

THE PATHOGENESIS OF HERPES VIRUS ENCEPHALITIS
II. A CELLULAR BASIS FOR THE DEVELOPMENT OF RESISTANCE WITH AGE

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The development of resistance to disease with increasing age is recognized in many viral infections of man and experimental animals (see reviews by Burnet, reference 1, and Sigel, reference 2). Metabolic and hormonal changes, antibody response, inhibitory substances, anatomical developments, and interferon production have all been suggested to explain this development of resistance (2, 3). Resistance to viruses causing encephalitis often develops only to virus inoculated extraneurally, and the adult remains susceptible to intracerebral inoculation (4-6). The elusive "blood-brain barrier" has been invoked to explain this selective resistance.

The most extensive study of the development of resistance to encephalitis with age was carried out with vesicular stomatitis virus in mice and guinea pigs by Sabin and Olitsky (7-10). They concluded that "barriers" developed with age and that these were located in the anterior olfactory region of the brain and at the myoneural junction. Extending their studies to Eastern and Western equine encephalitis viruses, they suggested barriers at the level of blood vessels (11, 12). Precisely which cells constituted the barriers, however, could not be determined with the methods employed.

In herpes simplex virus infection age is an important host determinant in the development of encephalitis. Andervont (13) first showed in mice that resistance to herpetic encephalitis developed only to extraneurally inoculated virus, and his finding has been confirmed by others (5, 14, 15). Since fluorescent antibody staining proved successful in demonstrating the pathogenesis of herpes virus encephalitis in the suckling mouse (16), the present study was undertaken to determine at what cellular level the spread of extraneural infection was arrested in the adult. With this method the location of the "age barrier" was deter-

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mined, and the humoral and cellular factors which might constitute this barrier were investigated.

Materials and Methods

The HFEM strain of herpes simplex virus was used. Mice of the multicolored, outbred Hall Institute strain were used in most studies; for experiments involving the transfer of cells or cell extracts, mice of the inbred, genetically homogeneous Bagg strain were used. Methods of inoculation, titration, and fluorescence microscopy were the same as those previously described (16).

Macrophages.—Macrophages were obtained from peritoneal cavities of normal mice without preceding stimulation and were maintained in chambers as described by Mackaness (17). Macrophages from suckling mice were obtained using an adjusted quantity of solution to wash out the peritoneal cavity in order to give a yield of approximately $10^{6.5}$ macrophages per ml of exudate. Peritoneal washings were introduced into chambers and incubated for 4 hours at 37°C in a humidified incubator with 5 per cent CO₂. Chambers were then washed vigorously with a jet of normal saline to remove excess macrophages and all other cells. After virus inoculation, cells were left for 1 hour at room temperature to allow adsorption. Chambers were filled with 20 per cent calf serum in Hanks' solution and incubated. Medium was changed every 24 hours. For virus titrations cells were harvested with the medium by freezing and thawing and treatment with an ultrasonic drill.

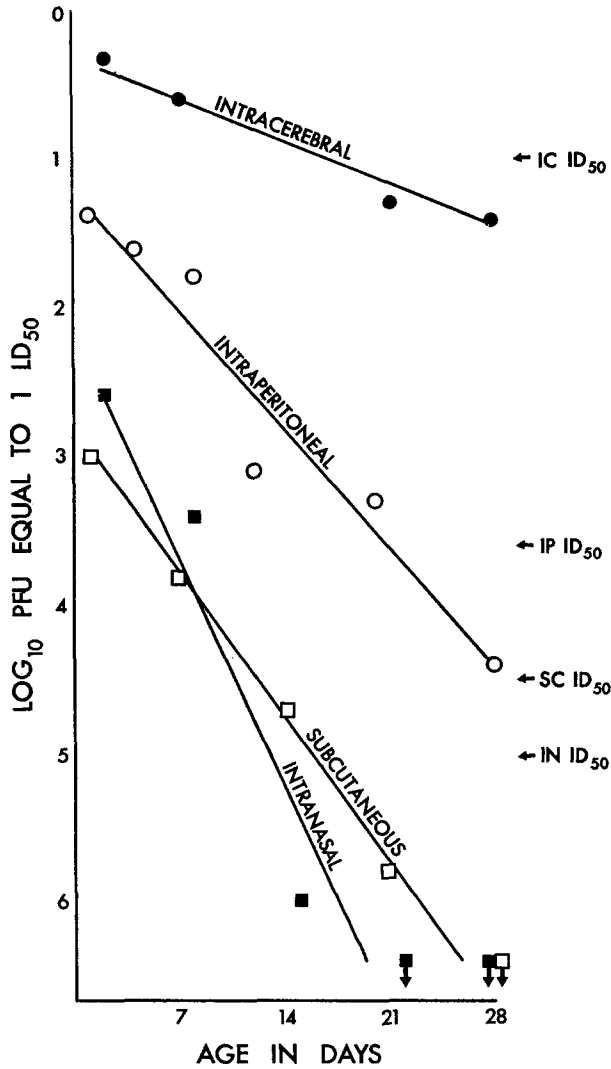
Electron Microscopy.—Macrophages were fixed *in situ* on rings with 1 per cent OsO₄ buffered fixative, dehydrated in graded alcohols, and embedded in araldite (Ciba Products Corporation, Fair Lawn, New Jersey). Rings were then removed from slides, and appropriate areas of clumped cells were selected and cut from the block. Thin sections were cut with a diamond knife using a LKB microtome, stained with uranyl acetate, and examined in a Siemens elmiskop I electron microscope.

