The Action of Cytotoxic Antisera on the HeLa Strain of Human Carcinoma

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Tissue culture affords a method for the study of the direct effects of cytotoxins separated from the cellular defense, vascular reactions, clotting mechanism, and other mediators of resistance and homeostasis that exist in vivo. Lambert and Hanes in 1911 were the first to utilize this technic for such study (19). They reported that rat sarcoma would not grow in plasma coagulum from guinea pigs immunized with rat sarcoma tissue. Since that time a number of observations have been made on the in vitro effects of cytotoxic antibodies. However, divergent views remain on the specificity of antisera formed by heteroplastic transplantation, and certain observations have not been made on the nature of the injury inflicted upon cells by antiserum.

Foot (4) reported that rabbit antichicken bone marrow serum was organ-specific, in that it affected only the bone marrow of the chicken, and that these cytotoxins damaged bone marrow from other species as well. Verne and Oberling (34) claimed that rabbit antirat kidney serum damaged epithelium and fibroblasts of kidney in tissue culture but had no effect on such cells from other organs of the rat. Lumsden and co-workers went even further in claiming specificity for antibodies formed in heterologous species (34–36). It was their conclusion that antiserum from rats, rabbits, and other mammals injected with mouse carcinoma was toxic to that neoplasm in vitro and not to other mouse tissues. In addition to being tissue-specific, it was claimed that this antiserum was cancer-specific and would damage malignant but not normal cells from other species. Harris (9) demonstrated complete specificity with antiserum prepared by injecting chick and mouse tissues into rats, but he found partial organ specificity, in that antichick heart serum was more damaging to chick heart in vitro than to chick spleen or kidney. Pomerat (32) observed that antirat spleen serum, while it was damaging to both rat sarcoma and spleen in vitro, had a greater titer of activity with the homologous tissue. Grunwalt (7), in the same laboratory, found that an alcohol-soluble fraction of chicken brain was capable of eliciting antibodies with a high degree of specificity for cultures of nervous tissue. Kimura (17) reported that rabbit antichick embryo brain serum had a weak cytotoxic effect on chick fibroblasts but a marked effect on cells migrating from cerebral tissue.

There are observations in disagreement with some of the conclusions suggested in the aforementioned work. Lambert (18) injected three sets of guinea pigs each with a different rat tissue. He found that antiserum from the three sets was overlapping in effect. It was his conclusion that “cytotoxins formed by injection of different body tissues into foreign species are to no extent specific for the tissue injected.” Phelps (31), attempting to duplicate some of Lumsden’s work, encountered no evidence for organ-specific cytotoxins or heterogenetic cancer-specific antibodies. Using antiserum from rabbits immunized with mouse tumor, Pybus and Whitehead (33) were unable to show any difference in effect upon normal or malignant mouse tissue. In addition, with antiserum from rabbits injected with human carcinoma, they were unable to evoke any specific activity on malignant tissue of the mouse.

The problem of specificity has been the chief concern of most of the early workers studying the action of cytotoxins in vitro. Indeed, the action of the antiserum was determined by its ability to inhibit outgrowth of an explant. In 1929, Niven made the first study of the damage inflicted upon growing cultures of cells by cytotoxic antiserum (30). The effect of rabbit antimouse embryo serum upon hanging drop cultures of mouse embryo tissues in vitro was studied. Harris (8, 9) also de-
scribed in detail the cellular effects of cytotoxins on cultures of chick and mouse tissues.

In the present investigation, the problem of specificity has been re-examined with human material, the HeLa strain of an epidermoid cervical carcinoma (6). It has also been our purpose to observe the cellular effects of cytotoxic antisera with the advantages afforded by phase contrast microscopy, alone and combined with time-lapse cinematography. In addition, the use of a perfusion chamber permitted continuous observation of a culture during the successive administration of normal nutrient medium, normal serum from the species used to prepare the cytotoxins, the antiserum, and finally the return to normal nutrient medium.

