STUDIES IN SENSITIZATION TO SKIN

I. The Production of Antibodies to Skin by Means of the Synergistic Action of Homologous Skin Antigen and Staphylococcus Toxin*

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In recent years the concepts of sensitization to species' own (homologous) products and of autosensitization (autogenous or autochthonous sensitization, *i.e.* to products of the own body) have been receiving an increased share of attention. And, more important still, there has been a recrudescence of experimental studies tending to substantiate the existence of sensitization to homologous organ products, and even to suggest that there may be instances of sensitization to the individual's own organs and tissues. Many of the immunologic changes produced by homologous organs have been shown to be organ-specific rather than species-specific; *i.e.*, the changes observed have been directed towards the particular organ employed as allergen, more or less regardless of the species from which the organ originated. The aggregate of this work has suggested that, under certain conditions, individuals may become sensitized to the slightly denatured substances of their own organs; and that organ-specific antibodies may thus be produced; and that these antibodies may, in turn, damage the particular organ or organs *in vivo* and *in situ*, and may thus produce clinical disease. Some examples of homologous and autogenous organ-specific sensitization can be cited in the work of Burky (1) and of Swift and Schultz (2) employing the crystalline lens, and of Schwentker and Rivers (3) employing the brain and cord. This work together with that of Masugi (4) and of Smadel and Swift (5) with kidney, and of Rivers and Schwentker (6), and of others, has shown that in experimental animals the organ-specific antibodies can, in all probability, produce disease in the particular organ or organs concerned.

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With this experimental background, it still remains necessary to demonstrate whether or not the chain of circumstances, consisting of: (1) autogenous organ-specific sensitization, (2) organ-specific antibody formation, (3) attack of organ-specific antibody on the organ *in vivo*, is actually at the basis of any of the known clinical diseases in man.

To date there has been little or no convincing evidence that organ-specific sensitization to homologous *skin* occurs or can be produced. However, Milbradt (7) has reported finding sensitivity to human skin in certain human beings. And the urticarial skin reactions which allergic individuals show on tests with human dander should be cited in this connection (Storm van Leeuwen (8), Hampton and Cooke (9), and others). It is also necessary to recall that dermatologists have suspected that autosensitization to skin occurs in certain dermatoses. It has been postulated that numerous observed clinical facts might best be explained by the hypothesis that the individual had become sensitized to the products of his own skin, and that the continuation and spread of the skin eruption was dependent upon this sensitization. Among the early proponents of this hypothesis was the English dermatologist Whitfield. He discussed this phenomenon under the title: "Autosensitization eczema" (10).

Every dermatologist has encountered numerous cases of eczematous and eczematoid dermatoses in which new lesions continue to develop long after the causal exposures have apparently ceased, and in areas remote from the sites of the original exposure. This well known but unexplained appearance and reappearance of eczematous and eczematoid reactions at unexpected times and in unexpected localizations has been designated by J. Jadassohn "*das Springen des Ekzems.*"

In addition to these observations, there is the common phenomenon that a skin which shows a certain pathological entity, such as psoriasis or lichen planus or eczema, is likely to develop at the sites of any banal trauma or inflammation, lesions identical with those of the original dermatosis (Koebner's isomorphic response) (11).

It occurred to us that the apparently spontaneous continuation or spread of eczematous, urticarial, and other types of lesions, the jumping about, waxing and waning, and the appearance of new lesions at sites of scratching or other traumatization, as well as the Koebner phenomenon in many other dermatoses, might perhaps be explained as follows: At the inception of the dermatosis the skin is damaged and some of its constituents denatured; the new products are absorbed and are antigenic; and thus, the individual becomes sensitized to his own skin with result that there are later lesions due to the action of antiskin antibodies which react locally with skin antigen liberated at sites of trauma or inflammation.

In order to test this hypothesis, which has been discussed at some length by one of us (11), we inaugurated a series of experiments designed to stimulate production of antiskin antibodies with a view to a study of their effects upon the skin.

The rabbit was chosen as the first experimental subject because of the ease

with which it produces precipitin antibodies. In preparing and administering the skin antigen, we adhered as closely as possible to the methods which previous observers have found to be successful in eliciting organ-specific antibodies to organs other than the skin (Spinka and Weichselbaum (12), Mann and Welker (13), H. R. Cohen (14 *a* and *b*)). Moreover, we took advantage of the experience of Burky (1), Swift and Schultz (2), Schwentker and Rivers (3), Smadel and others (5, 15), which has shown that the synergistic action of certain toxins and viruses with the organ allergen potentiates the antigenic effect and stimulates the production of organ-specific antibodies.

