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# MORPHOLOGY AND DISTRIBUTION OF IMMUNE DEPOSITS IN THE SKIN. THE ACTIVE ARTHUS REACTION USING HORSERADISH PEROXIDASE AS ANTIGEN*

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## Abstract

A model of the active Arthus reaction was studied immunohistochemically in the skin using a horseradish proxidase-anti peroxidase system, comparing with the results of the reversed passive Arthus reaction in the skin of rabbits. Fine or coarse granular deposits and lumpy aggregates of peroxidase-positive substances are considered to represent immune deposits, while the homogeneous staining of peroxidase may show the non-reacted free peroxidase. In the present study it was found that immune complexes precipitate not only on the vascular walls, but also widely in the skin, i.e., along the collagen fibers, in the dermo-epidermal junction and in the perifollicular areas in the active Arthus reaction, and that they adhere to the surfaces of histiocytes, polymorphonuclear leukocytes, fibroblast-like cells and also of epidermal cells.

## INTRODUCTION

Clinically in the skin of lupus erythematosus (DLE and SLE), several vasculitis, dermatitis herpetiformis or lichen planus, deposits of immunoglobulins and complements are observed in the wide distribution, i. e., in the dermo-epidermal junction, in the vascular walls, in the perifollicular areas or on the epidermal cells, suggesting to be deposits of immune complexes (1～4). However, the nature of such immune complexes and the mechanism of the deposition in the skin remain to be further clarified.

Using bovine serum albumin (BSA) as antigen, Cochrane (5) has induced the active Arthus reaction in the skin, and presented the deposits of antigen-antibody complexes (immune complexes) in the vascular walls, which were phagocytized by polymorphonuclear leukocytes, causing prominent inflammatory vascular damages. Experimental results obtained

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by Straus (6), Ueki et al (7), Weber et al (8) and Masuda et al (9) have clearly revealed that immune complexes can precipitate not only on the vascular walls, but also in the perifollicular areas, in the dermo-epidermal zones and along the collagen fibers under certain conditions, applying the active or the reversed passive Arthus reaction to the skins of rabbits using horseradish peroxidase (HRP) as antigen.

The purpose of this study is to know further the morphology and the distribution of immune complexes in the skin, comparing results of the reversed passive Arthus reaction and the active Arthus reaction using HRP as antigen.

## MATERIALS AND METHODS

*1. Antigen and Animal:* Horseradish peroxidase (HRP) (type VI from Sigma Chemical Company, St. Louis.) was used as antigen. Five mg of HRP was dissolved in 0.01 M phosphate buffered saline (PBS) pH. 7.2 and injected with the incomplete Freund's adjuvant into 4 footpads of albino rabbits weighing 3 kg. Four to Six weeks later, 2 mg of HRP was boostered subcutaneously into the same rabbits. The sera of each animal were obtained 10 days later, and tested for the semi-quantitative antibody titers in agar-gel precipitation.

*2. Induction of the active Arthus reaction:* Active Arthus reactions were induced in the back skin of immunized rabbits by intracutaneous injections of HRP (type VI from Sigma Chemical Company). Intracutaneous injections of 0.1 ml of antigen solution containing 1, 1/2, 1/4 mg HRP respectively were given into each of 4 sites per animal. Skin biopsy specimens were obtained from the skin sites 30 minutes, 1, 2, 4, 8, 12 and 24 hours after the induction of the Arthus reaction.

*3. Control experiments:* PBS was injected into the skin of the immunized animals as control, while HRP solution was administered into the non-immunized normal rabbits.

*4. Preparation of the tissue:* Skin specimens were quickly frozen at $-70°C$ and 6 $\mu$ sections were cut in a cryostat at $-20°C$. Sections were classified into two groups. One group was washed 3 times for 15 minutes in PBS solution after the air-drying and then fixed in 2.5% glutaraldehyde solution for 20 minutes. The other group was immediately fixed in the same fixative after the air-drying.

