

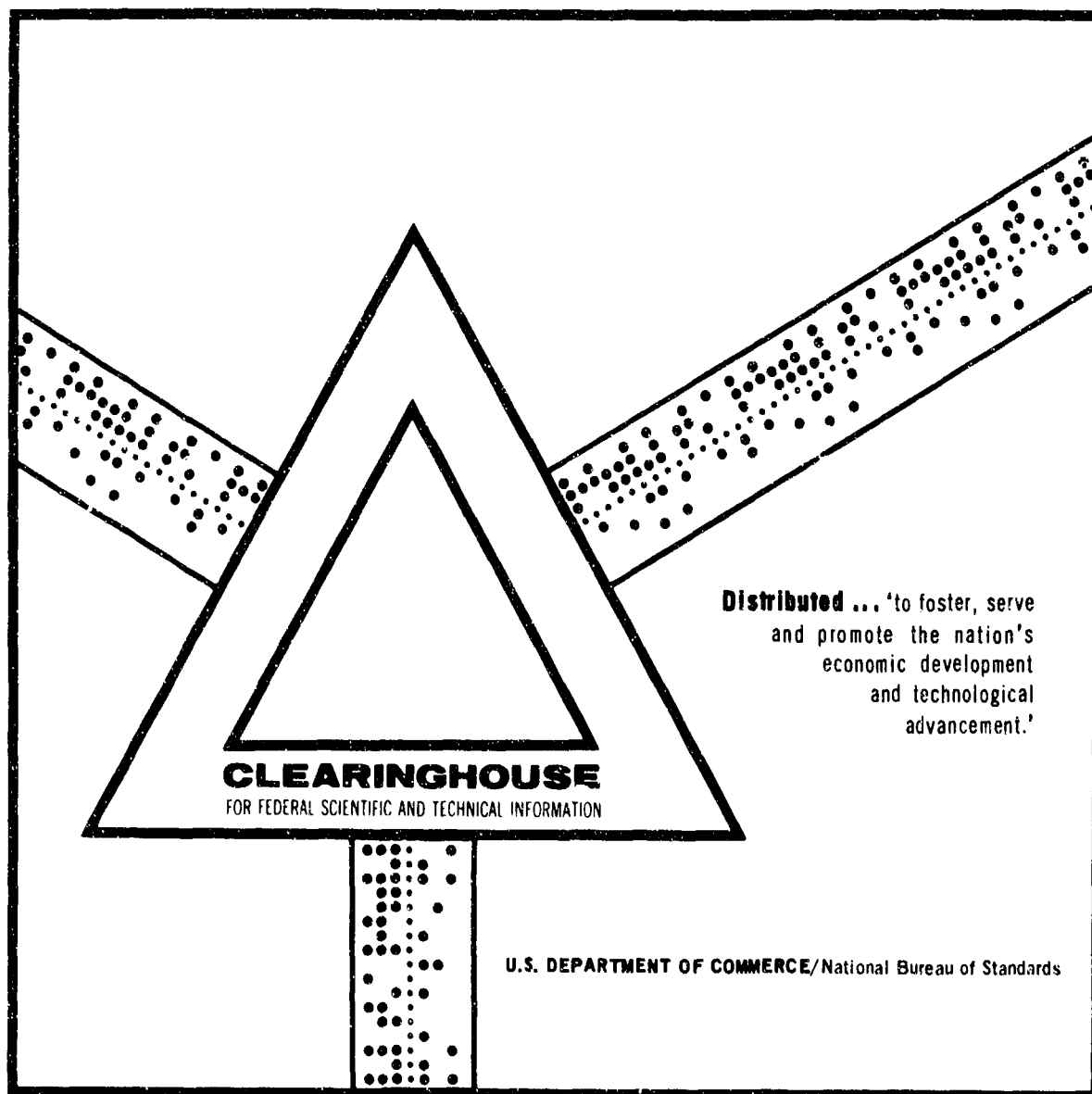
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METHOD OF LUMINESCENCE RADIOAUTOGRAPHY FOR  
STUDYING THE NATURE OF SYNTHESIS OF NUCLEIC  
COMPONENTS IN THE PROCESS OF DEVELOPMENT OF  
ORNITHOSIS VIRUS