Methods

Preparing and Administering the Skin Antigen.—Rabbits were shaved in an operating room and the skin surface cleansed with soap and water followed by applications of alcohol and ether. We avoided the use of strong antiseptics, such as mercurials and iodine, because we feared that these agents might denature the proteins of the skin and alter their antigenic qualities too radically. The animals were placed under deep narcosis by means of intraperitoneal injections of barbituric acid derivatives and then the cleansed skin was removed by careful dissection to free it of muscle and the peculiar, loose, mucinous connective tissue which is present under rabbit skin. Then the skin was placed in a sterile saline solution, cut into half-inch squares, and then introduced into autoclaved tubes containing Burky's beef heart infusion (hormone broth). The tubes were placed in the incubator; and upon examination in 24 hours, it was found that there was bacterial growth in each tube.

We wished to follow Burky's method as closely as possible, *i.e.* the obtaining of sterile antigen in the hormone broth with subsequent inoculation of "H. A." staphylococci (a strain of toxin-producing staphylococci isolated by Burky), and hence we tried various other means of attempting to obtain sterile skin. One of them was to drop pieces of the skin prepared as above described into boiling water for a few seconds; but this did not result in a sterile product.

In other tests we put official U.S.P. tincture of iodine or 1:1000 solution of bichloride of mercury on the skin prior to removal, but again it proved infected. Next we dropped the skin into a solution containing 0.1 per cent of merthiolate; and submitted other specimens to 5 per cent aqueous solution of sodium sulfathiazol. In neither case were we successful in obtaining a sterile product.

Finding that we could not obtain a sterile skin material, doubtless because of the microorganisms always deeply ensconced in crevices, ducts, and glands, we abandoned the attempt and contented ourselves with preparing the skin by the first mentioned shaving and cleansing with soap and water followed by ether and alcohol.

It would have been desirable of course to obtain sterile skin material, to place it in broth, to then inoculate the broth with staphylococci, and to have used the supernatant fluid containing both the toxin and the soluble antigenic skin material in solution, thus reproducing Burky's successful experiment with lens. Since this was impossible, we resorted to the intramuscular injection of whole rabbit skin obtained as described and then adsorbed on aluminum cream according to the method of Spinka and Weich-

selbaum (12). This skin antigen was injected intramuscularly at one site and the staphylococcus toxin intradermally and at other sites in the same animal.[1]

In order to prepare the skin material in a finely divided state, we experimented with many different methods of grinding, freezing, etc. It was finally found that the best product was obtained by the use of an ordinary hardwood board and a very sharp heavy butcher knife. The finely divided cutaneous material thus prepared was added to a colloidal solution of aluminum cream, as originally described by Welker (13). The proportion of skin to aluminum cream was about 1 to 1. This material was injected into rabbits in 20 cc. quantities into the muscles of the hind legs at various sites, to form *depots* of skin antigen which would be slowly absorbed. No more skin was injected during the course of the experiment.

Method of Preparing and Administering Staphylococcus Toxin.—The H. A. strain of staphylococci, kindly supplied to us by Dr. Burky, was inoculated into hormone broth and incubated for 10 days. The material was then filtered through a Berkefeld filter according to his directions, and the potency was determined by injecting various quantities into rabbits. We found that our material regularly killed the rabbits when 0.1 cc. per kilo of body weight was injected intravenously. This toxin was first diluted 1:10; and was injected daily in gradually ascending doses ranging from 0.05 cc. to 0.5 cc.; then the undiluted toxin was injected daily in doses ranging from 0.05 cc. to 0.5 cc. until a total of about 1.8 cc. of original toxin solution had been administered to each animal. The toxin injections were all given intracutaneously, and to two groups of rabbits. In group A, both toxin and the above described skin antigen were administered. In group B, the animals received only skin antigen; and in group C only toxin (Table I).

Methods of Preparation of Soluble Skin Antigen for Percipitin Tests.—Soluble antigen for precipitin tests was prepared in the following manner: Rabbits were shaved and skinned, and the skin was chopped up very finely as for injection. An equal volume of sterile saline solution was added to this material, and the whole was placed in the incubator. Samples were withdrawn at half-hour, 1 hour, 2 hour, 4 hour, 6 hour, 8 hour intervals and longer up to 1,000 hours. This material was filtered, placed in small bottles, covered with toluol, and put in the refrigerator until ready for use. In our latest preparations of autolysates, we omitted the early samples, since we had found that none of these contained soluble antigen. When we now prepare skin autolysates, we begin with the 24 hour sample.[1]

Method of Performing the Precipitin Tests.—Blood was drawn from the heart of the rabbits at weekly intervals, and serum was obtained from this blood. Precipitin

[1] As the skin antigen we used for sensitizing as well as the skin autolysates used for the precipitin reaction was not sterile, there is the possibility that the precipitin reactions observed might be due, wholly or in part, not to antiskin antibodies but to antibodies to certain of the contaminating microorganisms. However this possibility appears remote in view of many of our subsequent findings. These include the observation that the animals with circulating antibodies of the sort we have here demonstrated develop special kinds of lesions when their skins are subjected to various forms of trauma and irritation; and that normal animals, *i.e.* those not having these circulating antibodies, fail to develop these types of lesions in response to identical forms of skin trauma.

tests were made with this serum and the autolysates obtained from the skin-saline suspensions just described. The precipitin technique employed was the "ring"

TABLE I

Precipitin Tests

Anti-rabbit-skin rabbit sera and other control sera tested against rabbit skin autolysates which were incubated from 24 to 1000 hours.