Autoradiographs.—Macrophage monolayers were treated with tritiated thymidine (Schwarz BioResearch, Inc., Orangeburg, New York), 0.5 μ c per ml of maintenance media, for 1 hour at 37°C. Medium was removed, and excess cold thymidine was added for 5 minutes before fixation. Cells were washed in Hanks' solution, twice in acetone, and overnight in methanol. Kodak ARIO stripping film was applied to slides and exposed at 4°C for 14 days. Slides were developed in D19b (Eastman Kodak Company, Rochester, New York) and stained with hematoxylin and eosin.

RESULTS

Limitation of Virus Spread in Adult Mice.—The magnitude of resistance to fatal herpes virus infection which develops with age is shown in Text-fig. 1. By intracerebral inoculation young adult mice were killed by 10 times the amount of virus lethal to newborns; by extraneural inoculation 1000 times or greater amounts of virus were required to kill adult mice than newborn mice. Resistance did not develop at a particular age but developed progressively from birth.

Intracerebral inoculation of adult mice: Fluorescent antibody staining of brains at daily intervals after intracerebral inoculation failed to show any difference in the pattern of virus growth from that previously shown in suckling mice (16). Virus multiplication occurred first in the meninges and ependyma. Infection then spread into the underlying parenchyma, infecting neural



TEXT-FIG. 1. The development of resistance to herpes virus encephalitis after intracerebral and extraneural inoculation. Virus titers are expressed as the number of pock-forming units (pfu) equal to a 50 per cent lethal dose (LD₅₀) determined in mice at various ages from 1 to 28 days. Fifty per cent infectious dose (ID₅₀) as determined by subsequent intracerebral challenges was equal to the LD₅₀ in newborn mice. ID₅₀'s in 28-day-old adult mice are shown on the right.

and glial cells. In adult mice all central nervous system (CNS) cells remained susceptible to infection.

The small decrease in susceptibility of adult mice to intracerebral inoculation might be explained in part by dilution of virus in a larger volume of cerebrospinal fluid (CSF) and thermal inactivation.

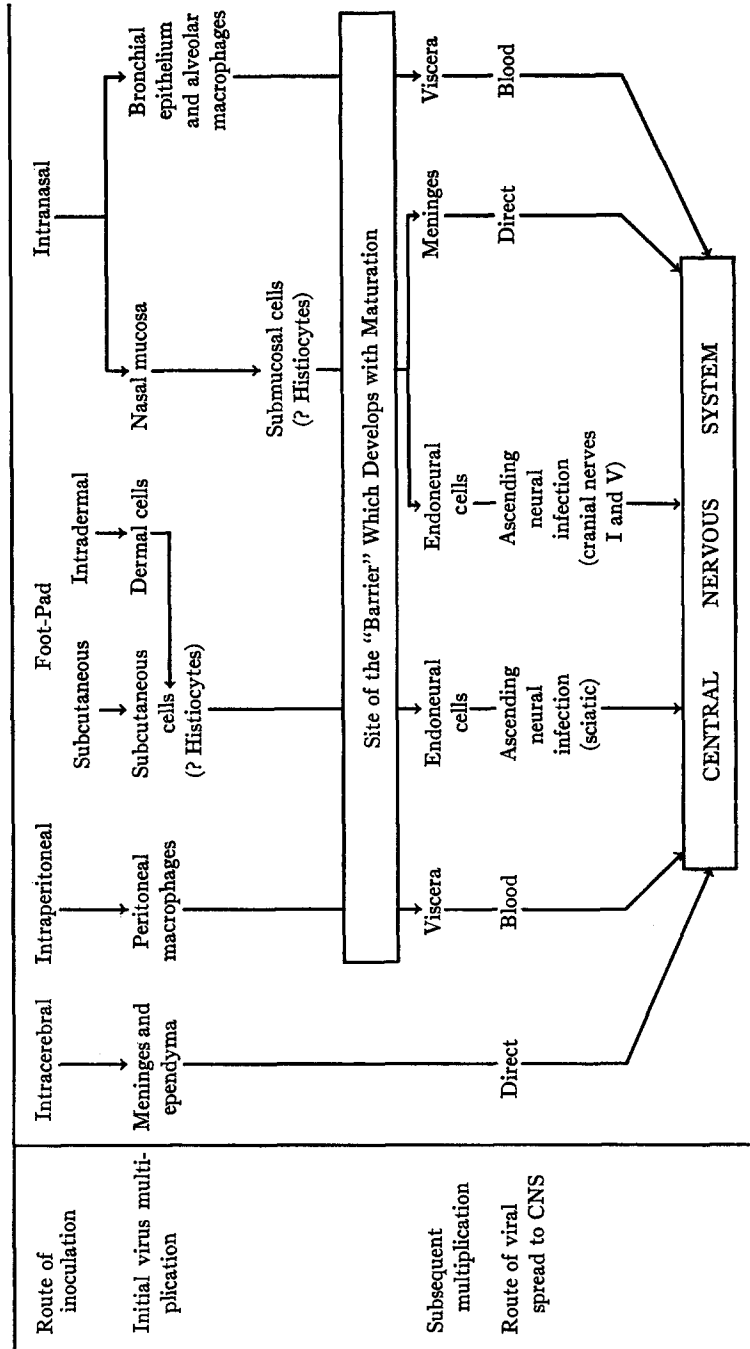
Extraneural inoculation of adult mice: Following intraperitoneal, subcutaneous, intradermal, and intranasal inoculation of herpes virus in adult mice, fluorescent antibody staining of tissues showed that initial virus multiplication took place in the same cells as were initially infected in suckling mice. However, this infection failed to spread to the CNS when 100 times the suckling mouse 50 per cent lethal dose (LD_{50}) was given. The site of the "barrier" which develops with age is shown diagrammatically in Text-fig. 2.

Four-day-old and 4-week-old mice were inoculated intraperitoneally with 100 suckling mouse LD_{50} . Six hours later free peritoneal macrophages were washed out, incubated 4 hours in chambers, washed to remove other cells, and stained with fluorescent antibody.

