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Failure to Sensitize to Autologous Skin.* (23715)

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The possibility that human beings could develop allergic sensitivity to their own skin, either alone or in combination with foreign materials, has been considered for many years. In clinical dermatology this concept is so ingrained that the diagnoses of autosensitization dermatitis and autoeczematization are in common use in some dermatological departments. Detailed descriptions of the phenomena which denote "autosensitization" have been given (Whitfield(1), Haxthausen (2)) and studies by means of skin and serologic tests using extracts of skin have been carried out (Templeton(3), Cormia(4)). There is, however, a scarcity of basic immunologic studies in this highly important segment of dermatologic investigation. Hecht, Sulzberger and Weil(5) produced precipitating antibodies in rabbits against homologous skin by injecting them with skin adsorbed on aluminum cream, and with staphylococcus toxin. They found that rabbits with these antibodies in their blood developed skin lesions when their skin was subjected to certain non-specific traumas, while rabbits not having such circulating antibodies did not react in such a manner. Voisin and Maurer (6) injected 6 rabbits with homologous skin and adjuvants. When subsequently they grafted the injected rabbits with skin from the donor animals, they observed the development of peculiar cutaneous lesions. Such lesions did not develop in animals which had received the skin-adjuvant mixture, but no skin grafts. Furthermore, 4 of the 6 test animals subsequently rejected autografts, an event which Voisin and Maurer attributed to autosensitization to skin.

In the present studies in guinea pigs we used technics similar to those which have been reported to have been successful in engendering experimental sensitization to homologous or autologous organs in experimental animals, using a variety of tissues: kidney(7), lens(8), nervous tissue(9-12), tumor(13) and testes (14).

Method. Emulsions of autologous skin were prepared as follows: guinea pigs, after having been clipped and shaved on the flank using a germicidal soap lather, were placed under general anesthesia with sodium pentothal. After sponging the skin with 0.1%

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cetyl trimethylammonium bromide in 70% ethyl alcohol, a full thickness piece of skin was removed from the prepared area of each animal by raising the skin with a forceps and slicing across with a razor blade. The skin sample obtained was oval in shape and averaged about 20 mm long, 15 mm wide, 2 to 3 mm thick at the center and 300 to 400 mg in weight. The skin was weighed to the nearest 0.1 g, minced with scissors and added to 4 volumes of sterile saline or staphylococcus toxin diluted 1:5 with saline. This crude skin suspension was fragmented in a high speed homogenizer† for 10 minutes while being cooled in an ice-bath. The resulting 'skin broth' except for a few remaining larger fragments of skin was taken up through a 20 or 21 gauge needle and emulsified in a syringe with 2 volumes of a mixture of Bayol F and Arlacel A containing 1 mg of killed, dried tubercle bacilli‡ per ml. The ratio of Bayol F to Arlacel A was either 8½:1½ or 2:1. Thus each ml of skin-adjuvant emulsion contained approximately 67 mg of skin. Skin-adjuvant emulsions which were used for more than 1 injection were kept in a freezer at a temperature of approximately −12°C, but none were kept longer than 1 week. Test animals received injections of their autologous skin emulsion with adjuvants, while control animals were given an emulsion of saline or staphylococcus toxin diluted 1-5 with saline, with adjuvants. Injections of skin-adjuvant emulsions were made intraperitoneally (0.1-0.3 ml), intracutaneously into multiple sites on the back (0.3-0.6 ml) or intramuscularly into the limbs (2 ml). A total of 89 guinea pigs were injected with autologous skin emulsion and 38 with control emulsion. Animals were autografted 28 to 64 days after the first injection of skin or control emulsion, following closely the pinch graft technic of Billingham and Medawar(15). All autografts were taken from sites which previously had been tattooed with India ink, in order to permit easier identification of accepted grafts. Grafts were taken from the right posterior flank and placed in a prepared site on the center of the side of the thorax. The graft, after it had been placed in its bed, was covered with sterile, petrolatum-impregnated gauze, followed by a dry gauze pad. Finally a plaster cast was applied surrounding the entire thorax to prevent the graft from slipping from the bed. The grafted sites were inspected at 7, 10, 14, 21, and 28 days, or until the graft had permanently healed in or had been rejected. In some instances, after the autologous skin graft experiment had been concluded, the skin of both test and control animals was tested for its response to physical trauma, as follows: one flank of the animal was clipped and traumatized by 1) superficial scraping of a skin area approximately 2 cm² using 10 strokes of a sharp scalpel blade, and 2) by burning another skin site using the Henriques-Moritz burn apparatus at 100°C for 60 seconds. Reactions to these forms of physical trauma were observed after 1, 2, and 7 days.

