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*).

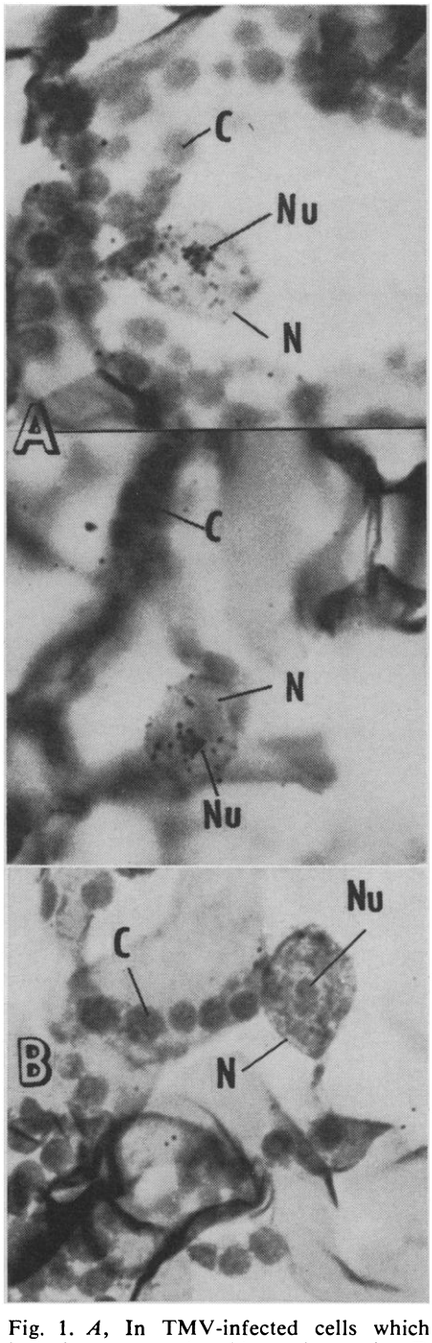

Fig. 1. *A*, In TMV-infected cells which had been treated with actinomycin D showing the localization of uridine-H^3 over the nuclei (*N*), and in particular, the nucleoli (*Nu*), there appears to be no uridine-H^3 in the chloroplast (*C*). *B*, Comparable healthy cell, which shows no incorporation of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

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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

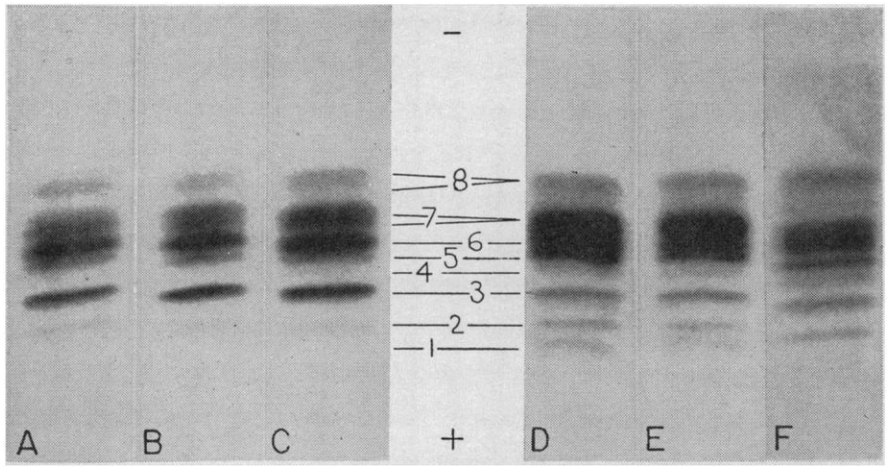

Fig. 1. Electrophoretic patterns of the histones of rat organs: *A*, Adult brain; *B*, adult liver; *C*, adult kidney; *D*, newborn brain (within 24 hours of birth); *E*, newborn liver; *F*, thymus gland (250-g rat).

changes in enzyme pattern and composition of the brain that are observed during maturation might be accompanied by changes in histone composition. If this were the case, the adult brain might be expected to contain a characteristic complement of histone entities. Therefore we compared the histone patterns of brain and other organs in the adult, as well as during development, in several mammalian species.

