A Cellular Reaction to Antibody in Tissue Culture Studied with Electron Microscopy*

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ABSTRACT

The reaction of embryonic chick heart cells grown in tissue culture to specific guinea pig antiserum has been studied with electron microscopy. Heart fragments from chick embryos were cultured with a plasma clot. After being tested with antiserum or normal serum, they were fixed with buffered osmium tetroxide and embedded in butyl methacrylate before removal from the glass culture chamber. Thin cells found by phase microscopy to have reacted were sectioned in a plane parallel to the glass surface on which they had grown. The results confirm and extend observations made previously while the reactions were occurring. The plasma membrane, like that of the red cell, becomes disrupted or less resistant to trauma following the action of antiserum. The membranes of mitochondria and endoplasmic reticulum vesiculate and swell. Before nuclear shrinkage becomes prominent, the outer nuclear membrane separates over a large portion of the nuclear envelope and forms one or more large swollen blebs. Thus, the outer nuclear membrane shows a reactivity similar to endoplasmic reticulum. It is suggested that the various physical and chemical changes observed to follow the action of antibody and complement on fibroblasts may be explained by osmotic pressure differences between various cell components. Some basic similarities to the action of hemolytic agents on red cells are noted.

INTRODUCTION

The reaction of cells to antibody and complement is fundamental to understanding the pathogenesis of many disease problems involving immunity and hypersensitivity. To simplify experimental conditions, the use of isolated cells seemed necessary. This study constitutes a sequel to an electron microscopic study of red blood cells and the changes in their surfaces with various agents including antiserum (8, 12).

To study the reaction of other body cells, the controlled conditions of tissue culture were utilized. This enables processes to be studied with the highest resolution of the phase microscope while they are occurring in a living system (11). Phase microscopy facilitates correlation with changes observed in the electron microscope and avoids interpretation of many of the latter as artifactual. The present electron microscopic studies are correlated with these previous studies. Some of the results have been reviewed in a symposium on the mechanisms of hypersensitivity (9).

METHODS

Guinea pig antiserum against chick embryo heart tissue was prepared as described previously (11). To obtain thin growing fibroblasts, explants from 6 to 11 day old chick embryo hearts were placed in a drop of chicken plasma which was clotted with a drop of solution consisting of chick embryo extract to which an equal volume of Hank’s balanced salt solution (BSS) had been added. The tissue was covered with reconstituted human cord serum (Difco) diluted with an equal volume of BSS. Tissue culture conditions were changed from those used previously (11), because, for reasons not entirely clear, satisfactory growth with the former conditions could not be obtained in a new laboratory with the eggs available from different sources. Plasma
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Observed growth was obtained when plasma clots were used and the cells did react to antiserum, but the reaction was slower than in previous experiments (11). The reaction began after an interval of about 30 to 45 minutes and usually took 90 to 120 minutes to progress to a point where there was well developed evidence of nuclear shrinkage.

Normal cells show denser structures than reacting cells (Fig. 1). The dense portions of the cell membranes of two adjacent control fibroblasts are about 280 A apart at the narrowest places where they can be identified as separate. This corresponds to about 140 A or less for the thickness of each cell membrane. Fibers with the periodicity of collagen can be found close to or touching the cell surface. These are quite different in appearance from the fibrin of the clot. In the cytoplasm rod-like mitochondria, lipid droplets, irregular membranes of endoplasmic reticulum, and small granules can be identified. Other as yet unidentified cytoplasmic bodies are also found. Where the nuclear membrane can be seen as "double," the spacing is about 300 A or more. In sections tangential to the surface of the nucleus, nuclear pores about 500 to 700 A in diameter are visible. The nucleoplasm is sparsely granular and the nucleoli appear as irregular accumulations of granules. The less well preserved fine structure in tissue culture cells is similar to that in cells in the outer layers of "well fixed" tissue blocks, and dissimilar in some respects to cells in a deeper zone of optimal fixation. The same unknown factors probably obscure or destroy detail in the exposed cells in each case.

After exposure to fresh normal serum, no change is apparent in the electron micrographs, except for one experiment in which normal serum at a 1:8 dilution proved to be toxic.

Following exposure to antiserum and fresh normal serum, the changes in the affected cells are dramatic (Figs. 2 and 3). Studies of electron micrographs confirm and extend the observations previously made by phase microscopy while the reactions were occurring (9, 11). An extensively altered cell was frequently found lying next to a relatively normal cell (Fig. 3), as was observed in the phase microscopic studies. The plasma membrane is frequently disrupted.