*5. Peroxidase reaction:* The method of Graham and Karnovsky was applied (10). A yellowish-brown precipitates were indicated to show peroxidase-positive substances. The reacted sections were also poststained with the hematoxylin or the 0.1% methylgreen solution.

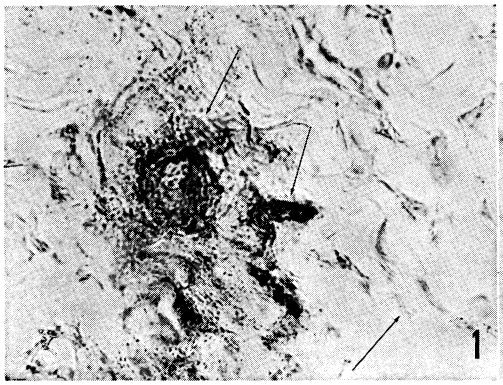
## RESULTS

*1. Immunized animals.*

The titers of the sera from the immunized rabbits against HRP showed 4 to 8 units in agar-gel precipitation. Unit means the highest dilution of sera, which showed a positive precipitin line.

*2. Active Arthus reaction.*

In the sections obtained 30 minutes, 1 and 2 hours after the induction of the Arthus reaction, the brownish-stained, peroxidase-positive deposits were detected in the vascular walls, in the perivascular spaces (Fig. 1, 2), along the collagen fibers, in the perifollicular areas and also



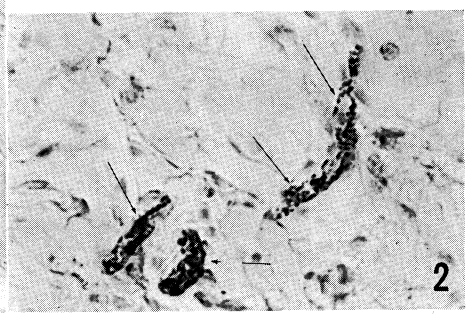




Fig. 1. Reaction for peroxidase activity in the skin obtained 30 minutes after the induction of the active Arthus phenomenon. The fine and coarse granular aggregates of the peroxidase-positive substances are seen on the vascular walls and in the perivascular spaces (arrows). Frozen section. Original magnification: X 400.

Fig. 2. Reaction for peroxidase activity in the skin 1 hour after the induction. The coarse granular deposits are detected on the vascular walls, mainly contacting with the surfaces of the endothelial cells (arrows). Frozen section. Original magnification: X 400.

in the dermo-epidermal junctions (Fig. 3, 4). These peroxidase-positive substances were composed of the fine or coarse granular, the large lumpy deposits and the homogeneously stained or linearly stained materials. Most of the homogeneously stained deposits and some of the granular deposits dissappeared after the washing of sections before fixation, but the coarse granular and the large lumpy deposits were not washed away by PBS.

In the sections 4 hours after the induction, most of the granular deposits were decreased in number and many polymorphonuclear cells

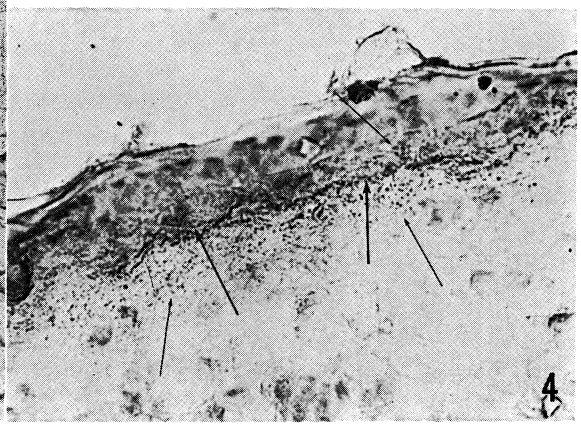


Fig. 3. Reaction for peroxidase activity in the skin. Biopsy from the challenged skin 1 hour after the antigen injection. The granular reaction products indicating the presence of immune deposits are demonstrated under the dermo-epidermal junction (arrows). Frozen section. Original magnification: X 400.

