Cytotoxic Action of an Antiserum to Soluble Collagen on Tissue Culture of Fibroblasts (34441)

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(Introduced by G. B. Pierce)
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A large amount of information has been accumulated on the physical chemistry of the collagen molecule, but the exact mechanisms by which the fibroblast synthesizes and secretes this protein are incompletely known (1). In our previous work (2), the localization of neutral salt-soluble collagen in tissue cultures of fibroblasts was studied by means of immunofluorescent techniques.

In the present paper the effect of an antiserum to neutral salt-soluble collagen on tissue cultures of fibroblasts was investigated in order to determine specificity and cellular site of action of this antibody. Various authors (3–6) have studied the mechanism of cytotoxic action in other systems, emphasizing the importance of complement in the antibody action on the cellular membrane and the dynamic equilibrium of several cellular components with the incubation medium.

Materials and Methods. As already described, neutral salt-soluble collagen from chicken tendons was purified and injected into rabbits, in order to induce the corresponding antibody (2).

The anticollagen serum had a titer of 1:1600 by complement fixation and the species specificity and collagen-fraction specificity were established by immunodiffusion, complement fixation, and immunofluorescent tests (2).

To study the cytotoxic effect of this anticollagen serum, tissue cultures of fibroblasts obtained from 9- to 11-day chick embryo were used. Trypsinized fibroblasts were grown as monolayers on coverslips in Leighton tubes for 30, 48, and 96 hr at 37°. The nutrient media consisted of Eagle's (90%) and lactalbumine hydrolyzate (10). After 30 hr of incubation, when the monolayer was formed, the cultures were washed in Hanks' solution and the immune serum was added at different dilutions with or without complement (fresh guinea pig serum, diluted with Hanks' 1:4). Cells were incubated with the immune serum from 30 min to 20 hr at 37°. The immune serum used was either inactivated at 56° for 30 min, or not. In each tube 0.3 ml of serum, 0.3 ml of Hanks' and 0.3 ml of complement were added.

As antibody controls, both normal rabbit serum and immune serum previously absorbed with the neutral salt soluble collagen, were used. The absorption procedure was accomplished by incubating 15 mg of the collagen in 1 ml of the immune serum for 30 min at 37°, then 24 hr at 4°, and centrifuged before use.

To study the tissue and species specificity of the cytotoxic action of this antiserum; tissue cultures of liver and mesonephros of 9- to 11-day chick embryo and fibroblasts of 11-day mouse embryo were used in the same experimental conditions.

To study the reversibility of the cell damage caused by the immune serum, some tissue cultures after a 30-min incubation with the antiserum were washed in Hanks' and reincubated with nutrient solution for 20 hr.

Microscopic observations were made in a phase-contrast microscope. Other cultures were fixed in 10% formalin and stained with hematoxylin-eosin or by the trichromic Mallory technique.

The percentage of dead cells was determined based upon their inability to exclude a vital dye. After the incubation with the serum, cells were trypsinized (trypsin 0.25%

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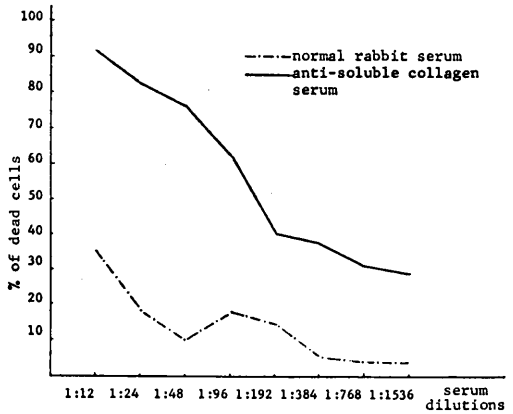


FIG. 1. Cytotoxic action of an anticollagen serum on tissue culture of fibroblasts. Percentage of dead cells after 2 hr of serum incubation. Nigrosin staining.

at 37°), centrifuged, and the pellet washed in Hanks' and stained with nigrosin 0.5%. The percentage of dead cells stained with nigrosin was counted in a hemocytometer.

