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## Effect of Actinomycin D on HeLa Cell Nuclear RNA Metabolism. (28521)

HILTON B. LEVY
*U. S. Department of H.E.W., Public Health Service, Nat. Inst. Health, Nat. Inst. of Allergy and Infectious Diseases, Bethesda, Md.*

Actinomycin D inhibits DNA-dependent RNA snythesis, both in intact cells(1) and in cell-free systems(2). The mechanism suggested for its action is a combination with DNA, so that the DNA is not capable of functioning as a template(1,3). Actinomycin D can stop all or virtually all RNA synthesis, and this has been interpreted as meaning that all cell RNA synthesis is DNA mediated.

It has been shown by autoradiography that tritiated uridine is first incorporated into the nucleus of cells, and then appears in the cytoplasm. It was thought desirable to test the effect of actinomycin D on this migration of RNA from the nucleus to the cytoplasm.

*Experimental.* Two types of experiments were performed. In the first HeLa cell monolayers on coverslips in Leighton tubes, grown in Eagle's medium, were exposed at 37°C to actinomycin D, 1 μg/ml for 20 minutes, then to tritiated uridine 1 μc/ml for 10 minutes. Controls were not treated with the antibiotic. Radioautograms on the washed, acid extracted cells were made with Kodak Ltd. AR 10 film, by standard methods. Previous exposure to actinomycin D virtually abolished the incorporation of uridine into acid insoluble polynucleotide (Fig. 1, 2). The tendency to nucleolar concentration in the untreated cells can be seen. This is in agreement with previously published reports(4).

In the second type of experiment, HeLa cells were exposed to tritiated uridine, 1 μc/ml for 15 minutes to allow incorporation into nuclear and nucleolar RNA. Further incorporation of the labelled compound was then stopped by diluting the radioactivity by addition of 50 μg/ml unlabelled uridine. Actinomycin D was then added to some of the cultures at a final concentration of 1 μg/ml, equivalent volume of diluent was added to control cultures, and both types of cultures were incubated for an additional 2 hours. After extracting acid soluble components, radioautograms were made and are shown in Fig. 3 and 4.

In the cells which were not treated with Actinomycin D there was the normal expected migration of radioactivity from the nucleus to the cytoplasm. In the actinomycin treated cultures there was virtually no migration to the cytoplasm of the radioactivity that was present in acid insoluble form in the nucleus prior to addition of the actinomycin. The nucleolar concentration of radioactivity that had been present prior to addition of actinomycin was no longer present. The total amount of acid insoluble radioactivity was about the same in both treated and untreated cultures.

*Discussion.* The data presented here indicate that actinomycin inhibits the transfer from nucleus to cytoplasm of radioactivity that appears associated with ribosomes, a process not previously reported to be DNA dependent. Three hypothetical explanations for this observation are: 1) continued synthesis of DNA-dependent RNA is needed for

transfer to the cytoplasm of RNA made in the nucleus; 2) the conversion of an early form of RNA such as the 45 S RNA desribed by Scherrer & Darnell(5) to the other forms

