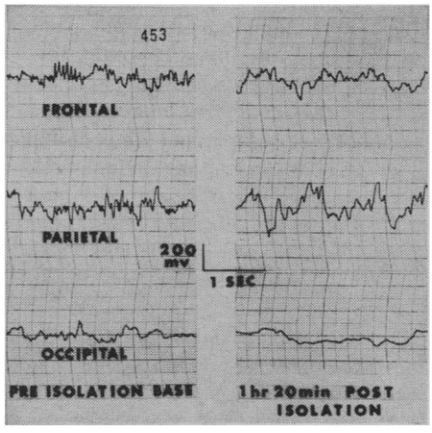

Fig. 2. A sampling of electrocortical activity from the frontal, parietal, and occipital lobes of the first monkey brain successfully maintained as a totally isolated organ is reproduced. The first recording was obtained prior to neurogenic and vascular isolation; the second was made 1 hour and 20 minutes after total isolation and the institution of extracorporeal donor perfusion. The slowing and reduction in electrical activity (particularly over the occipital regions) in the post-isolation tracing was considered to be due, in part, to the brain's low temperature (31°C).

was established between the reservoir and the donor monkey the arterial pressure rose to preperfusion levels of 160/120 to 190/140 mm-Hg. Internal carotid pressure measurements via the "T" cannula (Fig. 1) of the isolated brain were 20 to 30 mm-Hg lower than the donor's systemic arterial pressure during perfusion. Perfusion rates in the totally isolated monkey brain (average weight 83.5 g) ranged from 20 to 60 ml/min as measured by venous return.

Serial electrocortical tracings revealed persistence of electrical activity for periods ranging from 30 to 180 minutes during isolated brain perfusion (Fig. 2). With total cessation of perfusion the electrocortical tracings became flat within 30 seconds. Direct inspection of the cortex of the brain during perfusion demonstrated normal appearing cortex, arteries, and veins. This last observation was supported by the mean $A\text{-}V_{O_2}$ and $V\text{-}A_{CO_2}$ differences across the isolated brain, which averaged, respectively, 5.8 vol. percent and 5.0 vol. percent after 2 hours of perfusion. Intracerebral temperature recordings made in the frontal and parietal lobes of the isolated brain indicated a persistent hypothermia (30° to 35°C) with an excellent agreement (within 0.5°C) between the anterior, posterior, superficial, and deep areas of cerebrum.

The major problem in the preparation of the isolated monkey brain has been the design of an operation which would completely isolate the brain and yet permit the total organism to be maintained in a satisfactory physiological state until circulatory bypass was begun. This was achieved by meticulous surgical technique and careful maintenance of circulating blood volume. In each of the five isolated-brain perfusions, the experiment was terminated because of marked reductions in the hematocrit (mainly due to repeated arterial blood sampling) of the total circulating system. The desire here was to secure the survival of the large, costly donor monkey for future experiments. While there is reduction in the electrical activity recorded from the isolated brain (Fig. 2), particularly over the occipital cortex, some of this reduction was felt to be due to the existing cerebral hypothermia and the post-perfusion finding that the cortex, particularly the occipital cortex, fell away from the skull. Further evidence of active metabolism in the isolated monkey brain was demonstrated by the significant $A\text{-}V_{O_2}$ and $V\text{-}A_{CO_2}$ differences, which compare favorably with those reported for the monkey brain *in situ* (*6*).

Previous attempts to vascularly "isolate" the mammalian brain *in situ* or as an isolated head preparation have principally relied upon simple ligation of the extracranial vessels in order to eliminate arterial and venous contamination. In the ingenious "isolated" cat brain of Chute and Smyth (*2*) extracranial tissues were responsible for 14 percent of the measured metabolism of this preparation. Likewise, the metabolic data obtained from the *in situ* "isolated" cat brain of Geiger (*7*) has been criticized because of incomplete vascular isolation and the use of heterologous perfusion media (*8*).

These experiments demonstrate for the first time the feasibility of protracted survival of the subhuman primate brain as a totally isolated organ utilizing extracorporeal donor perfusion (*9*).