Results. The effect of an antiserum to soluble collagen on tissue cultures of fibroblasts in the presence or absence of complement, was studied. In the presence of complement the immune serum showed a toxic and lethal effect on fibroblasts. The cells sequentially presented irregularities in shape and vesiculation and swelling of the cytoplasm, which progressed to complete fragmentation in more advanced stages. Finally, nuclear damage, pyknosis, and lysis could be observed. The action was predominantly cytoplasmic; only when high concentrations of the immune serum were used, could nuclear changes be seen (Figs. 3–6). When the immune-serum was very dilute, the cell injury was as described above, but no damage was observed in cells near the center of the coverslip. In the preparations stained by the Mallory technique, thin collagen fibrils were observed, even in the cultures incubated with high concentrations of the immune serum. Cellular damage was already observed after 30 min of incubation with the antiserum. The degree of the injury increased both with concentration of antiserum and time of incubation, reaching a maximum at 15–20 hr.

Complement was demonstrated to be indispensable for the cytotoxic activity. In all cases, fresh guinea pig serum diluted 1:12 (final dilution) was used. More concentrated solutions were toxic. The normal rabbit serum used as a control, was also cytotoxic, with the same characteristics of cellular damage as above. However, this effect appeared only when high concentrations were used (final dilution 1:64), while the immune serum retained its action up to a dilution of 1:768 (Figs. 1, 2). The cytotoxicity of the immune serum previously absorbed with its specific antigen disappeared at a dilution of 1:128. The cytotoxic action was the same whether the serum was heat-inactivated or not.

The cell damage caused by the immune serum was irreversible, as shown by removing the antiserum, washing the cells, and then adding nutrient media for 20 hr or longer. In order to determine the tissue and species specificity, liver and mesonephros cells of 9- to 11-day chick embryo and fibroblasts of

FIG. 2. Normal chick embryo fibroblasts, exposed to normal rabbit serum (dilution 1:64) and complement for 20 hr. ×128.

FIG. 3. Normal chick embryo fibroblasts, same conditions as Fig. 1. ×400.

FIG. 4. Culture of chick embryo fibroblasts, exposed to antisoluble chicken collagen serum (ASC) (dilution 1:64) for 20 hr. ×100. Clumps of agglutinated and pyknotic cells instead of a cell monolayer as control (Fig. 2).

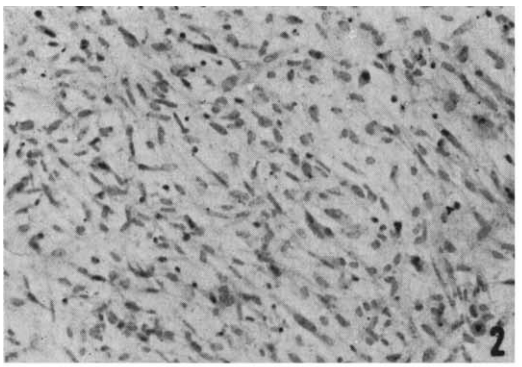
FIG. 5. Same as Fig. 4. ×400. Marked blistering of the cell surface.

FIG. 6. Same as Fig. 4. ×1000. Pyknosis and presence of large and numerous vacuoles in the cytoplasm.

FIG. 7. Same as Fig. 6. ×1000.

FIG. 8. Culture of mouse embryo fibroblasts, exposed to ASC (dilution 1:24) for 20 hr. ×100. No cytotoxicity is observed.

