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CELLULAR SUSCEPTIBILITY TO ENTEROVIRUSES[1]

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INTRODUCTION

The outcome of virus infections in animals can be influenced by dose of virus, route of administration, humoral immunity, barriers to invasion, and production of interferon, but the limiting determinant must be the relative abundance and distribution in the host of cells capable of synthesizing virus. Animals whose cells are incapable of manufacturing a specific virus should be considered as passively "indifferent" to the agent rather than actively "resistant," since the challenge virus is not recognized as anything more than a particulate foreign macromolecule. Similarly, organs or tissues which possess either few or no susceptible cells might be expected to undergo an abortive, asymptomatic infection, or none at all.

An important property of virus-sensitive cells is the presence of specific receptors, or virus attachment sites, on the surface membrane. A cell may contain adequate virus synthetic machinery but remain insusceptible to infection with intact virus if it lacks such sites. The mucopolysaccharide-neuraminic acid receptor systems of myxoviruses are well known (12). Enterovirus cellular receptors have been characterized more recently by McLaren, Holland, and Syverton (16, 26) and by Philipson and his associates (28). Enterovirus receptors are lipoproteins which appear to be fairly specific for individual members of this large group of agents. The experiments of Holland, McLaren, and Syverton (15) and of Alexander and coworkers (1), demonstrating that the ribonucleic acid of poliovirus and other enteroviruses can infect and induce virus synthesis in nonprimate cells which lack receptors and which are ordinarily insusceptible to these agents, underscores the primacy of the specific receptor site in determining cellular susceptibility to intact virus. The presence of receptor activity on a cell surface does not insure, however, that the cell will be capable of stripping the virus of its protein coat or able to synthesize it. Thus, erythrocytes are known to contain surface receptor for myxoviruses and some enteroviruses, but they do not possess virus synthetic machinery. Darnell and Sawyer (7) demonstrated that a strain of HeLa cells capable of adsorbing poliovirus was unable to synthesize it because of a defect in the eclipse mechanism. Nonetheless, tissues which lack cells containing virus attachment sites would not be expected to be able to synthesize virus.

One of the first questions to be answered when seeking to explain why a particular animal or tissue produces little or no virus after exposure is whether virus-susceptible cells are present and, if so, how abundant they are. Examination of the tissue or cells for specific receptor-like activity is a convenient method of determining the presence of potentially virus-sensitive cells. Enterovirus receptor-like activity may be determined by examining minces or homogenates of tissues for capacity to remove the specific virus from the suspending medium.

This approach has been used to explore a

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number of important problems, such as (i) the remarkable species specificity encountered in many virus infections, (ii) organ and tissue tropisms, (iii) age-specific susceptibility, (iv) derivation in tissue culture of virus-sensitive cells from virus-insensitive organs, (v) selective sensitivity of neoplastic tissue to certain viruses, and (vi) virus virulence.

The examples used in this paper will be limited to enteroviruses, but the phenomena to be discussed are by no means restricted to this group of agents.

SPECIES SPECIFICITY

The host range of human enteroviruses (poliomyelitis, Coxsackie, and ECHO viruses) is almost entirely limited to man, apes, and old world monkeys. Rodent species, particulary mice, can be infected with some of the human enteroviruses (Coxsackie viruses and mouse-adapted strains of poliovirus), but do not appear to be natural hosts. The major feature which distinguishes cells of susceptible from those of nonsusceptible species is the presence of specific receptors. Kaplan (18) found that cultured renal epithelial cells from newborn rabbits, hamsters, and capuchin monkeys do not adsorb or synthesize poliovirus. McLaren, Holland, and Syverton (26) characterized the attachment mechanism of poliovirus to sensitive human and primate cells, all of which contained receptor; no receptor activity was found in virus-insensitive cells from dog, cat, swine, calf, guinea pig, mouse, chick, or rabbit.

