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Properties of Poliovirus Inhibitor from Monkey Brain. (28291)

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Tissue suspensions of the central nervous system of rhesus monkeys have been reported to inhibit infectivity of all 3 types of poliovirus(1). Rapid sedimentation of the inhibitory activity, its heat lability and its virus specificity(1) suggest a possible relationship to the virus-adsorbing or receptor sites on the cell surface which have been reported for a number of viruses(2-11). Inhibition of virus by suspensions of receptor material has been shown to result from binding of virus to receptor which prevents virus from attaching to intact cells(2-11). In contrast the preliminary study of inhibitory suspension of monkey nervous tissue suggested that the antiviral action might be mediated through host cells and not by direct binding of virus because inhibition of poliovirus could be reversed by simple dilution(1). The present study of properties of the inhibitory factor, from the central nervous system tissues of monkeys, was undertaken to help determine its relationship to receptor sites, mechanism of action and possible importance in the pathogenesis of poliovirus infection.

Materials and methods. Suspensions of various organs, diluted in Medium 199, were prepared by homogenizing tissues with glass beads, glass tissue homogenizers or in an electrically driven blendor. Homogenates were stored at −25°C. Poliovirus inhibitor activity of tissue suspensions was determined by mixing equal volumes of organ homogenate and type 1, Mahoney strain poliovirus, and assaying for poliovirus plaque forming units (pfu) in monkey kidney bottle cultures (MK). A decrease of 50% or more, when compared with control plaque counts, was considered significant inhibition. The end point of antiviral activity of monkey nervous tissue homogenates occurred at a 1/20 dilution (5% suspension) of the original tissue and complete suppression of plaques occurred at 1/3 dilution (30% suspension). In the present study 10 or 20% suspensions were used.

Results. Effect of chemical and physical agents on inhibitory factor.

Organic solvents. Normal monkey brain suspension was shaken with an equal volume of ether or an equal volume of 2 parts ethanol and 1 part ether at room temperature. Aqueous phase, organic solvent phase and interphase were separated, the organic solvents allowed to evaporate at 4°C and the fractions brought back to original volume by addition of Medium 199. Assay of fractions revealed complete loss of the 80% plaque inhibiting activity of the untreated suspension. Inactivation by ether is a property shared by the inhibitory factor and previously studied receptor substance(6).

Acid. Monkey brain suspension was dialyzed overnight against 60 volumes of citric acid—disodium phosphate buffer solution at pH 2 then redialyzed against Earle's balanced salt solution at pH 7.4. This procedure resulted in loss of the 80% plaque inhibitory activity of the untreated suspension. Inactivation of inhibitory factor by acid is consistent with, but not necessarily due to the same mechanism as, the reported release of poliovirus from receptor at acid pH(9).

Trypsin. Incubation of inhibitory suspension for 1 hour at 37°C in the presence of 0.001% trypsin (twice recrystallized) resulted in loss of the 75% plaque inhibition which oc-

TABLE I. Binding of Poliovirus to Fractions of Homogenate of Monkey Nervous Tissue.

CNS preparation	pfu/0.4 ml at dilution 10^0	10^{-1}	10^{-2}	10^{-3}
Homogenate	8, 10	3, 3	0, 2	0, 0
Supernatant	3, 6	0, 0	—	—
Sediment	7, 9	4, 5	0, 2	0, 0

curred in the inhibitor controls. Destruction by proteolytic enzymes has also been reported for receptor material(3,4,9).

Thus poliovirus-inhibitory suspension from monkey brain and previously studied receptor site material shares the properties of inactivation by organic solvents, trypsin and heating at 56°C(1) as well as sedimentation at low speed(1). These results suggested that the inhibitory factor was similar to receptor site material. If receptor and inhibitory factor are related, as is indicated by their physical and chemical properties, then inhibitory factor should bind virus directly as does receptor. To test this possibility, experiments were carried out to determine ability of inhibitory factor to bind virus and to affect host cells.

