failure of a portion of an erythrocyte population to hemolyse when exposed to α-hemolysin (*14*).

The usual differences between rabbit and human erythrocytes were confirmed. The course of hemolysis of rabbit erythrocytes exposed to 25 to 65 HU of purified α-hemolysin per milliliter of cell suspension was identical to that described previously (*2–4*), the release of potassium ions in the prelytic lag phase being followed by a period of rapid linear release of hemoglobin (Fig. 1). Specific fluorescence (*9*), manifested by erythrocytes and red cell debris showing fluorescence of variable intensity (Fig. 2), was first demonstrated near the peak of the hemolytic curve, became most intense at the time of maximum hemolysis, and gradually lessened in brilliance and disappeared (Fig. 1). Fluorescence was no longer demonstrable at 25 and 120 minutes when the cells were exposed to 25 and 65 HU, respectively. Exposure to at least 15 HU of α-hemolysin was necessary before the phenomenon could be demonstrated. Specific fluorescence was never noted in control suspensions.

Fluorescence was not demonstrated after human erythrocytes had been exposed to 45 or 65 HU of α-hemolysin for periods as long as 4 hours. When incubated with 5000 HU, fluorescein-labeled cells could be detected after 5 minutes; the fluorescence was most intense after 60 to 120 minutes and then gradually diminished. Minimal fluorescence was still visible at 4 hours. It was evident that, when fluorescence could be seen, hemolysis was also occurring. Fluorescence could not be demonstrated in either rabbit or human erythrocytes exposed to α-hemolysin toxoid.

Our inability to demonstrate the adsorption of toxoid to rabbit red cells suggests that the addition of formalin to staphylococcal α-hemolysin may change the structure of the toxin molecules so that, despite the similarity to unaltered toxin evident in its ability to precipitate the specific antitoxin and to induce active immunity, the α-hemolysin can no longer attach to the erythrocyte surface.

The failure of human erythrocytes to fluoresce with small quantities of α-hemolysin and the high degree of fluorescence observed with very large doses are compatible with the natural resistance of these cells to purified staphylococcal α-hemolysin (*15*). This may reflect the relative unavailability of appropriate receptor sites or the necessity for interaction with a large number of toxin molecules before damage occurs. The latter is improbable since, if it were true, it should have been possible to demonstrate fluorescence during the early stages of hemolysis. The data indicate that adsorption of the toxin to the surface of the human erythrocyte is transient and that detachment gradually takes place.

The specific fluorescence observed in these studies must be interpreted as a visual demonstration of the interaction of staphylococcal α-hemolysin and the erythrocyte surface. The results suggest that this is probably a transient event, the hemolysin becoming detached from the erythrocyte at some time after cell damage occurs. Because of the early release of potassium ions, it is likely that the hemolysin reacts with the erythrocyte membrane in the prelytic lag period. However, we could not demonstrate this by the fluorescent antibody method. The presence and intensity of fluorescence appear to be directly related to the quantity and duration of exposure to α-hemolysin. Failure to demonstrate specific fluorescence during the early stages of hemolysis may have been due to adsorption of a number of toxin molecules large enough to initiate cell damage, but too few to be seen.

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29 June 1964

Trachoma and Inclusion Conjunctivitis Agents: Adaptation to HeLa Cell Cultures

Abstract. *The Mita-, Bour-, and Kami-strains of trachoma and inclusion conjunctivitis agents became adapted to serial passages in HeLa cell cultures after alternating passages between yolk sacs and tissue culture.*

The adaptation of trachoma and inclusion conjunctivitis (TRIC) agents to tissue culture is difficult (*1*). A Chinese strain of trachoma agent, TE-55, was adapted to HeLa cells by Stoker in 1959, as described by Collier (*1*). So far, this is the only strain of the trachoma agent adapted to growth in a cell line. Furness *et al.* (*2*) were able to adapt one British strain of inclusion conjunctivitis agent, LB-1, to HeLa cells, but failed in attempts to adapt the trachoma agent. Other investigators (*3*) described the cultivation of trachoma agents in tissue culture, but no mention was made of serial passage.

In our early attempts to adapt TRIC-agents to HeLa cells, the agents grew well in tissue culture during the first passage from yolk sacs, as shown by the formation of typical cytoplasmic inclusions. After several passages, however, the agents disappeared. After the TRIC-agents were alternated between yolk sacs and tissue culture, they became adapted to serial passage in tissue culture.

The Mita- and the Bour-strains of trachoma agent (*4*) became adapted to serial passage in tissue culture after 74 and 118 passages in yolk sacs, respectively. The Kami-strain of inclusion conjunctivitis agent (*4*) became adapted after 60 passages through yolk sacs. The tissue culture system consisted of HeLa cell monolayers grown in a yeast–lactalbumin hydrolysate (YLH) medium (*5*) containing 20 percent human serum in 250-ml bottles at 37°C.

