ent as to the length of time a rabbit will remain sensitized after the protein injections have been discontinued.

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Studies on Sensitization. IV. Antitoxin Immunity Contrasted with Tissue Hypersensitiveness.

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In connection with our studies of tissue hypersensitiveness following repeated injections of protein in rabbits, it seemed of interest to obtain a picture of antitoxin immunity in the same animal that is sensitized to some protein. A quantitative method for measuring skin immunity to diphtheria toxin, based on the Schick test, and for measuring the amount of antitoxin present in the serum of the immune animal, following intracutaneous injections of toxoid has been described.[1] Briefly, the least quantity of diphtheria toxin, in a 0.1 cc. volume, that is capable of producing an erythematous response following an intracutaneous injection in a normal rabbit is determined. This quantity usually ranges from 0.000003 MLD to 0.0000003 MLD. A given amount, such as 0.5 cc. of toxoid is then injected intracutaneously in fractional doses of 0.25 cc. in the same rabbit. After about 3 weeks, it will be found that the least quantity of diphtheria toxin that will produce erythema following intracutaneous injections of 0.1 cc. amounts, is considerably higher than 0.000003 MLD. With an additional injection of toxoid, the amount of toxin necessary to produce an erythematous response might be 0.1 or 1 MLD. With continued injections of toxoid, a skin response may not be obtained until 5 or 10 MLD of toxin are injected intracutaneously.

Parallel with this picture of the development of skin immunity to the toxin is the increase of antitoxin in the blood serum of the immunized animal, also measured by intracutaneous means, and determined as follows: A small dose of diphtheria toxin is chosen that invariably produces a definite inflammatory response in the skin of a normal rabbit when given intracutaneously in a 0.1 cc. amount. This dose, 0.0003 MLD, is mixed in series with varying dilutions

[1] Kahn, R. L., *J. Bact.*, 1933, **25**, 81.

of immune rabbit serum. Thus, 0.1 cc. of the toxin dilution containing 0.0003 MLD is mixed with 0.1 cc. of undiluted immune rabbit serum. Similarly, 0.1 cc. of the same toxin dilution is mixed with 0.1 cc. of 1:10 dilution of the immune serum. A third mixture is prepared with 1:100 dilution of the immune serum. Similar mixtures with still higher dilutions of the immune serum are prepared when indicated. The 0.2 cc. amounts containing the toxin and immune serum are injected intracutaneously in a normal rabbit with proper controls. The highest dilution of the immune serum capable of neutralizing the dose of toxin gives a measure of the amount of antitoxin in the immune serum. By utilizing this method, it will be found that whereas the rabbit before the toxoid injections showed no antitoxin in its serum, after the toxoid injections, sufficient antitoxin may be present in the serum to neutralize the 0.0003 MLD of toxin in a serum dilution as high as 1:10,000 or more.

Table I gives a record of a rabbit which, following a series of intracutaneous injections of toxoid* and, at a later date, intravenous injections of serum (previously heated at 56°C. for 30 min.), shows the presence of a highly developed skin immunity to diphtheria toxin as well as a high toxin neutralizing capacity of the

TABLE I.

Immunization and Sensitization of Rabbit (108) Following Injections of Toxoid and of Human Serum.

Date 1932	Intracut. injections of toxoid; fractional doses 0.25 cc. Injection, cc.		Intraven. injections of serum Injection cc.		Immunization response to toxin		Sensitization response to serum	
					Smallest amt. diphtheria toxin producing erythema MLD.	Highest dilution rabbit serum neutralizing 0.0003 MLD. toxin	Titer of skin sensitivity	Precipitin titer rabbit serum
6/16	1st	1						
6/20					0.0000003	no neutralization		
7/ 6					0.001	undiluted		
7/11	2nd	0.5						
8/ 2					1.0	1:100		
8/15	3rd	0.5						
10/ 4					2.5	1:1,000		
10/14								Negative
10/16			1st	1				
10/22	4th	0.5						
10/25					2.5	1:1,000	1:100	1:100,000
10/26			2nd	0.5				
11/ 9					7.5		1:100	1:100,000
11/22	5th	0.5						
12/ 1			3rd	1				
12/12					7.5	1:10,000	1:100	1:100,000

* The diphtheria toxin, toxoid and antitoxin were kindly furnished by the Bureau of Laboratories, Michigan Department of Health, Lansing.

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.

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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.