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Fort Detrick  
Frederick, Maryland

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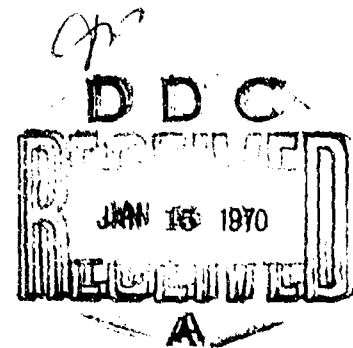
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METHOD OF LUMINESCENCE RADIOAUTOGRAPHY FOR STUDYING THE NATURE OF  
SYNTHESIS OF NUCLEIC COMPONENTS IN THE PROCESS OF DEVELOPMENT OF  
ORNITHOSIS VIRUS

[Following is the translation of an article by  
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Virology imeni D. I. Ivanovskiy, AMN USSR, Moscow,  
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Virusologii (Problems of Virology) No 1, 1969, page  
110. It was submitted on 22 Apr 1968.]

A study of the development of the ornithosis virus in a cell culture with the help of luminescence microscopy makes it possible to clearly determine the stage of development (maturation) of cytoplasmic viral inclusions based on cytochemical indices of the presence of viral RNA and DNA [1]. The method of radioautography makes it possible to establish the intensity of synthesis of viral and cellular DNA [2]. The combining of the merits of both methods would simplify the work and, by excluding parallelism, make it possible to consider the findings of radioautography by comparing them with cytochemical indices. We achieved this in the following manner.

A culture of human amniotic cells was infected with a 20% virus-containing vitelline suspension of ornithosis virus (strain No 15). The cover glasses with infected monolayer were incubated in medium No 199, containing  $H^3$ -thymidine  $10 \mu Ci/ml$  ( $8.6 Ci/m-mole$ ) for 40 minutes, and  $H^3$ -uridine  $5 \mu Ci/ml$  ( $2.7 Ci/m-mole$ ) for 20 minutes. After treatment with a 3% solution of perchloric acid (at a temperature of  $4^\circ$  for 30 minutes) they were rinsed with water and a photographic emulsion of the type M(NIKFI) was applied. Then with polystyrene they were attached to a microscope slide (with the monolayer up). As is known, fine autographs are obtained in the event that the layer of the emulsion is very thin and closely adjacent to the cells. In order to achieve this the emulsion was diluted with distilled water in a ratio of no less than 1:7 and applied with a platinum loop 3 cm in diameter. After a 2-week exposure in the dark at  $4^\circ$  the autographs were developed in amidol developer.

The preparations were stained with an 0.01% solution of acridine orange (pH 3.8) for 10 minutes, then they were rinsed in 3 changes of McIlveyn buffer, pH 3.8, and covered with a cover glass in such a manner that there was a buffer between the glasses. The edges were sealed with paraffin. The autographs were examined in incident light on an ML-2 luminescence microscope with a 90X objective with oil immersion (liquid petrolatum) with light filters FS 1-4, SZS 7-2, and BS 8-2.

Already with the 14th day after infection in the cytoplasm

of the cells orange-red inclusions were clearly apparent, and above them - black granules of silver. The nuclei were darker as a result of the more intensive incorporation of  $H^3$ -thymidine. Subsequently the intensity of synthesis of viral DNA increased, this was expressed in the fact that the label (amount of granules over the inclusion) became more intense; at the same time there was a change of color of the cytoplasmic inclusions to yellow, and then to green, which corresponded to the intensity of synthesis of DNA according to the findings of radioautography. The onset of synthesis of viral RNA was noted 10 hours after infection.

The method is simple and does not require removal of the emulsion layer. It is suitable for work with other isotopes and may be used during investigations not only of the causative agents of the group ornithosis - lymphogranuloma - trachoma, but also of other viruses, when it is necessary to compare the findings of two methods and with them confirm the nature of synthesis of nucleic components.

#### Literature

1. Terskikh, I. I., Zairov, G. K., Vopr. virusol., 1964, No 6, p 674.
2. Gaydamovich, S. Ya., Zhdanov, V. M., Virology, Moscow, 1966, p 117.