surface (Fig. 3) were actually small membranous structures (Fig. 4). Portions of the broad membranes were resting on the surface of the cover slip (Fig. 4) and were continuous with the main body of the cell.

Although the membranous structures on the surface of the cell appear well preserved and intact, there are areas at the edge of the cell, where the membrane is attached to the cover slip, that appear to have been torn from the cell. The cause of this tearing is unknown, but it is presumed to be the result of the method of preparing the cells.

If time-lapse microcinematography of the cells in the living state had been performed (Figs. 2 and 3), results very similar to the picture of the macrophage in figure 2.3 in (*1*) would probably have been obtained. Our findings are compatible with the electron micrographs of other workers (*1*, *2*) who have shown the macrophage to be rough-surfaced with microprojections. If the cells in Figs. 2 and 3 had been sectioned in the proper plane, they would undoubtedly look very similar to the cell in figure 2.6 in (*1*). The microvilli mentioned by Nelson (*1*) may be the result of sectioning through the surface membranes.

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Specific Inhibition of Nuclear RNA Polymerase II by α-Amanitin

Abstract. *α-Amanitin, a toxic substance from the mushroom* Amanita phalloides, *is a potent inhibitor of DNA-dependent RNA polymerase II (the nucleoplasmic form) from sea urchin, rat liver, and calf thymus. This compound exerts no effect on the activity of polymerase I (nucleolar form) or polymerase III (also nucleoplasmic). The inhibition is due to a specific interaction with polymerase II or with a complex of DNA and polymerase II.*

We have described the physical separation of three distinct DNA-dependent RNA polymerases from sea urchin embryos and two polymerases from rat liver (*1*, *2*). Polymerase I resides in the nucleolus, and polymerases II and III reside in the nucleoplasm (*2*). The enzymes exhibit distinctive variations in activity with magnesium, manganese, and salt concentration changes. It is inferred that the polymerases have different functions, but for definitive investigations on this point specific inhibitors would prove highly useful. We have tested the effect of α-amanitin [the toxic bicyclic octapeptide from the mushroom *Amanita phalloides* (*3*)] on the various nuclear polymerases. Administration of this compound to mice results in fragmentation of liver nucleoli (*4*) and an overall decrease in nuclear RNA content (*5*). Furthermore, in the presence of Mn++ and high concentrations of salt, α-amanitin partially inhibits the polymerase activity of mouse liver nuclei (*6*) and of crude nuclear preparations (*7*).

We now report that α-amanitin specifically inhibits polymerase II from the nuclei of several organisms although polymerase I and III activities are not affected. We also provide substantial

