a value of 38×10^{15} liters as the annual river and ice-cap runoff and an upper limit of 0.1 ppb of mercury in rivers (*7*), an upper limit of transfer of 3.8×10^9 g of mercury per year is obtained.

The amount of mercury produced throughout the world in 1968 was 8.8×10^9 g. A substantial loss of mercury to the atmosphere occurs in chlor-alkali production, and about one-third of the mercury consumed annually in the United States is used in that process (*8*). If we apply this factor of ⅓ to the world consumption, the resultant flux is 3×10^9 g/year, a fraction of the flux involved in the degassing process.

On the basis of recent data on fossil fuel combustion, it has been computed that an upper limit of 1.6×10^9 g of mercury per year can be released to the environment through the burning of coal, oil, and lignites (*9*). Other industrial activities yield potential atmospheric fluxes of this order of magnitude. The amount of cement produced throughout the world in 1960 was 33×10^{13} g. Since cement, during its production, is heated to temperatures of 1400° to 1500°C, the mercury release from the limestone and shale components of the raw materials could conceivably discharge significant quantities of mercury to the environment. Since the mercury content of shales clusters around several hundred parts per billion and since the mercury content of limestones is usually less than 100 ppb, an upper limit of 300 ppb is taken as a conservative estimate of the mercury content of the building materials for cement. Thus, cement manufacture might result in the release of 10^8 g of mercury per year. Perhaps the roasting of sulfide ores releases significant quantities of mercury to the environment. Such mining operations involve 2×10^{14} g of ore per year (*10*). But even with a mercury content of 10 parts per million, this flux is only 2×10^9 g/year. Hence, a survey of industrial activity has not revealed mercury releases to the atmosphere that can rival that of the natural degassing rate, estimated to range between 2.5×10^{10} and 1.5×10^{11} g/year.

The following explanation is proposed to account for the doubling of the mercury content in a Greenland ice sheet. The background concentration of mercury in the atmosphere arises from the degassing of the upper mantle and lower crust. This view is supported by the observations that the mercury is markedly enriched in sediments as compared to igneous rocks (*11*). Moreover, the atmospheric mercury concentration is a function of barometric pressure (*6*). Increased exposure of such crustal materials can result in increased fluxes of mercury to the atmosphere. The variety of activities of man that result in greater exposure of the earth's crust through alteration of terrestrial surfaces allows more mercury vapor and more gaseous compounds to enter the atmosphere.

In conclusion, the recently measured mercury concentrations in such pelagic fish as tuna and swordfish are probably not far removed from the norm. The input of twice as much mercury to surface waters of the ocean in recent times can only increase the amount of mercury in the lower trophic levels by, at best, a factor of 2.

This argument can be approached in another way. There are 10^{12} g of mercury in the mixed layer of the ocean, if we assume a depth of 100 m and an average mercury content of 30 ng/liter. The increased mercury flux, 1.5×10^{11} g, presumed to result from an enhancement of earth degassing, would add annually only 15 percent to the mercury burden of this water body. With a residence time of mercury in this layer of as much as 5 years, the mercury content would be augmented by a factor of only 0.75. The increased mercury content in surface waters, if transmitted through the food web to such upper trophic levels as those of the swordfish and tuna, would, at best, double the mercury contents in these organisms.

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Viral RNA Polymerases: Electron Microscopy of Reovirus Reaction Cores

Abstract. *The Kleinschmidt technique has been used to observe reovirus cores that have synthesized messenger RNA. Some individual viral cores probably synthesize all ten messenger RNA molecules in vitro. Each messenger RNA molecule appears to be attached to a different site on the core surface, implying that there are probably a number of different enzymic sites in each core.*

Virus particles that contain enzymes capable of transcribing the viral genetic information have been subjected to intensive biochemical study. Reovirus (*1*, *2*), vaccinia virus (*3*), influenza virus (*4*), vesicular stomatitis virus (*5*), and the RNA tumor viruses (*6*), contain enzymes of this type. These enzyme activities are usually studied by measuring the incorporation of labeled ribo- or deoxyribonucleoside triphosphates into the acid-insoluble product synthesized by the enzyme. This provides a sensitive and convenient method for characterizing the average product synthesized in vitro by the viral cores or nucleoids. However certain aspects of the mechanism of action of these enzymes are not readily investigated by these methods—for example, an assessment of the number of sites at which nucleic acid is synthesized.

