autoradiographs *A* and *B* in Fig. 1 were obtained from a plasmodium that was incubated for 3 hours before the beginning of prophase. During this time less than 5 percent of the nuclei incorporated thymidine-H^3 (*10*).

Degenerating nuclei could not have accounted for the presence of the numerous labeled particles observed, because in smear preparations obtained from growing plasmodia such nuclear forms accounted for less than 0.5 percent of the total number of nuclei. Similarly, contaminating bacteria could be ruled out as a possible site of thymidine incorporation since the cultures were maintained under rigidly controlled, axenic conditions. We believe therefore that the labeled particles were cytoplasmic particles.

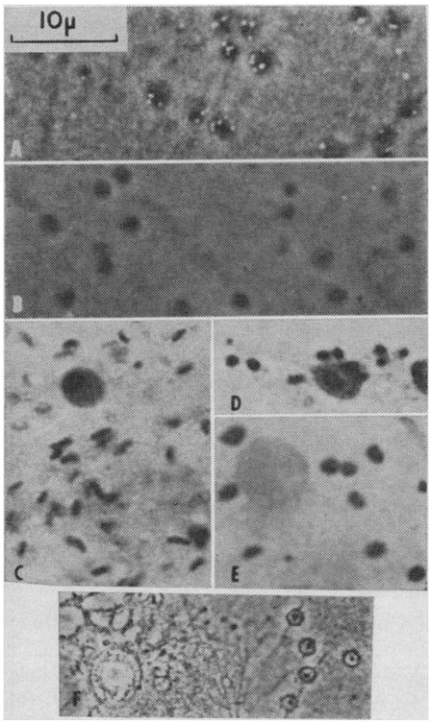

Fig. 1. (*A*) and (*B*) Autoradiographs of unstained smear preparations from a plasmodium of *Physarum polycephalum*. Smears were fixed in ethanol (95 percent) followed by ethanol–acetic acid (3 : 1); phase-contrast microscopy. The plasmodium was exposed to thymidine-H^3 during late interphase. Prior to the application of the film emulsion, both preparations were treated with ribonuclease. This treatment was followed in (*A*) by McIlwain buffer, and in (*B*) by the same buffer containing deoxyribonuclease. The mitochondria in (*B*) are slightly out of focus so that the few silver grains can be seen. (*C*) Section through a plasmodium during late interphase. The mitochondria appear as rod-shaped bodies (thickness of section, 3.0μ). Fixed with Champy's liquid; stained with acid fuchsin by Altmann's procedure. (*D*) and (*E*) Two areas of different thickness (*D*, "normal"; E, extremely thin) in the same smear preparation from a plasmodium during mid-interphase, fixed with Champy's liquid, and stained with acid fuchsin (Altmann). Nucleus and mitochondria in (*D*) appear small in comparison with the structures shown in (*E*). (*F*) Thin area in a smear preparation showing mitochondrial ghosts. Fixed with ethanol followed by ethanol–acetic acid; phase-contrast microscopy.

Cytological evidence for our assumption that the comparatively large labeled particles were mitochondria is presented in Fig. 1, *C* to *F*. In sections from explants fixed with Champy's liquid and stained with acid fuchsin according to Altmann's procedure, the mitochondria (Fig. 1*C*) appeared as rod-like elements with a maximum length of approximately 2 μ. In smear preparations fixed and stained as above (Fig. 1, *D* and *E*), the mitochondria appeared as small globules which varied in diameter within the same smear preparation from approximately 1 μ in the thicker parts (Fig. 1*D*) to 2 μ in extremely thin parts (Fig. 1*E*). There was a continuous transition between these extremes in sizes. After fixation of smear preparations with ethanol (95 percent) followed by ethanol-acetic acid (3 : 1 by volume), the mitochondria were preserved as ghosts (Fig. 1*F*). The autoradiographs in Fig. 1, *A* and *B*, were taken from areas similar to those shown in Fig. 1, *E* and *F*.