Homogenates and minces of tissues from various species have also been examined for enterovirus-adsorbing properties. Francis and Chu (11), in detailed studies of the distribution of receptor-like substance for the Lansing strain of poliovirus, found activity in gray matter of rhesus monkey brain, but not in mouse brain. Sabin (33) confirmed and extended these findings by demonstrating that highly virulent poliovirus combined with spinal cord and brain of cynomologous monkeys, chimpanzees, and man, but not with homologous dog tissues. Similar results have been reported by others (2, 14, 24).

ORGAN AND TISSUE TROPISMS

Kunin and Jordan (24), Holland (14), and Baron et al. (2) have explored the possibility that receptor affinities of tissues are major determinants of tissue tropisms in susceptible animals. Kunin and Jordan (24) reported that minces of all tissues of the rhesus monkey studied, as well as brain, liver, skin, gut, and amnion of a 3-month-old human fetus, contained a heat-labile substance which inactivated poliovirus. No such activity could be demonstrated in mouse or rabbit tissues. Holland (14) found that only the gray matter of the brain, spinal cord, and small intestine of adult rhesus monkeys and human fetal tissues regularly adsorbed poliovirus. Some activity was occasionally observed in kidney, liver, and lung. Baron et al. (2) reported monkey brain and adrenal to be more active than other tissues. All workers agreed that primate central nervous tissue was most active.

Thus, the tissues of monkey and man known to support poliovirus multiplication best had the most readily demonstrable receptor activity. Our observation of the capacity of a wide variety of noncultured rhesus monkey tissues to adsorb poliovirus are in accord with those of Wenner et al. (42) of the widespread distribution of virus in tissues of cynomologous monkeys experimentally infected by intramuscular or alimentary routes.

Further support for the notion that tissue tropisms may largely depend upon the abundance and distribution of receptors is provided by the observation that a neurotropic strain of Coxsackie B_1 (Conn. 5) is adsorbed only by mouse brain and not by other mouse tissues, whereas the pantropic Nancy strain of Coxsackie B_3 is adsorbed by a wide variety of newborn mouse tissues (23).

AGE-SPECIFIC SUSCEPTIBILITY

One of the major characteristics of the Coxsackie viruses is their marked virulence for the newborn mouse (6). Group B Coxsackie viruses produce severe, frequently generalized infections of the human newborn, whereas they are responsible for relatively mild or subclinical infection in adults (19). Several other viruses, including herpes simplex (20), mumps (22), arthropod-borne (32), salivary gland (41), and polyoma (8), exhibit similar properties. Various hypotheses have been advanced to explain the phenomenon of age-specific susceptibility, including impaired antibody formation, increased adrenocortical activity (4, 5), the presence of tissue

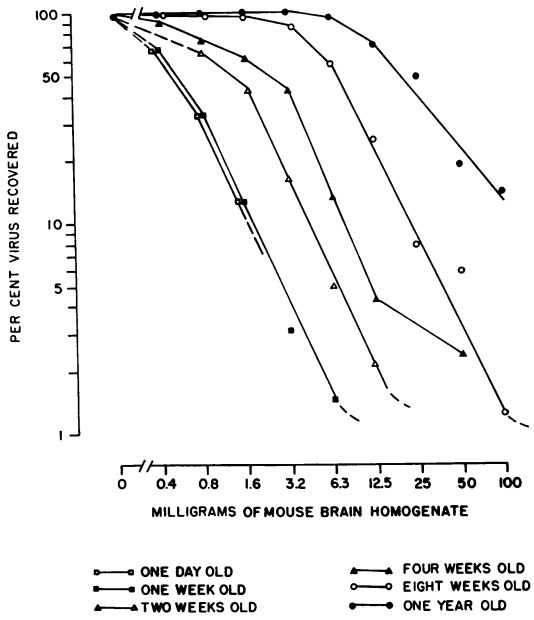

FIG. 1. *Recovery of Coxsackie B_1 virus after incubation with various amounts of brain homogenates from mice of different ages. Inoculum size, 1,300 plaque-forming units. Reading from left to right, curves are for mice 1 day, 1 week, 2 weeks, 4 weeks, 8 weeks, and 1 year old. Reproduced from the Journal of Immunology (23).*

barriers to invasion (32), differences in capacity to form interferon (3, 13), and the development of immune tolerance (17).