We report here experiments designed to make possible electron microscopic observation of RNA transcription in vitro. Reovirus cores have been used as an experimental model because the core contains a very active RNA tran-

scriptase that synthesizes copies of all ten segments of genome double-stranded RNA in vitro (*1, 2*). We define "cores" as virus particles from which the outer layer of subunits have been removed. "Reaction cores" (*1*) are those core particles engaged in the synthesis of messenger RNA. In the work reported here, reovirus reaction cores were fixed in formaldehyde, spread over a hypophase containing formaldehyde, and studied by electron microscopy. Individual active cores appear capable of synthesizing up to ten messenger RNA molecules that are attached to a number of different sites on the surface of the core.

Reovirus type III (Dearing strain) was propagated in mouse L cells and purified by banding on CsCl gradients (*7*). Purified virus in 1 × SSC (0.15*M* NaCl, 0.015*M* sodium citrate, *p*H 7) was first incubated with α-chymotrypsin (50 μg/ml) at 39°C for 30 minutes to convert the intact virus to cores. RNA transcriptase reaction components (after being mixed at 0°C) were added to the cores to give a final volume of 0.5 ml. The complete reaction mixture, based on that described by Skehel and Joklik (*1*), contained tris-HCl buffer, *p*H 8.5, 30 μmole; $MgCl_2$, 5.0 μmole; adenosine, guanosine, cytidine, and uridine triphosphates (ATP, GTP, CTP, UTP), 0.5 μmole each; 2-mercaptoethanol, 1.0 μmole; [^{3}H]CTP, 1 μc; Macaloid, 100 μg; reovirus cores, 200 μg or more. The reaction mixture was incubated at 39°C for an appropriate time (usually 15 minutes), and the reaction was terminated by chilling to 0°C and adding formaldehyde to a final concentration of 1 percent. (Formamide proved unsuitable for use as a spreading agent since it induced extensive lysis of the core particles and the release of double-stranded RNA.)

Reaction cores and native core particles were freed from contaminating double-stranded RNA and capsid proteins by sedimentation through a discontinuous sucrose-CsCl gradient. The lower (0.5 ml) layer of the gradient contained 40 percent sucrose in 0.05*M* tris, *p*H 7, 0.1 percent formaldehyde, and CsCl to a final density of 1.48 g/ml. The upper (4.0 ml) layer of the gradient contained 15 percent sucrose, 0.05*M* tris, *p*H 7, and 0.1 percent formaldehyde.

The formaldehyde-treated reaction mixture (0.5 ml) was applied to the top of the gradient and the mixture was