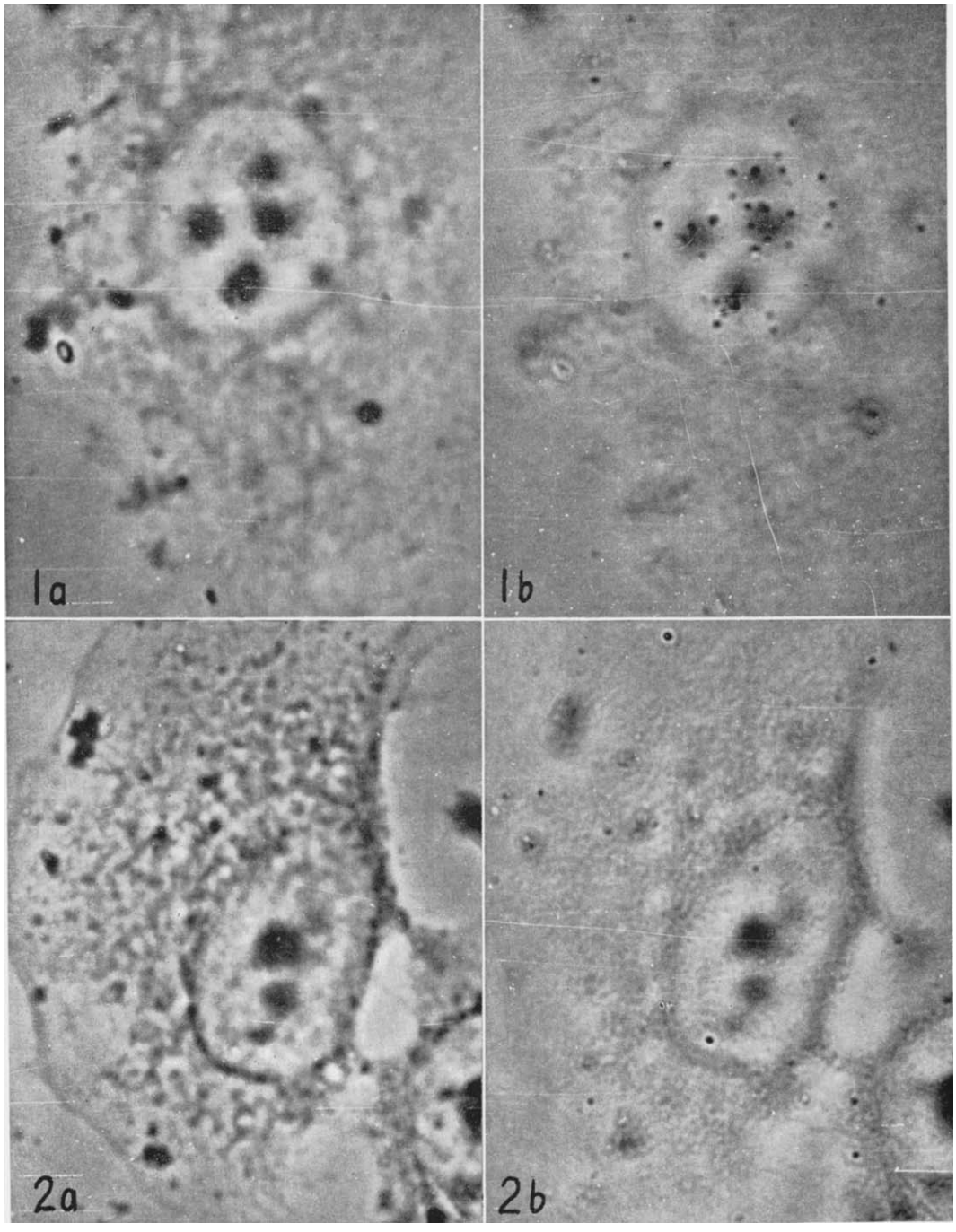


FIG. 1. Radioautogram of normal HeLa cells exposed to $H^3$-uridine for 10 min. a) Cells in focus. b) Grains in focus.

FIG. 2. Radioautogram of HeLa cells exposed to Actinomycin D for 20 min prior to exposure to $H^3$-uridine. a) Cells in focus. b) Grains in focus.

of RNA is DNA dependent; 3) actinomycin acts on some other mechanism in the cell responsible for RNA transport in addition to its effect on DNA. The present data do not