The possibility that age-specific susceptibility might be explained on a cellular level was explored in the following experiments (23). Homogenates of brains obtained from mice of various ages were serially diluted and tested for their capacity to remove neurotropic Coxsackie B_1 from the suspending medium (Fig. 1). Newborn mouse brain was most active; progressively larger amounts of brain were required to remove a standard amount of virus with increase in age. Almost 38 times more brain from 1-year-old mice than from newborn mice was required to inactivate 50% of the inoculum. Adult mouse brain appears to contain only about one-eighth as much receptor activity as newborn mouse brain, when differences in mass are considered.

In similar experiments, it was shown that a variety of tissues of newborn mice readily took up the pantropic Nancy strain of Coxsackie B_3 virus, whereas tissues of adult mice, with the exception of limited uptake by brain, were inactive. Progressive loss with age of receptor-containing cells may be the mechanism which accounts for the relative indifference of older animals to Coxsackie viruses. In experimental Coxsackie virus infections studied by Eggers and Sabin (9) and Lerner et al. (25), the crucial factor governing production of disease appeared to be the rate of synthesis of a critical mass of virus before the host either became unresponsive to virus or manufactured sufficient antibody to limit infection. The development of such a critical mass would depend upon abundant virus-sensitive cells. Certain situations, such as administration of cortisone, cold, or stress, may effect host response to Coxsackie infection, but have not been clearly demonstrated to augment virus synthesis (21). Manifestation of disease is not necessarily a direct reflection of virus growth.

DERIVATION OF VIRUS-SENSITIVE CELLS IN TISSUE CULTURE

Three possible explanations of the mechanism by which virus-sensitive cells emerge in tissue culture from organs not thought to be virus sensitive in vivo are as follows. (i) A small population of virus-sensitive, relatively undifferentiated cells already present in the tissue grow abundantly in in vitro culture, and form the virus-sensitive population. (ii) Receptors are actually present in vivo, but are in some way masked, incomplete, or inaccessible until the cells are dispersed and grown in vitro. (iii) Receptors are synthesized *de novo* in tissue by cells previously lacking this property.

Primary monkey-kidney and human amnion cells have been the systems most extensively studied thus far. Kaplan (18), Holland (14), and Quersin-Thiry (30) were unable to demonstrate receptor-like activity for poliovirus in preparations of uncultured monkey cells, but receptor-like activity could be demonstrated soon after the cells were cultured. These results are at variance with the finding of Kunin and Jordan (24) of receptor-like activity in uncultured monkey and human fetal kidney. Accordingly, another experimental approach was necessary to resolve the question of whether a small population of receptor-containing cells might be present in the organ prior to cultivation.

Trypsin-dispersed, noncultured cells from

rhesus monkeys were obtained under refrigeration from a commerical source (Microbiological Associates, Inc., Bethesda, Md.) Methods used in these experiments were previously published (23). The cells were washed three times in balanced salt solution (BSS) and exposed for 1 hr at 37 C with constant agitation to a high multiplicity of type 1 (Mahoney) poliovirus. Excess virus was removed by washing the cells three more times with BSS. Cells were then diluted in BSS or medium 199, without serum, and were dispersed in culture bottles or test tubes and incubated at 37 C. Various inhibitors of cell proliferation, known not to affect poliovirus synthesis (31, 34), including mitomycin C, colchicine, and 5-fluoro-2-deoxyuridine, were incorporated into the medium.

