ANTIGEN-ANTIBODY REACTIONS IN ALLERGIC HUMAN TISSUES*

II. STUDY BY FLUORESCENCE TECHNIQUE OF THE LOCALIZATION OF REAGINS IN HUMAN SKIN AND THEIR RELATION TO GLOBULINS

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The first of these studies (1) described the conjugation of skin-sensitizing antibodies (reagins) with fluorescent dyes. Judged by the criteria recommended by Coons and Kaplan (2), the conjugated reagins specifically stained atopic human skin which had been challenged by antigen. When antigen was absent the atopic skin did not stain with conjugated reagins. Therefore, no staining occurred in atopic skin from unchallenged or histamine-tested skin sites.

In the atopic skin challenged with specific antigen, all epithelial cells showed evidence of intracellular antigen-antibody reaction. After staining with conjugated anti-egg reagins the cells of hair follicles, of sebaceous and sweat glands had a narrow zone of fluorescence immediately inside the cell border. The purpose of the present study was to determine (a) whether reaginic antibody occurs in cells only in response to specific antigen or whether it is also present in unchallenged and histamine-tested tissues, and (b) whether the intracellular antibody is globulin.

Materials and Methods

1. Histologic Preparations.—Four patients who were highly sensitive to hen egg albumin clinically and by scratch test each provided skin biopsies from three sites: 1. an area which was not tested (unchallenged site); 2. an area tested by scratch method with 1-1000 histamine; 3. an area tested by scratch method with 1-1000 hen egg albumin (challenged site). One of the four patients was sensitive to cottonseed as well as egg albumin. The biopsies were immediately frozen in isopentane cooled with liquid nitrogen to \(-165^\circ\text{C}\). and dried \textit{in vacuo} at \(-30^\circ\text{C}\). The tissues were prepared for staining as described in the first study (1), \textit{i.e.} 1 and 4 micron sections were cut from the wax-impregnated tissue, post-fixed for 1 minute in 1 per cent formaldehyde containing 50 per cent dioxane (3), transferred to a drop of water on a scrupulously clean slide and dried at 40\(^\circ\text{C}\). Adherence of the section to the slide was facilitated by pressure with a rubber covered finger.

2. Antigen Coating.—As previously noted, the tissues from the unchallenged and histamine-tested skin sites were placed in a pool of 1-1000 hen egg albumin and kept at \(-30^\circ\text{C}\). for 24 hours.

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tested sites did not stain with conjugated reagin because they did not contain specific antigen. In order to study the antigen-antibody reaction the sections were coated for 30 minutes with a 1–2000 solution of egg albumin, and then layered with conjugated reagin. The concentration of egg albumin was not critical since dilutions to 1–50,000 could be used without any decrease in staining intensity. It was essential, however, that sections coated with egg albumin should be thoroughly washed with constant shaking for 20 minutes in buffered saline which was changed every 2 minutes before staining with the conjugated reagins. When coated sections were not adequately washed, subsequent staining with reagin-isothiocyanate conjugate gave a spotty orange color, rather than the characteristic yellow to apple-green hue.

3. Preparation of Conjugated Reagins.—The method for preparation and purification of conjugated reagins described in the first study (1) was modified by the use of 50 per cent ammonium sulfate at 4°C. to precipitate the serum globulins. The precipitate was dissolved and dialyzed against M/15 sodium chloride in M/100 phosphates at pH 7.4 to remove the sulfate ions. The reagins were then conjugated with fluorescein isothiocyanate according to the method of Riggs et al (4).

4. Preparation of Conjugated Rabbit Anti-human Globulin Antibodies.—Three rabbits were given 4 intramuscular injections at weekly intervals of 66 mg. human globulin (Poliomyelitis, Lederle) in Freund’s adjuvant. The pooled serum from the two rabbits with the highest titer contained 0.808 mg. antibody nitrogen per ml. The globulins from this were precipitated at half-saturation of (NH₄)₂SO₄; they were conjugated with fluorescein isothiocyanate and purified in the manner to be presently described.¹

5. Purification of Conjugated Antibodies.—The method described by Marshall (3) of repeated precipitation with 20 per cent alcohol at −10°C. was used in this as in our previous study. (1) for the removal of non-specific staining properties from the conjugates. A few preparations required, in addition, adsorption with tissue powder. For this, finely powdered epidermis, derived from human callouses, was found more effective than liver powder. Adsorption once or twice with 20 mg. of powder per ml. of conjugate yielded a satisfactory preparation.²