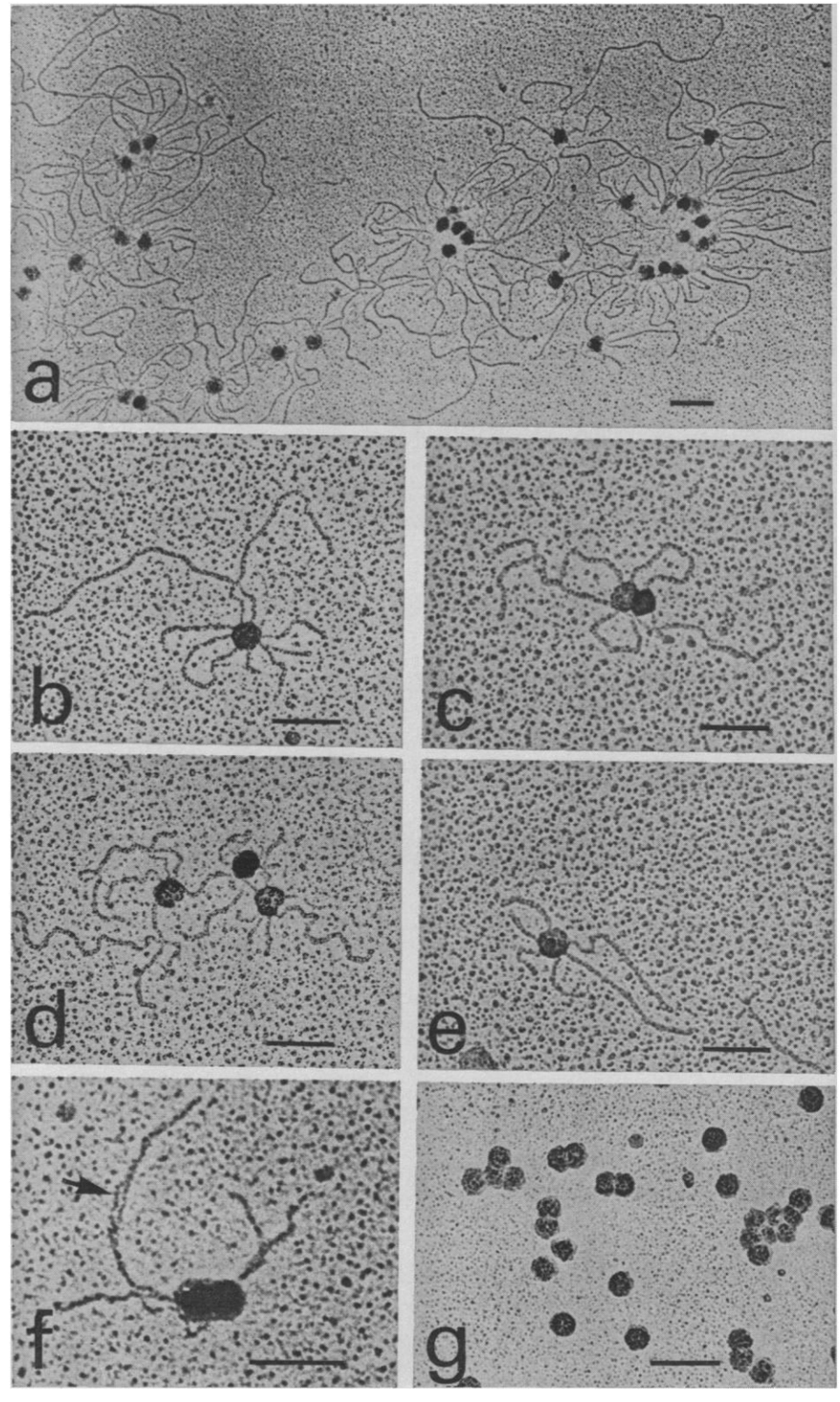

Fig. 1. Reovirus reaction cores observed by the Kleinschmidt surface spreading technique. Reovirus cores prepared by chymotrypsin digestion were incubated in the standard polymerase incubation mixture for 15 minutes. The resulting reaction cores were purified from the reaction mixture by centrifugation through a sucrose gradient and spread by the Kleinschmidt technique (*8*). The hyperphase contained the reaction cores diluted in 0.25 percent formaldehyde, 0.01 percent cytochrome c. The hypophase was 0.2*M* ammonium acetate containing 0.5 percent formaldehyde. All films were rotary shadowed with platinum-palladium from an angle of 3 to 5 degrees, and photographed in a Philips EM 200 electron microscope. (a–f) Examples of reaction cores formed in the presence of all four ribonucleoside triphosphates. The arrow in (f) indicates a pair of messenger RNA strands coiled together. (g) Cores incubated in the absence of ATP. Reaction cores treated with pancreatic ribonuclease prior to spreading gave an appearance similar to (g). The bar included in each frame represents 0.2 μm.

centrifuged (45,000 rev/min, 1 hour, SW-50 rotor, Spinco L2 ultracentrifuge). The single mixed band of cores and reaction cores that formed in the lower portion of the tube was collected by piercing the side of the centrifuge tube with a hypodermic syringe of small bore; the sample was then diluted fourfold with 1 percent formaldehyde. Cytochrome c was added to give a final concentration of 100 μg/ml, and the mixture was immediately spread (*8*) over a hypophase of 0.2*M* ammonium acetate, *p*H 6.8, containing 0.5 percent formaldehyde. The monolayer was transferred to grids that had been coated with collodion and stabilized with a carbon film. The grids were then rotary shadowed with platinum-palladium from an angle of approximately 5 degrees.