FIG. 9. Same as Fig. 8. ×400.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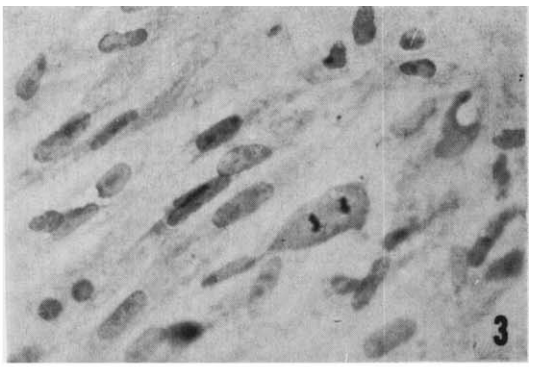
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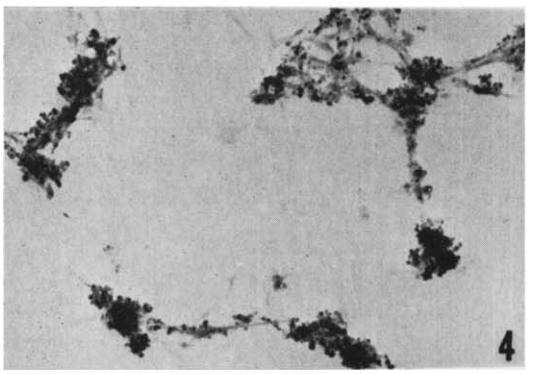
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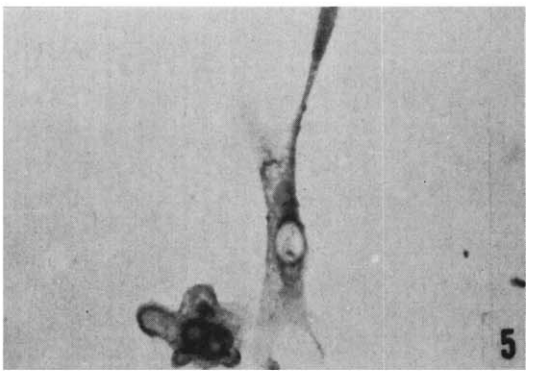
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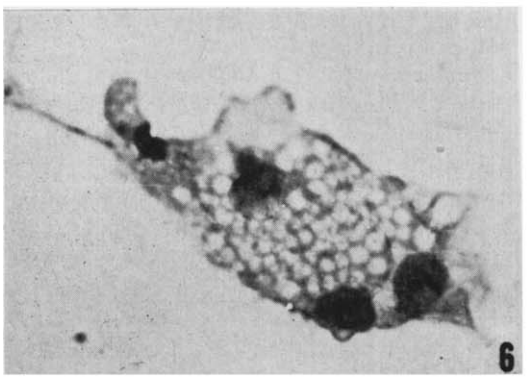
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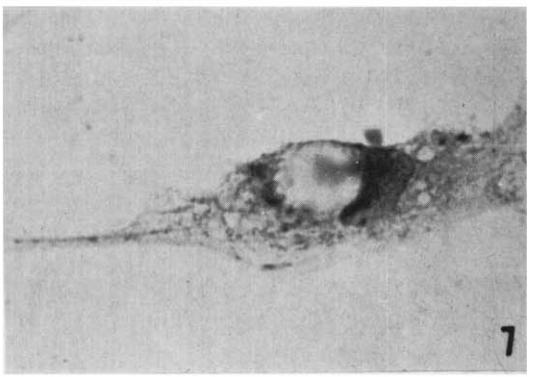
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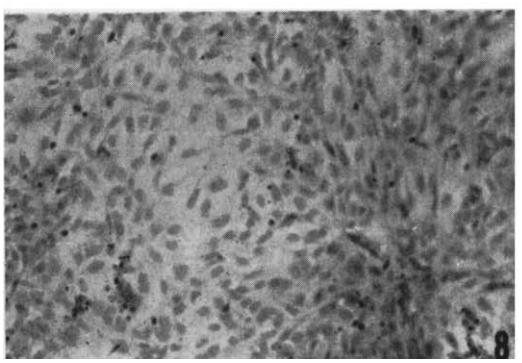
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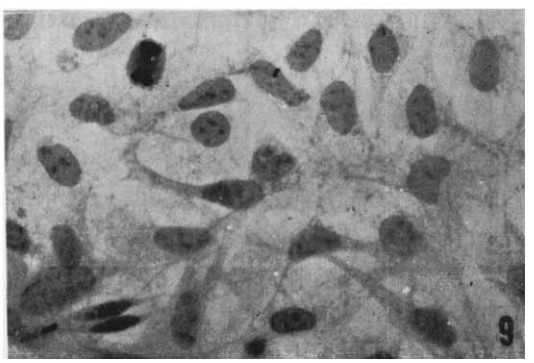
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11-day mouse embryo were cultured for 24 and 48 hr and incubated with the anticollagen serum in the presence of complement. In the three cases, no cytotoxicity was shown, even using high concentrations of the serum (Figs. 7, 8). The percentages of dead cells of cultures exposed to normal serum and anticollagen serum, stained with negrosin, are shown in Fig. 1. When incubation was performed with the antiserum, a larger percentage of dead cells was observed.