Approximately 1 macrophage per 100,000 was infected in both suckling and adult mice. Twenty-four hours after inoculation infected peritoneal macrophages could no longer be recovered from suckling or adult mice. In contrast to the rapid growth of virus in livers and spleens of suckling mice after intraperitoneal inoculation, only a few pock-forming units (pfu) of virus were recovered from adult livers and spleens at 24 hours, and no virus was detected at 48 hours. No fluorescent cells were found in livers and spleens of adults at any time. On the other hand, after intraperitoneal inoculation of 10^5 to 10^6 pfu small foci of infected cells were found in liver, spleen, and adrenal cortex. In approximately one-half of these adult mice virus was demonstrated in the brain by egg inoculation or fluorescent antibody staining. Areas of infection in the brain were identical with those shown after hematogenous spread of virus to the CNS of suckling mice (16). Thus, when the barrier was overcome by a large dose of virus, the route of spread to the CNS of adult mice was the same as the route in suckling mice.

Following subcutaneous inoculation in foot-pads of adult mice a few scattered cells in the subcutaneous tissue showed fluorescence, as in suckling mice, but in adults no fluorescent endoneural cells were found even with massive dosages of virus. After intradermal inoculation vesicles developed on the foot-pad of adult mice after 3 to 5 days. Fluorescent antibody staining showed areas of infected dermal cells (Fig. 1), but again infection did not spread beyond occasional subcutaneous cells. The location and cytology of these subcutaneous cells infected in adult and suckling mice after subcutaneous and intradermal inoculation suggested that they were probably tissue macrophages (histiocytes).

Intranasal inoculation of adult mice with 100 suckling mouse LD_{50} resulted in infection of nasal mucosa similar to infection in suckling mice, but, unlike



TEXT-FIG. 2. Diagram of the pathogenesis of herpes virus encephalitis in mice. In young suckling mice infection spread readily to the CNS; this spread was arrested with increasing age at the site shown by the barrier. When the barrier was overcome in young adult mice by massive intraperitoneal or intranasal inocula of virus, infection followed the same pathways to the CNS.

suckling mice, submucosal tissues and endoneural cells of cranial nerves were not involved. In contrast to the rapid and widespread growth of virus in lungs of suckling mice after intranasal inoculation, lungs of adult mice contained only small amounts of virus for the first 3 days, and the titer remained unchanged. Four to 5 days after inoculation virus could no longer be recovered from lungs. Fluorescent antibody staining of lungs showed patches of fluorescence in bronchial and bronchiolar epithelium (Fig. 2), occasional submucosal cells, and some single crescent-shaped cells thought to be alveolar macrophages. The extent of infection in the lungs appeared unchanged from 24 to 72 hours after inhalation of virus, and, thereafter, fluorescence faded. Of 62 adult mice given large intranasal inoculations of virus ($10^{4.7}$ pfu or more), 7 developed encephalitis. Fluorescent antibody staining of 1 mouse brain showed scattered fluorescent foci of probable hematogenous origin; 2 other mice were examined and both had fluorescent cells in the trigeminal nerve and brainstem. The brainstem lesions were identical with those found in suckling mice, but in the trigeminal nerve and in the gasserian ganglion fewer Schwann cells and ganglion cells were infected.

In summary, the growth of herpes virus in the CNS and in surface tissues (*viz.* dermis and nasal and bronchial epithelium) was similar in suckling and adult mice. In adult mice the subsequent spread of virus was limited, and virus growth in viscera and endoneural cells was not readily established. When the barrier was overcome by giving very large amounts of virus intraperitoneally and intranasally, the route of virus spread to the CNS was the same as in suckling mice.

Factors Limiting the Spread of Virus in Adult Mice.—A difference in antibody response seemed an unlikely explanation of the barrier, since the progress of infection was retarded within 24 hours after inoculation. Also a general cellular maturation could not explain the limitation of virus spread, since cells of ectodermal, mesenchymal, and endodermal origin remained highly susceptible to infection.

Two factors which might explain the barrier were apparent: First, a "natural antibody," which would not have ready access to virus in the CNS or superficial tissues, could inactivate virus as it penetrated beyond these tissues. Secondly, a change in macrophages might limit the spread of virus; macrophages are uncommon in CSF, where no barrier was apparent, and appeared to be involved at the site of the barrier after intraperitoneal, subcutaneous, and intranasal inoculation (Text-fig. 2). Therefore, serum inactivation of virus and *in vitro* infection of macrophages were investigated.

Serum inactivation:

Herpes virus was mixed with undiluted fresh, uninactivated serum of adult and suckling mice and with buffered gelatin saline. Mixtures were incubated for 2 hours at 4°C, and then

titrated intracerebrally in adult mice, intraperitoneally in 4-day-old suckling mice, and on chorioallantoic membranes of embryonated eggs. Results are shown in Table I.

Both adult and suckling mouse sera appeared to enhance the titer of virus in the *in vivo* titrations, and the difference between them was not significant. Serum from adult mice 24 hours after intraperitoneal inoculation of herpes virus and serum from suckling mice 24 hours after subcutaneous inoculation (non-viremic sera) were tested in the same way with similar results. Resistance in the adult mouse could not be explained by development of natural or early humoral antibody.

Fluorescent antibody staining of macrophages: Normal peritoneal macrophages from 8-week-old adult and 4-day-old suckling mice were infected with herpes virus *in vitro*. Virus antigen was first apparent in cytoplasm 4 hours after

TABLE I
Effect of Normal Mouse Serum on Infectivity of Herpes Virus (Strain HFEM)

Inoculum	Log ₁₀ of virus per 0.05 ml inoculum determined in		
	Adult mice intracerebral	Suckling mice intraperitoneal	Embryonated eggs chorioallantoic membrane
	<i>LD</i> ₅₀	<i>LD</i> ₅₀	<i>pfu</i>
HFEM + adult mouse serum	4.0	4.2	4.7
HFEM + suckling mouse serum	4.2	4.5	5.1
HFEM + diluent (control)	3.2	3.3	4.7

inoculation, and by 6 hours maximum fluorescence of cytoplasm was present (Figs. 3 and 5). Inoculum containing 10⁶ pfu of virus infected 10 to 20 per cent of the cells; 10⁵ pfu infected 1 to 2 per cent of the cells. There was no difference in the ease of infecting adult and suckling mouse macrophages.