MATERIALS AND METHODS

Antigen.—The antigen employed in the preparation of the cytotoxic antiserum was prepared exclusively with cells of the HeLa strain. A stock of these cells was maintained in roller tubes. No clot was utilized, since the cells grew well on the glass surface. Five to six culture colonies were contained in each roller tube. The fluid nutrient medium consisted of 80 parts of Gey’s balanced salt solution, 45 parts of human ascitic fluid, and 5 parts of chick embryo extract. Penicillin was added to make a final concentration of 1,000 units/ml. Two ml. of this solution was placed in each roller tube and replaced twice a week. The explants were subcultured every 2 weeks.

In preparing the antigen for injection, cultures 7–10 days of age were removed from the roller tubes, pooled and washed 3 times with the balanced salt solution, then ground with a Potter tissue grinder. The entire suspension was employed as the antigen and used immediately after its preparation.

Immunization procedures.—Two rabbits and two roosters were used for the production of antibodies. The immunization schedule for the young adult white rabbits was four intravenous injections/week for 4 weeks. Each injection was prepared with the HeLa contents of five roller tubes. Ten days after the last injection the animals were bled to obtain serum for preliminary tests. One rabbit was discarded, because its serum failed to show any cytotoxic effects. The second rabbit was kept for 6 months and was the subject of the given booster injections. One subcutaneous injection of the contents of one roller tube was followed the next day, 1 week, and 2 weeks later by intravenous injections with the HeLa cells contained in twenty tubes. The animal was bled a week after the second and third intravenous injections.

Two Rhode Island Red roosters, each weighing 5 pounds, were also used to produce antiserum. Each animal received two subcutaneous injections, 1 week apart, of the cellular contents of ten roller tubes. Two months later they were given one subcutaneous and two intravenous boosters in the manner described above. Bleeding followed 5–7 days after the last two inoculations. One chicken died of an unrelated illness during the course of immunization. For subsequent bleedings of rooster or rabbit, if more than a month had elapsed since the last inoculation, the animal received one subcutaneous and one intravenous booster injection. It was found desirable to precede the intravenous injections with one subcutaneous dose in order to reduce the hazard of anaphylactic response to the intravenous injection.

Test materials.—Tissue cultures to be used as test antigens were first set up on cover slips in roller tubes without a clot. However, it was later found that the presence of the clot did not interfere with the action of the antiserum. Although many explants grew well on the cover slips in the roller tube, they had a tendency to wash off and attach to the wall of the roller tube. Subsequently, all cultures were prepared in a clot made from the mixture of chicken plasma and chick embryo extract in equal parts. Each 12 X 60-mm. cover slip contained three or four explants in a clot, and two cover slips, back to back, were placed in a roller tube. Fluid nutrient containing 50 parts Gey’s balanced salt solution, 45 parts horse serum, and 5 parts chick embryo extract was used to replace the medium containing protein from human sources. This medium was also used as the diluent for the antiserum. For experiments, HeLa cultures 1–2 weeks of age were employed. In addition to HeLa cells, cultures were prepared similarly with human tonsils (from which fibroblasts and epithelial cells were derived), human fetal muscle, and the BS mouse mammary adenocarcinoma (1). Human white blood cells were also studied by preparing explants of buffy coats on hanging drop slides in a mixture of chicken plasma, chick embryo extract, and suitably diluted antiserum in equal parts.

Tests were carried out in roller tubes, closed chambers, and perfusion chambers, all kept at 37° C. More than 150 cultures were studied. Observations and photographic recordings were made by phase contrast microscopy, with American Optical Co. equipment. Still photographs were made with Ansco Isopan film. Motion pictures were taken with Kodak Super-X film, and selected frames were enlarged on DuPont 506 x-ray film.

RESULTS

Titer of the Cytotoxic Antibodies

The rabbit antiserum showed cytotoxic activity in a dilution of 1:60; and chicken antiserum, 1:20. No effort was made to increase the titer through the use of adjuvants or other special procedures.1 Although Wolfe (35) reported that keeping chicken antiserum at 40° C. for several days increased the activity of precipitins, the opposite was the case for cytotoxins. However, the antiserum was satisfactorily stored in a deep-freeze unit until used.