Binding of virus to inhibitor. One ml of monkey brain suspension and 200 pfu of poliovirus in 0.2 ml were incubated at 37°C for 2 hours and then centrifuged at 1000 rpm for 30 minutes to sediment the inhibitory factor and any attached virus. As shown in Table I assay of supernatant revealed loss of 90% of viral infectivity which could be recovered from the sediment by dilution. Since virus was bound to inhibitory factor its antiviral action more likely occurs by direct action on virus rather than being mediated by an effect on host cells.

Effect of inhibitor on cells. Further to determine whether the inhibitory factor affects host cells, cultures of MK were incubated with 0.3 ml of monkey brain suspension for 90 minutes at 36°C. The suspension was then decanted and cultures washed with Earle's balanced salt solution before challenging with virus. Brain suspension, which was collected from the first set of cultures, was similarly applied to 3 groups of MK cultures in serial fashion and aliquots were set aside at each transfer. Challenge of serially treated and washed MK cultures revealed no antiviral activity. Assay of inhibitory material which had been serially incubated with as many as 4 sets of MK cultures revealed undiminished antiviral activity (70% plaque reduction). The results indicate that the inhibitory factor does not irreversibly decrease ability of cells to support growth of virus and that inhibitor is not taken up by cells.

Additional evidence that the inhibitory activity does not act through the cell was obtained by inoculating 0.1 ml containing 24 pfu of poliovirus onto MK cultures and incubating at 36°C for 0, 15, 30 and 60 minutes before applying an equal volume of monkey brain suspension and overlaying. As early as 15 minutes after inoculation of virus the inhibitory action was no longer significant (Table II). The result indicates that inhibitory material cannot affect virus after virus adsorbs to or penetrates the host cell.

Presence of the inhibitory material in various organs and species. Presence of receptors in various species and organs has been correlated with susceptibility to virus infection(2,3, 4,6,7,9,10), presumably because virus attachment to cells is a necessary first step in infection. To determine whether the inhibitory factor was similarly associated with susceptibility to infection, 10-20% organ suspensions from monkey, man and guinea pig were assayed for antiviral activity. Monkey brain gave 60-90% inhibition of 30 pfu poliovirus, adrenal gave 70% inhibition, testis gave 50-70% inhibition and kidney, liver, muscle, spleen, heart, lung and thymus gave less than

TABLE II. Virus Growth in Relation to Time of Addition of Inhibitory Suspension to Infected MK.

Time inhibitor added after virus inoculum (min)	Pfu	% inhibition
0*	8, 8, 12, 15	54
0†	13, 17	38
15	18, 20	21
30	25, 25	0
60	25, 26	0
No inhibitor	23, 24	—

* Inhibitor mixed with virus just before inoculation of MK.

† Inhibitor added to MK immediately after virus.

50% inhibition. Monkey brain and probably adrenal gland are able to support poliovirus multiplication *in vivo*(12), although monkey testis, which does have receptor activity, does not support growth of poliovirus(13).

Tissue suspensions of 2 human brains were found inhibitory for poliovirus. The inhibitory activity was sedimentable at 3000 rpm and destroyed by heating to 56°C for 30 minutes, indicating that the inhibition was not due to contaminating antiviral antibody. Seventy to 80% of virus was inhibited by suspensions of human spinal cord and grey matter from brain, while white matter and cerebral spinal fluid inhibited less than 20% of virus. Similar results were obtained by others, and were interpreted to suggest that virus and adsorbing sites in the central nervous system are associated mainly with cell bodies rather than axones(4).

Suspensions of guinea pig brain, kidney, muscle and spleen were found not to be inhibitory for poliovirus. This result is consistent with insusceptibility of guinea pig cells to poliovirus infection.

In general, the present results are in agreement with the reported correlation between presence of receptor sites and susceptibility to infection(2-5,7,8,10,11).

Discussion. The properties of poliovirus inhibiting factor from central nervous system tissues of monkeys coincides with those properties described for viral receptor sites on surface membranes of cells susceptible to enteroviruses(4-9,11), myxoviruses(2,4,8), pneumonia virus of mice(3) and arbor viruses(8). Also receptor site material has been detected in the central nervous system of monkeys (5, 7,14). The similarity of properties and location suggests that poliovirus inhibitor from rhesus monkeys may be receptor site material. A related material has been found in the brains of mice recovering from infection with mouse poliomyelitis virus but unlike receptor site material it did not bind with virus and was not present in uninfected brains(15).