When purified formaldehyde-fixed reaction cores were spread and examined by election microscopy, RNA strands were clearly visible (Fig. 1, a–f). The following evidence strongly supports the contention that the structures shown in Fig. 1, a–f, are reaction cores.

1) Lysed particles and free double-stranded RNA derived from them were removed prior to spreading and electron microscopy by sedimentation into the density gradient.

2) RNA strands were not observed associated with cores if transcriptase reactions were carried out in the absence of ATP [RNA synthesis by cores required the presence of all four ribonucleoside triposphates (Fig. 1g)].

3) RNA strands were not observed associated with reaction cores which had been digested with pancreatic ribonuclease A after incubation and prior to fixation and spreading (10 μg/ml for 10 minutes at 39°C, 0.15*M* salt, $10^{-3}M$ $MgCl_2$).

4) When single-stranded RNA and purified cores were mixed and then spread, structures such as those shown in Fig. 1, a–f, were not observed, implying that released single-stranded RNA does not reassociate with cores to form artifacts resembling reaction cores.

We have estimated the proportion of viral cores which participate in the polymerase reaction by counting particles which show evidence of attached messenger RNA. In a random count of 8100 particles were observed 695 (8.5 percent) reaction cores. The small proportion of active cores observed here is in agreement with other data (*9*) indicating that reovirus cores synthesize approximately eightfold less RNA than the amount expected from the known rate of chain elongation. However, some aspects of our preparative procedure might influence the accuracy of our estimate of the proportion of active cores. For example, reaction cores show a marked tendency to aggregate (Fig. 1a), and this might prevent observation of much of the RNA present.

Examination of reaction cores that showed visible strands gave an average of 2.8 strands (free-ends) per particle. Occasionally two or more strands were observed coiled around each other (Fig. 1f). Frequently this would result in their being scored as one strand. The maximum number of strands observed associated with a core particle was ten, a value in agreement with the number of messenger RNA molecules produced by the transcriptase (*1, 2*).

Further studies will be required to provide a more detailed structural analysis of reovirus reaction cores. However, examination of the electron micrographs presented in Fig. 1 permit two preliminary conclusions to be reached concerning the synthesis of messenger RNA by the reovirus RNA transcriptase. (i) At least some of the cores appear capable of synthesizing ten messenger RNA molecules. It is probable that these strands represent the ten individual messengers synthesized in vitro (*1, 2*). (ii) Nascent messenger RNA molecules appear to be attached to different sites on the surface of the core, suggesting that more than one enzymic site exists per core.

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Acridine Orange Potentiation of Actinomycin D Uptake and Activity

Abstract. *Acridine orange enhances the uptake of [^3H]actinomycin D in disrupted and intact human lymphocytes, as measured by liquid scintillation and autoradiography. Proflavine, quinacrine, chloroquine, and ethidium bromide are not effective. In mice, acridine orange increases the capacity of actinomycin to reduce spleen weight. Type II acridine binding to DNA may be a prerequisite for actinomycin enhancement.*

Actinomycin D is a potent inhibitor of DNA-dependent RNA synthesis and an effective agent in the treatment of certain malignant tumors. Although it has a marked effect on lymphoid tissues, autoradiographic studies of [^{3}H]-actinomycin D uptake have shown that only 5 to 28 percent of human lymphocytes in culture take up actinomycin (*1*). These studies also demonstrated that, 24 hours after stimulation of the lymphocytes with phytohemagglutinin, there occurred a large increase in the number of cells binding actinomycin. It is likely that an agent that could enhance the uptake of actinomycin would potentiate its activity and possibly its antitumor effect as well. This report shows that acridine orange is capable of enhancing the uptake of actinomycin in vitro and its activity in vivo. Unlike phytohemagglutinin, its effect appears rapidly and is not dependent on intact cellular processes.

Acridine orange was obtained from Calbiochem and the National Aniline Corporation; proflavine, chloroquine, quinacrine, and ethidium bromide from the Sigma Chemical Company.

Blood from normal donors was collected in phenol-free heparin. The red cells were sedimented with 5 percent dextran, and the leukocyte-rich plasma was passed through a column of washed Fenwal Leukopak nylon. The yield of approximately 95 percent lym-