The Cytotoxic Effect of Antiserum on HeLa Cells

Except when otherwise noted, the following observations were made with the use of rabbit antiserum but, in general, apply as well to the action of chicken antiserum.

The first observations were carried out with undiluted antiserum. Within 1 hour most of the cells were destroyed. When the antiserum was diluted 1:10 in the horse serum medium, the onset of injury was apparent in about 20 minutes and was complete in less than 2 hours. All experiments were carried out at this dilution, since it was well out of the range of some of the nonspecific reactions that are discussed below.

Not every cell showed all the effects noted, but they were all observed when a large population of cells was studied.

The cell membrane and cytoplasm.—The first effect was motion of the cell body followed by either...
swelling or contraction. Which effect occurred probably depended upon how the cell was damaged. The sheetlike outgrowth, characteristic of HeLa cells, was usually disrupted in many places by retracting cells. The cell processes in some cases were withdrawn. Pinocytosis ceased. Vacuoles within the cytoplasm coalesced and became larger. Activity within the cytoplasm decreased, except for certain granules that continued to move by Brownian motion, long after the cell was apparently dead. The general appearance of the cytoplasm was one of greater granularity (Fig. 1). Membranous extrusions from the cytoplasm resembling blisters were common (Fig. 2). They appeared to a greater degree following the application of rabbit antiserum than with the chicken material, but this may have been owing to the higher level of antibodies in the rabbit antiserum.

The mitochondria.—The cytoplasmic and nuclear alterations usually began in about 20 minutes and continued for about 1 hour. In contrast, while the effect of the anti-HeLa serum on the mitochondria became manifest at about the same time the other changes were beginning to occur, it was completed in less than 5 minutes. The to-and-fro motion of the filamentous mitochondria ceased. This was followed by fragmentation and contraction, the mitochondria first thickening and then forming small dense spheres which enlarged, as if swelling, so that a less dense central portion was apparent. The final appearance then was that of dark rings or spheres with a light center. These damaged mitochondria remained rigidly fixed in the granular cytoplasm.

The nuclei.—The nuclear changes were striking. There was an increase in the density of the nucleus. All motion within the nucleus ceased, but in some cases intense Brownian movement was seen. The nucleus became more discrete, rounded up, and decreased in size. Under phase-contrast observation this pyknosis resulted in a halo of light in the perikaryon. The nucleolus changed its refractivity and usually appeared more dense. But in some cases it disappeared altogether.

The cellular damage was to some degree reversible if the antiserum was replaced by normal nutrient medium but not after nuclear damage became apparent. Some cells within the population appeared to escape the effects of the antiserum. An estimated 3–5 per cent of cells under continual observation for up to 7 hours appeared uninjured, despite the frequent addition of fresh antiserum.

Eleven cinematographic sequences were made by perfusing the antiserum through a chamber containing living HeLa cells. Each motion picture sequence was made in the following way: The culture was set in a perfusion chamber with its original medium. The activities of the cells were recorded for 1 hour, eight exposures being made each minute. Normal rabbit or chicken serum (1:10) was then perfused. Exposures were taken at the same rate for another hour, and anti-HeLa serum (also 1:10) was introduced. The culture was again perfused with its regular nutrient fluid at least 1 hour after all or a part of the cells in the photographic field had shown cytotoxic effects.

The sequence shown in Figs. 3–10 was selected to illustrate the action of the antiserum on living cells, and several frames were enlarged and reproduced to show the appearance of the same group of cells under various treatments. Figure 3 shows a field of HeLa cells 7 days after subculturing in their regular nutrient medium. Figure 4 represents the same field of cells in normal rabbit serum (1:10). No demonstrable changes can be noted in this medium. The introduction of the anti-HeLa serum revealed no change for approximately 25 minutes (Fig. 5). The nuclei then very rapidly became pyknotic, and the cytoplasm appeared coagulated (Figs. 6–8). After 45 minutes in the antiserum there were no further changes (Fig. 9). The return to normal nutrient medium did not restore the normal activities of the injured cells (Fig. 10). The sequence shown in Figures 11–18 was chosen to illustrate the changes undergone by the mitochondria. This sequence, chosen because of its optical clarity, was made with chicken anti-HeLa serum on normal human tonsillar epithelium, but the action of chicken and rabbit antisera on HeLa cells was comparable. The effect of the antiserum on the mitochondria took place concomitantly with that on the nucleus. Figures 11 and 12 represent a part of the cell in regular nutrient medium and normal chicken serum (1:10), respectively. Figure 13 shows the appearance of the nucleus and the mitochondria 10 minutes after perfusion with chicken anti-HeLa serum. At this time no remarkable change can be observed. Twenty-one minutes after the introduction of the anti-HeLa serum, the nucleus became pyknotic, and the long filamentous mitochondria very rapidly rounded up (Figs. 14–16) and in a few minutes became spherical with a light refractive center (Fig. 17). This damage was not reversible by replacing the antiserum with normal nutrient fluid (Fig. 18).