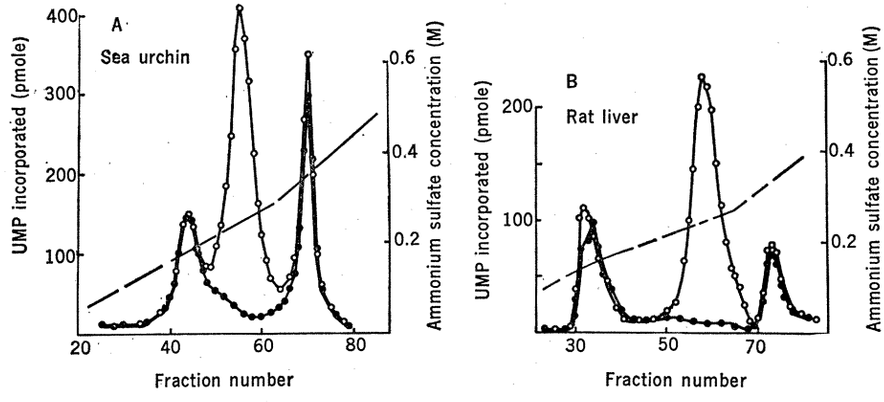

Fig. 1. (A) Inhibition of sea urchin (*Strongylocentrotus purpuratus*) and rat liver RNA polymerase II by α-amanitin. A sample containing 9.5 mg of protein was applied to a column (1 by 5 cm) of DEAE Sephadex (A-25) that was equilibrated with 0.05*M* tris-HCl (*p*H 7.9), 25 percent (by volume) glycerol, 5 m*M* $MgCl_2$, 0.1 m*M* EDTA, 0.5 m*M* dithiothreitol, and 0.02*M* ammonium sulfate. Polymerase activities were eluted with a gradient from 0.02 to 0.42*M* ammonium sulfate (40 ml total volume) with an additional 10-ml washing of 0.5*M*; 0.6-ml fractions were collected. Polymerase activities were assayed by the usual procedure and at $3.4 \times 10^{-6}M$ α-amanitin. The assay mixture contained 0.01 m*M* uridine triphosphate (UTP) (unlabeled) and sheared calf-thymus DNA, but otherwise it was identical to that of Roeder and Rutter (*1*). Reactions were stopped by pipetting 0.10 ml of this assay onto Whatman DE-81 filter disks (2.1 cm). Filters were washed six times, for 4 minutes each time, in 5 percent Na_2HPO_4, then twice in water, twice in 95 percent ethanol, and twice in diethyl ether, and air dried. Samples were counted after solubilization in a toluene-based scintillation fluid containing Ominifluor (4 g/liter) (New England Nuclear) and 2.5 percent NCS (Nuclear-Chicago solubilizer) in a Nuclear-Chicago Mark II at 60 percent efficiency (^3H). The RNA polymerase activity is expressed as the number of picomoles of uridine monophosphate incorporated into RNA per milliliter per 10 minutes. (B) Conditions were the same as those in (A) except for the following. The protein (7.4 mg) was applied to the column, and the column was washed with an additional portion of the equilibrating solution; then a gradient (0.1 to 0.5*M* ammonium sulfate) was applied in a total volume of 30 ml. Fractions of 0.3 ml were collected, and the assays were performed at 3 μ*M* UTP. Open circles, activity in the absence of α-amanitin; closed circles, activity in the presence of α-amanitin.

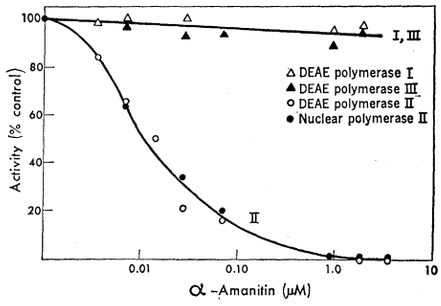

Fig. 2. Titration of the inhibition by α-amanitin of solubilized and nuclear RNA polymerase II from rat liver. Polymerases I, II, and III were isolated from DEAE Sephadex chromatography. Assays of polymerase I contained 0.2 unit of enzyme while those of polymerase II contained 0.16 unit and those of polymerase III contained 0.5 unit of enzyme. Assays of whole nuclei were performed at 0.24*M* ammonium sulfate and 0.1 m*M* unlabeled UTP. The effect of α-amanitin on nuclear RNA polymerase activity was determined, and a correction for the polymerase I and III contribution was made by subtracting the residual activity obtained at an inhibitory concentration of α-amanitin ($3.4 \times 10^{-6}M$) from the total activity. The resultant profile represents the action of α-amanitin on polymerase II. Assays of whole nuclei were stopped as described in the legend of Fig. 1.

evidence for the presence of polymerase III in rat liver. The typical resolution by diethylaminoethyl (DEAE) Sephadex chromatography of polymerases I, II, and III from sea urchin and rat liver nuclei are presented in Fig. 1. We have detected polymerase III activity from rat liver nuclei by fractionation of (nonfrozen) nuclei. Addition of α-amanitin to the assays of the column effluent results in specific inhibition of only the activity in the second peak. Kedinger *et al.* (*8*) have independently observed a similar effect of α-amanitin on calf thymus polymerase II (B) but not I (A).

A more complete titration of this effect of α-amanitin with whole nuclei and with purified polymerases I and III is shown in Fig. 2. There is no detectable inhibition of polymerase I or III activity at any concentration tested (up to $10^{-6}M$). In contrast, polymerase II is inhibited 50 percent at $10^{-8}M$ α-amanitin and completely at $10^{-6}M$ under our assay conditions. In assays of nuclei in the presence of Mn^{++} and high salt, nucleoplasmic RNA synthesis predominates (*9*). However, there remains a residual polymerase activity (20 percent of the total) that is not inhibited by α-amanitin. Knowing the salt concentrations for optimum activity of the purified enzymes (*1*), we can tentatively ascribe the activity at low salt concentrations (less than 0.04*M* ammonium sulfate) in the presence of α-amanitin to polymerase I and III, and the activity at high salt concentrations (0.2*M* ammonium sulfate) in the presence of α-amanitin to polymerase III. We have made the assumption that the characteristics of the polymerase activities in intact nuclei mimic those measured in vitro, and therefore the nuclear polymerase II activity shown in Fig. 2 is obtained by subtracting the residual α-amanitin resistant polymerase activity from the total nuclear polymerase activity.