The method chosen was that of disc electrophoresis on polyacrylamide gels, described by Ornstein and Davis (*2*) and modified by Reisfeld *et al.* (*3*) for application to basic proteins. This procedure was attractive because of both its high resolving power and the small quantity of protein required (*4*). However, several modifications of the system were required to obtain detailed, reproducible patterns (*5*).

In all experiments, histones were prepared from nuclei isolated from homogenates of fresh tissue in hypertonic sucrose (*6*). Relatively uncontaminated nuclei, as shown by phase-contrast microscopy, were obtained in a yield of approximately 20 percent from organs other than adult brain. The adult brain gave considerably smaller yields, perhaps because of trapping of nuclei by the lighter myelin fragments. The nuclei were washed exhaustively with isotonic sodium chloride–sodium citrate-solution, and the histones were extracted with either dilute hydrochloric acid (*7*) or 10 percent (wt./vol.) ammonium sulfate solution, followed by acid precipitation of the DNA. If the nuclei were thoroughly washed with salt-citrate solution, both methods gave identical results. If the washing was incomplete, however, histones prepared by the ammonium sulfate method gave additional bands on disc electrophoresis. Since extraction with acid appeared somewhat more selective, this method was used routinely.

Histones isolated from brain, liver, and kidney were indistinguishable within a given species (for example, rat, mouse, guinea pig, rabbit; Figs. 1 and 2). These conclusions are based on repeated electrophoretic runs of the same preparation, as well as on comparison of histones isolated independently from different animals of the same species. Histones were also applied to the gels at various concentrations which, within limits (30 to 300 μg per gel), did not influence the electrophoretic patterns.

When different species were compared, rat and mouse tissues gave very similar electrophoretic patterns, the principal difference being a single faint band, characteristic of rat organs (Fig. 1, band 4). In contrast, histones of rabbit and guinea-pig appeared to differ significantly both from each other and from those of the rat and mouse (Fig. 2). As yet, however, there is no evidence that each band shown in the gel pattern represents a single molecular species. At present we are attempting to isolate histone fractions corresponding to individual gel bands in order to obtain evidence bearing on this point.

The patterns of histones isolated from brain and liver of newborn rats appeared to be indistinguishable from each other, but they differed significantly from those of the adult animal in both intensity and spacing of the bands (Fig. 1). In particular, bands 2 and 3 were of approximately equal intensity in the newborn, whereas in the adult band 3 appeared considerably more intense. Band 1, while always present in newborn organs, may or may not be seen in adult histones. Band 6 is more intense in adult preparations. Bands 7 and 8 appear to be split into two bands in the adult. The question of whether the differences between the histones of newborn and adult are of a quantitative or qualitative nature can be resolved only by further characterization of individual bands. Also to be considered is the possibility that the observed differences result from differences in extractability of the histones from young and adult organs.

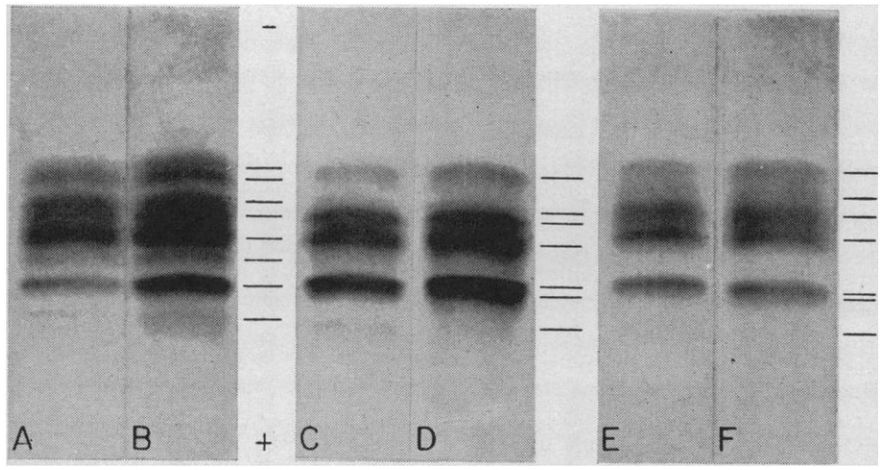

Fig. 2. Electrophoretic patterns of the histones of brain and liver of various species: *A*, Rat brain; *B*, rat liver; *C*, guinea pig brain; *D*, guinea pig liver; *E*, rabbit brain; *F*, rabbit liver.