The Effect of Cytotoxins on Cells in Mitosis

Niven (30) noted that a strong dose of cytotoxin "fixes" the cells and that mitotic cells would thus appear normal. Furthermore, she stated that "the process of mitosis stops abruptly." The effect of anti-HeLa serum on mitosis of HeLa cells was
studied in eight motion picture sequences, but in most cases the mitotic cells seemed to be more resistant to the antisera than were the interphase elements. Usually many interphase cells appeared damaged by the time mitotic cells began to show any evidence of injury. In two cases mitosis was not interfered with, but the cells died when they reached interphase. The mitotic cells that were injured showed cessation of chromosome activity, and in some cases there was clumping of the chromosomes on the metaphase plate. Blister formation occurred, but it was infrequent.

The Effect of Anti-HeLa Serum on Normal Human Tissues in Vitro

The antisera (1:00 dilution) was tested against several types of normal human tissues in vitro, including fibroblasts and epithelium from human tonsil and skin, fibroblasts from fetal muscle, and human leukocytes. The effect of the antisera on the white blood cells was estimated by two criteria: the degree of migration of the cells from the explant and the motility of the cells. Within 8 hours the hanging-drop preparation of white cells displayed marked agglutination in large clumps. The individual leukocytes lost the characteristic amoeboid appearance that they have in vitro, and all cell motion ceased. The controls, in contrast, showed uniform and diffuse migration from the central explant, and about 50 per cent of the cells continued to show motility for 24-36 hours. No blister formation or pyknosis of the nuclei was noted in the case of the leukocytes. At one time 18-week-old human fetal tissue was available for experimentation. Good outgrowth of fibroblasts was obtained from cultures of striated muscle, but despite repeated attempts our rabbit antisera failed to injure this tissue. In the case of the fibroblast and epithelial cells derived from adult and newborn skin, and from the tonsils of children, the reaction to the antisera was the same as with the HeLa cells, the characteristic nuclear and cytoplasmic changes described above.

The Effect of Anti-HeLa Serum on a Mouse Carcinoma in Vitro

The B3 mouse mammary carcinoma (1) growing on coverslips was placed in roller tubes. Inactivated antisera in 1:2 and 1:10 dilutions in 2 ml. of fluid nutrient was added to a total of twelve cultures. The coverslips were removed at 2- and 4-hour intervals and examined by phase contrast microscopy. There was no apparent injury to the cultures.

Absorption Experiments

Absorption with components of the fluid nutrient media.—Since the HeLa cells that were used as antigens had been grown in nutrients containing chick embryo extract, human ascitic fluid, and horse serum, the anti-HeLa serum was tested for precipitation with these substances. Aside from the fact that these proteins might not have been washed away in preparing the cells for injection, Langman (20) has reported that cells in tissue culture may incorporate antigenic substances from the culture medium and that these will be manifested by precipitation tests. In our case precipitation tests were negative with chick embryo extract, but the anti-HeLa serum gave a positive ring test with undiluted human ascitic fluid. With horse serum a positive ring test was obtained with the antisem diluted 1:2. One ml. of antisera was absorbed by the addition of 0.1 ml. each of human ascitic fluid and of horse serum and left overnight at room temperature. Precipitation tests were then negative. The absorbed serum was tested for cytotoxicity. Its activity against HeLa cells remained unchanged. Epithelial cells and fibroblasts from human tonsillar tissue were also injured following application of the absorbed antisera.