TABLE I. Results of Autografts in Guinea Pigs Injected with Autologous Skin in Emulsion with Adjuvants.

Route of inj.	Wt of skin inj., mg	No. of successful autografts/autografts done: Test animals	Control animals
Intracut.	20–40	32/34 94%	19/21 90%
Intramusc.	134	28/30 93%	16/17 94%
Intraper.	7–27	25/25 100%	Not done

Results. The results of the autograft experiments are summarized in Table I. This indicates that there was no significant difference in the percentages of skin autografts accepted and rejected between the animals pretreated with an emulsion of their own skin and control animals. It may be assumed that the small percentage of failures to accept autografts in both groups was due to technical factors rather than specific immunologic alterations. The tests with physical trauma did not produce any unusual reactions, no significant differences being noted in the response between the test and control animals. Thus neither the autograft experiments nor the irritation tests produced any evidence indicating that sensitization to autologous skin had taken place after the intracutaneous, in-

† "Vir-Tis 45" made by the Vir-tis Co., Yonkers, N. Y.

‡ The tubercle bacilli were kindly supplied by Dr. Jules Freund.

tramuscular or intraperitoneal injections of autologous skin and adjuvants.

Summary. 1) Eighty-nine guinea pigs were injected intracutaneously, intramuscularly or intraperitoneally with autologous skin in emulsion with adjuvants. Thirty-eight guinea pigs were injected intracutaneously or intramuscularly with control emulsion, not containing skin. Subsequently an autograft was done on each animal. No significant differences were noted in acceptance of autografts between animals injected with skin emulsion and control emulsion. 2) Irritancy tests using scraping and burning of the skin did not produce different results in the test animals and the control animals. 3) Under the conditions of these tests no autosensitization to skin could be demonstrated in guinea pigs.

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Chromatographic Differences Between Radioiodinated Albumin Preparations and Normal Human Serum Albumin. (23716)

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Radioiodinated human serum albumin has been extensively used to investigate albumin metabolism in the normal and in diseased states(1-3). Albumin preparations used in these studies have been indistinguishable from normal human serum albumin in many respects but not in all(1,2,4,5). The recent introduction of anion-exchange adsorbents(6) which have proved very useful in the chromatographic separation and characterization of serum proteins(7,8), made possible further evaluation of radioiodinated human serum albumin preparations. In the present study, utilizing the anion-exchange adsorbent, diethylaminoethyl-cellulose(6), the chromatographic distribution of radioiodinated human serum albumin was investigated and found to differ markedly from the distribution of native serum albumin.

Materials and methods. The radioiodine-labeled human serum albumin preparation used in these studies has been commercially prepared by the following procedures. Albumin was separated at the Cutter Laboratories from pooled plasma by a modification of the cold ethanol technic of Cohn(9). Following addition of stabilizers (acetyltryptophan and sodium caprylate) the albumin was heated to 60°C for 10 hours to minimize the possibility of transmission of homologous serum hepatitis(10). Iodination of the albumin was performed at the Abbott Laboratories(11) and included exposure of the albumin to potassium iodide and sodium hypochlorite solutions and passage over an ion-exchange column. This preparation, referred