Six such experiments are summarized in Fig. 2 and 3. Increase in virus content could be demonstrated as early as 8 hr, and maximal titers were achieved at 20 to 24 hr. Inhibitors of cell replication had no effect on production of virus. Chick-embryo cells or human amnion cells dispersed by trypsin and treated in the same manner showed a steady decline in virus, indicating that elution of virus was probably not responsible for the apparent increase noted in the monkey-kidney experiments. Maximal increase of virus was limited to only 2 "logs," suggesting that very few cells actually were capable of synthesizing virus. This may have been due, in part, to inactivation of receptors by trypsin used to disperse the cells. Similar, but less regular, synthesis of Coxsackie B_1 virus could be demonstrated in "noncultured" monkey cells (Fig. 4). Virus synthesis, however, could not be detected

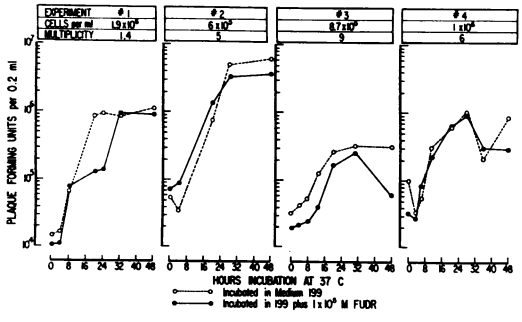

FIG. 2. *Synthesis of type 1 poliomyelitis virus (Mahoney) by "noncultured," trypsin-dispersed rhesus monkey kidney cells incubated in medium 199, with and without 5 fluoro-2-deoxyuridine.*

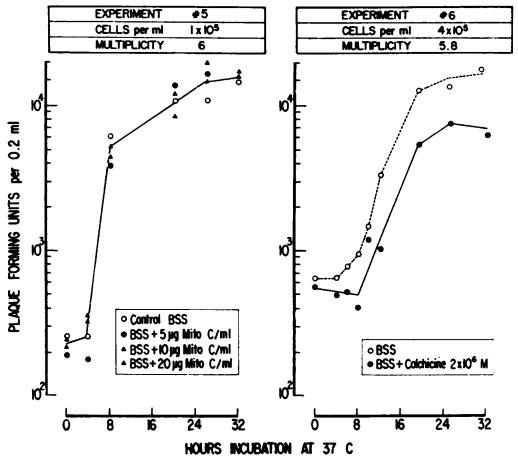

FIG. 3. *Synthesis of type 1 poliomyelitis virus (Mahoney) by "noncultured," trypsin-dispersed rhesus monkey kidney cells incubated in Hank's balanced salt solution, with and without mitomycin C or colchicine.*

prior to 16 hr with Coxsackie virus, suggesting that many fewer cells susceptible to this virus were present.

A number of other tissues of human origin were studied in similar fashion. Growth of both type 1 poliovirus and Coxsackie B_1 virus could be demonstrated with freshly dispersed human fetal kidney cells (Fig. 5 and 6). The marked differences shown in Fig. 6 between fetal kidney and lung are even more striking when one considers that there were 20 times as many lung as kidney cells available. Very little virus was synthesized under the same conditions by adult human kidney cells.

The most virus-sensitive tissue studied, thus far, was a Wilms tumor removed at operation from the lung of a 4-year-old boy. It was trypsinized, for 4 hr at room temperature, stored in BSS overnight at 4 C, and infected with type 1 poliovirus and Coxsackie B_1 virus the next morning. Synthesis of virus in cells from this tumor, compared with that in cells from a human metastatic carcinoma of the lung, is shown in Fig. 7.