Macrophages from 8-week-old mice showed no further change 72 hours after inoculation; the fluorescent cells remained intact and surrounding cells were not infected (Fig. 4). However, macrophages from 4-day-old mice infected other cells after the first 6 hours. Eight hours after inoculation slight fluorescence was found in cells adjacent to the initially infected macrophages, and by 10 hours bright fluorescence was often present in 2 or 3 neighboring cells. Twelve hours after inoculation macrophages had infected as many as 10 neighboring cells (Fig. 6). This process continued until giant clumps of fluorescent cells had formed 72 hours after inoculation (Figs. 7 and 8).

Macrophages from mice 1, 2, 3, and 4 weeks of age were similarly examined. With maturation secondary infection occurred more slowly and involved fewer cells. For example, infected macrophages from 3-week-old mice showed no

infection of other cells at 24 hours, but by 48 hours about one-half had infected adjacent cells, and by 72 hours clumps of 2 to 5 fluorescent cells were common.

The number of infected foci did not increase in adult or suckling mouse macrophage monolayers even after 7 days of incubation. When antiserum to herpes prepared in rabbits was added to media after initial virus adsorption, no change occurred in the number of foci or number of cells in each focus. Therefore, after the initial growth cycle, infection of macrophages took place only by cell-to-cell transfer of virus. Since large areas around infected foci were cleared of cells and since no mitotic figures were found, it was concluded that clumps of fluorescent cells formed by secondary infection of uninfected macrophages rather than division of infected macrophages.

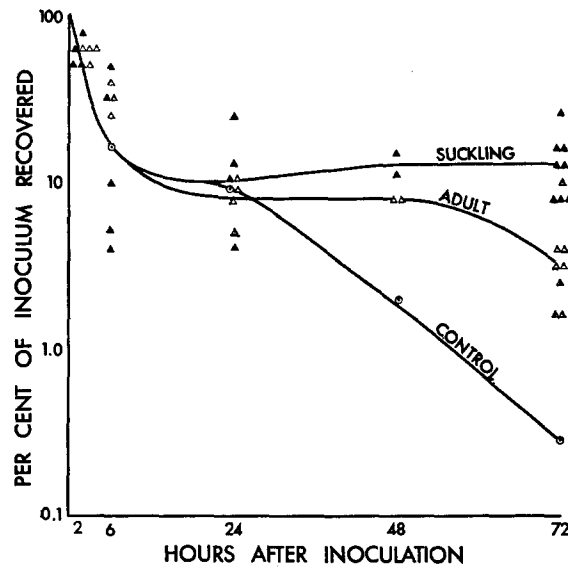
The observed difference in infected adult and suckling mouse macrophages might be due to a difference in the infected cell or to a difference in the phagocytic or other properties of the uninfected macrophages around it. Therefore, small numbers of adult mouse macrophages (10^4) were placed in rings and inoculated with 10^6 pfu of virus; after 2 hours cells were washed thoroughly, and sufficient suckling mouse macrophages were added to form a monolayer (about 10^5). Similarly, suckling mouse macrophages were infected and adult macrophages were added. Infected adult mouse cells failed to infect surrounding suckling mouse cells, but infected suckling mouse cells did infect surrounding adult mouse cells. Using the same method, human carcinoma (HeLa) cells were added to rings containing small numbers of infected adult and suckling mouse macrophages; again, adult mouse macrophages failed to spread infection (Fig. 9), while suckling mouse macrophages infected the HeLa cell monolayer (Fig. 10). Thus, the process was initiated by the infected macrophage and appeared to be independent of the nature of adjacent cells.

Titration of virus in macrophages: Virus growth curves in 8-week-old adult and 4-day-old suckling mouse macrophages are shown in Text-fig. 3. Initial inactivation of virus in macrophage cultures followed the rate of thermal inactivation in cell free controls. The difference in virus yield from adult and suckling mouse cells after 72 hours incubation was slight but consistent and significant ($p = <0.01$). Since the number of foci was the same in adult and suckling mouse macrophage monolayers, the large clumps of infected suckling mouse cells (Fig. 7) contained only 7 times as much infectious virus as the solitary infected adult cell (Fig. 4). The paucity of infectious virus recovered from macrophages was unexplained; however, the lack of recoverable herpes virus from infected HeLa cell syncytia presents a similar phenomenon (18).

Electron microscopy of infected macrophages: Thin sections of clumps of infected suckling mouse macrophages 72 hours after inoculation were examined. Cells were tightly packed together, but plasma membranes were intact showing that polykaryocytosis was not occurring. A number of dead cells were present within clumps. These were at times partially encircled by infected but other-

wise healthy appearing macrophages suggesting that dead cells were being phagocytosed. Large numbers of apparently normal virus particles were present in the cytoplasm of macrophages.

