completely, or nearly so, at some stage in the course of the subcultures.

No attempts have been made to study the genetic pattern of the emergence of the resistant and dependent variants and of the "back mutations" to more sensitive and more resistant variants. The possible significance of these variants with respect to the mechanism of action of antibiotics has been discussed previously in relation to the streptomycin-dependent variants(4).

Summary and conclusions. The resistance of a strain of *Klebsiella pneumoniae* to chloramphenicol was enhanced 128-fold by serial subculture on the surface of agar plates containing graded concentrations of this antibiotic.

A chloramphenicol-dependent variant also emerged during the course of these subcultures. This variant grew best in the presence of a critical concentration of chloramphenicol; as the concentration of antibiotic was either increased progressively beyond this critical level or decreased below it there was a steady decline in growth until complete inhibition of growth occurred.

The chloramphenicol-dependent variant was first recognized soon after the resistance of the strain had been enhanced to a point where it was completely inhibited by this critical concentration.

Evidence was also obtained of possible "back-mutation" to variants which were as sensitive as the parent strain.

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Hemorrhagic Skin Lesions Produced by Intradermal Meningococcus Toxin in Rabbits following Treatment with ACTH or Cortisone.*(18060)

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Inhibition of the Shwartzman phenomenon by ACTH has been described by Soffer, Shwartzman, Schneierson, and Gabrilove(1). These authors reported that the phenomenon was prevented in 8 out of 10 rabbits when 12.5 mg of ACTH was injected intramuscularly 2 hours prior to the intravenous or "provocative" injection of meningococcus toxin. Comparable amounts of ACTH had no inhibitory effect when given on the preceding day, before the intradermal or "preparatory" injection of toxin.

In preliminary experiments, a similar result was obtained in this laboratory. When ACTH was administered to 8 rabbits in 2 separate doses of 10 mg Armour Standard ACTH, 6 and 4 hours before the intravenous injection of toxin, the gross intradermal hemorrhage which characterizes the Shwartzman phenomenon failed to occur in every case, while typical reactions were produced in each of 4 control rabbits. However, it was noted that the skin sites prepared with

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1. Soffer, L. J., Shwartzman, G., Schneierson, S. S., and Gabrilove, J. L., *Science*, 1950, v111, 303.

TABLE I.
Occurrence of Hemorrhagic Skin Reactions in Rabbits Following Intradermal Injection of Meningococcus Toxin and Intramuscular Injection of ACTH or Cortisone.

Exp. No.	No. of rabbits	Drug	Time of injection (hr) Before prep. 24	4	After preparation 0	4	8	12	18	20	24	28	Results Hr after preparation	Lesions†
1	2	ACTH		10*	10	10	10						18	2/2
2	3	”							10	10			24	3/3
3	8	”			10			10					16	8/8
4	2	”			10	10	10		10	10			18	2/2
5	3	”				10			10				18	3/3
6	2	Cortisone		30	30	30	30						30	2/2
7	2	”	10	10		10							20	2/2
8	4	”				10	15			15			30	4/4
9	2	”							10	10	10	10	48	2/2
10	26	Untreated controls											36	0/26

* Numbers refer to dosage, in mg, of Armour Standard ACTH or of Cortisone, at the indicated hour.

† Numerator shows number of rabbits with reaction of pallor and petechiae at prepared skin site. Denominator shows total number in group.

meningococcus toxin became extremely pale within a few hours after ACTH treatment, and many small petechiae appeared in these areas. In order to determine whether the lesions were the result of partial inhibition of the Shwartzman phenomenon, or were brought about by ACTH, rabbits were given intradermal injections of meningococcus toxin and treated with ACTH or Cortisone, without being given the customary intravenous injection of toxin. The present report is concerned with the skin lesions which were observed following such treatment.

Method. Intradermal injections of 0.5 cc of meningococcus "agar washings" filtrates, prepared by the method of Shwartzman(2), were made in the shaven abdominal skin of male adult hybrid rabbits weighing approximately 2 kg. Two strains of Group I and II meningococci were used as sources of toxin, and an additional sample of standard toxin (44-B) was obtained through the kindness of Dr. Gregory Shwartzman. In most experiments, 2 dilutions (1:1 and 1:4) of 2 different toxins were injected in separate areas on each side of the abdomen. The toxins were shown to be effective in the usual Shwartzman reaction before being tested with ACTH and Cortisone; the results observed with different toxins in the experiments to be described were qualitatively similar.