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Circulating Interferon in Mice after Intravenous Injection of Virus

Abstract. *Circulating interferon was detectable in mouse serum within 1 hour after the intravenous injection of various types of virus and it reached maximum levels in about 4 hours. Rapidly produced interferon may play a role in the pathogenesis of viral infection and in viral interference.*

Interferon is thought to be an important antiviral factor in the recovery of animals from viral infection (*1*). Although the production and antiviral action of interferon is generally localized at the site of viral infection (*2*), indirect evidence suggests that small amounts of interferon may pass from infected tissues to distal sites (*3*). However, circulating interferon was not consistently detected in these studies. To test the possibility that circulating interferon might be produced as a result of viremia occurring during infection, mice were injected intravenously with virus and subsequently their serums were assayed for interferon.

Interferon was assayed by the plaque-reduction method (*4*) in which primary cultures from mouse embryos were used; the cultures were treated for 24 hours with 3 ml of two- to four-fold serial dilutions of interferon and then challenged with 20 to 60 plaque-forming units (pfu) of vesicular stomatitis virus (VSV). Interferon titers, expressed as units per 3 ml, were determined as the dilution of the original material required to reduce the plaque count to 50 percent of that of the controls. Newcastle disease virus and Sendai virus were grown in the allantoic sac of chick embryos aged 9 to 11 days; vaccinia virus was propagated in

HeLa cell cultures; Sindbis virus was grown on primary cultures from chick embryos; and vesicular stomatitis virus was cultured on primary cultures from mouse embryos.

Two-tenths of a milliliter of allantoic fluid containing $10^{7.9}$ pfu of Newcastle virus was injected into the tail vein of male Swiss mice weighing 12 to 14 g. The amount of virus which was absorbed by the cells may be considered equal to the inoculated dose, since it has been determined that the major portion of intravenously injected virus is absorbed by cells within 30 minutes (*5*). At different times groups of four or more mice were bled; the serum obtained from each one was acidified to *p*H 2 with HCl to inactivate the virus, held at 4°C for 1 to 7 days (depending on the type of virus which was injected), and neutralized with NaOH. The serums were then assayed for antiviral activity. As shown in Fig. 1, interferon-like, antiviral activity was detected within 1 hour of inoculation of virus and the highest concentration occurred after about 4 hours. In a confirmatory experiment, no antiviral activity was detected in serum collected after ½ hour, 6 units of antiviral activity were present after 1 hour, and 2000 units were present in the serum collected after 4½ hours.

To confirm that the antiviral material in the serum was interferon, its properties were compared with a known preparation of mouse interferon. The antiviral substance in the serum samples collected at each time interval remained stable at *p*H 2 for 7 days, was active against a heterologous virus (VSV), and was not contaminated with live Newcastle virus after acid treatment. The antiviral property of both the 7-hour serum and the known mouse interferon was stable at 44°C; both were partially inactivated at 56°C and 60°C; and both were fully inactivated at 80°C for 1 hour. Incubation of the serum with 0.05 mg/ml of crystalline trypsin at 37°C for 1 hour resulted in loss of antiviral activity. Inoculation of the serum into the allantoic sac of embryonated eggs did not result in the production of Newcastle virus hemagglutinin, indicating that interference was not caused by the live virus. Centrifugation at 105,000*g* for 1 hour in the presence of 0.06-percent gelatin (*6*) did not cause the activity of the supernatant to be diminished, showing that the antiviral effect was not caused by inactivated virus. The antiviral activity of the serum was effective in cultures

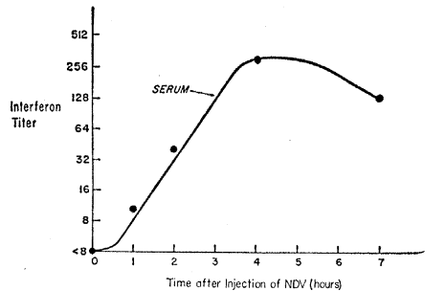

Fig. 1. Interferon titers, expressed as units per 3.0 ml, in mouse serums collected at intervals after intravenous inoculation of $10^{7.9}$ plaque-forming units of Newcastle disease virus.