Absorption with extracts of normal human skin.—From the observation that anti-HeLa serum damaged normal human cells, it appeared that there were antigens common to HeLa and normal cells. The important question remained: was there any anti-HeLa activity remaining after absorption with normal human skin extract? The extract was prepared by mincing infant foreskin, then grinding the mince in a mortar and pestle with sand and a small amount of Gey’s balanced salt solution. The suspension was then centrifuged and the supernatant used as the skin extract. A precipitation test was performed with the anti-HeLa serum and the extract. There was heavy precipitation, a 4-plus ring test, within 2 hours. At a dilution of 1:10 there was also a distinct ring at the interface, but the reaction took approximately 4-5 hours. The rabbit anti-HeLa serum was absorbed with this extract (10 parts of antisem with 2 parts of extract) for 24 hours. Tests of this absorbed antisem with HeLa cells showed that it had lost its cytotoxicity even in the dilution of 1:2. The chicken anti-HeLa serum could similarly be neutralized by absorption with human skin extract.

Cytotoxic Action of Normal Serum on HeLa Cells

It has been demonstrated that normal rabbit serum may damage cells of other species in tissue culture (25, 30, 38) and that this cytotoxic effect can be removed by heating the serum at 56° C. for 30 minutes. Our experiments with HeLa cells showed similar results. In addition, it was found...
that serum from adult rabbits was more toxic than that from immature animals. At the dilution of 1:2 approximately 2 per cent of the cells were damaged in 1 hour, showing pyknotic nuclei and cytoplasmic blebs or blisters, but when diluted 1:10 the rabbit serum exerted no deleterious effects, even without heating. An attempt was made to restore the cytotoxic activity to normal rabbit serum that had been inactivated by heating, by the addition of guinea pig serum with known quantities of complement. The complement titer of normal rabbit serum was 16 units or less. Experiments were made in duplicate, adding guinea pig serum with 2, 4, 8, and 16 units of complement to inactivated serum diluted 1:2 with balanced salt solution. The results were completely negative for damage to the HeLa cells. Guinea pig complement alone titrated at 2, 4, 8, and 16 units in Gey's solution also produced no toxic effect. When the rabbit antiserum was heated to 56° C. for 30 minutes, it retained its cytotoxic effect. Chicken antiserum, when inactivated, retained a considerably weakened cytotoxic effect.

**THE EFFECT OF ANTI-HELA SERUM ON HELA CELLS**

**In Vivo**

The outstanding disadvantage to the use of cells of the HeLa strain for the study of cytotoxins is the absence of a natural host for in vivo experiments. In some small measure this may be made up by heterologous transplantation in the form of anterior chamber grafts. When cells from tissue culture are to be used, the technic differs from that classically employed, since a mass of tissue cannot be poised in the bevel of a needle for introduction by a stylette. Instead, the cell colonies are washed in balanced salt solution and cut into small pieces. This suspension is then taken into a tuberculin syringe and concentrated to about 0.1 ml. The cellular material is then manipulated to lie within or at the entrance to the needle. A sharp, short-beveled, #25 needle is used. The injection into the anterior chamber is made at the corneoscleral junction. The intra-ocular transplantation of HeLa cells was first reported by Gey, Bang, and Gey (8). In the present experiments growth was rapid and continued until vascularization took place. The first procedures were done with rats. After 4-6 days the eyes were removed. Stained sections showed actively growing tissue. Since our antiserum was prepared in rabbits, it was felt that it would be desirable to use this animal for anterior chamber experiments. The use of the same species would eliminate the possibility of a foreign protein reaction. In five out of six rabbit eyes used, the cells grew rapidly in the anterior chambers. The injection of 0.1 ml. of antiserum into the anterior chamber was sufficient to cause regression and eventual disappearance of the implanted tissue. Growth of the transplant continued in the non-treated eyes until the graft became vascularized. It was then slowly resorbed. Normal rabbit serum was without effect on HeLa cells growing in the anterior chamber of the rabbit eye.

