(arborvirus, Bunyamwara group) rapidly gives rise to circulating interferon; and (ii) the circulating interferon is protective in mice against intraperitoneal challenge with Germiston virus or intracerebral challenge with encephalomyocarditis virus (*12*).

That interferon may be produced rapidly in the cells of species other than the mouse is indicated by the detection of interferon in fibroblast cultures from the chick embryo within 2 hours of infection by the Chikungunya strain of group A arborvirus (*13*). The finding by many investigators that a substantially longer time, 4 hours to 2 days, is required for interferon production (*14*) may be due to the different cell-virus systems which are used for production and for assay of interferon. A variety of viral infections associated with the vascular system of the mouse fulfil the cell-virus relationship necessary for the rapid production of interferon. Infection of chick embryo cell cultures with Chikungunya virus induces the rapid production of large amounts of interferon, although the virus has little or no cytopathic effect. A possible interpretation is that nonlethal infections of cells by viruses (for example, chick embryo cells infected with Chikungunya virus, WS amnion cells infected with Sindbis virus, and human thyroid cells infected with Newcastle disease virus) give rise to rapid production of interferon and poor yield of virus. Some types of cells (for example, leukocytes) within the vascular system of the mouse may be resistant to the lethal action of a given virus and thus rapidly produce circulating interferon (*15*). Other types of cells, either within the vascular system or in infected tissues, may be more severely disrupted by viral infection and as a result produce interferon in a delayed and decreased manner (*1*).

The onset of interference between two viruses, within a few hours, may not be mediated by interferon because none was detected until after the beginning of interference (*16*). The present finding, that cells may produce interferon rapidly, suggests that amounts of interferon below levels of detection may play an important role in mediating viral interference of rapid as well as delayed onset.

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## Actinomycin D Effects in Frog Embryos: Evidence for Sequential Synthesis of DNA-Dependent RNA

Abstract. *Early gastrulae of* Rana pipiens *exposed to actinomycin D for 2 days were then cultured in saline. The resulting larvae were immotile; histological examination revealed impaired development of axial nerve and muscle tissues, but normal differentiation of sensory, pronephric, heart, and digestive tissues; this suggests sequential synthesis of the different tissue-specific molecules of DNA-dependent RNA.*

Actinomycin D combines with DNA and prevents the synthesis of nuclear RNA (*1*). Exposure of developing sea urchin eggs to concentrations of actinomycin D above 20 $\mu$g/ml prevents differentiation and causes an abnormal distribution of cleavage planes; however, cell division can continue after exposure to concentrations as high as 100 $\mu$g/ml (*2*). Normal cleavage of amphibian eggs (*Pleurodeles* and *Xenopus*) in actinomycin D (10 $\mu$g/ml), has been observed (*3*), but the *Xenopus* embryos exogastrulated while development of *Pleurodeles* ceased at the neurula stage. Whereas the *Pleurodeles* embryos survived for several days, the nervous system was practically absent, and the notochord and somites were poorly differentiated. Actinomycin D (2.5 to 5 $\mu$g/ml) allowed differentiation of the nervous system, but the embryos were usually microcephalic and the eyes were small or missing. The fact that cell division of amphibian and sea urchin eggs is unaffected by actinomycin D has led Brachet and Denis (*3*) to suggest that significant production of nuclear RNA synthesis does not begin before gastrulation. They presume that this nuclear RNA is messenger RNA (mRNA), but they caution that extensive biochemical information is needed before it can be identified as mRNA. Denis (*4*) has examined by cytochemical methods explants of chorda mesoderm and ectoderm treated with actinomycin D and found that the nuclei of the ectoderm cells had less RNA than those of untreated explants.

When polyuridylic acid is added to cell-free systems of unfertilized and developing sea urchin eggs, the stimulation of incorporation of $C^{14}$-phenylalanine into polypeptides is greater in the systems derived from unfertilized eggs (*5*, *6*), although Wilt and Hultin (*6*) found the degree of incorporation into homogenates of unfertilized and fertilized eggs (2 hours after fertilization) similar with higher concentrations of polyuridylic acid.

Nemer (*5*) attributes this to the newly synthesized endogenous mRNA on the ribosomes of the developing eggs which interferes with the action of the synthetic mRNA (polyuridylic acid). Recent work by Wilt (*7*) indicates that RNA synthesis is activated shortly after fertilization and this RNA is then found on the ribosomes. The fact that the sedimentation properties of this RNA differ from ribosomal RNA suggests that it might be mRNA. However, it is not known if this mRNA acts as the code for the synthesis of

ubiquitous proteins participating in cell division, or for the production of tissue-specific proteins that will later characterize cells as differentiated. Although some mRNA may be produced before gastrulation (*5*, *7*, *8*), or beginning at that time (*3*), the question remains unanswered whether the nuclear RNA, which acts as a code for the synthesis of the various tissue-specific proteins, is produced before the time of differentiation (at or before gastrulation) or at the time of the synthesis of the tissue-specific proteins, that is, at the time of differentiation.

