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IN-VITRO FIXATION OF SKIN-SENSITIZING ANTIBODIES TO SKIN CELLS AND MESENCHYMAL TISSUE

By

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The localization of the skin-sensitizing antibodies (atopic reagents) in the skin forms the basis of positive cutaneous tests; in the mucous membranes they are responsible for the allergic manifestations. If they are circulating in the blood, they may be demonstrated by the method of passive transfer introduced by the German workers *Prausnitz & Küstner* (3) in 1921.

In the literature on allergy and serology, it is frequently stated that: The antibodies have marked affinity for skin and mucous membranes, the antibodies are fixed to elements or cells in the skin. *Feinberg* (1) has advanced the theory that the antibodies are bound to the endothelial cells in the skin capillaries, as the vessels play an important rôle in the processes that take place during antigen-antibody reactions. In 1957 it was stated by *Hans Schmidt* (5) that the substance to which the skin-sensitizing antibodies become fixed is presumably mucoproteid and that most mesenchymal cells must be able to fix these antibodies.

We have investigated whether skin-sensitizing antibodies, grass pollen reagents, can become fixed to skin *in vitro*. Moreover, we investigated whether they can be fixed to skin treated in various ways and to other tissues, viz. muscle fascia and peritoneal connective tissue.

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MATERIAL AND METHODS

As antibody we used a 10 % solution in physiological saline of a dry serum containing grass pollen reagents. This dry serum was made at Statens Seruminstitut, Copenhagen, in 1945 and was derived from 15 patients with hay fever. Despite many years' storage it possesses high biological activity, i.e. its ability of passive sensitization is marked.

The skin was removed as skin grafts from 20 children all of whom were oligophrenic (hydrocephalic and mongoloid). On the average, 10 grafts were obtained from each child, and the specimens were of the size used e.g. in autotransplantation of leg ulcer (2), comprising the epidermis and a small portion of corium. The specimens were cut into small pieces, practically minced, and after being rinsed in several changes of physiological saline they were placed in dwarf test tubes.

The skin from the first 10 children was used untreated for the binding experiments; that from the next 5 was frozen in a mixture of acetone-carbon dioxide snow before the fixation experiments, while those from the last 5 were heated on a water bath for half an hour at 60° C.

Muscle fascia and peritoneal connective tissue were received fresh from the operative theatre of the surgical department¹, derived from abdominal surgery on a total of 13 patients. After being cut and rinsed in several changes of physiological saline, this tissue was used without further treatment in quantities corresponding to about 10 skin grafts.

Binding experiments were then carried out as follows: To each tube was added 4 drops of the reagent-containing serum solution, using an ordinary drop counter. This secured intimate contact between serum and tissue. Control tubes holding serum without tissue specimens were run to match each experimental

¹ For which our thanks are due to Dr. K. Trautner, head of the department.

tube. After incubation in a refrigerator for 24 hours, 6 drops of physiological saline was added to all tubes in order to obtain fluid enough to perform passive transfer by the method of Prausnitz-Küstner, as this reaction forms the basis of the evaluation as to whether antibody has been fixed to the tissue pieces.

Passive transfer was in all cases applied to the volar aspects of the forearms of a number of big children and a few of our colleagues. A quantity of 0.1 ml "absorbed" serum and 0.1 ml control serum was slowly injected intracutaneously using tuberculin syringes and Mantoux platinum needles. At the end of 24 hours, we elicited the Prausnitz-Küstner reactions by intracutaneous injection of 0.3 ml 1 % grass pollen extract. Fifteen minutes later, we measured the diameters of the immediate reactions with a plastic ruler and calculated the areas as shown in the following tables for each experimental group.

TABLE 1.

Areas (square mm) of passive transfer reactions elicited from skin sites sensitized with a serum solution incubated for 24 hours with skin cells, and from skin sites sensitized with serum solution only (control).

Exp. No.	1	2	3	4	5	6	7	8
Serum sol. + skin cells	28	64	50	20	20	20	28	7
Serum sol. (control) ...	95	154	79	79	50	113	95	79

TABLE 1 (cont.)