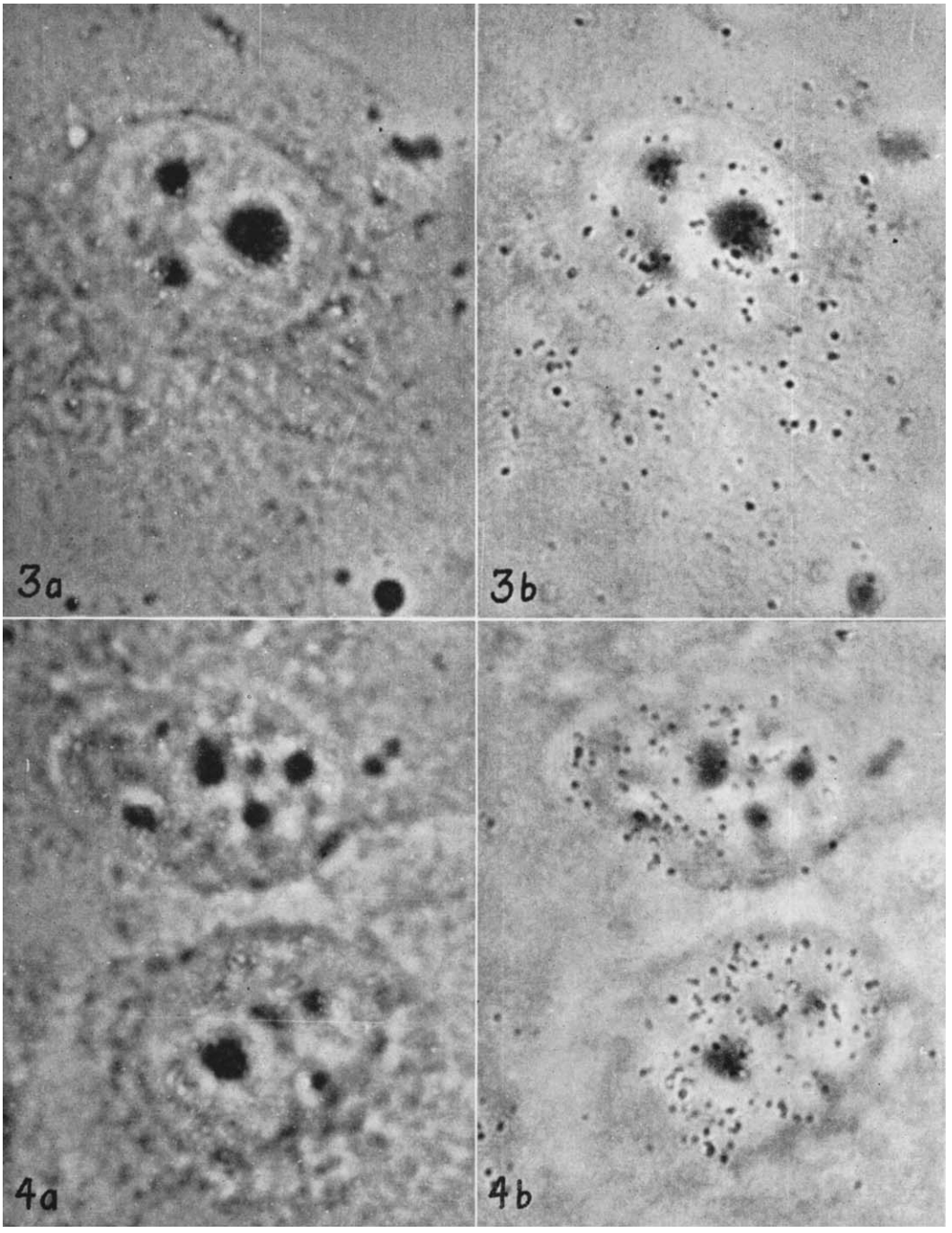


FIG. 3. Radioautogram of normal HeLa cells, exposed to $H^3$-uridine for 10 min, then excess non-radioactive uridine for an additional 2 hr. a) Cells in focus. b) Grains in focus.

FIG. 4. Radioautogram of HeLa cells exposed to $H^3$-uridine for 10 min, then excess non-radioactive uridine and Actinomycin D for an additional 2 hr. a) Cells in focus. b) Grains in focus.

allow of a choice among these possible explanations.

Incubation with actinomycin of cells that had already developed the characteristic concentration of $H^3$ uridine into nucleolar RNA leads to a loss of this concentration. This would mean both that the loss of RNA from the nucleolus is not inhibited by actinomycin, and that the synthesis of nucleolar RNA is so inhibited.

*Summary.* Actinomycin D, in addition to preventing incorporation of uridine into cellular RNA, also prevents the "migration" of previously incorporated radioactive uridine in acid insoluble form from the nucleus to the cytoplasm of HeLa cells.

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## Dose-dependent Responses to Endotoxin in the Dog.* (28522)

VINCENT FIORICA AND GORDON E. FUNKHOUSER (Introduced by L. B. Hinshaw)
*Environmental Physiology Branch, Civil Aeromedical Research Institute, Federal Aviation Agency, Oklahoma City, Okla.*

The sudden arterial hypotension that develops in the anesthetized dog after intravenous injection of gram-negative bacterial endotoxin is well-documented(1-5). The primary circulatory derangement contributing to this form of hypotension in the dog is the rapid pooling of blood in the hepato-splanchnic bed and concomitant reduction in circulating blood volume(6-8). Since total peripheral resistance is not increased under these circumstances(9,10), reduction in venous return is reflected by decline in systemic arterial pressure. The evidence that this mechanism underlies the early stages of the endotoxin response is convincing(11). The initial depression in arterial pressure is generally followed by a return to near normal levels. If a sufficiently large dose of endotoxin is involved, a progressive hypotension develops that may terminate in death.

This course of events is consistently observed with a variety of gram-negative endotoxins at dose levels exceeding one mg/kg. Experiments reported here extend previous observations to include responses to small, graded doses of the purified lipopolysaccharide (LPS) from *Serratia marcescens*. Systemic arterial pressure, portal venous pressure, blood pH, hematocrit and rate of mortality were measured.

*Materials and methods.* Twenty-five adult mongrel dogs of both sexes were used (12-21 kg). The animals were divided into 5 equal groups according to dose given. Control group 0 received an injection of normal saline; groups 1, 2, 3, and 4 received, respectively, 0.001, 0.01, 0.10 and 1.0 mg/kg LPS in saline. LPS was prepared by the procedure of Boivin *et al.*(12) from the non-chromogenic Bizio strain (no. 264) of *S. marcescens* grown on a synthetic medium.

Animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Polyethylene catheters were placed in the abdominal aorta and inferior vena cava through the femoral artery and vein. The portal vein was catheterized through a splenic branch vein. Arterial and portal pressures were continuously monitored with Statham pressure transducers and a Sanborn recorder. One mg/kg heparin was administered as needed to maintain catheter patency.

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