6. Immunologic Staining.—The staining methods described in the first report (1) were used in this study. Two sets of sections from each of the three tissues were stained for reagin localization with the conjugated reagins; the first set was coated with egg albumin, while the

¹ An interesting sidelight on the storage of fluorescent antibodies was provided by the following experience. The various lyophilized preparations of conjugated antibodies and the stock fluorescein isothiocyanate stain were stored at −28°C. Failure in the electric current for 12 hours resulted in defrosting of the deep-freeze. The incident was not disclosed until several weeks later when all conjugates, including freshly prepared ones from the stock staining material, failed to stain tissues specifically or non-specifically. This occurred despite the unchanged solubility on gross appearance of the lyophilized preparations or of the dye itself. The antibody titer of the precipitin preparations appeared unaffected despite the profound change in their staining properties. A new supply of staining reagent from the same batch was kindly supplied by Dr. Charles Griffin of Baltimore Biological Laboratories, Inc., Baltimore. This, like the original dye, gave satisfactory staining conjugates with both human and rabbit antibodies. The experience emphasizes the importance of storing the stain and the lyophilized conjugates in desiccators to avoid absorption of moisture.

² An observation which may deserve further study was the difference in avidity for fluorescent stain of skin from non-atopic and atopic individuals. Unchallenged skin from the four atopic patients continued to stain non-specifically with various conjugated antibodies after a non-atopic skin showed no staining. Additional purification was necessary to render the conjugated preparations clear of non-specific stain for the atopic sections.
second set was left uncoated. In addition, an uncoated section from each tissue, serial to the
reagin-stained section, was treated with rabbit conjugated anti-human globulin. Control of
staining specificity was achieved by the techniques described in the first study (1), consisting
of slight modifications in the methods of Coons and Kaplan (2).

RESULTS

A. Staining with Conjugated Human Reagins:

1. Challenged Tissue.—The sections coated with egg albumin stained more
intensely with conjugated reagins than the uncoated ones. Since no difference
was found in the localization of the antigen-antibody reaction in the coated
and uncoated preparations of the challenged tissue, they will be described
together. The following results were observed:

(a) Epithelial cells: The cells in all layers of the epidermis, of the hair follicles,
of sweat and sebaceous glands showed a zone of fluorescence 1 or 2 microns in
width limited to the region just within the cell border. Neither the nucleus nor
the remaining cytoplasm was stained (Figs. 1, 5, 6). The intercellular cement
substance was also unstained.

(b) Dermis: Two zones of different staining intensities were present in the
dermis. The outer region, contiguous with the epidermis and extending for
about 0.5 mm. in depth, was less fluorescent than the deeper dermis. The
staining intensity of each region was roughly proportional to its content of
macrophages and perivascular cells (pericytes). Both stained and unstained
macrophages were present in any given section; the former, while abundant,
were much less numerous than the latter. Stained macrophages about blood
vessels were more numerous than in the rest of the dermis. The intensity of
fluorescence of the stained macrophages was similar to that of the epithelial
cells (Fig. 3); in the macrophage, however, the entire cytoplasm was stained,
rather than only a zone at the cell periphery. The stained material was present
in the cytoplasm as granules of varying size (Fig. 7). Stained and unstained
macrophages in the same section also differed in both color and morphology.
The stained cell (Fig. 3 (m)) was larger and had rounded, poorly defined con-
tours, and an intense yellow fluorescence extending into its processes. The
unstained macrophage was smaller, had sharply defined, angular outlines, an
intense auto-fluorescent turquoise color which extended into its processes
(Fig. 3 (u)).

The two types of macrophages could be distinguished even in unstained
sections from challenged tissue; one was enlarged with rounded, ill defined
borders, the other was smaller with sharply defined, angular borders. The
difference is most clearly shown in toluidin blue stained sections (Figs. 33 to
35).

The intensity of fluorescence of the small blood vessels in a given stained
section depended on the presence or absence of pericytes. The majority of the
capillaries did not show these cells and their endothelium was only lightly fluorescent. The large number and uniform distribution of such capillaries gave the antigen-challenged tissue a light yellow-green fluorescence which distinguished it from unstained or "control" sections.

