

which also gave the characteristic reaction with naphthoresorcin and benzol.³

While the vibrios contain carbohydrate factors so closely allied that they cross-react throughout the group at high dilution, yet the vibrios themselves are not identical, since their specific substances are different. So far as our present analysis goes, the chemical difference between the specific substances of the pathogenic vibrio and the water vibrio lies in the possession of galactose by the first and of arabinose by the second. It appears further that the smooth-rough transition, brought about by bacteriophage action, does not change the character of the carbohydrate, nor does the change from the agglutinable to the inagglutinable type have any effect upon the specific substance.

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Studies on Sensitization. I. Skin Sensitivity and Serum Precipitin Response Following Intracutaneous Injections.*

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This report is the first of a series undertaken with the aim of throwing light on the rôle of sensitization in infection and immunity. Our early studies deal with the tissue hypersensitiveness that results from repeated injections of protein substances in animals, a condition generally classed under the Arthus phenomenon. Albino rabbits served as the experimental animals and the sensitizing injections were made, in most instances, intracutaneously.¹ The white skin in these animals rendered the effects of the injections readily discernible. This fact, in turn, permitted quantitative studies of the skin (local) response and its ready correlation with the serum (general) response.

The present study utilizes the method for measuring skin sensitivity recently reported.² Briefly, into the hair-clipped skin of an

³ Abderhalden, E., *Handbuch d. biol. Arbeitsmethoden*, Abt. I, Tl. 5, Kohlenhydrate, 99.

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¹ A review of the literature on immunization by cutaneous means is given by Tuft, L., *J. Immunol.*, 1931, **21**, 85.

² Kahn, R. L., *J. Bact.*, 1933, **25**, 81.

albino rabbit are injected 0.1 cc. quantities of a series of dilutions of some protein with physiological salt solution. The dilutions usually employed are: undiluted, 1:10, 1:100, 1:1,000, 1:10,000, and 1:100,000. The skin response is read 24 hours after the intracutaneous injections, and the highest dilution producing an inflammatory area, is termed the skin sensitivity titer of the rabbit.

If, for example, human serum previously heated for 30 minutes at 56°C. is used as the protein, the skin of a normal rabbit will show practically no response to this sensitivity test; only the area wherein the undiluted serum had been injected will show a slight transitory edema. A rabbit previously sensitized to human serum, however, will be likely to show, after a similar sensitivity test, a marked inflammatory response with central necrosis in the area where the 0.1 cc. of undiluted serum had been injected; a somewhat less marked response in the area where the 0.1 cc. of the 1:10 serum dilution had been injected, with gradual reduction in the response corresponding to the higher dilutions of serum, showing perhaps only a small erythematous area in the 1:10,000 dilution, with no response in the 1:100,000 dilution. A rabbit showing this picture is recorded as having a skin sensitivity titer of 10,000 to human serum.

The diameters of the inflammatory areas, resulting from skin sensitivity tests with human serum, in 4 rabbits having sensitivity titers of 10,000, 10,000, 100,000, and 100, respectively, are illustrated in Table I. It should be emphasized that while necrosis accompanies inflammation in the low dilutions of serum, the responses in the higher dilutions are limited to inflammation.

TABLE I.
Skin Reactions in Rabbits Sensitized to Human Serum.

Rabbit	Serum Dilutions						
	Und.	1:10	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000
	Diameter of inflammatory area expressed in cm., 24 hours after intracutaneous injections of 0.1 cc. doses of serum dilutions.						
1	5.5	5	3	2	1	—	—
2	3	3	2.5	1.5	1	—	—
3	4	2	1.5	1.5	1.5	1.5	—
4	2.5	1.5	1	—	—	—	—

It is not aimed to imply that the skin sensitivity titer is an absolute criterion of the degree of sensitization in rabbits. It is believed, however, on the basis of our present knowledge, that a rabbit showing a skin response to a 1:1,000,000 dilution of a protein is probably sensitized to a greater degree, for example, than a rabbit showing a skin response to a 1:1000 dilution.