The degree of inhibition by α-amanitin is not affected by prior incubation of the inhibitor with the enzyme or components (or both) of the reaction mixture. Increasing the concentration of DNA in the assay has no influence on the degree of inhibition, thus the inhibitor does not react with the DNA itself. This result is consistent with the fact that α-amanitin does not inhibit the activity of polymerase I and III or of *Escherichia coli* polymerase on a variety of templates. The action of α-amanitin therefore appears to be on the protein itself. Addition of α-amanitin to an enzyme actively engaged in RNA synthesis results in abrupt cessation of activity as if chain elongation were effected (Fig. 3). More definitive experiments, however, are required to determine the specific nature of the α-amanitin inhibition. This compound could react with the free enzyme to inhibit binding with the template, or it could interact with the enzyme-DNA complex to block initiation, chain elongation, or enzyme release.

Our experiments allow a number of conclusions and also raise a number of questions. The specific inhibition of polymerase II by α-amanitin implies a structural difference between this polymerase and polymerases I and III. This confirms the conclusion that polymersases I, II, and III are distinct molecules. However, we do not yet know whether the three enzymes are unique or whether they contain any common subunits. The results suggest that the toxicity of α-amanitin is due to the specific inhibition of polymerase II activity and a resultant blockage of the synthesis of certain RNA species in the nucleus. If the transcription of all RNA species could be effected by either polymerase I, II, or III, then α-amanitin should produce only a mild functional impairment rather than a cellular catastrophe (*4*). Because nucleolar polymerase I is unaffected by α-amanitin the nucleolar fragmentation observed in liver after administration of this compound may reflect a dependence of nucleolar structure on polymerase II function.

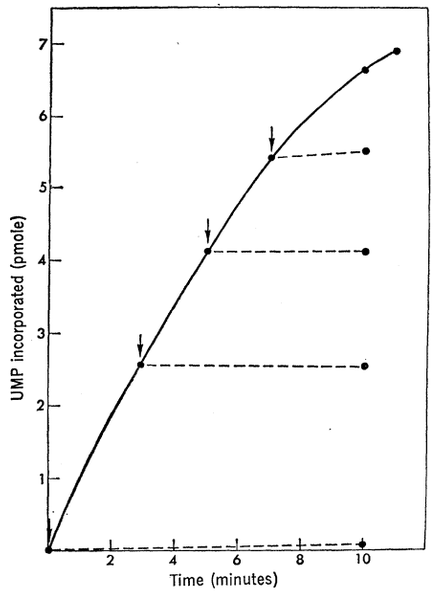

Fig. 3. Abrupt inhibition of rat liver RNA polymerase II activity by α-amanitin. During the course of an RNA polymerase II catalyzed reaction, duplicate portions were removed at various intervals (0, 3, 5, and 7 minutes). One was stopped in the usual manner (control), and the other was treated with α-amanitin ($1.4 \times 10^{-6}M$) and incubation continued to 10 minutes. Assays were stopped as described in the legend of Fig. 1. Solid lines indicate control, and dotted lines denote the α-amanitin–inhibited reaction.

Our observations have immediate practical consequences. Measurements of RNA polymerase activity at different salt concentrations in the presence and absence of α-amanitin allows the determination of the relative proportion of polymerase I, II, and III activities in isolated nuclei. Variations in the levels of these polymerases have already been observed in regenerating rat liver (*10*), during sea urchin development (*11*), and in cortisone and estrogen induced functional transitions in rat liver and uterus, respectively. α-Amanitin may prove to be a most useful inhibitor to probe the transcriptive function of the various nuclear polymerases.

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Echo-Ranging Neurons in the Inferior Colliculus of Bats

Abstract. *Bats measure the distance to an object in terms of the time lag between their outgoing orientation sounds and the returning echo. For measurement of the time lag, the latency of response of a neuron to a stimulus must be nearly constant regardless of the stimulus amplitude and envelope. Otherwise, a large error would be introduced into the measurement. Bats have neurons that are specialized for echo ranging.*

For echolocation, some insectivorous bats emit tone pulses which are variously modulated in frequency and amplitude (*1*). Echoes coming back from objects at different places overlap and show complex envelopes and structures which are quite different from those of the outgoing sounds. The bats analyze these complex echoes from different aspects in order to echolocate. One of the fundamental aspects is echo ranging. A basic clue for measurement of the distance to an object is given by the time lag between the outgoing orientation sound and the returning echo (*2*). This time lag is presumably coded in the difference in time between the response of a neuron to the outgoing sound and that to the echo.