Those vessels whose walls contained pericytes showed intense staining. The pericytes, large ovoid or elongated cells, were more numerous in the capillaries of the deep dermis than in the superficial layers. Groups of stained macrophages were frequently present about vessels with pericytes.

The fibrous tissue bundles showed distinct fluorescence. No staining, however, could be detected in the ground substance or basement membrane zones.

2. Histamine-Tested Site.—Sections from the histamine-tested site which were not coated with egg albumin did not show fluorescence when exposed to conjugated anti-egg reagins (Figs. 9 and 11). The morphology of the macrophages differed from those in unstained challenged tissue by the absence of the rounded, swollen cells with ill-defined borders. Preparations which were coated for 30 minutes with a 1:2000 solution of crystallized egg albumin and then treated with the conjugated reagins were similarly but less intensely stained than sections from the challenged tissue site (Figs. 8, 10, 13, and 14).

In the coated sections from the histamine-tested site, the cells of epithelial origin, i.e., of the epidermis, the hair follicles, sebaceous and sweat glands, had cytoplasmic fluorescence in a narrow circumferential zone which was similar to that in epithelial cells of the challenged tissue site.

Compared with sections from the challenged site, coated histamine-tested sections contained fewer and less intensely stained macrophages and pericytes. In addition, the stained macrophages in the histamine-tested tissue differed from those in antigen-challenged tissue by their smaller size and sharply defined, angular outlines. Fibrous tissue and capillaries lacking pericytes did not stain.

3. Unchallenged Skin Site.—The uncoated unchallenged tissue, like the uncoated histamine-treated site, did not stain (Figs. 16 and 18). In the antigen-coated, unchallenged tissue, specific staining was found in the epidermal cells (Fig. 15), the hair follicles, the sebaceous and sweat glands, the macrophages and the perivascular cells. The intensity of fluorescence, its localization and the morphology of both stained and unstained cells, was similar in the unchallenged and histamine-tested skin.

4. Sections from normal (non-atopic) human skin and from an atopic individual who was not sensitive to egg albumin which had been coated with egg albumin did not stain with conjugated anti-egg reagins.

5. Control of Staining Specificity.—Staining of the uncoated and coated challenged skin sites (Figs. 2, 4, and 14), of the coated histamine-tested site (Fig. 12), and of the coated unchallenged sites (Fig. 20) was largely or completely inhibited when the sections were covered with unconjugated anti-egg reaginic
globulin for 1 hour at room temperature and then layered for 20 minutes with the conjugated anti-egg reagins.

No inhibition of staining occurred in the challenged or antigen-coated tissues after layering with unconjugated non-reaginic human globulin for 1 hour and then staining with anti-egg conjugated human antibodies.

No staining occurred in the challenged or antigen-coated tissues after layering with conjugated heterologous (anti-cottonseed) human reagins. The ability of the conjugated anti-cottonseed reagins to stain homologous antigen was demonstrable in the tissues of the one patient who was sensitive to cottonseed as well as egg albumin.²

6. Cross-Inhibition with Unconjugated Reagins and Unconjugated Rabbit Antibodies.—One set of antigen-challenged sections was covered for an hour with unconjugated anti-egg reagins, washed, and then stained with conjugated rabbit anti-egg antibodies; and second set of sections was covered with unconjugated rabbit anti-egg antibodies, washed, and then stained with conjugated anti-egg reagins. Inhibition of staining was good in both sets of sections.

B. Staining with Conjugated Rabbit Antibodies:

The procedures carried out with conjugated reagins were repeated on the various tissues with conjugated rabbit anti-egg albumin antibodies. The staining effect of the conjugated rabbit antibodies was the same in all tissues as with conjugated reagins.

Staining with conjugated rabbit antibodies gave the opportunity for additional control techniques other than those used for demonstrating the specificity of the conjugated reagin stain. Thus, after precipitating the antibodies from the conjugated preparation the supernatant failed to stain the tissues specifically; also, when the antibodies in the unconjugated preparation were immunologically precipitated the supernatant was no longer able to inhibit specific staining with conjugated anti-egg antibodies.