A number of other freshly dispersed human tissues, including amnion, hypernephroma, carcinoma of the breast and colon, and tonsil, showed little or no synthesis of poliovirus or Coxsackie virus when similarly studied. With many of the tissues, days elapsed before a few

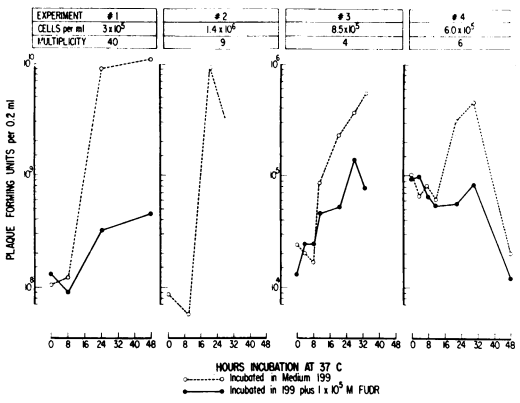

FIG. 4. *Synthesis of Coxsackie B_1 virus (Conn. 5 strain) by "noncultured" trypsin-dispersed rhesus monkey kidney cells incubated in medium 199, with and without 5 fluorodeoxyuridine.*

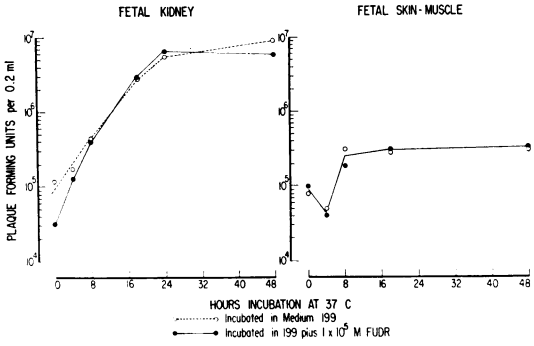

FIG. 5. *Synthesis of type 1 poliomyelitis virus (Mahoney) by trypsin-dispersed "noncultured" cells from kidney and skin muscle of a 4-month-old human fetus. Cells were dispersed by trypsinization overnight at 4 C. Kidney cells (160,000/ml) were exposed to virus multiplicity of 16 for 1 hr at 37 C. Skin muscle cells (80,000/ml) were exposed to multiplicity of 32.*

colonies of viable cells appeared in culture. Synthesis of poliovirus and Coxsackie B_1 virus could not be demonstrated with nontrypsinized minces of various rhesus monkey tissues. Nor was Coxsackie B_1 virus synthesized in vitro by minced newborn mouse brain. In the latter instances, failure to synthesize virus in vitro is obviously misleading when used to interpret ability to support virus growth in vivo, since newborn mouse brain is highly susceptible to this virus in vivo.

These experiments indicate that some of the dispersed, noncultured cells of monkey and human fetus and certain other tissues do have the capacity to support virus multiplication prior to cultivation, and favor the first hypothesis of origin of cells in culture. These studies also support the earlier report of Kunin and Jordan (24) of receptor-like activity in uncultured primate kidney tissues. Holland (14) has

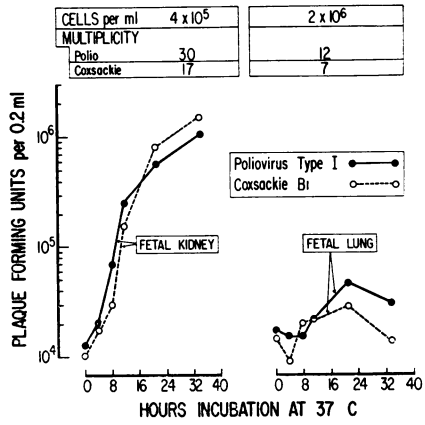

FIG. 6. *Synthesis of type 1 poliomyelitis virus (Mahoney) by trypsin-dispersed, "noncultured" cells from kidney and lung of a 5-month-old human fetus incubated in medium 199 at 37 C.*