In addition to mitochondria, *P. Polycephalum* contains only two other groups of cytoplasmic particles which can be distinguished with the light microscope, either in stained preparations or by phase contrast. These particles are the pigment granules and polyphosphate granules (see *6*, Figs. 8, 10). Neither of them is similar, in size or distribution, to the granules shown in Fig. 1. The pigment granules are of irregular size, and their diameters vary from 0.2 μ to 2 μ. In smear preparations they are distributed in irregular clusters. The diameters of the polyphosphate granules are less than 0.2 μ in smear preparations.

The identity of the thymidine-incorporating material with DNA must be established by more direct procedures. The large amount of thymidine-H^3 incorporated by the mitochondria should make it possible to study the kinetics of this process in relation to the mitotic cycle of the plasmodia.

EDMUND GUTTES
SOPHIE GUTTES
Department of Biology, Loyola University, Chicago, Illinois 60626

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11. Supported by USPHS grant No. GM-08495-03.

27 May 1964

Incorporation of Uridine-H^3 into Nuclei of Virus-Infected Tobacco

Abstract. *Tritiated uridine is selectively incorporated into the nucleus and nucleolus of tobacco leaf discs infected with tobacco mosaic virus that has been treated with actinomycin D. Such incorporation did not occur in noninfected tobacco treated with actinomycin D. Nontreated, infected, and healthy tobacco incorporated substantially more tritiated uridine.*

Actinomycin D has been shown by Sänger and Knight (*1*) to inhibit the incorporation of uracil-2-C^{14} into the ribonucleic acid (RNA) of healthy tobacco but not to interfere with the incorporation into the RNA of tobacco infected by tobacco mosaic virus (TMV). Both the radioactivity and the infectivity were associated with the same RNA fraction. Actinomycin D has also been shown to have little effect on the multiplication of RNA-containing animal viruses (*2*, *3*). These observations suggested that autoradiography of infected tissues treated with actinomycin D and fed labeled uridine might provide evidence of the cellular localization of TMV-RNA synthesis.

In the present study uridine-H^3 was selectively incorporated into the nucleus of leaf discs treated with actinomycin D when the leaf discs had been infected with tobacco mosaic virus, but the uridine was not incorporated into

the noninfected leaf discs treated with actinomycin D. Previous cytological observations by Zech and Vogt-Köhne (*4*) and Bald and Solberg (*5*) have also indicated that the nucleus is implicated in plant-virus multiplication. However, with L-cells infected with Mengo virus (an RNA virus) the cytoplasm was the site of new RNA synthesis (*2*).

Turkish-tobacco leaf discs inoculated with TMV and discs from comparable healthy leaves were placed on distilled water in a light chamber (11,000 lu/m^2) at 26°C. After 24 hours actinomycin D (50 μg/ml) was added to the water, and the discs were floated an additional 12 hours to permit the penetration of the antibiotic and the inhibition of the normal RNA synthesis. Then tritiated uridine (specific activity 3.7 curies/mmole) in a final concentration of 25 μc/ml was added. Samples were taken 4 hours after the addition of the uridine and washed thoroughly; the cells were then killed and fixed in Randolph's solution (*6*) and embedded in paraffin. Sections (6 μ thick) were cut, the paraffin was removed, and the sections were washed with 2 percent perchloric acid for 20 minutes at 4°C to remove the unincorporated uridine. The sections were then stained with chlorazol black E (1 percent in 70 percent ethanol) for 12 hours and covered with Ilford K5 liquid emulsion. Slides were held at 4°C for an exposure period of 6 days and then developed by the method of Caro *et al.* (*7*).