**DISCUSSION**

Our observations on the cellular effects of cytotoxic antisera on human cells in vitro are generally in agreement with the findings concerning cells of lower animals reported by Niven (30) and by Harris (8, 9). There are two points, however, at which our results differ. Niven reports that, within 20 minutes after the application of the antiserum, “the mitochondrion cannot be distinguished.” Harris states that “the mitochondria become less evident or disappear.” With the ordinary light microscope the change of the mitochondria from a filamentous to a globular form would be difficult to perceive, particularly in cytoplasm that has assumed an all-over granular appearance.

In the present studies the antiserum did not appear to have a prompt antimitotic effect. In some cases cells continued to divide in the presence of antiserum and died only when they reached interphase. Mitotic cells were seen to undergo clumping of the chromosomes on the metaphase plate, but, since mitotic abnormalities are frequent in HeLa cells (11), the former condition is of greater interest. Does the mitotic cell, engaged in reproduction, temporarily escape injury because pinocytosis has ceased or the permeability of the cell membrane has been altered? This question must be left open in the absence of pertinent information.

Another striking feature of the effect of the antiserum is the great variety of cell responses, both sequentially and morphologically. In some cases the nuclear effects may be more apparent, in others the cytoplasmic. In point of time the nuclear changes are frequently seen first, but often these are delayed and are preceded by membrane and cytoplasmic alterations. This is not surprising in light of the nature of the materials employed in these tests. A suspension of crushed and broken-up cells was used for injection into the animals; a myriad of antigens was employed. The antiserum developed was no doubt correspondingly heterogenous. When one considers that this mixture of antibodies to an untold number of cell antigens was applied to a population of cells, young and old, dividing and dying, with varying abnormalities and at varying levels of physiological activity, the great variety in response is not unexpected.
Of great interest are the cells that were not apparently injured by the antiserum. Other workers have made similar observations. Kalfayan and Kidd (15) observed that 0.5 per cent of Brown-Pearce carcinoma cells, grown in vitro, seemed to escape injury by the application of a cytotoxic antiserum. Harris (9) noted that, with cultures of chicken heart, the greatest damage occurred at the periphery of the cultures, while cells more centrally located frequently escaped damage. Two possibilities present themselves as explanations for this phenomenon. Either the great number of cells in the culture constitutes an excess of antigen sufficient to deplete the antiserum, or, in a population of mammalian cells, as in a colony of microorganisms, there are genetic variants resistant to a noxious agent. The first possibility is weakened by the observation that these cells remained undisturbed despite several applications of fresh antiserum. The second possibility is strengthened by the observations of Law on the development of resistant strains of leukemic cells to chemotherapeutic agents (21, 22). There is also the consideration that, when primary explants are used for the study of cytotoxins, there will be a variety of cell types in the outgrowth and that these may vary in their susceptibility to cytotoxins or other agents. Ludford (23) was the first to point out this pitfall in interpretations made from tissue cultures. Therefore, the observation that this phenomenon occurred in a culture that is presumably of a single cell type is of particular importance. However, it must be admitted that continual observation of particular cells in closed chambers was limited to a few hours, and cells that appeared normal during this period might manifest abnormalities at a later time.

No evidence is found in this work to support the view that antiserum against malignant tissue, formed in heterologous species, has any degree of cancer specificity. Our antisera were species-specific, but not tissue-specific. To achieve a cancerspecific antiserum, we must first have an antigen that is unique to the malignant tissue. Again we must comment on the limitations of our method. When a mosaic of antigens is injected into an animal, antibodies will be formed only to some of them. Competition for antigen sites and masking of antibodies for fowl tumor agents were prepared similarly by Kabat and Furth (14). Kidd (16) states, however, that he was unable to isolate serologically unique components from other rabbit tissues, normal or malignant. Using lipoidal antigens obtained from certain mouse tumors, Hoyle (10) reported positive complement-fixation tests only with the sera of the animals bearing these tumors. It must be kept in mind that, in some cases at least, the differences between normal and malignant cells may be quantitative and not qualitative (2, 3, 27–29).