1 The author is indebted to Dr. Emil Borysko for suggesting this and discussing other aspects of technique.
This is not entirely artifactual, because the plasma membrane of adjacent more normal cells is intact. In the thin sections, retraction and swelling of the cytoplasm can not be demonstrated, but there is a marked loss in density and granularity of the cytoplasm, corresponding to the loss of ribonucleic acid (RNA) previously suggested by the Jacobson and Webb staining technique (11). Endoplasmic reticulum may become quite swollen and irregular, assuming bizarre shapes. The many small, circular structures seen here are assumed to represent vesiculation of the reticulum (19). All vestiges of rod-like mitochondria disappear. This happens rapidly, so that morphologic transitions are difficult to trace. Some of the moderate-sized circular forms which remain have remnants of an internal structure and seem to be produced by vesiculation and swelling of mitochondria. Lipide droplets stain more lightly and irregularly, which may indicate swelling or a loss of lipide. It is difficult to tell from these electron micrographs whether the apparent increase in size seen with phase microscopy is due to swelling or to an amalgamation (Fig. 4). Some lipide droplets appear to be surrounded by a membrane.

The most striking and unexpected changes following antiserum appear in the nucleus. As found with phase microscopy, the visible nuclear reaction starts after the cytoplasmic changes are well under way. The granules of the nucleoplasm become coarser than normal and, with shrinkage of the nucleus, accumulate on the inner surface of the inner nuclear membrane (Fig. 3), accounting for the increased optical density found there by phase microscopy. No change could be found in the nucleoli, to correspond to the shrinkage seen with phase microscopy.

The nuclear membrane undergoes separation of its inner and outer layers (Figs. 3 and 5). The inner membrane is obscured by the granules of the nucleoplasm piling up on it, but in some places projections of it into the nucleus can be seen. The outer membrane may separate over a large portion of the nucleus. In some nuclei separation of the outer membrane over a large area forms one or two huge vesicular blebs (Fig. 6). These areas obviously correspond to the large spherical vesicles frequently seen in the phase microscopic studies (11) and even in the stained sections (9), lying next to and apparently indenting the nucleus. The nuclear pores, which appear as rings in tangential section (Fig. 1), show in cross-section the continuity of the inner and outer membranes (Fig. 7). The frequency with which they are seen in normal nuclei (Fig. 1) suggests that they may disappear under the action of antiserum or toxic serum, when the membranes separate to form blebs (Figs. 3 and 7). In some pictures what may be the remnants of pores are seen at the inner nuclear membrane under a large bleb formed by the outer membrane (Fig. 7).

**DISCUSSION**

Plasma clots had previously been avoided in culturing cells to enable determination of the sequences of an immediate hypersensitive reaction of cells (11). The slower reaction in the present experiments is probably due to the diffusion time of antibodies through the clot. This may explain why different stages of reaction can be found at different places in the same growth zone.

The thickness of the plasma membrane of fibroblasts is of about the same magnitude as some measurements of the outer portion of the red blood cell (8). The disruption of the plasma membrane observed after the action of antiserum is not seen in control cells or in adjacent cells protected from the action of antiserum. Whether this physical change appeared during the reaction to antiserum or during the preparation for microscopy, it indicates a weakening of the membrane structure that follows and is etiologically associated with the action of antiserum.

The swelling and fragmentation of mitochondria have long been recognized as reactive processes in cells and have been found in isolated mitochondria (23). The disappearance of the small granular component from the cytoplasm could be due partly to such swelling of the cytoplasm as may occur, but it also correlates with an apparent decrease in RNA (11) and with evidence relating RNA to such small cytoplasmic granules (18). Although the changes in the endoplasmic reticulum seen in these experiments are more bizarre than those usually observed, the lability of this “reticulum” (or α-cytomembranes) is a characteristic stressed by Porter (19).

The accumulation of the granules of the nucleo-
plasm at the inner nuclear membrane as the nucleus decreases in volume could be merely the result of the passage of fluid from the nucleus. It may be associated also with depolymerization of desoxyribonucleic acid (DNA) suggested by previous evidence (11) or with collapse or solvation of a nuclear gel structure (3).

The separation of the outer nuclear membrane from the inner membrane demonstrates the remarkable lability of the potential perinuclear space or cisterna between the membranes. That the nuclear envelope in most cells studied is a double structure formed by two membranes separated by about 300 A is a general finding reported by a number of investigators (22). In several cell types the outer nuclear membrane has been found to be continuous with membranes of endoplasmic reticulum (18, 19, 22), making the perinuclear space continuous with the cavities or cisternae of the reticulum. The cisternal space in more nearly normal cells is considerably smaller than it appears in this pathological condition.