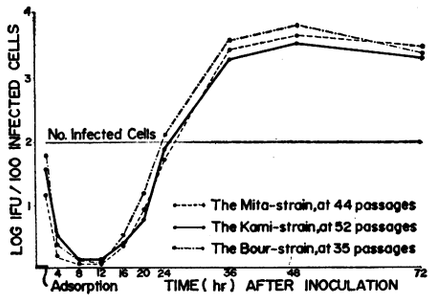

Fig. 1. One-step growth curve of the TRIC agents in HeLa cell monolayers grown on coverslips in Leighton tubes. Cells were infected with 10 inclusion forming units (IFU) per cell. For virus assay, subcultures were made at intervals in new monolayers grown on coverslips with a dilution capable of infecting 1 to 5 percent of the cells. After incubation for 72 hours, the coverslips were stained with Giemsa and the inclusions counted. From the number of the cells containing inclusions in original- and sub-cultures, the IFU titer of the culture was calculated (*6*).

The tissue culture was kept at 35°C after inoculation with the TRIC-agents.

To inoculate the tissue culture, an emulsion of infected yolk sacs prepared in YLH-medium (*5*) containing 10 percent bovine serum was centrifuged at a low speed. Three milliliters of the supernatant, with an ELD_{50} (50 percent egg lethal dose) of about 10^4 per milliliter, was added to each bottle containing the HeLa cells which had been washed twice with YLH-medium (*5*). After 4 hours the cells were again washed with YLH- medium and 10 ml of the same medium containing 2 percent bovine serum was added. For inoculation back into yolk sacs, the cells were suspended in the culture medium by the aid of a rubber spatula 72 hours after they had been inoculated, and 0.4 ml of the suspension was injected into each yolk sac of eggs which had been incubated for 6 days. The embryos then died only 8 to 12 days after inoculation. Before proceeding to the next inoculation into tissue culture, therefore, passages through yolk sacs had to be repeated until the embroys died within 5 days.

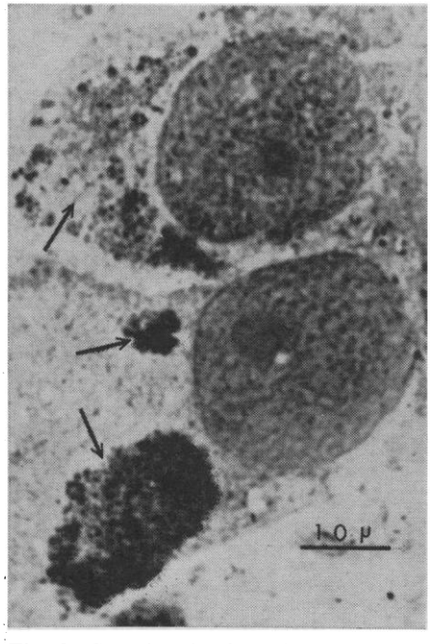

Fig. 2. Cytoplasmic inclusions (arrows) in a smear from a HeLa cell monolayer grown in a 250-ml bottle, 72 hours after inoculation with the Mita-strain in 23rd passage in cell culture. Giemsa stain.

After two (Kami-strain) or three (Mita- and Bour-strains) such alternating passages, serial passage in tissue culture was attempted. Cells were suspended in the culture medium by means of a rubber spatula, 72 hours after inoculation. The suspension was homogenized in a motor-driven Pyrex homogenizer, and was then centrifuged. Three milliliters of the supernatant was added to each bottle containing the HeLa cells which had been washed twice as before. After 4 hours, 7 ml of YLH-medium containing 2 percent bovine serum was added to each bottle without withdrawing the inoculum.

In experiments with the Kami- and the Bour-strains, 10 percent or less of the cells were infected before 10 passages in tissue culture had been completed, as shown by the examination of smears stained for cytoplasmic inclusions. The proportion of infected cells increased thereafter and, after 20 passages, 80 percent of the cells became infected. At the same time, a cytopathic effect became evident. Infected cells assumed spherical shape with granularity of the cytoplasm as observed in unstained preparations.

The Mita-strain became adapted to tissue culture more rapidly than the other strains. After five passages, the proportion of infected cells increased up to 50 percent and, after eight passages, there was an apparent inclusion formation in up to 80 percent of the cells.

Figure 1 shows one-step growth curves of the three strains in tissue culture as determined by the method described by Furness *et al.* (*6*). Figure 2 shows typical cytoplasmic inclusions as observed in a smear from a HeLa cell monolayer infected with the Mita-strain. The Kami-, Mita-, and Bour-strains have now survived more than 80, 50, and 40 passages in tissue culture, respectively. In our laboratory we have never received the TE-55 and the LB-1 strains. The present results are, therefore, not due to a contamination with known strains adapted to tissue culture.

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Hormone Excretion Patterns in Breast and Prostate Cancer Are Abnormal

Abstract. *In patients with breast and prostate cancer hormone excretion patterns differ in a similar way from patterns in persons without cancer.*

Patients with advanced cancer of the breast or prostate are given palliative hormone therapy ranging from administration of estrogens and androgens to ablation of endocrine glands. Remission occurs in some cases. Similar effects have not been observed with such regimens in cancers at other sites. The initial observation by Beatson that inoperable cancer of the breast in premenopause women responded to castration (*1*) has been extended, and the findings suggest that carcinoma of both breast and prostate may be hormone-dependent (*2*). In studies of the urinary excretion of hormone metabolites in women with cancer of the breast the concentration of androgens was abnormally low, and this difference was useful in discriminating between nonresponders