Although the rabbit and chicken antisera were lacking in specificity for the HeLa cells, the cytoxic efficacy of antiserum from different species points up another limitation in the therapeutic use of antiserum and suggests a solution. Any antiserum, no matter how specific, if prepared in a heterologous species, is still a foreign protein, and, if used repeatedly, sensitization and anti-antibody formation will occur. It is possible that this difficulty could be overcome by preparing a battery of antiserum from different species of animals to be used successively as sensitization develops.

**SUMMARY**

1. The cytopathogenic effects of rabbit and chicken anti-HeLa sera have been described. The action of the antisera was observed on HeLa cells, normal human cells, and a mouse mammary carcinoma in tissue culture.

2. Rabbit and chicken anti-HeLa sera were shown to be cytotoxic for a variety of human tissues and not specifically directed against HeLa cells.

3. Anti-HeLa serum, under the conditions described, had no effect on the in vitro growth of a mouse mammary carcinoma.

4. The efficacy of rabbit cytotoxic antiserum upon HeLa cells "in vivo" has been demonstrated.

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REFERENCES


FIGS. 3–10 are selected frames from motion picture sequence 41 XIII B, showing the cytological effects of rabbit cytotoxic antiserum on cells of the HeLa strain. The number of the figure is at the lower right corner of each photograph. The time-lapse (in minutes) between successive frames is given at the upper left corner of each photograph. For magnification see scale on Fig. 3.

FIG. 3.—Appearance of cells in normal nutrient medium.

FIG. 4.—Twelve minutes after perfusion with normal rabbit serum (1:10). There are no demonstrable changes in cellular structure.

FIG. 5.—Twenty-two minutes after perfusion with rabbit anti-HeLa serum (1:10). No remarkable evidence of injury can be noticed at this time.

FIG. 6.—Beginning of the cytotoxic effect. Note the discreteness of the nuclei and the change of refractility of the lower nucleolus of the binucleate cell on the right.

FIG. 7.—Retraction of the cytoplasmic processes of the cell on the left. The binucleate cell shows further changes of refractility of nuclei and nucleoli. The cytoplasm appears more vacuolated and changes its refractility also.

FIG. 8.—The nuclei have apparently reached the point of maximum damage, showing extreme pyknosis and enclosure by brightly refractile halos. The beginning of blister formation at the lower portion of the binucleate cell is evidenced.

FIG. 9.—There is enlargement of the blister, which is somewhat indistinct. Other parts of the cell show no further changes at this time.

FIG. 10.—The return to normal nutrient medium brings no recovery to the damaged cell. The morphologic changes in the cell on the left are less apparent. However, increased granularity of the cytoplasm is seen in Figs. 9 and 10. On projection of this sequence this cell was seen to cease movement; it was regarded as being severely damaged.
FIGS. 11–18 are selected frames from motion picture sequence 50 XIV L2, showing the response of the nucleus and the mitochondria of a cell derived from human tonsillar epithelium to treatment with chicken anti-HeLa serum. The number of the figure is at the lower right corner of each photograph, and the time-lapse (in minutes) between successive frames is given at the upper left corner of each photograph. For magnification see scale on Fig. 11.

Fig. 11.—Appearance of a nucleus and mitochondria in normal nutrient medium.

Fig. 12.—Sixty-two minutes after perfusion with normal chicken serum (1:10). There is no evidence of injury.

Fig. 13.—Ten minutes after perfusion with chicken anti-HeLa serum (1:10). There is no evidence of injury at this time.

Fig. 14.—The beginning of cellular damage. Note that one of the nucleoli has disappeared, leaving a ghost spot to indicate its former position. The mitochondria have become shorter, thicker, and in some cases fragmented.

Fig. 15.—Further rounding up of the mitochondria.

Fig. 16.—Practically all the mitochondria are globular, and some are beginning to swell.

Fig. 17.—Most of the mitochondria have assumed a ring-like appearance. Damage to the nucleus and mitochondria in the adjacent cell in the upper left portion of the frame is also apparent.

Fig. 18.—Perfusion with normal nutrient medium brings no recovery. Pyknosis of the nucleus is most evident at this time.
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