The tendency of the blebs formed by the ballooning of the outer membrane to be spherical when the whole cell is examined (9, 11) suggests osmotic pressure as a cause and, moreover, suggests that the osmotic pressure of the perinuclear space is or becomes relatively higher than that of the nucleus and cytoplasm. It is noteworthy that the swollen outer membrane covers a much larger area than the inner membrane. It is also denser than the normal outer or inner membrane. The density seems more than would be expected with a tangential cut. As the perinuclear cisterna swells, the outer membrane could adsorb material from the cytoplasm or even incorporate parts of the endoplasmic reticulum continuous with or adjacent to the outer nuclear membrane. However, most of the reacting nuclei show a general loosening of the two membranes rather than the latter feature. More in conformity with the idea of a transport mechanism from nucleus to cytoplasm is the hypothesis that the swelling perinuclear cisterna pulls part of the inner nuclear membrane out through nuclear pores into the cytoplasm and some nuclear material along with it. The swelling of both the outer nuclear membrane and the endoplasmic reticulum is evidence for a similar reactivity of the two structures.

The presence of rings or pores in the nuclear envelope of most cells of 300 to 700 A diameter is well established (22), but their function under normal or pathological circumstances is a matter of speculation. Study of the normal fibroblasts in the present experiments shows such pores to be numerous. The size of the holes in the living state, of course, might be smaller or they might even have a single thin membrane across them (22). However, it is not necessary to look for a semipermeable membrane at the nuclear envelope to explain shrinkage of the nucleus (see below). The permeability of some isolated nuclei to proteins (2) suggests that nuclear pores may be of considerable size and electron micrographs also show pores of relatively large size. Nuclei, however, do not appear to be permeable to proteins under all circumstances, and the degree of permeability of the unaltered nucleus in the cell remains a question (4).

Considerable lability of the nuclear pores is suggested by their infrequency and apparent disappearance in some of the reacting cells. Although the inner and outer membranes seem to be attached at frequent points in the early stages of the reaction, they subsequently separate over much larger areas which may involve the whole circumference of a sectioned cell nucleus.

These studies offer further electron microscopic evidence to support a dynamic concept of the nuclear envelope, such as proposed recently (18, 19, 22). Indeed, lability of the nuclear envelope has long been indicated by its dissolution and reformation during cell division and by early tissue culture observations on nucleolar extrusion in fibroblasts (13).

Studies of isolated liver nuclei have added considerable information about nuclear behavior (1, 2). Anderson concludes that nuclear behavior may be explained by the action of ions (or their absence) on a predominantly anionic gel (3). However, if the nucleoplasm is considered to react as a gel without the nuclear envelope presenting an osmotic barrier, this gel reactivity can still be explained on an osmotic basis (10).

Blebs on isolated nuclei have been produced by distilled water and dilute salt solutions (2). Large blebs were produced by CaCl₂ or MgCl₂ solutions. By light microscopy these appear similar to the blebs which occurred in these experiments under the influence of antiserum. The requirement of Ca²⁺ and Mg²⁺ for hemolysis by antiserum (21) suggests similar possible mechanisms.
Other experiments (10) show the production of similar changes in both cytoplasmic and nuclear structures by hypotonic solutions and distilled water. These support the concept that such changes may be due to osmotic pressure differences between the various cell components.

The mechanism responsible for osmotic pressure differences and the movement of water between cell structures remains obscure. The osmotic pressure of liver and kidney cells is normally considerably above that of the blood, but may fall after treatment with chloroform (17). Opie suggests that this normally high pressure is of use to the cell in maintaining its volume while secreting fluid. If this is true of cells in general, the antiserum effects might be brought about simply by damage to a secretory mechanism at the surface of the cell, rather than by a process of increasing the osmotic pressure of some components within the cell.

That antiserum acts at the surface of cells has long been a postulate of immunology (7). The reactivity of the red cell surface found by a previous electron microscopic study seemed to support this postulate (8). The surface ultrastructure of erythrocytes undergoing hemolysis by different agents showed correspondingly different changes in the normally elastic surface. The changes following the action of antibody and complement suggested a two-step process in which an increase in intermolecular forces at the cell surface was accompanied by a change in permeability and a probable consequent increase in osmotic forces within the cell. Confirmation of osmotic factors operating at a semipermeable cell membrane during hemolysis was obtained with another hemolytic agent, resorcinol, with a less rapid action than antiserum (12). Although these experiments do not exclude antiserum from having some action at the interior of the cell, they show changes at the surface that could be responsible for the sequence of events within the cell. To explain these events it is not necessary to call upon more than a surface action for antiserum. On the other hand, it is noted that plasma membranes, mitochondrial membranes, and nuclear membranes can have common antigens (5). An antibody to one, if it could enter the cell, might act on all three.

The effects of antiserum observed by electron microscopy are similar to those seen in light microscopy by several investigators (6, 11, 15, 16, 24) but unlike some effects of antiserum found by others (7, 20). The delayed appearance of changes and their relatively slow development in those cells covered by a fibrin clot, in contrast to the rapid events in thin cells exposed directly to the antiserum, indicate that the reaction does not necessarily automatically progress once started and that variations in degree of response may be readily produced. Antisera may vary in ability to produce injury and in the degree to which that injury is manifest. Such a diversity in degree of action may be analogous to the diversity noted both in antigens and in antibodies (21). The similarity of the reactions to distilled water and to one toxic normal serum indicates that the pattern of reaction to antisera is not specific. The general significance of this type of cellular reaction to antiserum has been discussed in previous papers (9, 11). While other cellular reaction patterns are known, the reaction to this antiserum has many similarities to the pyknotic type of necrosis seen by light microscopy.