Fig. 4. The active Arthus reaction. Biopsy from the injected site of antigen 1 hour after the challenge. The granular deposits (narrower arrows) are found in the dermo-epidermal junction and also in the lower part of the epidermal cell layer. Wider arrows show the dermo-epidermal junction. Frozen section. Original magnification: X 400.

laden with the granular peroxidase-positive material on their surfaces were found widely in the injected sites. In the sections 24 hours later, most such deposits were taken up by the above-mentioned infiltrating cells.

*3. Distribution of the granular and the lumpy deposits.*

The distributions of the granular and the lumpy deposits were found to be different in the skin sites injected with the antigen of different concentrations. In the skin received 1 mg of antigen, the granular and the lumpy deposits were observed mainly in the vascular walls or in the perivacular spaces. When the antigen solution was diluted to 0.25 mg, the granular deposits became smaller and more fine and located widely along the collagen fibers, in the perifollicular areas and also in the dermo-epidermal junctions. Some of them were detected in the lower

portions of the epidermal layer. In these sections, the large lumpy aggregates could be scarcely observed in the skin.

In the sections 8 hours after the induction, most of the granular deposits dissappeared from the upper dermis, but some of them remained only in the perifollicular areas and in the dermo-epidermal zones.

*4. Adherence of the granular deposits to the cells.*

In almost all sections, many granular deposits were found to adhere to the surfaces of several cells in the skin, i.e., to the endothelial cells, to the histiocytes, to the fibroblast-like cells, (Fig. 5) to the polymorphonuclear cells and also to the epidermal cells (Fig. 6). Such aggregates adhering to the histiocytes and the polymorphonuclear leukocytes appeared to be phagocytized by them relatively quickly, but those adhering to the endothelial cells, the fibroblast like cells or the epidermal cells seemed to remain on the surfaces of cells longer till 24 hours after the induction.

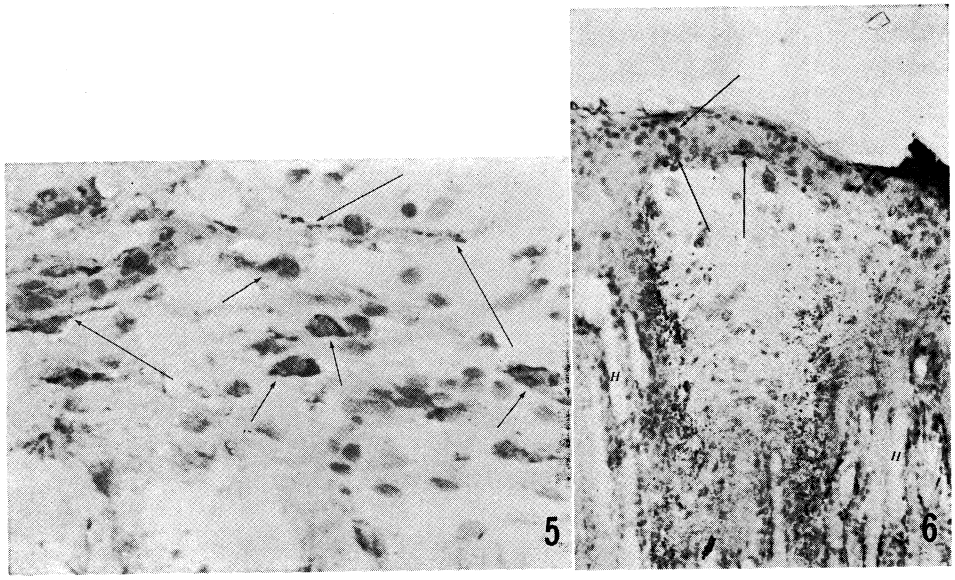



Fig. 5. Reaction for peroxidase activity in the skin of the active Arthus reaction, 1 hour after the induction. The granular peroxidase-positive substances are found to adhere to the cell surfaces of fibroblast-like cells (longer arrows) and to the histiocytes (shorter arrows). Frozen section. Original magnification: X 400.