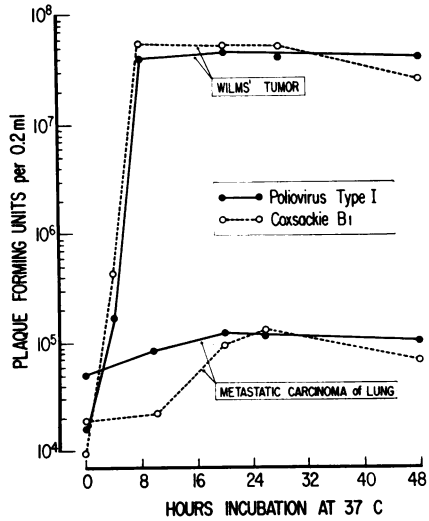

FIG. 7. *Synthesis of type 1 poliomyelitis (Mahoney) and Coxsackie B_1 viruses by trypsin-dispersed, "noncultured" cells from a human Wilm tumor and metastatic carcinoma of the lung. Wilm tumor, cell count = 6 × 10^6/ml, virus multiplicity = 1; carcinoma of lung, cell count = 5 × 10^5/ml, multiplicity = 10.*

argued that massive growth of one cell type from kidney cells cultured in vitro is unlikely, since most trypsinized cells attach to the glass and grow rapidly. Youngner (43), however, reported that only 5 to 10% of the original count of trypsinized monkey-kidney cells could be found attached to the glass by the third day. Some of these may actually have grown from an even smaller original viable population. Much smaller numbers of cells remain attached to the glass in slower-growing cultures, such as those from adult human tissues. Furthermore, one of the charactistics of the tissue-culture cells, even in primary culture, is their lack of differentiated functional activity, suggesting that parenchymal cells do not readily grow in culture. Sato et al. (35) have examined the problem of the origin of tissue culture populations from rat liver. These workers demonstrated that differentiated liver cells do not grow in culture, but that an undifferentiated cell type, antigenically distinct from liver parenchymal cells and selected under in vitro conditions makes up the cultured population. Thus, the first hypothesis seems, at this time, to account best for the appearance of virus-sensitive cells from tissues such as monkey kidney and human fetal tissues, and certain tumors.

The situation appears somewhat different in the case of the development of virus-sensitive cells by primary human amnion cells. Neither Holland (14) nor Kunin and Jordan (24) were able to detect receptor activity in uncultured human amnion, nor has either group been able to demonstrate an inhibitor to poliovirus receptors in uncultured amnion. Freshly trypsinized amnion cells did not synthesize poliovirus in our experiments. Holland (14) has examined this problem in a series of elegant studies. Poliovirus-sensitive cells appeared in slow-growing amnion cultures in vitro only when the cells were dispersed. Minces of amniotic membranes, in which cells remained in close contact, did not develop receptor activity or virus-sensitive cells. Holland concluded that contact inhibition imposed by the immediate environment of differentiated cells repressed synthesis of receptor protein. Hypothesis two or three seems to explain best these observations on acquired susceptibility of human amnion cells in culture.

Tissue injury, induced by incision of monkey skin (10) or kidney transplantation (30), appears to stimulate the proliferation of virus-sensitive cells very much like dispersion of cells in tissue culture. Fibroblasts growing in granulation tissue may be analogous to cultured cells. It is not clear, however, which of the three hypotheses best explains the origin of these virus-sensitive cells.

SELECTIVE VIRUS SENSITIVITY OF NEOPLASTIC TISSUES

Several groups of investigators have been able to demonstrate oncolytic activity with adenoviruses, Coxsackie, and other viruses (27, 37). Kunin and Jordan (24) reported receptor-like activity of poliovirus in human carcinomas of the breast, stomach, and rectum. The virus synthetic capacity of freshly dispersed Wilms tumor cells shown in Fig. 7 is dramatic evidence of abundant virus-sensitive cells in this tissue. Neoplasia in itself, however, is not synonymous with virus sensitivity, since many of the adult human tumors studied in the same fashion and referred to above were not capable of very much virus synthesis, nor was receptor activity to Coxsackie B_1 virus found in a wide variety of mouse tumors (23). The capacity of cells from the Wilms tumor, believed to be of embryonic origin, may reflect the relative abundance of enterovirus receptor-containing cells in human embryonic tissue, and supports the notion that virus-sensitive cells are abundant at an early age.