Intracutaneous Injections of Serum and Egg White. An intracutaneous injection of these protein solutions in amounts from 0.5 cc. to 2 cc. in rabbits, will produce after 6 to 14 days a precipitin titer ranging from 1,000 to 100,000 and a skin sensitivity titer from 10 to 1,000. A repeated sensitizing injection of the protein, in about 2 weeks will frequently raise the skin sensitivity titer to about 100,000 with an equally high or higher serum precipitin response. After a week or more, the latter response is likely to be considerably reduced while the skin sensitivity response usually remains at a high level for a considerable period.

Intracutaneous Injections of Gelatin and Split Protein Products. Gelatin, as is well known, lacks cystine, tyrosin and tryptophane, 3 important aminoacids. This incomplete protein, according to Wells,³ does not produce anaphylactic antibodies in guinea pigs. Neither, apparently, does it produce complement fixing substances in rabbits.⁴ It is generally accepted also that split protein products do not produce antibodies. Intracutaneous injections of 1 cc. and 0.5 cc., in series, 14 days apart, of a 7.5% solution of gelatin of high purity, given in fractional doses of 0.2 cc., did not produce serum precipitins but the skin gave a mild sensitivity response. Before the sensitizing injections, the rabbit's skin gave no response to 0.1 cc. containing 7.5 mg. of gelatin. Two weeks after the second injection, the rabbit showed a skin sensitivity titer of 10, corresponding to 0.75 mg. of gelatin. Racemized casein⁵ gave no skin sensitivity (nor precipitin) response after 2 intracutaneous injections, 17 days apart, of 3 cc. quantities, each containing 60 mg. Negative results were also obtained following intracutaneous injections of similar quantities of: (1) proteose formed during preparation of racemized casein; (2) proteose formed from gliadin by acid digestion; (3) proteose formed in preparation of racemized zein; (4) proteose soluble in alcohol, obtained during racemization of edestin† It is possible that larger amounts of these products and continued injections might bring forth a skin sensitivity response. It should be mentioned, however, that 2 injections of 20 mg. each of casein (Kahlbaum), 17 days apart, gave, 2 weeks after the second injection, a skin response of 100, corresponding to 0.007 mg. of this protein. Previous to the sensitizing injections, the same rabbit gave

³ Wells, H. G., *J. Infect. Dis.*, 1908, **5**, 449.

⁴ Kahn, R. L., *J. Immunol.*, 1918, **3**, 277.

⁵ Ten Broeck, C., *J. Biol. Chem.*, 1914, **17**, 369.

† These split proteins were kindly furnished by the late Dr. Frank P. Underhill.

no skin response to 0.7 mg. of the same protein. The question of the sensitizing capacity of gelatin and split proteins merits further investigation.

Intracutaneous Compared with Intravenous Injections. No essential differences in skin sensitivity and in serum precipitin response were noted following these 2 methods of sensitization (Table II).

TABLE II.
Serum Precipitin and Skin Sensitivity Titers

Rabbit	Method of Injection	Dilutions human serum 1 cc. amounts injected 14 days apart	Response days after 2nd injection		
			0 days serum p'tin	14 days skin s'tivity	serum p'tin
95	Intraeut.†	1:10	100,000	100	1,000,000
161	Intraven.	1:10	10,000	100	1,000,000

† The 1 cc. amount was given in fractional doses of 0.2 cc.

This table also shows that high precipitin titers can be obtained with small sensitizing doses of the proteins, contrary to a widely held belief that it is necessary to give multiple injections of comparatively large amounts to produce high titers. It should be emphasized, however, that variation in skin sensitivity and precipitin titers occurs in rabbits even when the sensitizing injections are made under identical conditions.

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Studies on Sensitization. II. Skin Sensitivity and Serum Precipitin Response Following Injections of Unheated and Heated Serum.

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Fresh, unheated human serum is toxic to the rabbit's skin. If 0.1 cc. of such serum is injected intracutaneously, a marked inflammatory response, boil-like in appearance, measuring from 1 to 2 cm. in diameter, follows with subsidence in about 10 days or longer. When human serum is previously heated for 30 minutes at 56°C., however, it has practically no toxic effect on the rabbit's skin. Intracutaneous injection of 0.1 cc. produces a slight transitory thickening of the skin which usually disappears in 24 hours. The question arose, whether the inflammation accompanying the injection of