Incorporation of Tritiated Actinomycin D into Drug-Sensitive and Drug-Resistant HeLa Cells

Abstract. *HeLa cells, sensitive and resistant to actinomycin D, were treated with the tritiated antibiotic; only the nuclei of the drug-sensitive cells incorporated the tritium.*

The development of resistance to actinomycin D by sensitive strains of *Bacillus subtilis* is accompanied by a change in the permeability of the cell wall to this antibiotic (*1*). Although the antibiotic cannot enter the resistant bacterial cell, it can bind to the DNA of protoplasts of resistant cells and to the isolated DNA of both sensitive and resistant cells. *Escherichia coli* is normally resistant to this antibiotic, but its DNA can bind the drug. We now report on the incorporation of actinomycin D into drug-sensitive and drug-resistant HeLa cells.

The cells sensitive to actinomycin D, HeLa-S, are lysed within 48 hours in growth medium containing 0.1 μg of actinomycin D per milliliter. Cells of the resistant line, HeLa-R-4, have been propagated for over 1 year in growth medium containing 0.4 μg of actinomycin D per milliliter. The resistant line was developed by intermittent exposure of HeLa-S cells to increasing concentrations of the antibiotic (*2*). In our study both cell lines were grown in medium composed of medium 199 (70 percent) and calf serum (30 percent). In order to determine whether HeLa cells that were resistant to actinomycin D destroyed the antibiotic, actinomycin D (0.1 μg/ml) was added to 5 ml of medium in which 6 to 8 $\times$ 10^6 cells of both sensitive and resistant lines were grown for 18 to 20 hours. The medium was removed from the cultures, centrifuged, diluted with an equal volume of fresh medium, and tested for toxicity on HeLa-S. The toxicity of the medium was reduced when the medium containing actinomycin D, which had been exposed to the sensitive cells, was reincubated with HeLa-S cells. The medium in which resistant cells had already been incubated was as toxic as fresh medium similarly diluted. This indicated that over a period of 18 to 20 hours the resistant cells did not break down actinomycin D.

HeLa-R-4 cells were grown for 51 to 80 days in medium that contained no actinomycin D to be certain that no actinomycin remained within or on the surface of the cells. HeLa-S or HeLa-R-4 cells (3×10^6) cells growing in 5 ml of medium in 50-mm petri dishes were treated for 1 hour with 1.0 μc of H^3-actinomycin D (1.0 μc = 7 μg/ml = 80,000 count/min) per milliliter. The cells were washed five times in Hanks solution, removed from the glass with 0.02 percent ethylenediaminetetraacetate (EDTA) solution in 0.9 percent saline and centrifuged at 1500 rev/min for 10 minutes. The supernatant was discarded, and the pellet was processed in a Virtis freeze-dryer, dissolved in 1.0 ml of hydroxyhyamine during a 5-day period, and diluted to 10 ml with Packard scintillation counting fluid; the radioactivity was recorded with a 500-D Packard scintillation counter. All counts were corrected for background.

Autoradiographic studies were done on HeLa-S and HeLa-R-4 cells grown on coverslips in 30-mm plastic petri dishes. The cells were treated for 1 to 5 hours with H^3-actinomycin D (0.1 μc/ml to 0.5 μc/ml), rinsed in Hanks solution, and fixed in 2 percent glutaraldehyde in Hanks solution. Autoradiographs were prepared with Kodak AR-10 stripping film or Ilford L4 liquid emulsion. The coverslips were stored for 12 to 14 days at 4°C before they were developed and stained with 0.02 percent crystal violet.