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REFERENCES

10. Latta, H., to be published.


**EXPLANATION OF PLATES**

**Abbreviations**

- c, fibers suggesting collagen.
- cm, cytoplasmic membrane.
- er, endoplasmic reticulum.
- f, fibrin of plasma clot.
- l, lipide droplet.
- m, mitochondria.
- n, nucleus.
- nc, nucleolus.
- ne, nuclear envelope.
- np, nuclear pores.
- onm, outer nuclear membrane.

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**FIG. 1. Normal.** Chick embryo heart fibroblasts grown in clot for 48 hours and washed in salt solution before fixing. The cytoplasmic membranes (cm) are close together but they can be recognized as "double" in higher magnifications of some areas. Fibers (c), sometimes showing the periodicity of collagen, are found between cells and, in some areas, closely applied to the surfaces of fibroblasts. The fibrin (f) of the chicken plasma clot has a different pattern. Endoplasmic reticulum (er), mitochondria (m), and lipide droplets (l) are seen in the cytoplasm. Small granules, supposedly representing nucleoprotein, are distributed through the cytoplasm and nucleoplasm. A nucleolus (nc) appears as an irregular grouping of similar granules. The nuclear envelope (ne) is seen as double in some areas. Where the nuclear envelope is cut tangentially, nuclear pores (np) are found spaced close together. \( \times 6,100 \).
(Latta: Cellular reaction to antibody)
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Fig. 2. Antiserum—early reaction. The more superficial cell on the left, next to the fibrin clot (f), shows an early stage of reaction to antiserum. This may be compared to the deeper and more protected cell on the right. The reaction of the cell on the left involves mainly the cytoplasmic components, while the nucleus shows little change. The cytoplasm seems to have lost small granules. The mitochondria (m) appear as rounded forms representing fragments of the original rods. The cristae are disrupted. The membranes with little or no visible internal material, especially the larger irregular forms, are considered to be derivatives of the endoplasmic reticulum (er). Here and in Figs. 3, 5, and 6 fibroblasts have been exposed to antiserum (1:16) and fresh normal serum (1:16) for 3 hours. X 4,500.

Fig. 3. Antiserum-late reaction. The cell on the left next to the plasma fibrin clot shows dramatic changes when compared to the deeper more normal cell on the right. The cytoplasmic membrane (cm) is disrupted. The endoplasmic reticulum has assumed bizarre shapes. Loss of the small cytoplasmic granules is quite apparent. Mitochondria and lipide droplets appear like those in Fig. 2. The nucleus shows considerable shrinkage. The outer nuclear membrane (onm) has separated widely from the inner nuclear membrane, increasing the perinuclear space greatly. No nuclear pores are visible, suggesting that they may have disappeared. The granules of the nucleoplasm are heaped up on the inner nuclear membrane, while the nucleoli are still evident. Although the cells are from the same culture as those in Fig. 2, they show a later stage of the reaction. X 5,100.
(Latta: Cellular reaction to antibody)
FIG. 4. Antiserum. A structure (arrow) with components of the same density as lipid droplets (l). This suggests that fusion of some of the lipid droplets may explain the apparent increase in size seen in light microscopy. Other cytoplasmic structures and a nucleus are seen. This culture was exposed to the action of antiserum (1:16) for 2 hours, in this case without the addition of normal serum. X 7,700.

FIG. 5. Antiserum. The nuclear reaction begins to develop with swelling of the perinuclear space. The points of attachment of the nuclear membranes may be at nuclear pores. X 8,300.

FIG. 6. Antiserum. Nuclear blebs have been formed by the outer nuclear membrane (omn) at both poles of the nucleus. Swollen mitochondria are shown also. X 8,300.

FIG. 7. Toxic normal serum. With permanganate fixation, nuclear pores (arrows) and the continuity of the inner and outer nuclear membranes are evident. The nuclear blebs are clearly continuous with the outer nuclear membrane. The two structures at the base of the bleb on the right may represent remnants of nuclear pores. Although normal serum from most unimmunized guinea pigs produced no reaction visible in the electronmicrographs, the serum from one guinea pig proved to be toxic. This picture shows the effect of this serum diluted 1:8, after 3 hours. The cytologic reaction resembles the reaction to antiserum. (This figure is reproduced with permission from Mechanisms of Hypersensitivity, Little, Brown, and Co. See reference 9.) X 8,600.