Exp. No.	9	10	11	12	13	14	15
Serum sol. + skin cells	28	0	3	3	39	50	20
Serum sol. (control) ...	50	79	133	95	177	236	133

TABLE 2.

Areas (square mm) of passive transfer reactions elicited from skin sites sensitized with serum solution incubated for 24 hours with frozen skin cells and from skin sites sensitized with serum solution only (control).

Exp. No.	1	2	3	4	5	6	7	8	9	10
Serum sol. + frozen skin cells	79	20	113	0	13	3	0	13	28	64
Serum sol. (control) ...	177	79	177	102	154	95	79	154	95	154

TABLE 3.

Areas (square mm) of passive transfer reactions elicited from skin sites sensitized with serum solution incubated for 24 hours with heated skin cells and from skin sites sensitized with serum solution only (control).

Exp. No.	1	2	3	4	5	6	7	8
Serum sol. + heated								
skin cells	154	20	198	189	79	130	64	64
Serum sol. (control) ...	154	20	198	177	79	78	64	79

TABLE 4.

Areas (square mm) of passive transfer reactions elicited from skin sites sensitized with serum solution incubated for 24 hours with muscle fascia and peritoneal connective tissue and from skin sites sensitized with serum solution only (control).

Exp. No.	1	2	2	4	5	6	7	8	9	10
Serum sol. +										
muscle fascia	86	113	64	79	95	64	71	113	94	140
Serum sol. + periton.										
connect. tissue ...	94	113	79	79	79	—	—	—	—	—
Serum sol. (control)	86	113	79	79	113	79	79	113	85	140

TABLE 4 (cont.)

Exp. No.	11	12	13	14	15	16	17	18
Serum sol. +								
muscle fascia	153	130	177	—	—	—	—	—
Serum sol. + periton.								
connect. tissue ...	—	—	—	133	141	122	122	133
Serum sol. (control)	153	140	189	122	153	94	95	146

RESULTS

In 15 experiments there was a pronounced weakening of the passive transfer reactions derived from serum incubated with untreated skin (Table 1). In one experiment the reaction was entirely extinguished and in a few present only as a trace.

The same weakening of the skin reactions was observed in the 10 experiments in which the skin had been pre-treated with cold (Table 2), while in the 8 experiments in which the skin had been pre-treated with heat there was no definite weakening (Table 3). Lastly, 13 fixation experiments using muscle

fascia and 10 using peritoneal connective tissue showed no definite weakening of the cutaneous reactions.

DISCUSSION

Investigations of this nature have not been carried out previously except by *Shermann & Seebohm* (6) who used pieces of skin, cut in a microtome, and ragweed pollen reagins. They could not demonstrate any fixation of the antibodies *in vitro*, but on the other hand they could bind the reagins or passively transferable factors encountered in some cases of cold urticaria. Later, one of us (4) confirmed *Shermann & Seebohm's* results in respect to the fixation of these cold reagins to skin *in vitro*.

Our investigations, which are merely preliminary, permit but a cautious conclusion, viz. that the skin-sensitizing antibodies may be fixed to skin *in vitro*. Their fixation also to cold-treated skin may be explained by the fact that proteins are not denaturated by cold, but by heating as indicated by the fixation experiments presented in Table 3.

Nor do our studies allow a conclusion as to the site in the tissues where the antibodies are bound. Evidently, however, they cannot be bound to all kinds of connective tissue, since according to the binding experiments the passive skin reactions could not be weakened either by muscle fascia or peritoneal connective tissue.

SUMMARY

A solution of serum containing skin-sensitizing antibodies was incubated *in vitro* with skin (untreated, treated with cold, and heated), muscle fascia, and peritoneal connective tissue. Subsequent passive transfer reactions by the method of Prausnitz-Küstner appeared to indicate *in-vitro* fixation of the antibodies to untreated and frozen skin. No fixation occurred to heated skin (denaturation of protein?) or to mesenchymal tissue, such as muscle fascia and peritoneal connective tissue.

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