There was very little incorporation of tritium into cells of the resistant line. Scintillation counting indicated that less than 1 percent of the antibiotic was incorporated into the resistant cells when compared with the sensitive line. For example, 1×10^6 HeLa-S cells showed 11,800 count/min after 1 hour of treatment with tritiated antibiotic (1 μc/ml) while the same number of HeLa-R-4 cells only had 115 count/min. The autoradiographs showed that H^3-actinomycin D was incorporated only into the HeLa-S cells and was localized in the nuclei (Fig. 1*A*). Cultures labeled for 1 hour contained many cells in metaphase with a dense concentration of grains over the chromosomes (Fig. 1*B*). Labeling was also observed over the reconstituting nuclei of cells in late telophase. HeLa-S cultures treated for 2 to 5 hours contained cells which entered cell division, but most of the cells treated for more than 2 hours in this concentration of actinomycin D did not complete mitosis. Many of the cells entered metaphase, but did not complete cell division, and the cell membranes degenerated. Label was concentrated over the condensed chromosomes in these degenerating cells. Cells of the resistant line contained no label even though these cells were treated for as long as 5 hours with five to ten times the concentration of actinomycin normally used in medium to grow the resistant line.

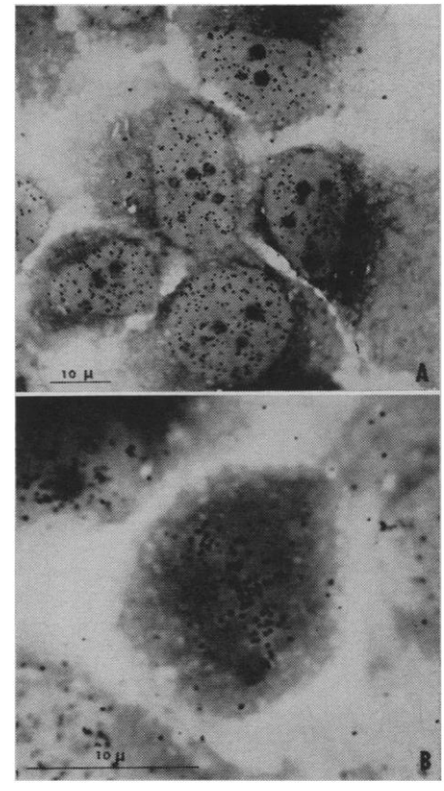

Fig. 1, *A* and *B*. Autoradiographs of HeLa cells sensitive to actinomycin D, after a 1-hour pulse with the tritiated antibiotic.

HeLa-S and HeLa-R-4 cells were rinsed in Hanks solution and incubated for 20 minutes in double-strength (hypertonic) Hanks. The cytoplasm of cells of both the resistant and sensitive lines condensed, the limiting membranes were ruffled, and the nuclei were smaller and denser. The cultures were quickly rinsed in normal medium and immediately treated with normal medium containing tritiated actinomycin D. During the 1-hour pulse period, almost all of the cells returned to their normal morphology, but only the cells of the sensitive line contained labeled nuclei. Another series of cultures was treated with a 0.5 percent sodium chloride solution for 20 minutes with marked swelling of the cells and the formation of many blisters along the limiting membranes of the sensitive and

resistant cells. The cultures were rinsed and placed in media containing the tritium-labeled actinomycin D, and, as in the previous experiment, again only the sensitive cells were labeled in spite of the fact that marked alterations had occurred to the surface of the resistant cells.

Bacterial and mammalian cells which become resistant to purine analogs develop alternate pathways for the synthesis of essential purine metabolites. Development of resistance to amethopterin, an analog of folic acid, is in part due to the increase in folic acid reductase within the cell which then binds amethopterin (*3*). In both of these cases the development of resistance does not appear to alter the manner in which these analogs enter the cell. Actinomycin D was not incorporated into the nuclei of drug-resistant cells after damage to the cell membranes by treatment with hypertonic or hypotonic media. This may indicate that an active mechanism is necessary for the incorporation of the drug and that this mechanism is absent from the membranes of resistant cells. Another alternative is that the drug can enter the cytoplasm but is unable to enter the nuclei because of a change in permeability of the nuclear membranes of resistant cells. Subak-Sharpe (*4*) has reported that hamster cells which were resistant to actinomycin D were also more resistant to puromycin. Since actinomycin D is used as an antitumor agent, investigators should be aware of the change it can induce in the permeability of the limiting or nuclear membranes of surviving tumor cells, and of its effect on further chemotherapy by other antitumor agents.