The finding in the present study that receptor site substance from monkey brain can dissociate from virus following simple dilution is in contrast to strong binding reported for other poliovirus receptor material(14). The difference suggests that receptor from various species and tissues differ in certain properties. This interpretation is supported by the observed variation in stability at −70°C of some receptor materials(7).

Athough tissues, *in vivo,* may lack receptor sites and lack ability to support growth of virus, *in vitro* cultures of these tissues often acquire the ability to support growth of virus. As might be expected the development of viral susceptability by these cultures is accompanied by the appearance of receptor site material in cultured cells(8,10).

It is generally thought that presence or absence of receptor material within various tissues and its concentration exerts a major influence on the pathogenesis of virus infection *in vivo*(2,3,4,7,8,10,11). Cells which lack receptor sites do not become infected and cells with receptor sites become infected if they also possess a suitable internal environment for growth of virus. If cells have receptor sites and can eclipse virus but cannot support virus multiplication, then virus is rendered non-infectious without the production of new virus(16,17). As observed here or reported previously, the major portion of amounts of virus from $10^{1.5}$ to $10^{8.0}$ may be adsorbed to receptors and rendered noninfective(1-8). Binding of virus may become complete as concentration of suspension approaches that of undiluted nervous tissue. During recovery from virus infection an increasing percentage of cells lose ability to support virus growth as a result of interferon, temperature elevation, local hypoxia and acidity, but retain ability to adsorb and eclipse virus(18,19,20). In the tissues of animals recovering from virus infection, not only is virus unable to multiply but the virus-binding and eclipsing capacity of newly resistant cells may be quantitatively sufficient to account for elimination of virus. Such a non-immunological process for rendering virus non-infectious might explain the observed elimination of virus during recovery of the infected animal in the absence of detectable antibody(20,21).

Summary. Properties of monkey brain suspensions which are inhibitory for polioviruses

were studied and found to coincide with properties of cell receptor site material. Inhibitory material did not directly affect host cells but was bound to virus. A possible role of receptor in elimination of virus during recovery from infection is discussed.

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Rate of Metabolism of 9,10-Dimethyl-1,2-Benzanthracene in Newborn and Adult Mice.* (28292)

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During investigations in this laboratory it was demonstrated that the newborn mouse is particularly susceptible to the action of carcinogenic chemicals(1,2). This has since been confirmed by others(3,4). In particular, the polycyclic aromatic hydrocarbon 9,10-dimethyl-1,2-benzanthracene (DMBA) has been found to induce a high incidence of lymphomas and other tumors in newborn mice at dosages, on a body weight basis, that would induce a few such tumors in the adult. In addition to this quantitative difference there is a qualitative difference in the neoplasms induced. The majority of tumors that have been recorded in animals treated at birth have been lymphomas, lung adenomas and a scattering of other interesting neoplasms(1,2,3,4). In the adult, injection of DMBA by the subcutaneous route results in induction of sarcomas, a tumor seen rarely in mice treated when newborn(5). A number of other carcinogens have been tested in the same system including the related polycyclic aromatic hydrocarbons 3,4-benzpyrene,1,2,5,6-dibenzanthracene and 20-methylcholanthrene, in addition to the unrelated compound urethan(2,6,7). All of these compounds manifested an enhanced response in the newborn with the same characteristics, but in none was the difference so marked as with DMBA.

A variety of reasons for the susceptibility of the newborn mouse to carcinogens has been advanced, but none have been tested experimentally. The experiments referred to were originally prompted by the demonstration of the usefulness of the newborn mouse in studies of viral carcinogenesis. The particular susceptibility of the newborn animal to various oncogenic viruses has been the foundation of the recent upsurge of work in this field. It is still unclear why the newborn responds in this manner, although the ill-informed nature

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