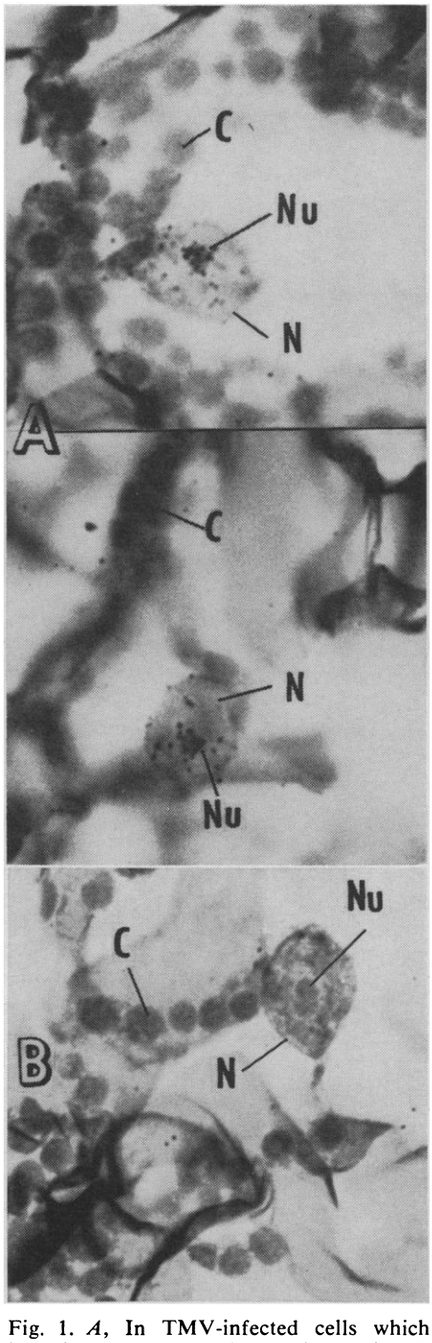

Fig. 1. *A*, In TMV-infected cells which had been treated with actinomycin D showing the localization of uridine-H^3 over the nuclei (*N*), and in particular, the nucleoli (*Nu*), there appears to be no uridine-H^3 in the chloroplast (*C*). *B*, Comparable healthy cell, which shows no incorporation of uridine-H^3 in any structure.

Uridine-H^3 was incorporated into the nucleus, and particularly the nucleolus, of the actinomycin D treated leaf discs infected with TMV, but it was not incorporated into the comparably treated healthy tissue (Fig. 1*A* and 1*B*). No other structures in the treated cells showed accumulation of the H^3. Sections of healthy and TMV-infected nontreated leaves, also exposed for 6 days, appeared similar to each other, although they incorporated substantially more uridine-H^3 than did the inhibited cells. Biological assays of the homogenates of the TMV-infected, treated tissues showed that virus synthesis had taken place in the leaf discs, although the final amount of infectivity was 25 to 30 percent lower than in nontreated discs. A similar effect was reported by Sänger and Knight (*1*).

Results, reproducible in each of six experiments, were consistent with the earlier evidence by Sänger and Knight (*1*) showing that the synthesis of tobacco mosaic virus RNA does not require a DNA template. Although our data do not indicate the chemical form in which the uridine-H^3 was incorporated, they do indicate a specific function of the nucleus in uridine metabolism of infected cells. In summary, the inhibitor prevents the incorporation of uridine-H^3 into any structures in healthy cells, yet permits considerable incorporation into the nuclei of cells in which virus is multiplying. Thus it seems reasonable to suggest that the nuclei may take part in at least one step in the synthesis of tobacco mosaic virus RNA.

SAMUEL H. SMITH
DAVID E. SCHLEGEL
Department of Plant Pathology, University of California, Berkeley

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Histones: Species and Tissue Specificity

Abstract. *Within a given species (rat, mouse, guinea pig, rabbit) the histones of brain, liver, and kidney are indistinguishable on disc electrophoresis in polyacrylamide gels. However, characteristic species differences were observed. In the immature rat, the histones of brain and liver, while very similar to each other, differ from those of the adult. The histones of the thymus of the immature and adult rat exhibited a pattern corresponding to that of the immature brain and liver.*

A possible function of histones as regulators of information transfer from DNA to RNA has been investigated in various laboratories (*1*). The underlying hypothesis suggests that certain sequences of DNA are activated by the removal or alteration of masking histones, and that as a result the synthesis of messenger RNA complementary to the now accessible DNA becomes possible. For such a mechanism to be operative it is necessary to postulate specific interactions between DNA and histones. Differentiation and development might then be accompanied by changes, qualitative or quantitative, in the histones of a particular tissue.

From our studies of the biochemical development of the nervous system, the question arose whether the drastic