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References and Notes

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5. Dr. H. Weissbach of NIH donated, from his dwindling supply, the H^3-actinomycin D. Supported by the American Lebanese Associated Charities, and from PHS grant CA 07477-02 from the National Cancer Institute.

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Chromosome Changes Induced by Infections in Tissues of Rhynchosciara angelae

Abstract. *The main effects of two infections, one by a protozoan and the other by a virus, in cells of* Rhynchosciara angelae (*Diptera, Sciaridae*) *are an increase in cell size and changes in the size, shape, and behavior of the chromosomes. The X chromosome of some cells reacts differently from the autosome to the protozoan infection. Some chromosomes show specific, easily traceable points after infection by the virus. Some of the effects of these infections may be similar to the effects of infective agents in other organisms.*

Diaz and Pavan have described two infections in the larvae of *Rhynchosciara angelae* (Diptera, Sciaridae) (*1*); these infections, one by a protozoan and the other by a DNA virus, induce hypertrophy of the affected cells and their chromosomes. We now describe two infections (probably the same as those described by Diaz and Pavan) recently found in several cultures of *R. angelae*.

In *R. angelae* most of the cells of the larvae and of the adults have polytene chromosomes. The increase in size of these multistranded elements after these infections is normally represented by an increase in the number of chromonemata that form each individual chromosome (*2*). In addition to this increase in diameter, some cells, after a certain stage of infection, show both an increase in the total length of the chromosome and specific changes in some of the chromosomal bands.

The polytene chromosomes of Diptera function as though they are in a permanent interphase, since they are normally very active and have a great part of their DNA in a distended phase. The relatively great size, the polytene structure, and the fact that these chromosomes occur in various tissues of the larva and of the adult of many species of Diptera permit observations on the behavior of these chromosomes and of specific chromosomal regions in different physiological stages of the organism as well as in different stages of development (*2, 3*). When either of the two infections occurs in cells of different tissues, with an accompanying increase in chromosome size, it is possible to observe the reaction of the whole chromosome or of individual bands to the infective agents.

The protozoan (probably a microsporidian) infects the cytoplasm and was found in cells of such tissues of the larvae and adults of *R. angelae* as salivary gland, intestine, muscle, fat body, and other glands (Fig. 1). This infection, when affecting only a few tissues, is not lethal to the larva or the adult. Frequently an infected larva is transformed into an adult whose morphology is apparently normal. Some infected larvae, however, give rise to phenocopies, many of which do not pass the beginning of metamorphosis. These abnormal developments may be due to an effect of the infected cells on the development of the embryonic cells or to an infection in some of the cells which would normally give rise to the organs.

In some infected cells (Fig. 1) the polytene chromosomes grow so that they are very similar to the salivary-gland chromosomes, which are frequently the largest polytene chromosomes of normal larvae. In other cases, however, the hypertrophied chromosomes induced by the infection (Fig. 2) are very different in shape from normal polytene chromosomes and resemble instead the peculiar chromosomes found in some cells of supposedly normal larvae of a Cecidomydae, *Lestodiplosis* sp. (*4*). In some cells infected by the protozoan, the X chromosome shows a greater increase in size than the other chromosomes of the same nucleus (Fig. 2). We still do not have measurements of the DNA content of these enlarged X chromosomes, but the pale pink color they show after Feulgen staining, when compared with the autosomes, suggests that the larger volume attained by the X chromosomes is due to a higher activity (production of RNA) of this chromosome induced by the infection.

In heavily infected cells, the surface of the chromosomes frequently has a coat of RNA, which shows strong incorporation of H^3-uridine from 20 to 40 minutes after the larva is injected. A rather disperse multibranched nucleolus can frequently be found in the base of the X chromosomes of in-