serum. At the same time it shows also a moderate skin sensitivity titer and a high precipitin titer. No skin sensitivity test was given this rabbit before administering the sensitizing doses of serum, because this experiment was part of a group where sensitization was attempted purely by the intravenous route.

Employing this method for demonstrating the immunity of the skin to diphtheria toxin and the capacity of the serum to neutralize this toxin, combined with tissue hypersensitiveness and precipitin production, it appears that the immunity has little effect on the sensitivity, and vice versa.

6594

Studies on Sensitization. V. Fixation of Diphtheria Antitoxin in Skin of Rabbits Sensitized to Horse Serum.

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The element of fixation that accompanies sensitization was well demonstrated by Opie,[1] who cut out the inflammatory area resulting from subcutaneous injection of the specific protein in a sensitized rabbit, extracted the ground tissue with salt solution, and established the presence of the antigenic substance in the fluid by testing it with the serum of a specifically sensitized rabbit. In this laboratory, the aspect of fixation was studied from another point of view. The question was raised, whether an animal sensitized to horse serum would fix diphtheria antitoxin at the point of injection. Accordingly, the following conditions were experimentally established: (1) 50 MLD of diphtheria toxin injected intracutaneously in rabbits of about 3 kg. weight, cause death in from 2 to 4 days. (2) The same dose of toxin and 50 units antitoxin injected intracutaneously 1 inch apart, result in the survival of the animals.

A group of rabbits previously sensitized to horse serum was given the toxin and antitoxin injections intracutaneously, employing non-sensitized rabbits as controls. It was found that the control animals survived this treatment while the sensitized animals, showing a marked inflammatory response at the area wherein the antitoxin was injected, succumbed within 2 to 4 days, unquestionably

[1] Opie, E. L., *J. Exp. Med.*, 1924, **39**, 659.

because the fixation of the antitoxin at the point of injection prevented its neutralizing action on the toxin. Table I gives the results obtained with 11 rabbits.

TABLE I.
Fixation of Diphtheria Antitoxin in the Skin of Rabbits Sensitized to Horse Serum.

Rabbit	Method of Sensitization cc. serum	Interval bet. last sensitizing injection and administration of toxin and antitoxin days	50 mld toxin and 50 units antitoxin given intracutaneously 3 cm. apart. Response to antitoxin injections	Condition of rabbit
77	1, horse intracutaneously	15	Marked inflammatory area 2x5 cm. diam.	Died on 4th day
77[1]	Non-sensitized control		Slight erythematous area 1 cm. diam.; negative after 24 hours	Survived
78	1 and 0.5, horse intracutaneously 3 weeks apart	2	Marked inflammatory area 3 cm. diam.	Died in 24 hours
78[1]	Non-sensitized control		Slightly thickened area 1.5 cm. diam., negative after 24 hours	Survived
80	1 and 0.5, horse intracutaneously 3 weeks apart	5	Marked erythematous area 3 cm. diam. with 1 cm. diam. blackened center	Died on 2nd day
80[1]	Non-sensitized control		Thickened area 2 cm. diam.; subsidence after 24 hours	Survived
117	1, human intracutaneously	16	Slightly thickened area, 1 cm. diam.; subsidence in 24 hours	"
118	" "	16	Slightly thickened area, 1 cm. diam.; subsidence in 24 hours	"
121	1, horse intravenously	14	Marked erythematous area 4 cm. diam.	Died on 2nd day
123	" "	14	Marked erythematous area 2.5 cm. diam.	Died on 4th day
125	" "	14	Mild response, 2 cm. diam. thickening of skin	Died on 4th day

Three rabbits sensitized intracutaneously with horse serum and 3 others sensitized intravenously, succumbed to the toxin and antitoxin injections. Three normal rabbits and 2 others sensitized to human serum survived the same treatment. Four of the rabbits sensitized with horse serum received but one injection of this reagent. One additional rabbit (79) sensitized to horse serum and one non-sensitized control (79[1]) received 50 units of antitoxin intracutaneously 48 hours before the injections of 50 units of antitoxin and 50 MLD of toxin. The sensitized animal died in 2 days, whereas the non-sensitized one survived. Autopsies were made of

the animals that had succumbed, with special attention to the gross appearance of the adrenals, which were found to be enlarged and hemorrhagic.

This study corroborates the generally accepted observation that the antitoxin molecule is bound to the protein (globulin) constituents of the immune serum. The study throws light also on conditions one frequently meets in children exposed to diphtheria, who after receiving diphtheria antitoxin for passive immunization, contract this disease some weeks later and are then given antitoxin intramuscularly. A severe local inflammatory response usually follows. The results of our experiments indicate that the children, when highly sensitized to the horse serum, do not derive any curative benefit from the antitoxin since this reagent undoubtedly remains fixed at the point of injection.

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Studies on Sensitization. VI. Specific Desensitization of Rabbits Sensitized to Protein Mixtures.

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It is generally believed that while it is possible to desensitize rabbits injected with a purified protein such as crystalline egg albumin, it is not possible to desensitize these animals when injected with a complex mixture of proteins such as serum or egg white. On reinvestigating this problem, it was found that rabbits can be readily desensitized to serum and egg white, the desensitization, however, being of short duration. In the early experiments, large quantities, such as 10 or 20 cc. of these proteins were injected intravenously to produce desensitization. Later experiments indicated that relatively small amounts, such as 1 cc. or 0.5 cc. per kg. of body weight of rabbits, were sufficient to desensitize.

Table I illustrates desensitization in 2 rabbits, sensitized to human serum that had previously been heated for 30 min. at 56°C. Rabbit 173 had a skin sensitivity titer of 1,000, but no serum precipitins. By injecting intravenously 0.5 cc. of human serum per kg. of body weight, the skin sensitivity tests given 1 hour and 6 hours later, were practically negative, the skin response being limited to the injections of undiluted serum—a response commonly given by