VIRUS VIRULENCE

Sabin (33) found quantitative differences in adsorption of virulent and attenuated type 1 poliovirus by central nervous system tissues of man, chimpanzee, and cynomologous monkeys. His data strongly suggested that neurovirulence was associated with more avid binding to neural cells. Holland (14) obtained similar results with human fetal spinal cord. I (23) was unable to detect significant differences in adsorption to rhesus monkey brains between Mahoney and LSc strains of type 1 poliovirus.

Eggers and Sabin (9) found that the Hill strain of ECHO 9 virus was not pathogenic for newborn mice or adsorbed by mouse tissues. Kunin (23) studied two strains of Coxsackie B_3 virus for mouse virulence. The virulent Nancy strain was adsorbed by a variety of newborn-mouse tissues. An avirulent strain, which had been isolated in HeLa cells and which had never

been passaged in mice, was only moderately adsorbed by newborn-mouse brain.

It should be emphasized that virus virulence and ability to produce large amounts of virus are not necessarily synonymous. For example, mice may be persistently infected with lymphocytic choriomeningitis without production of disease (17). Thus, the receptor hypothesis need only be used to explain virus synthesis and not necessarily the consequence of virus production.

ALTERNATIVE EXPLANATIONS OF CELLULAR SUSCEPTIBILITY TO VIRUSES

An attempt has been made to explain a variety of complex phenomena on the basis of the relative abundance in tissues of cells containing specific surface receptors. Factors governing distribution of virus and host response to infection are not pertinent to the present discussion, and have been mentioned only briefly. An example, however, of the importance of barriers to distribution of virus to sensitive cells is the study of Sellers and Lavender (36) on the enhancement of paralysis and death in mice given CO_2 during viremia with type 2 poliomyelitis virus. Carbon dioxide augmented transport of virus to brain by virtue of its effect on cerebral blood flow and cerebral vascular permeability. Susceptibility of mice to intracerebral injection of virus was not altered by CO_2, indicating that the effect of this pharmacological agent was on delivery of virus to brain rather than on cellular resistance.

Interferon production is well known to alter cellular susceptibility to a variety of viruses (40). Baron and Isaacs (3) proposed that increased sensitivity to interferon with age may account for the decreased lethal effect of various viruses on chick embryos. Heineberg, Gold, and Robbins (13) reported a direct relationship to exist between age, production of interferon, disappearance of virus from tissues, and survival from infection with Coxsackie B_1 virus in mice. They proposed that the young mouse succumbs to Coxsackie infection because of an inability to produce adequate interferon, in contrast to older animals which produce interferon and survive. Entirely opposite results were reported by Vilček with Sindbis virus (39). He compared virus and interferon production in brains of newborn and adult mice inoculated intracerebrally. The highest interferon titers were found in brains of newborn mice, which synthesized the largest amounts of virus and succumbed to the infection. Thus, the simple fact of formation of interferon in an organism during a viral infection is insufficient to establish its role in host resistance. Similar results were reported by Vainio, Gwatkin, and Koprowski (38), who studied interferon production by genetically sensitive and resistant mice infected with West Nile virus. Interferon production closely paralleled virus synthesis rather than resistance. Postic et al. (29) were also unable to demonstrate that interferon played a crucial role in central nervous disease induced by Sindbis virus in adult mice.

Current concepts require that foreign nucleic acid must enter a cell to induce formation of interferon (40). Under natural conditions, specific receptor sities are necessary to permit the cells to become infected and to be stimulated to produce interferon. It is possible that virus infection can occur so rapidly in a highly susceptible animal given a large infective dose that cells are destroyed before they can manufacture interferon. Thus, the "abundance of susceptible cells" and the interferon theories are not necessarily incompatible, and may actually be complementary.

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