C. Staining for Human Globulins:

Sections from the following frozen-dried skin preparations were stained with conjugated rabbit anti-human globulin antibodies. 1. Rabbit skin to demonstrate the absence of non-

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² In a personal communication Dr. Maxwell Richter of the Division of Immunochemistry and Allergy Research, Royal Victoria Hospital, Montreal, calls attention to the fact that in the first of these studies (1) no mention was made of the effect of heating at 56°C on the staining properties of the conjugated human anti-egg antibodies. Conjugation of heated antibodies was tried but to date we have been unable to rid the preparation of intense, nonspecific staining properties. However, in the studies planned for the future, comparison will be made of the relative effectiveness of heated and unheated reagins for inhibiting staining with conjugated homologous antibodies.
specific staining by the conjugated antibodies. 2. Normal (non-atopic) human skin to provide a frame of reference for the study of fluorescence in stained atopic skin. 3. The three atopic sites from a patient sensitive to egg albumin and from one sensitive to egg albumin and cottonseed.

The results were as follows:

1. Rabbit skin stained with the conjugated anti-human globulin antibodies showed no fluorescence in either the epidermis or dermis.

2. Normal (non-atopic) human skin stained for human globulin (Figs. 21 and 22) showed no fluorescence in the epithelial cells of the epidermis, the hair follicles, the sebaceous or sweat glands. The dermis showed slight, diffuse, general fluorescence due to the light staining of the endothelium of most of the capillaries. Only an occasional lightly stained pericyte or macrophage was present in the dermis of the non-atopic human skin.

3. Atopic skin sites: The localization of the stained globulins was identical in the atopic tissues from the three different sites of the egg-sensitive patient. The epithelial cells of the epidermis, hair follicles, sebaceous and sweat glands contained fluorescent material after staining with the conjugated anti-human globulin antibodies. As in the comparable tissues stained with conjugated anti-egg reagins, the fluorescence was limited to a narrow zone immediately within the epithelial cell border (Figs. 23, 25, 27, 29 and 32).

In the dermis also the stain with conjugated rabbit anti-human globulin antibodies produced results in the three atopic sites which were indistinguishable from the stain with conjugated reagins. The stained macrophages, while abundant, were not as numerous as the unstained ones. Significantly, the proportion of stained to unstained macrophages was much greater in the tissues of the patient who was sensitive to both cottonseed and egg albumin than in the tissues from the patient who was sensitive only to egg albumin (compare Fig. 30 with Fig. 31). And, like the macrophages stained with reagins, those stained with rabbit anti-human globulin antibodies showed granules of varying size in the cytoplasm.

The intensity of staining following the use of conjugated anti-human globulin antibodies was as great as that after the use of conjugated reagins.

The intensity of staining in the three atopic sites after the use of conjugated anti-human globulin antibodies differed in the same manner as that following the use of conjugated anti-egg reagins. In both types of stains the tissue from the site challenged with egg albumin was more fluorescent than that from the histamine-tested or the unchallenged site.

4. Inhibition of fluorescence: The sections from the three atopic sites were treated as follows:

(a) Coating for 1 hour with unconjugated globulins of rabbit anti-human globulin antibodies followed by layering for 20 minutes with conjugated rabbit
anti-human globulin antibodies. No fluorescent staining occurred in any of the tissues (Figs. 24, 26, and 28).

(b) Coating for 1 hour with unconjugated globulins from normal rabbits followed by layering for 20 minutes with the conjugated rabbit anti-human globulin antibodies, resulted in only a slight diminution in the intensity of the fluorescent stain.

(c) After precipitation of the antibodies from the conjugated antibodies by the addition of human globulins, the supernatant failed to stain sections from the various tissues. And the supernatant from the unconjugated anti-human globulin antibodies to which homologous antigen had been added was much less effective than the unprecipitated material for inhibition of the stain with conjugated antibodies.

DISCUSSION

The similarity in the localization of fluorescence in the epithelial cells and dermis of atopic skin after staining with conjugated anti-human globulin antibodies and with conjugated reagins indicates that anti-egg reagin is identical with, or a moiety of, human globulin. It is noteworthy that in normal (non-atopic) skin no globulin in sufficient concentration to be stained by the procedures used is demonstrable in the epidermis and only minimal amounts were shown in the macrophages and endothelium of the dermis. The relationship of reagins and human globulins is emphasized in the tissues from one patient who was sensitive to both cottonseed and egg albumin. The proportion of stained macrophages in the tissues from this patient was greater than in the tissues from the patient sensitive to egg albumin alone. Presumably, the number of macrophages containing sufficient globulin to show a positive stain is related to the number of antigens to which a patient is sensitive. A high degree of specific skin sensitivity to the antigen may be necessary for demonstrating the presence of reagins in the tissues by fluorescence techniques.