We have also isolated histones from rat thymus. It has been reported (*8*) that thymus and spleen histones differ from those of other rat tissues. The histone patterns of thymus from both 40-g and 250-g rats (Fig. 1*F*) appear very similar to those of the organs of newborn animals and are therefore different from those of brain, liver, and kidney of the adult animal. The histone pattern of rat thymus is also quite distinct from that of calf thymus.

Similarity in the histone composition of various tissues of the same species has been pointed out (*7, 9*). Our studies of histone patterns of brain and other organs, obtained by electrophoresis in polyacrylamide gels, are consistent with this conclusion. The complexity of the histone pattern appears to vary among species, from the relatively simple pattern found in the guinea pig and rabbit to the more complex patterns of rat and mouse. This observation may be of interest in view of the highly complex patterns reported for calf thymus histones (*8, 10*) and of the speculation that this complexity may be related to histone function (*11*). Our results with rat tissues suggest changes in histone composition with maturation; changes in which the histones of the thymus gland do not participate. Our findings impose considerable restrictions as to the function of histones in the regulation of information transfer from DNA. Whereas our findings, and those of other laboratories demonstrating similarity of histones in various tissues, do not exclude a direct role for these basic proteins in regulating DNA expression, they point to the need for additional mechanisms to provide the specificity required to control the transcription process. It would seem that such a control mechanism is not reflected in a differential rate of breakdown and resynthesis of individual histones in the adult animal.

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Transcription of a Repressed Gene: Evidence That It Requires DNA Replication

Abstract. *It is known that the synthesis of DNA is not essential for constitutive or induced enzyme synthesis. Our studies indicate that DNA synthesis is required for the basal level synthesis (that is, for synthesis in absence of inducer) of two inducible enzymes, a finding which supports an earlier speculation that a messenger-RNA transcription event may normally accompany DNA replication. Studies with cultures of* Escherichia coli *TAU-bar in which DNA replication is synchronized suggest that the β-galactosidase gene is transcribed at a particular time in the sequential replication of the bacterial chromosome.*

The induced synthesis of a number of inducible enzymes has been demonstrated in bacteria under conditions of thymine starvation (*1–3*). As initially reported by Cohen and Barner (*1*), the induction process itself can occur in the absence of thymine in a thymine-requiring bacterium. Thus, the synthesis of DNA is not essential to either the induction process or to induced enzyme synthesis. However, the *rate* of induced enzyme synthesis is dependent on the number of copies of the particular gene in the cell (*4*) and there is some evidence from synchronous growth studies that the enzyme synthesizing *capacity* doubles as the particular gene is duplicated (*5*). In the studies described here we examined specifically the synthesis of an inducible enzyme in the *repressed* state and the dependence of its synthesis on the simultaneous replication of DNA.

Escherichia coli strain TAU-bar (*6*) was cultured aerobically in a glucose-salts synthetic medium at *p*H 7.4 and 37°C with the required supplements (*7*). Changes in the medium were accomplished by the rapid filtration technique (*8*). Induced synthesis of β-galactosidase required the use of 0.5 percent lactose in place of 0.5 percent glucose as energy source in the growth medium. Bacteria were lysed for enzyme assay by treatment of a 5-ml suspension for 1 minute at 0°C with a Branson Sonifier adjusted for resonance at power setting No. 7. Control studies indicated better than 95 per-

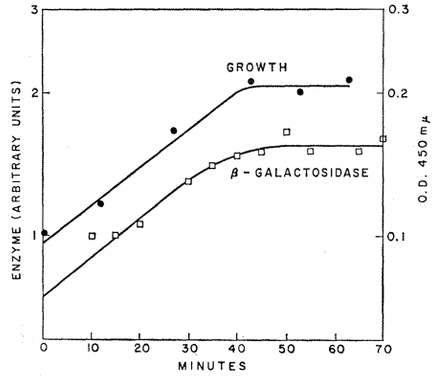

Fig. 1. Effect of thymine deprivation on induced enzyme synthesis and growth in *E. coli* TAU-bar. Culture grown in lactose medium. Thymine removed at 0 minutes. Growth followed by optical density (O.D.) readings (*15*). β-Galactosidase activity in samples from lysed culture determined by hydrolysis of ONPG (*16*) and optical density readings at 420 mμ (*17*).