The latency of response of a neuron to a stimulus generally changes as a function of stimulus amplitude and rise time. If the amplitude of a stimulus such as an outgoing sound is very large, and quickly reaches the threshold of a neuron, the latency of response of the neuron will be short. If the amplitude of a stimulus such as an echo is just above the threshold of the neuron, or if it slowly increases up to the threshold, the latency of response will be long. When this occurs, a large error will be introduced into distance measurement. For echo ranging, there must therefore be neurons which show relatively constant latency regardless of stimulus amplitude and rise time. If such neurons discharge multiple impulses in response to a single stimulus, there will be no way to distinguish between one strong echo and a few weak echoes. There must therefore be neurons with constant latency which do not fire repetitively. Furthermore, the neurons must be spontaneously inactive. Any neurons which satisfy the above requirements may be considered to be specialized for the measurement of distance, and may be called echo-ranging neurons.

Neurons in the cochlear nucleus of bats fire repetitively in repsonse to a tone burst, and some of them are spontaneously active (*3*). Therefore, echo-

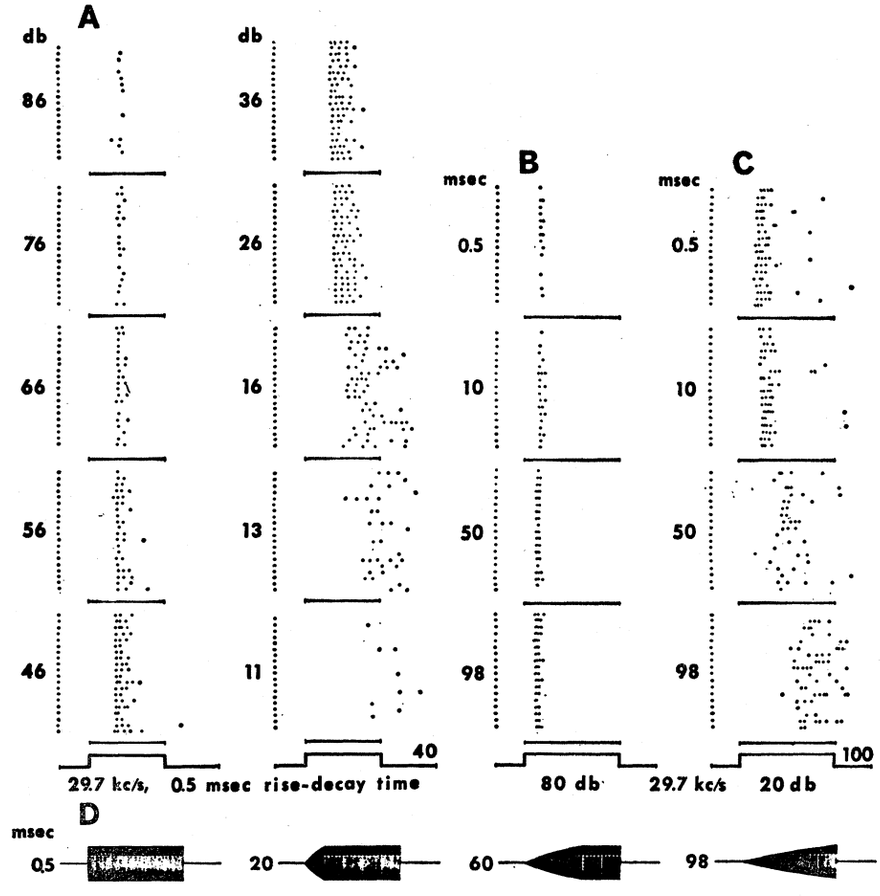

Fig. 1. Responses of an inferior collicular neuron which was not specialized for echo ranging. Responses to repeated presentations of the same tone pulse are shown by a dotted pattern in which one dot corresponds to the peak of one action potential. The sweep of a cathode-ray oscilloscope was displaced vertically before each stimulus. The dots to the left mark the start of the sweep. The sound simulus is represented by a horizontal bar and square wave at the bottom of each dotted pattern. (A) The amplitude of a 29.7-khz tone burst with a 0.5-msec rise-decay time and a 40-msec duration was attenuated from 86 db to 11 db. (B and C) The rise time of a 29.7-khz tone burst with a 100-msec duration was changed, as indicated by the number to the left of each dotted pattern. The decay time was always 0.5 msec. The peak amplitude of the tone burst was (B) 80 db and (C) 20 db. Note the changes in the latency of response and number of impulses. (D) Four samples of the 100-msec tone bursts used are shown.