Discussion. The results obtained indicate that the antiserum to neutral salt-soluble chicken collagen has a pronounced cytotoxic action on tissue cultures of chick embryo fibroblasts, effective up to a 1:768 dilution of antiserum. The degree of the cellular damage depends on the concentration of the immune serum used and the time of incubation. The cytotoxic action is complement-dependent and the antiserum retains its action even after heat inactivation (30 min at 56°). The injury is present after 30 min of exposure to the antiserum. Moreover, it is irreversible as shown when cells were incubated with the serum, rinsed, and reincubated in nutrient media for 20 hr. The morphological changes caused by the antiserum are not specific for this antigen-antibody reaction, but are similar to cytotoxic effects of other antisera in other cell systems. According to the results obtained with the cultures of mesonephros and liver of chick embryo and mouse embryo fibroblasts, a species and tissue specificity is apparent. With normal rabbit serum there was a cytotoxic action present up to a dilution of 1:64 and with the antiserum previously absorbed with its specific antigen, cytotoxicity was present up to an antiserum dilution of 1:128.

Comparing these results with others reported in different systems, the cytotoxic action of the normal rabbit serum in high concentrations has already been demonstrated (7). The complement-dependency of cytotoxic antigen-antibody reactions were also observed in different cell systems by Bitensky (8), Goldstein (7, 9), Green and Goldberg (3), Taylor (10), and Wissler (11). The characteristics of cellular damage observed by these authors were very similar to our observations. The irreversibility of the cell damage has also been reported (6). In most of the cases a species specificity in the cytotoxic action has been claimed (as in 6, 7, 12–14) but tissue specificity was only observed by Pulvertaft incubating human thyroid cells with the serum of patients with Hashimoto's disease (15). It is important to emphasize that the majority of the authors used complex antigens, such as tissue or cell culture homogenates, in order to induce antibodies.

Only Robbins and co-workers in a brief note (16) reported the cytotoxic action of an antiserum against an acetic extract of chicken collagen on chick dermis explants.

The use of a more specific anticollagen serum, restricting the number of antigenic determinants present in heterogeneous antibodies, enhances the significance of the cytoxicity.

The collagen species specificity observed here as a cytotoxic action is in accordance with our previous work and those of other authors (2, 17–19) in which specificity was judged by complement fixation, hemagglutination, double agar diffusion, and immunofluorescent tests.

Summary. The cytotoxic effect of an antiserum to neutral salt-soluble chicken collagen on tissue culture of chicken fibroblasts was studied. The cytotoxic action was present up to a final dilution of the antiserum of 1:768, and was complement-dependent. No cytotoxicity was observed when tissue cultures of liver or mesonephros of chick embryo or mouse embryo fibroblasts were incubated with the antiserum. The action was, therefore, tissue- and species-specific. The damage observed consisted of irregularities in the cell membrane followed by vesiculation and swelling of the cytoplasm. Nuclear changes were observed only when high concentrations of the antiserum were used.

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Received Sept. 12, 1969. P.S.E.B.M., 1970, Vol. 133.