Two lots of ACTH† were employed with activity corresponding to 20% and 30%, respectively, of Armour Standard ACTH. The dosages employed are expressed below in terms of Standard ACTH. In all experiments, the material was injected subcutaneously or intramuscularly in the thigh, with 10 mg suspended in 5 cc sterile saline.

Cortisone (11-dehydro-17-hydroxycorticosterone-21-acetate, Merck) was suspended in sterile saline in a concentration of 10 mg in 2 cc, and injected intramuscularly in the thigh.

Effect of ACTH on skin areas prepared with meningococcus toxin. Eighteen rabbits were prepared by intradermal injections of meningococcus toxin and injected with ACTH at varying intervals of time, as shown in Table I. In 2 animals, 4 injections of 10 mg each were given at 4-hourly intervals, beginning 4 hours before skin preparation. In the remainder of the group, total amounts ranging from 20 to 50 mg were given in divided doses at the time of preparation and thereafter.

Twenty-six control rabbits received intra-

† Supplied through the cooperation of Armour and Co., Chemical Research and Development Department, Chicago, Ill.

dermal injections of meningococcus toxin and were observed during the next 36 hours without further treatment.

Results. In all of the 26 control animals, the skin areas injected with toxin became reddened and slightly edematous after approximately 4 hours, and after 24 hours the lesions in every instance consisted of pink, slightly elevated, indurated swellings measuring 3-4 cm in diameter (Fig. 1-A). No hemorrhages or dilated vessels were grossly visible in the lesions.

In contrast, the rabbits receiving ACTH exhibited the following changes in the skin areas prepared with toxin: When ACTH was given before or at the time of the intradermal injection, edema and erythema were slight or failed to occur at all. When ACTH was started after edema had already appeared, the edema subsided within the next few hours. In 15 rabbits (Table I, Exp. 1, 3, 4, and 5) approximately 8 hours after the injection of toxin, oval or circular areas of pallor were seen at the injected skin sites, corresponding in size to the erythematous zones in control rabbits. At this time, small dilated blood vessels formed a rim around the periphery of each area of pallor. After 16-18 hours, the central portions exhibited dark blue discolorations caused by a superficial meshwork of engorged small blood vessels, and scattered petechiae appeared at the periphery (Fig. 1-B). In all 18 rabbits, between 18 and 30 hours after skin preparation, petechiae of varying sizes appeared throughout the injected areas and in some instances became confluent, resulting in grossly hemorrhagic lesions (Fig. 1-C). The lesions differed from the typical Shwartzman reaction in that they were flat and possessed irregular margins, and the spots of hemorrhage were usually distributed unevenly through the involved area, in contrast with the diffusely swollen, uniformly purple appearance of the Shwartzman phenomenon. Moreover, the development of the lesions was gradual, often continuing over a period of 36 hours with increasing degrees of vascular engorgement and ecchymosis, in contrast with the abrupt and rapid development of hemorrhage in the Shwartzman reaction.

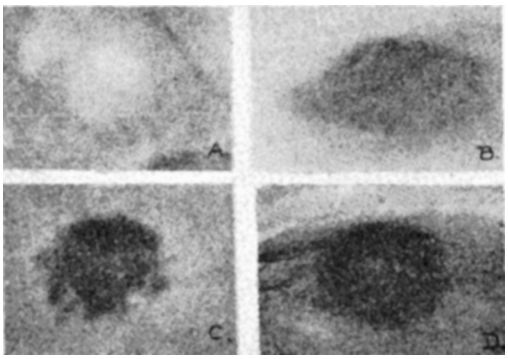

FIG. 1.
Reactions in rabbit skin 24 hr after intradermal injection of meningococcus toxin.
(A) Control. (B) After 20 mg ACTH. (C) After 30 mg ACTH. (D) After 30 mg Cortisone.