Autoradiographs of macrophages: One hour pulses of tritiated thymidine were given to adult and suckling mouse macrophages 6 and 24 hours after inoculation with sufficient virus to infect approximately 5 per cent of the cells. At 6 hours after infection 6 per cent of adult and 4 per cent of suckling mouse cells had



TEXT-FIG. 3. Virus recovered from cultures of 4-day-old and 8-week-old mouse macrophages. Results of titrations of suckling mouse cultures are shown by solid triangles and adult mouse cultures by open triangles. Mean titers of cell free controls are shown by circles and represent thermal inactivation of virus. Determinations after inoculation of both 10^8 and 10^6 pfu per culture are included.

diffuse label over nucleus and cytoplasm. The number of grains was similar over infected adult and suckling mouse cells and was assumed to represent viral DNA production. At 24 hours no label was taken up by infected adult macrophage monolayers; in suckling mouse monolayers label was present over all clumps of cells indicating that viral DNA was continuing to be produced by the newly infected macrophages. Infected cells incorporated tritiated thymidine as would be predicted from the fluorescent antibody studies; viral DNA was produced equally in adult and suckling mouse cells during the first growth cycle, but subsequent cycles of DNA production were seen only in suckling mouse macrophages.

Controls showed that 10 per cent of uninfected suckling mouse macrophages

had dense label over nuclei after 6 hours on glass, but no cells showed evidence of thymidine uptake after 24 hours on glass. Less than 2 per cent of uninfected adult cells showed evidence of nuclear DNA production at 6 hours and none at 24 hours. Nuclear label was seen in infected monolayers but was readily distinguishable from less dense, diffuse label over infected cells. There appeared to be a basic difference in normal adult and suckling mouse macrophages, in that suckling mouse cells were more active in nuclear DNA production. However, nuclear thymidine uptake stopped within 24 hours of being placed on glass, and there was no evidence of mitotic division of either adult or suckling mouse macrophages in monolayers.

Interfering substances and macrophage infection: It might be suggested that the observed differences in infected macrophages *in vitro* were due to inhibitory

TABLE II
Infection of Adult Mouse Macrophages with Herpes and Ectromelia Viruses after Initial Infection with Herpes Virus (Strain HFEM)

Initial inoculum	Inoculum at 24 hours	Fluorescent macrophages per 1000 at 48 hours
HFEM 10 ⁸ pfu	HFEM 10 ⁸ pfu	153
HFEM 10 ⁸ pfu	0	12
0	HFEM 10 ⁸ pfu	146
HFEM 10 ⁸ pfu	Ectromelia	102*
0	Ectromelia	95*

* Stained by direct method with fluorescein-labeled antivaccinia conjugate.

or interfering substances present in residual adult mouse serum or liberated by adult mouse macrophages. First, macrophages were grown in normal adult and suckling mouse serum; this had no effect on the pattern of infection or virus titers in macrophages. Secondly, adult macrophages were infected with herpes virus; 24 hours later cells were inoculated with 10 times the previous dose of herpes virus or with the Hampstead mouse strain of ectromelia virus. Results in Table II show that an initial growth cycle in adult mouse macrophages did not produce an interfering substance which blocked the infection of the previously uninfected cells.

Transfer of resistance and susceptibility: In order to investigate the possible transfer of resistance or susceptibility an inbred strain of mice was used (Bagg). Development of resistance to extraneural inoculation *in vivo* and the behaviour of infected macrophages *in vitro* were found to be similar in the inbred and outbred strains of mice.

Kantoch and Bang (19) and Kantoch *et al.* (20) recently reported the transfer of susceptibility from mouse macrophages susceptible to mouse hepatitis virus (MHV) infection to macrophages from a strain of mice resistant to MHV in-

fection by maintaining resistant macrophages in media containing extracts of susceptible macrophages. Using their method, peritoneal macrophages of 8-week-old and 4-day-old mice were maintained for 3 days in rings in the presence of extracts of adult and suckling mouse macrophages. Extracts were then removed and monolayers were infected with herpes virus; containment of infection by adult mouse macrophages and spread of infection by suckling mouse macrophages were the same as in controls maintained in rings without extracts.

Passive transfer of macrophages was also attempted. Macrophages from peritoneal washings collected at 4°C in silicone-coated tubes were concentrated by centrifuging at 1500 RPM for 10 minutes. Cells were resuspended in Hanks' solution and inoculated intraperitoneally into adult and suckling mice. Macrophages from peritoneal washings of 10 4-day-old mice were injected into each 4-week-old mouse; and macrophages from 1 4-week-old mouse into each 4-day-old mouse (*i.e.*, about $10^{6.6}$ macrophages in each transfer). Virus was titrated intraperitoneally in 5-fold dilutions immediately after transfer of cells, but no difference in titer was found.

Macrophages were similarly collected and incubated with virus *in vitro* at 20°C for 1 hour. Unadsorbed virus was then removed by washing 3 times in large volumes of Hanks' solution or by neutralizing extracellular virus with antibody. This method succeeded in infecting almost half of the macrophages as determined by fluorescent antibody staining. Infected macrophages were titrated intraperitoneally in 4-, 7-, 14-, 21-, and 28-day-old mice. No significant difference in fatal infection of adult and suckling mice was found. A few more deaths occurred in both adult and suckling mice given infected suckling mouse macrophages, but at no time was a 10-fold difference in titer obtained.

In summary, attempts to transfer susceptibility and resistance *in vivo* and *in vitro* were unsuccessful by the methods employed.

DISCUSSION

The emerging importance of macrophages in the pathogenesis of virus infections has recently been reviewed by Mims (21). Blood clearance studies suggest that most viruses are taken up by macrophages just as are other colloidal materials (21, 22); since macrophages move freely through tissues, it has also been suggested that they play a primary role in disseminating virus (23). Therefore, the susceptibility or resistance to disease could depend, in large part, on the macrophage-virus interaction.

Bang and colleagues (20, 24, 25) have shown that cultured macrophages from a susceptible strain of mice were selectively destroyed by MHV and that macrophages from a resistant strain of mice were not. Genetic differences in the susceptibility of macrophages from these strains of mice have also been shown with group B arboviruses by Goodman and Koprowski (26, 27). The present study is unique in showing for the first time that a change in macrophages from the same strain of mice can occur with age and that the change need not be in the susceptibility or resistance of macrophages to infection but can be a difference in the macrophages' capacity to disseminate virus.