The fact that reagins will inhibit the stain with homologous conjugated rabbit antibodies and vice versa suggests that these two antibodies have related or possibly overlapping antigen combining sites.

The greater intensity of fluorescence produced by the conjugated rabbit anti-human globulin antibodies in the antigen-challenged tissues, as compared with the unchallenged or histamine-tested sites, is probably due to the mobilization of antibodies in the challenged sites.

The evidence that antigen-antibody reactions occur in all epidermal cells of the skin poses the question whether antibody in such cells of atopic persons is present only as a result of challenge with specific antibody or whether it is also present in “resting” epithelial cells. The same question arises regarding the presence of antibody in macrophages and pericytes. The answer is found in the stained, antigen-coated sections from the histamine-tested and from the un-
challenged sites. While the uncoated sections from these areas did not stain with conjugated reagins, those to which specific antigen was added \textit{in vitro} showed staining which was identical in location with that seen in the tissues in which the antigen-antibody reaction was induced \textit{in vivo}. Additional evidence for the similar localization of antibodies in challenged, unchallenged, and histamine-tested sites is indicated by the localization of stained globulins after treatment with conjugated anti-human globulin antibodies.

The greater staining intensity of the \textit{in vivo}-produced reaction than of that produced \textit{in vitro} can be explained by the assumption that in the former the reaction is more complete, involving larger amounts of antigen within the various cells in the former and probably only a coating of antigen in the cell periphery in the latter. Mobilization of macrophages during \textit{in vivo} reactions is suggested by the greater number of such stained cells, especially about blood vessels, than are observed during \textit{in vitro} reactions.

In the tissue challenged with specific antigen it is noteworthy that the macrophages which participate in the reaction differ in morphology from those which are not involved in the reaction. Only the former stain with reagin and with anti-human globulin antibodies; their morphology is that of an edematous cell (Fig. 34). The cells not stained by reagins or anti-human globulin antibodies are smaller and have sharp, angular borders (Figs. 33 and 35). The cell enlargement cannot be reproduced by the use of histamine to induce a wheal reaction. One may occasionally observe in challenged but not in histamine-tested tissue two adjacent macrophages, the stained one with rounded, ill defined borders, the unstained cell with sharply delineated, angular outlines (Fig. 3 (\textit{m} and \textit{u})).

It is noteworthy that despite the close resemblance of the wheal produced by histamine and by specific antigen that the morphologic changes in macrophages are present only in the latter.

In the cells of the epidermis, of hair follicles, of sebaceous glands, and of sweat glands, the antigen-antibody reaction was localized to a narrow zone within the cell border. A rough approximation of the concentration of antibody present in the various parts of the skin, based on the amount and intensity of fluorescence in each region, suggests that the epidermal cells contain much more antibody than any other structure in the skin. The presence of intense fluorescence in the epithelial cells of the sebaceous and sweat glands also suggests that the sebum and sweat of atopic patients, and possibly of others as well, have an immunologic function. Antibodies in sebum and sweat may in part explain the self-disinfecting capacity of the skin described by Arnold and Bart (5).

Many authenticated experiments emphasize the importance of skin as an active immunologic organ. The best known illustration of this is probably the immunity resulting from smallpox vaccination. In a review of the evidence for immunologic importance of the skin, Sulzberger concludes with the following
statement: “So that reasoning by analogy and from the teleologic point of view it would not be astonishing that the skin has still another important duty: namely that of being the organ principally concerned in the mechanism of immunologic protection and of allergic alteration.” (6).

The only previous evidence that epithelial cells may participate in human reactions were the findings that the skin surface is capable of self-sterilization (5) and that the skin is the main site of reaction in contact-type dermatitis (6). Evidence of the presence of antibodies in epidermal cells, sebaceous glands, or sweat glands has not been previously reported.

The absence of staining of the ground substance is probably due to insufficient concentration of antigen-antibody complexes to produce visible fluorescence. The fluorescence of collagen fiber bundles in the site tested with egg albumin is probably due to antibodies in ground substance concentrated about the fibers rather than to the presence of antibodies in the collagen itself.