from the mouse embryo, but not in cultures from the chick embryo, indicating species specificity. Encephalomyocarditis virus and vesicular stomatitis virus were both inhibited, showing lack of viral specificity. There was no inactivation of $10^{6.0}$ pfu of VSV which was incubated with undiluted serum for 1 hour and then diluted $10^{-5.0}$ for virus assay, which suggested that the antiviral action did not affect the virus directly. These properties of the antiviral material in serum coincide with those of interferon which is produced by mouse cells (*7*), and exclude the possibility that the activity was due to live, incomplete, or inactivated virus.

To examine the possibility that nonviral material in allantoic fluid might be the stimulus for interferon production, the Newcastle virus was sedimented from the allantoic fluid at 105,000*g* for 3 hours in the presence of 0.06-percent gelatin. The sediment was diluted to the original volume with phosphate buffered saline, and the resuspended sediment and supernatant were each injected intravenously into mice. The serum collected 5 hours after intravenous injection of the sediment contained 600 units of interferon, while the serum collected 5 hours after injection of the supernatant contained less than 8 units, indicating that the substance which stimulated interferon production was deposited with the virus.

Table 1. Production of circulating interferon following intravenous inoculation of different amounts and types of virus.

Virus	Infectious units inoculated	Interferon titer at 4½ hours
NDV*	$10^{7.0}$	300
NDV	$10^{5.0}$	<8
NDV	$10^{3.0}$	<8
Sindbis	$10^{8.3}$	80
Sendai	$10^{7.7}$	30
Vaccinia	$10^{5.9}$	10

* Newcastle disease virus.

To estimate the minimum amount of Newcastle virus required to induce circulating interferon, mice were inoculated intravenously with varying amounts of virus, and serum samples were collected at the time of maximum interferon production, 4½ hours. As shown in Table 1, production of circulating interferon required the injection of more than $10^{5.0}$ pfu and less than $10^{7.0}$ pfu of Newcastle virus. Similarly, lower but detectable amounts of circulating interferon were induced by $10^{5.9}$ pfu of vaccinia virus, $10^{7.7}$ EID_{50} (egg infectious dose—50 percent effective) of Sendai virus, and $10^{8.3}$ pfu of Sindbis virus.

Interferon production was stimulated by the virus in the inoculum, and not by new virus resulting from replication, because serum samples for interferon were taken at 4½ hours after intravenous inoculation, a time too early for significant growth and release of virus. These results indicate that circulating interferon may be induced by a single intravenous dose of approximately $10^{6.0}$ or more infectious units of virus. Since noninfectious virus usually constitutes a large fraction of a live virus preparation, it is not clear whether stimulation of circulating interferon was due to live or dead virus. This question is under active investigation by others (*8*).

That viremia, sufficient to stimulate circulating interferon, may be produced during certain infections of animals is suggested by the finding that virus titers in the blood of up to $10^{4.0}$ infectious doses per ml of poliovirus, and up to $10^{10.0}$ per ml of arborviruses, may occur during experimental infections and persist for several days (*9*). When the amount of virus is corrected for total blood volume and for rapid elimination and replacement of virus (*5*), it then seems likely that sufficient virus to stimulate production of circulating interferon may occur in some infections. Consistent with this view is the reported detection of nonspecific antiviral factors in the blood of mice undergoing viral infection (*10*). If interferon is often produced within 1 to 2 hours of viral infection of most tissues in vivo and if it is equally rapidly absorbed into surrounding cells (*4*), then an antiviral reaction of the infected organism may occur while the first cycle of viral replication is still partly sensitive to the action of interferon (*4, 11*). These questions are being studied and preliminary results indicate that (i) the viremia which follows intraperitoneal infection of mice with Germiston virus

(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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26 June 1963

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 μg/ml prevents differentiation and causes an abnormal distribution of cleavage planes; however, cell division can continue after exposure to concentrations as high as 100 μg/ml (*2*). Normal cleavage of amphibian eggs (*Pleurodeles* and *Xenopus*) in actinomycin D (10 μg/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 μg/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