The present studies suggested that infected macrophages are an essential link in the dissemination of herpes virus infection in suckling mice. In contrast, in the adult mouse the infected macrophages (in peritoneal cavity and in subcutaneous and submucosal tissues) appeared to arrest the spread of infection. *In vitro* the ability of suckling mouse macrophages to spread infection to adjacent cells of various types was demonstrated; whereas, infected adult mouse macrophages failed to infect other cells. This *in vitro* phenomenon of infection was shown to gradually decrease with the age of the mice from which macrophages were obtained and to parallel the development of resistance *in vivo*.

The nature of the change in macrophages with maturation of the mouse was not determined. Both adult and suckling mouse macrophages were infected with equal ease *in vivo* and *in vitro*, and no difference in the development of antigen in the first growth cycle was found. Autoradiographs suggest that both adult and suckling mouse macrophages are capable of viral DNA synthesis, and infectious virus was recovered from both adult and suckling mouse cells. Possibly quantity or quality of virus produced in the first growth cycle in adult cells differed from virus produced by suckling mouse cells, but this could not be determined. The subsequent success or failure of macrophages to infect other cells did not appear to be affected by humoral or extracellular factors, since normal adult and suckling mouse serum and rabbit antiherpes serum had no effect on the outcome of secondary infection. Similarly, no evidence was found of interferon production by infected adult mouse cells, and cell extracts did not modify the macrophages' capacity to spread or contain infection. Since herpes virus spreads in monolayers solely by cell-to-cell infection, the change in macrophages might be explained by some alteration of the cytoplasmic membrane which accompanies maturation.

A classic explanation for the development of resistance to encephalitis with age has been that the blood-brain barrier develops with age. The precise location of this barrier has long been in question. In the case of herpes virus, intraperitoneal inoculation of susceptible young mice results in infection of peritoneal macrophages, followed by growth in viscera, viremia, and hematogenous spread of virus to the CNS. In resistant adult mice only peritoneal macrophages are infected; no detectable growth in viscera or viremia occurs and consequently no encephalitis develops. Thus, the barrier is not between blood and brain but appears to develop in peritoneal macrophages. However, no rash generalizations are possible, since subsequent studies of other neurotropic viruses to which resistance develops with age have not incriminated macrophages. Fluorescent antibody studies showed that neither fixed rabies nor Sindbis viruses grew in adult or suckling mouse macrophages *in vitro* or *in vivo* (28). It would also be naive to assume that macrophages represent the only barrier to the spread of herpes virus infection in adult mice. If they were, the

transfer of a few infected suckling mouse macrophages should result in the dissemination of infection in the adult; this did not occur. Subtle differences in the capacity of other cells to be infected and to spread infection could not be determined. Nevertheless, the complementary nature of the *in vivo* and *in vitro* studies supports a hypothesis that the resistance to herpes virus encephalitis which develops with maturation of the mouse is due, in part, to a change in the macrophage; this change with age tends to confine infection and thus limit virus spread.

SUMMARY

The resistance to herpes virus encephalitis which develops with age was studied in mice using fluorescent antibody staining. Adult mice remained susceptible to intracerebral inoculation, and the infection of the central nervous system was identical with that found in immature mice. A "barrier" to the spread of virus inoculated extraneurally developed with maturation, and the limitation of spread appeared to coincide with the infection of peritoneal and tissue macrophages.

In vitro, suckling and adult mouse macrophages were infected with equal ease. However, suckling mouse macrophages infected other cells in contact with them, while infected adult mouse macrophages did not. Studies failed to reveal the nature of this change in macrophages which developed with age.

The role of macrophages in the pathogenesis of herpes virus encephalitis is discussed. The hypothesis is made that an alteration in the macrophages of the maturing mouse plays an important role in its development of resistance to herpes virus encephalitis.