Fig. 6. Reaction for peroxidase activity in the skin of the active Arthus reaction, 1 hour after the induction. The granular substances are seen to adhere to some separate epidermal cells (arrows) and in the perifollicular areas. (H) shows the hair follicles. Frozen section. Original magnification: X 400.

In the epidermis, two different distributions of the deposits were observed. Some of such granular aggregates located widely in the lower portions of the epidermis and others solitary on the epidermal cells. Some histiocytes were found to migrate into the epidermal cell layer.

*5. Control experiments.*

In the sections from the non-immunized animals, no granular nor lumpy aggregates of peroxidase-positive substances were observed in the injected sites of HRP, but only the homogeneous distribution of HRP was found widely in the connective tissue.

## DISCUSSION

It is well known that immune complexes arise on the vascular walls in the Arthus type reaction or in the serum sickness, however there are very few reports on the locations of immune complexes in the skin except on the vascular walls in such Arthus reaction. In order to investigate the immune deposits in the skin, we have used HRP as antigen. The usefulness of HRP for such purpose can be confirmed as follows.

1). HRP has a molecular weight of 40,000, thus possesses a high antigenicity against rabbits.
2). The antibody against HRP can not inhibit the enzymatical activity of HRP (11, 12).
3). Free HRP can penetrate easily vascular walls, follicular basement membranes and also dermo-epidermal basement membranes (9).
4). The immunologically and enzymatically reacted meterials can be preserved longer on the sections.
5). The immune complexes composed of HRP and its antibody can be observed as fine or coarse granular and lumpy deposits in tissues under light microscopy, while free HRP as homogeneous pattern (6, 7, 8, 9).
6). HRP or its complexes with the antibody can be prepared and observed also under electron microscopy (8).
7). Purified HRP can be obtained commercially.

From the results of Straus (6, 12, 13), Ueki et al (7), Weber et al (8) and Masuda et al (9), it can be considered that the fine or coarse granular or the lumpy aggregates of HRP in tissues show the deposits of immune complexes. Moreover, the soluble immune complexes prepared in vitro showed the fine or coarse granular aggregates, while the insoluble ones the large lumpy aggregates of HRP (14). On the other

hand, the free non-reacted HRP in tissues showed always a homogeneous distribution (9, 14).

In our study, the presence of the endogeneous peroxidase should be also ruled out. Generally, the endogeneous peroxidase can be observed in polymorphonuclear leukocytes (granulocytes), but in the early stages of our Arthus reaction there were very few such cells containing the endogeneous peroxidase. Histiocytes and fibroblasts have no detectable endogeneous peroxidase (9, 13, 15, 16).

Using the active Arthus reaction, Straus (13) has demonstrated the immune deposits of HRP-Anti HRP system in the skin, i.e. in the vascular walls, in the perifollicular areas, along the collagen fibers and on the surfaces of fibroblasts. Applying the reversed passive Arthus reaction, Masuda et al (9) have observed at the first time such granular immune deposits in the dermo-epidermal junction of the skin. Our present findings show that immune complexes can precipitate in the dermo-epidermal zones in both the active and the reversed passive Arthus reaction under certain conditions. As Masuda et al have described, immune deposits can be found on the vascular walls, when antigen or antibody may be administered sufficiently into the skin. However, when scanty amount of antigen or antibody may be given into the skin, immune deposits could be found widely in the skin, i.e. in the perifollicular areas, in the dermo-epidermal junction, or along the collagen fibers.

The next interesting findings are affinities of immnue complexes to the cells. It is well known that some mesenchymal cells, i.e., human red cells, B-lymphocytes, polymorphonuclear leukocytes or macro-phages possess some receptors for immune complexes. Although it should be clarified more precisely, our present result suggests the possibility that some epidermal cells have receptors for immune complexes on their surfaces. It should be also clarified whether the immune complexes could penetrate the intact dermo-epidermal basement membrane or the antigen and the antibody could form the immune complexes within the epidermal layer, thus adhere to the surfaces of the epidermal cells under the participation of complement.

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