Whether antibodies are elaborated in epithelial cells, macrophages, and pericytes, or whether they are merely stored in these cells, is not disclosed by this study. The known functions of the epidermal cells, the macrophages and pericytes would make the latter seem more probable.

**SUMMARY**

The skin of atopic patients contains specific reaginic antibodies in all epidermal cells, in unchallenged as well as antigen-challenged areas. The reagins are also present in the epithelial cells of sebaceous glands, sweat glands, hair follicles and also in macrophages and pericytes. Judged by staining characteristics, their immunologic reaction in tissues with antigen is identical with that of rabbit antibodies.

Comparison of tissues stained with conjugated reagins and conjugated antihuman globulin antibodies demonstrates the close relationship of reagins and globulins.

In antigen-challenged tissues the macrophages and pericytes become enlarged and stain more intensely with toluidine blue. No such changes in morphology or staining are present in histamine-tested or unchallenged atopic tissues.

The technical assistance of Mr. B. F. Booker is gratefully acknowledged.

**BIBLIOGRAPHY**


EXPLANATION OF PLATES

The tissues illustrated in these plates are, except for Fig. 30, from the skin of an egg-sensitive patient. Fig. 30 is from a patient sensitive to egg and cottonseed. The term challenged tissues is applied to tissues obtained from the wheal produced by egg albumin; histamine-tested, from the wheal produced by histamine; unchallenged, from an untested site.

Staining was with either conjugated homologous reagins (anti-egg), heterologous reagins (anti-cottonseed) or, (Figs. 21 to 32), with rabbit anti-human globulin antibodies. Toluidine blue 1/1000 was used to stain for macrophages in Figs. 33 to 35.

Coating of sections with egg albumin before staining consisted of layering with 1-2000 egg albumin for 30 minutes and thoroughly washing.

Inhibition of specific staining was either with unconjugated anti-egg reaginic globulins or unconjugated rabbit anti-human globulin antibodies.

PLATE 54

Challenged skin stained with conjugated reagins.

Fig. 1. Epidermis: Only a narrow zone within the cell border is fluorescent. Dermis shows faint fluorescence of capillary network and intense fluorescence of macrophages. 1 micron × 400.

Fig. 2. Inhibition of staining: No specific fluorescence can be seen. Arrows point to epidermal-dermal junction. 1 micron × 400.

Fig. 3. Stained dermis: Note the rounding and marked enlargement of stained macrophages (m). Compare these with the unstained (u) autofluorescent macrophages. The latter are smaller, have sharply demarcated borders and angular outlines. 4 microns × 400.

Fig. 4. Inhibition of staining: note sharply demarcated, angular, autofluorescent macrophages (u). 4 microns × 400.

Fig. 5. Stained sebaceous gland: The epidermal cells and glandular debris are stained: Note stained macrophages within the gland (m). 4 microns × 400.

Fig. 6. Stained sweat gland: note that epidermal cells have a narrow peripheral zone of fluorescence. 4 microns × 400.

Fig. 7. 3 stained macrophages. Note discrete distribution of reagin-antigen complexes. 1 micron × 1000.
(Rappaport: Antigen-antibody reaction in allergic tissues. II)
PLATE 55

Staining of histamine-tested coated and uncoated tissues with conjugated reagins.

Fig. 8. Egg-coated tissue. Epidermal cells and macrophages stained. 4 microns X 400.

Fig. 9. Uncoated tissue. No fluorescence because antigen is absent. Arrows indicate epidermal-dermal junction. 4 microns X 400.

Fig. 10. Deep dermis of antigen-coated tissue. Marked fluorescence of macrophages (m) and pericytes (p). Note small size, angular shape, and distinct borders of macrophages. Compare with those in challenged tissue (Fig. 3). 4 microns X 400.

Fig. 11. Inhibition of staining in antigen-coated histamine-tested skin. Sections were covered with unconjugated anti-egg reagins before staining. No fluorescence. 4 microns X 400.

Fig. 12. Inhibition of staining in antigen-coated histamine tested tissue. No fluorescence. Arrows indicate epidermal-dermal junction. 4 microns X 400.

Fig. 13. Cross-section of portion of hair follicle of antigen-coated, histamine-tested tissue. Intense fluorescence of epithelial cells. 4 microns X 400.