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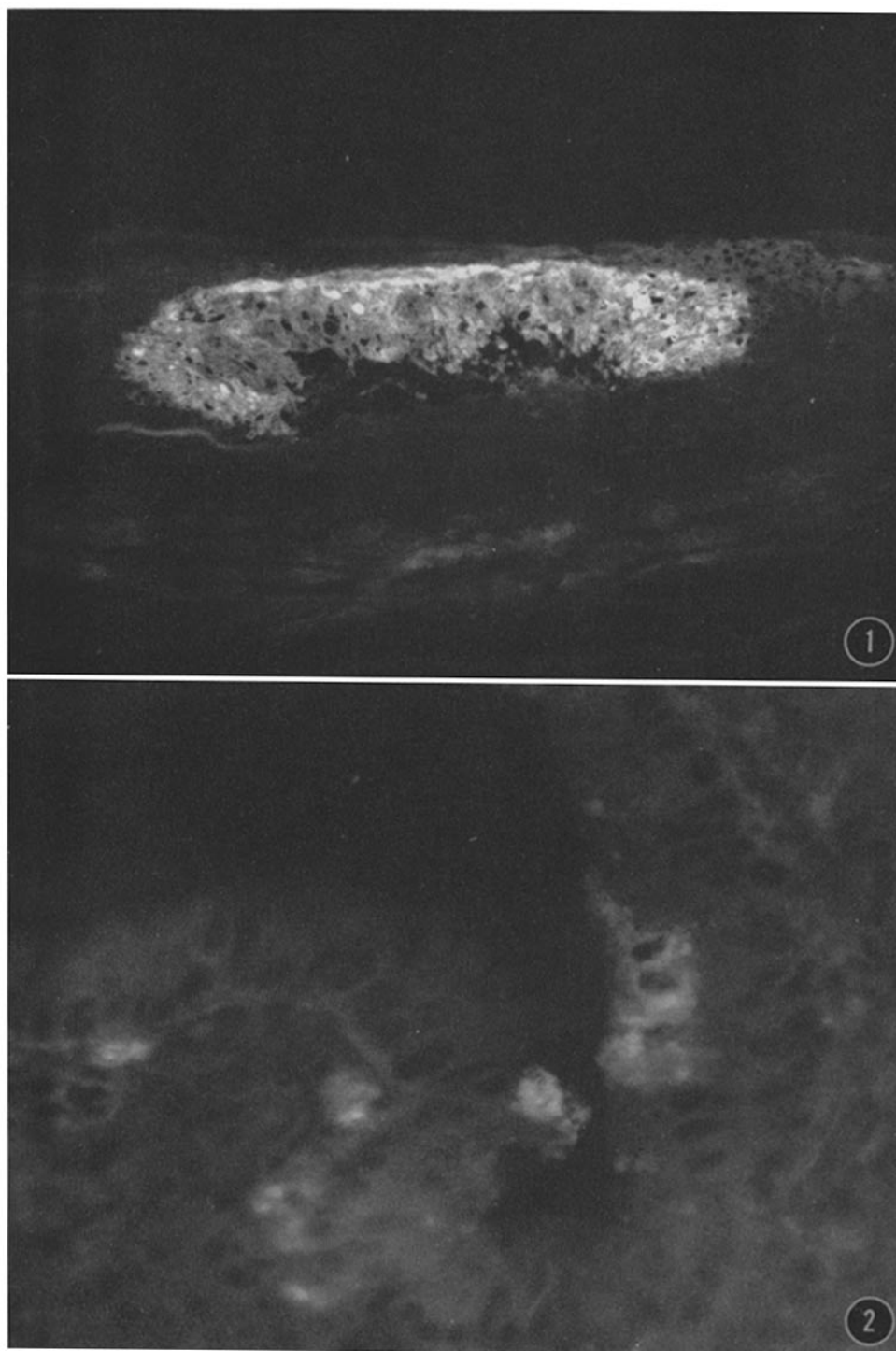
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EXPLANATION OF PLATES

PLATE 36

FIG. 1. Sagittal section of the foot of an adult mouse 5 days after intradermal inoculation with herpes virus. Section includes the edge of a vesicle where infected dermal cells have separated from the subcutaneous tissue. Fluorescent cells are limited to the dermis. $\times 120$.

FIG. 2. Section of lung of an adult mouse 3 days after intranasal inoculation with herpes virus. Fluorescence is present in the columnar epithelial cells of a bronchiole, several submucosal cells, and a free cell within the bronchiole. $\times 640$.



(Johnson: Pathogenesis of herpes virus encephalitis. II)

PLATE 37

Fluorescent antibody staining of cultures of mouse macrophages infected with herpes simplex virus (strain HFEM). Photomicrographs of representative infected foci of macrophages obtained from 8-week-old and 4-day-old mice were taken from the same test in which 1 to 2 per cent of the macrophages had been infected. $\times 480$.

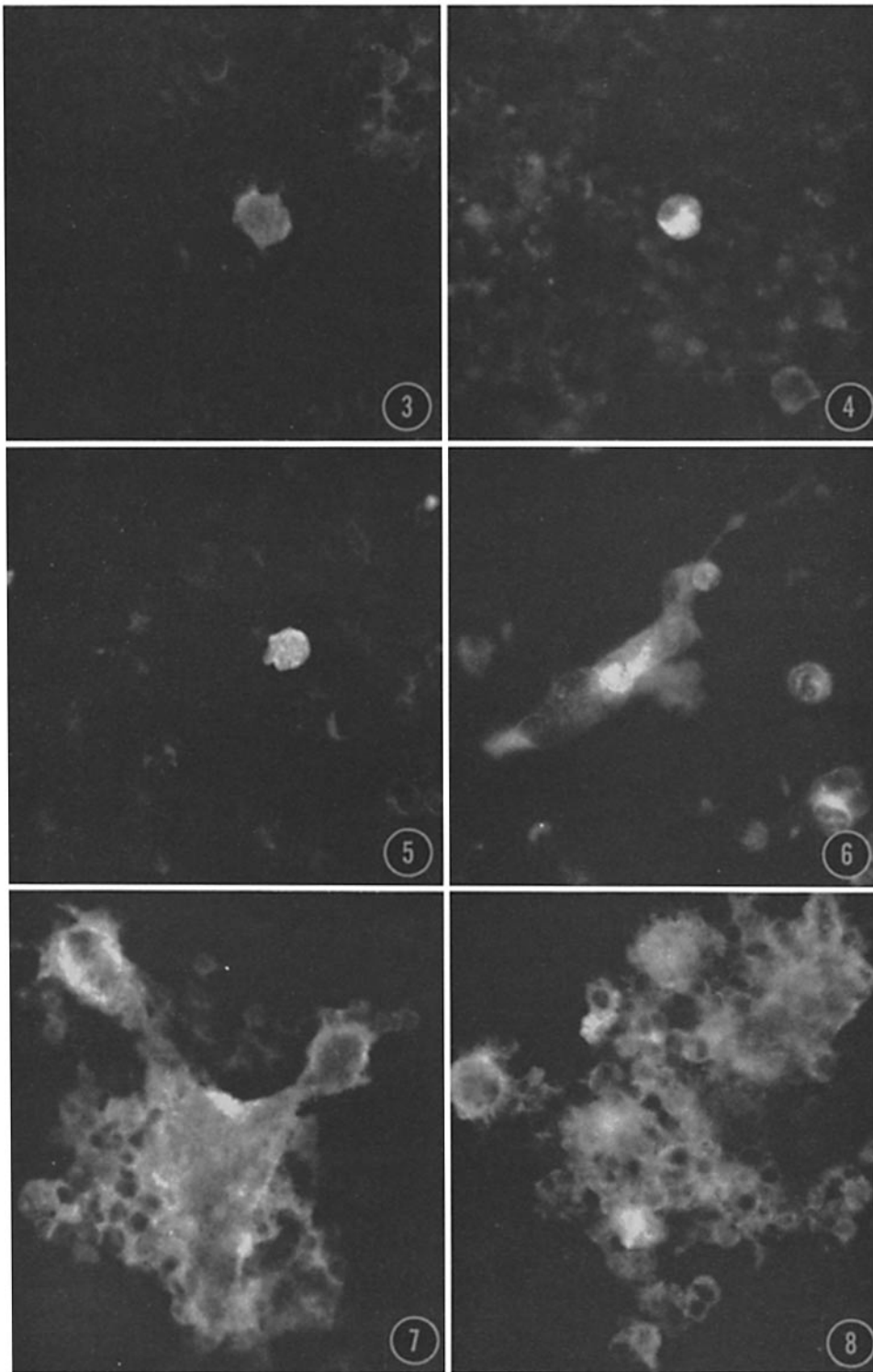
FIG. 3. Adult mouse macrophages 6 hours after inoculation.