The optimal dosage of ACTH for the production of lesions was not accurately determined, because of limitation of the supply of material. In 8 rabbits which received 2 mg each, before and after skin preparation, no lesions occurred. With 40 mg of ACTH the lesions were more deeply hemorrhagic than with 20 mg. When ACTH was given at or before the time of skin preparation and followed by 2 or 3 additional doses at 4 hour intervals, the eventual lesions were more extensive than when ACTH administration was started on the following day.

Effect of Cortisone on prepared skin. Ten rabbits were given intramuscular injections of Cortisone, in total doses ranging from 30 to 120 mg each (Table I). In 4 animals, Cortisone was given both before and after the intradermal injection of toxin; in 6 it was given only after the toxin.

Results. In all 10 animals, pallor and vascular engorgement followed by the appearance of petechiae were observed in the prepared skin sites. An illustrative lesion is shown in Fig. 1-D. The reactions were similar to those described above in ACTH-treated rabbits, but confluent hemorrhages were less frequently observed. A longer time was required for the development of lesions following Cortisone. In 8 rabbits petechiae were not observed until 30 hours or longer after skin preparation. In 2 animals which were given Cortisone 24 hours prior to skin injection, lesions developed in the prepared skin after 20 hours.

Comment. In untreated rabbits, hemorrhage does not occur in skin sites prepared with meningococcus toxin until after an intravenous injection of toxin or other suitable "provocative" material is given, 18 or more hours later. A variety of apparently unrelated substances are capable of producing the phenomenon by intravenous injection, including bacterial toxins, whole bacteria, starch, agar, glycogen, kaolin, and antigen-antibody mixtures(2,3). The possibility cannot be excluded that ACTH and Cortisone produced the observed reactions by virtue of a comparable "non-specific" property which is not related to the known functions of these materials. It is unlikely that bacterial contaminants in the substances were responsible for the lesions, since cultures of the ACTH and Cortisone preparations yielded no growth.

It is possible that the skin reactions in ACTH- or Cortisone-treated rabbits may be based on a mechanism different from the Shwartzman phenomenon itself. In the absence of edema and erythema, and in the gradual progressive manner of their development, the lesions differed sharply from the typical Shwartzman reaction. Conceivably, the abatement of the usual inflammatory reaction to locally injected toxin may have increased the vulnerability of skin tissue to a primary damaging property of the toxin. There is evidence which suggests that the vessels of skin become less permeable following an injection of meningococcus toxin(4), and the possibility that further interference with permeability may occur following the administration of ACTH and Cortisone is under investigation.

Summary. The observations indicate that under certain circumstances the treatment of rabbits with ACTH or Cortisone results in a type of local skin damage by meningococcus toxin which is not seen in untreated animals. The application of these findings to other varieties of tissue damage by bacteria and their products is a field which merits further study.

2. Shwartzman, G., *Phenomenon of Local Tissue Reactivity*, New York, Hoeber, Inc., 1937.

3. Thomas, L., and Stetson, C. A., Jr., *J. Exp. Med.*, 1949, v89, 461.

4. Thomas, L., and Stetson, C. A., Jr., PROC. SOC. EXP. BIOL. AND MED., 1949, v69, 409.

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A Method for Visualization of Kidney Blood Vessels Applied to Studies of the Crush Syndrome.* (18061)

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A variety of conditions such as crush syndrome, severe trauma to muscles, non-traumatic muscular ischemia, transfusions with incompatible blood, heat stroke, toxemias of pregnancy, sulfonamide intoxications, poisoning of different types, etc., have one clinical manifestation in common, oliguria or anuria. Furthermore, the pathological finding of lower nephron nephrosis seems to be typical for all the above mentioned conditions. A drop in blood pressure below the necessary filtration pressure would itself be expected to cause anuria as filtration in the glomeruli stops. Arterial blood pressure determinations in the conditions mentioned will usually, in the very early stages, show a lowered blood pressure, but not always a pressure below that believed adequate for ultrafiltration. Often an increase in blood pressure is seen as the condition develops; despite this the patient may be completely anuric.

Three main theories are generally accepted as being possible explanations of oliguria and

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