Fig. 14. Dermis of antigen-coated, histamine-tested tissue. Macrophages show discrete localization of reagin-antigen reaction. Note angular cell with sharp borders. Compare with cells in challenged tissue (Fig. 7). 1 micron X 1000.
PLATE 56

Staining of antigen-coated and uncoated unchallenged atopic skin with conjugated reagins.

Fig. 15. Antigen-coated tissue. Epidermal cells and macrophages stain. 4 microns × 400.

Fig. 16. Uncoated unchallenged tissue. No fluorescence because antigen is absent. Arrows indicate epidermal-dermal junction. 4 microns × 400.

Fig. 17. Antigen-coated dermis. Intense staining of macrophages. 4 microns × 400

Fig. 18. Uncared dermis. No fluorescence. 4 microns × 400

Fig. 19. Cross-section of hair follicle in antigen-coated tissue. Note fluorescence of narrow zone within epithelial cells. 4 microns × 400.

Fig. 20. Inhibition of staining in antigen-coated tissue. Arrows indicate epidermal-dermal junction. 4 microns × 400.
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PLATES 57 and 58

FIGS. 21 to 32. Relationship of reagins to human globulins. Staining of challenged, histamine-tested (uncoated) and unchallenged (uncoated) sections with conjugated rabbit anti-human globulin antibodies.

Note the similarity of localization of fluorescence in all sections stained with reagins and with the anti-human globulin antibodies. Inhibition with unconjugated rabbit anti-human globulin antibodies is definite. All sections 4 microns × 400.

PLATE 57

FIG. 21. Normal (non-atopic) human skin stained for human globulins. Note that epidermis and dermis stain much less than in atopic skin. 4 microns × 400.

FIG. 22. Deep dermis of normal (non-atopic) human skin stained for human globulins. Note that only a few macrophages are stained. 4 microns × 400.

FIG. 23. Antigen-challenged tissue stained for human globulins. Epidermis shows zone of staining just within cell border. Macrophages are intensely stained. 4 microns × 400.

FIG. 24. Inhibition of staining for human globulin in antigen-challenged tissue. 4 microns × 400.

FIG. 25. Histamine-tested tissue stained for human globulin. Note narrow zone of fluorescence within epidermal cells and stained macrophages. 4 microns × 400.

FIG. 26. Inhibition of staining for human globulins in histamine-tested tissue. Fluorescence is absent except for precipitated stain. Arrows indicate epidermal-dermal junction. 4 microns × 400.
PLATE 58

FIG. 27. Unchallenged tissue of egg-sensitive patient stained for human globulins. 4 microns × 400.

FIG. 28. Inhibition of staining in unchallenged tissue with unconjugated rabbit anti-human globulin antibodies. 4 microns × 400.

FIG. 29. Cross-section of hair follicle in unchallenged skin stained for human globulins. 4 microns × 400.

FIG. 30. Dermis of challenged tissue of egg and cottonseed-sensitive patient stained for human globulins. Note greater proportion of stained macrophages than in Fig. 3 (challenged tissue of patient sensitive to egg only). 4 microns × 400.

FIG. 31. Sebaceous gland from histamine-tested atopic tissue stained for human globulins. 4 microns × 400.

FIG. 32. Sweat gland from antigen-challenged tissue stained for human globulins. 4 microns × 400.
PLATE 59

Figs. 33 to 35. The difference in morphology and avidity for toluidine blue (1/1000 aqueous) of macrophages in unchallenged (Fig. 33), challenged (Fig. 34), and histamine-tested (Fig. 35) atopic skin.

The macrophages in the unchallenged and histamine-tested sites (m) are very similar in size and color. Their shape is generally angular, frequently rectangular, with sharply delineated borders. In this stain the histamine-tested differs from the unchallenged site only in the greater dispersion of the various elements because of the edema of the ground substance.

The egg-challenged tissue (Fig. 34) shows not only edema of the ground substance but marked changes in the morphology and stainability of the macrophages (m), and pericytes (p). Note how much larger these cells are than those in the other two sites. Note their bizarre, rounded shape, the absence of sharp definition of the cell borders.

With toluidine blue the macrophages and pericytes of the challenged tissue stain more intensely than do those in the histamine-tested or unchallenged sections.

All 3 sections 1 micron X 400.
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