FIG. 4. Adult mouse macrophages 72 hours after inoculation.

FIG. 5. Suckling mouse macrophages 6 hours after inoculation.

FIG. 6. Suckling mouse macrophages 24 hours after inoculation.

FIGS. 7 and 8. Suckling mouse macrophages 72 hours after inoculation.



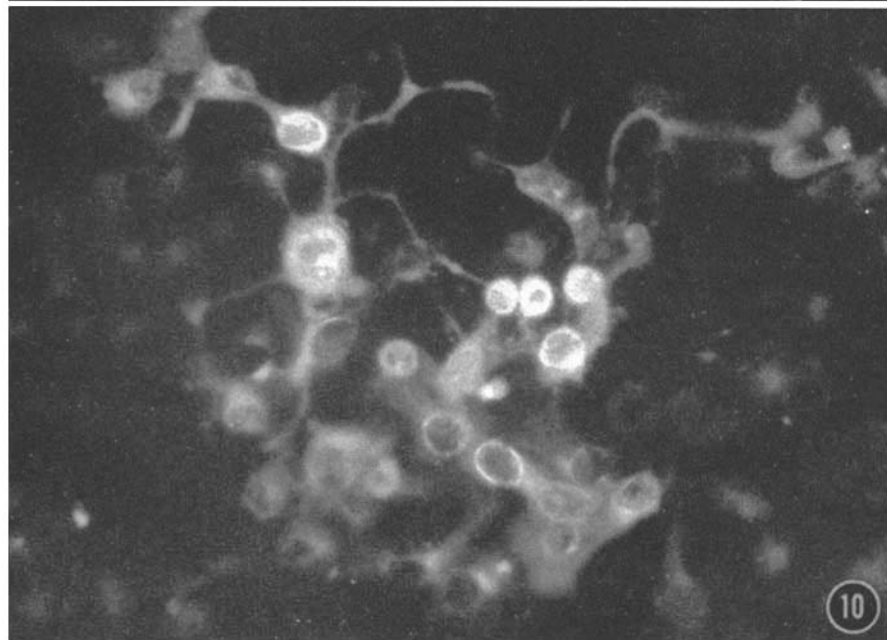
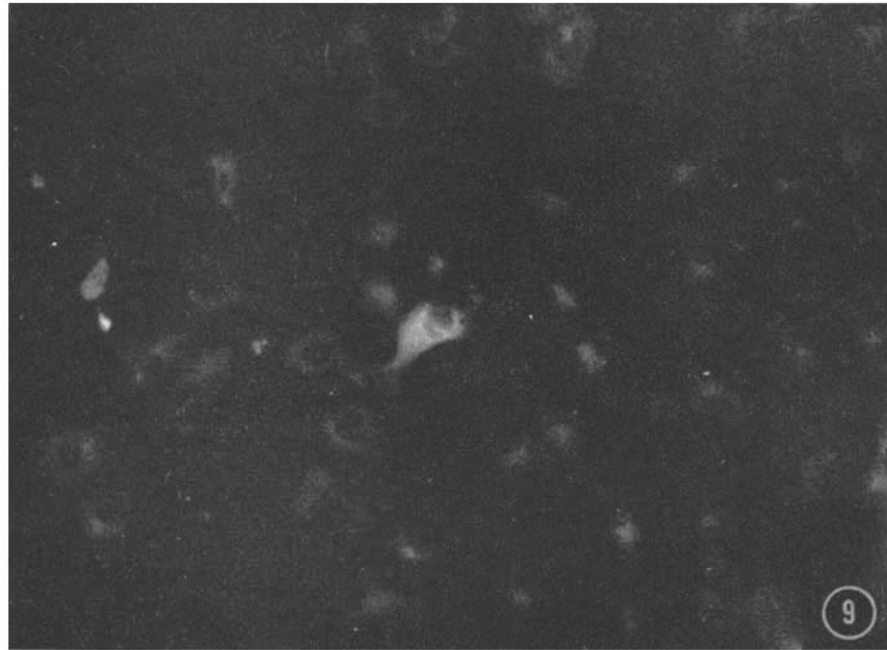
(Johnson: Pathogenesis of herpes virus encephalitis. II)

PLATE 38

Fluorescent antibody staining of mouse macrophages infected with herpes virus and in contact with a monolayer of HeLa cells. $\times 450$.

FIG. 9. Adult mouse macrophage 72 hours after addition of HeLa cells showing containment of infection by the macrophage.

FIG. 10. Suckling mouse macrophage 72 hours after addition of HeLa cells showing spread of infection to the HeLa cells.



(Johnson: Pathogenesis of herpes virus encephalitis. II)