Incubation of autolysates, *hrs*.....		24	50	100	150	200	300	400	500	600	700	800	900	1000
	Rabbit No.													
A. Rabbits injected intramuscularly with rabbit skin and intradermally with staphylococcus toxin	1	0	0	+	++++	+	+	0	0	0	0	0	0	0
	2	0	+	+	+++	++	+	0	0	0	0	0	0	0
	3	0	0	+	++	+	0	0	0	0	0	0	0	0
	4	0	+	++	+	+	+	+	0	0	0	0	0	0
	5	0	0	+	+	+	+	±	0	0	0	0	0	0
	6	0	0	+	+	+	+	0	0	0	0	0	0	0
	7	0	±	+	+	±	±	0	0	0	0	0	0	0
	8	0	+	+	+	±	0	0	0	0	0	0	0	0
	9	0	+	+	+	0	0	0	0	0	0	0	0	0
	10	0	0	+	+	0	0	0	0	0	0	0	0	0
	11	0	0	+	++	0	0	0	0	0	0	0	0	0
B. Rabbits injected intramuscularly with rabbit skin	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	±	±	0	0	0	0	0	0	0	0	0
	3	0	0	0	±	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0	0	0	0
C. Rabbits injected with staphylococcus toxin intradermally	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	?	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0	0	0	0
D. Untreated control rabbits	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0	0	0	0	0	0

technique. Antigen is placed into tiny test tubes (about 5 mm. in diameter) and the serum being studied for antibody content is introduced below the antigen by means of a capillary tube, producing a zone of contact between the two materials. The tests were read after standing at room temperature for one hour. All tests were controlled

by the demonstration of negative reactions between the sera and normal saline and between the sera and other unrelated protein extracts (similarly prepared autolysates of egg white and autolysates of organs other than skin); as well as by negative reactions when the autolysates were tested with normal rabbit serum.

TABLE II

Precipitin Tests

Antiserum from rabbits injected with staphylococcus toxin. Antigens were staphylococcus toxin and broth used to manufacture the toxin.

		Staphylococcus toxin	Broth
	Rabbit No.		
Staphylococcus toxin rabbit antiserum	1	+	0
	2	+	0
	3	+	0
	4	+	0
	5	+	0
	6	+	0

RESULTS AND DISCUSSION

Table I shows the results of precipitin tests made with four groups of rabbits. The groups consisted of 11, 6, 6, and 9 rabbits respectively. In group A the eleven animals had been injected intramuscularly once with skin adsorbed on aluminum cream followed at once and twice weekly thereafter by intradermal injections with ascending doses of staphylococcus toxin. In group B, the six animals were injected intramuscularly with the same amount of skin adsorbed on aluminum cream. In group C, the six animals received only intradermal injections of staphylococcus toxin. Group D consisted of nine untreated controls. The serum used for the tests was obtained between the 6th and 7th weeks after the injection of skin antigen.

Examination of Table I shows that the serum of the rabbits of group A, *i.e.* those injected with both skin and toxin, regularly gave precipitin reactions with the rabbit-skin autolysates (see footnote 1, page 62).

The serum from the rabbits of group C, injected with toxin alone, gave no reactions, except for a peculiar hazy ring in one tube (animal 3).

The sera of the untreated control animals of group D gave no reactions.

Table II shows precipitin reactions obtained when serum from rabbits of group C (injected only with staphylococcus toxin) was tested with staphylococcus toxin as well as with the broth used to manufacture this toxin. It will be seen that the solution of toxin gave positive reactions, while the broth alone gave none. This shows that these animals formed specific antibodies to staphylococcus toxin itself (antitoxins?). This result suggests that the synergistic effect of the staphylococcus toxin broth here observed is probably de-

pendent on the specific effect of the toxin rather than on the non-specific effects of the broth.

SUMMARY AND CONCLUSIONS

1. Techniques for the preparation of skin antigen suitable for intramuscular injection in rabbits, and of skin antigens (autolysate) for serological experiments are described.

2. A method was evolved which produced a soluble skin antigen (autolysate) suitable for performing precipitin tests.

3. Injection of the rabbit skin antigen and of staphylococcus toxin in rabbits resulted in the formation of antibodies (precipitins) to homologous skin.

4. When homologous skin alone was injected into rabbits, the antibody formation was questionable, or at most, slight.

5. The injection of staphylococcus toxin alone resulted in antibody formation, this antibody being specific for the toxin and not reacting with broth.

6. By utilization of the synergistic action of staphylococcus toxin and of homologous skin antigen, it has been possible for the first time to produce specific antiskin antibodies in experimental animals.

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