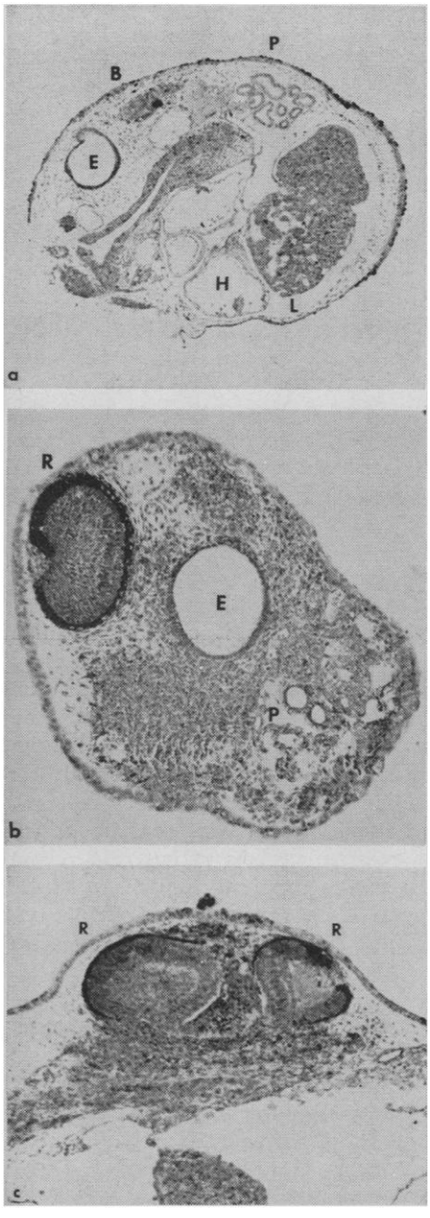


Fig. 1. Early gastrulae were exposed to actinomycin D (7.5 $\mu$g/ml) for 2 days, and then raised in a saline medium for 4 days. These larvae typically had poor differentiation of brain (B) and spinal cord, but pronephros (P), heart (H), liver (L), and ear vesicles (E), developed normally (Fig. 1*a*). Retinal (R), otic (E) and pronephric (P) development occurred in an explant of the dorsal half of a gastrula treated similarly (Fig. 1*b*), while abnormal location of retinas (R) sometimes occurred in the actinomycin D treated larvae (Fig. 1*c*).

This question was approached experimentally by exposing different stages of developing *Rana pipiens* embryos to actinomycin D for several days, raising the embryos in a saline medium (Niu-Twitty solution) for 6 to 8 days, and examining them histologically. If the synthesis of all the various tissue-specific nuclear RNA begins at gastrulation, one would expect actinomycin D at this time (in concentrations which do not block development completely) to interfere with the subsequent differentiation of all of the embryonic tissues and organs. However, if the synthesis of tissue-specific nuclear RNA occurs shortly before the time of differentiation of the tissue in question, then one would anticipate that exposure to actinomycin D for 2 days would interfere with the differentiation of the tissues that normally develop at that time, but not with tissues and organs that develop later in point of time. The results support the second possibility and complement experiments with ethionine which show that the synthesis of tissue-specific proteins occurs at the time of differentiation of the tissues in question (*9*).

The development of early gastrulae (stage 10) was unaffected by concentrations of actinomycin D less than 5 $\mu$g/ml in the media, but concentrations of 100 $\mu$g/ml caused development to cease by the mid-gastrula stage (stage 11). These embryos died even though they were transferred to saline. Concentrations of 40 to 60 $\mu$g/ml were also toxic. Exposure to media containing 7.5 to 10 $\mu$g/ml of actinomycin D for 2 to 3 days resulted in development of the gastrulae to early tail-bud stages (stages 16 to 17) at which time there were no twitching movements. If such embryos were left in actinomycin D, they did not develop further and cytolysis took place. If they were removed from actinomycin D after 2 to 3 days exposure and raised in saline, they resumed development to stage 25, when feeding normally begins. They were often microcephalic, but most conspicuously, they were usually immotile. They did have large tails, red blood cells, and beating hearts.

Histological examination of larvae exposed to actinomycin D (7.5 $\mu$g/ml) for 2 days and then cultured in saline showed that the brain, and the spinal cord in the trunk region, but not the tail, were often absent and axial muscle was poorly differentiated; but the notochord, sense organs, pronephros, liver, stomach, blood cells and heart had differentiated normally (Fig. 1*a*). Explants of dorsal halves of gastrulae similarly treated with actinomycin D (50 explants) showed differentiation of sensory and excretory structures, but not of brain, spinal cord, or muscle (Fig. 1*b*). In some of the larvae the spatial organization was abnormal, for example, two retinas located side by side (Fig. 1*c*).

Early neurulae (stage 14) were cultured in actinomycin D (7.5, 15, 20, 30, or 40 $\mu$g/ml) for 2 days and then in Niu-Twitty saline for 4 days. They showed twitching movements by stage 21. If the early neurulae were placed in concentrations of actinomycin D of 50, 60, or 80 $\mu$g/ml for 1 day, then raised in saline for 5 days, the resulting larvae (stage 22 were usually microcephalic and immotile, but they possessed beating hearts, red blood cells, differentiated pronephros, and digestive structures.

Tail-bud stages (stage 16) of *Rana pipiens* were cultured in actinomycin D in the media at concentrations of 15, 20, 30, or 40 $\mu$g/ml. After 4 days, they developed into motile larvae, stage 22. Similar concentrations of actinomycin D did not affect the motility of embryos at stage 18 or 22 after 2 days of exposure, but 100 $\mu$g/ml was toxic. This concentration also was lethal to feeding larvae (stage 25) after 4 days.

The results support the idea that the synthesis, which is sensitive to actinomycin D, of any particular tissue-specific nuclear RNA occurs shortly before the time of differentiation of that tissue.

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