# INFLUENCE OF HOST FACTORS ON NEUROINVASIVENESS OF VESICULAR STOMATITIS VIRUS

## IV. Variations in Neuroinvasiveness in Different Species*

By ALBERT B. SABIN, M.D., and PETER K. OLITSKY, M.D.

*(From the Laboratories of The Rockefeller Institute for Medical Research)*

(Received for publication, October 16, 1937)

The preceding investigations have thrown some light on the cause of variations in the capacity of vesicular stomatitis virus to give rise to encephalitis or myelitis in young and old animals of the same species (mice) (1). This virus, like a number of others, displays also distinct variations in its ability to cause disease of the central nervous system (CNS) in animals of different species under conditions of spontaneous infection or experimental peripheral inoculation. In nature it produces vesicular lesions in horses and cattle but is not known to involve the CNS. In guinea pigs suitable peripheral inoculation also gives rise to vesicular lesions unaccompanied by CNS disease, while in mice of proper age such injections are almost always followed by encephalitis or myelitis, depending upon the site of peripheral inoculation. The present investigation was undertaken to determine, for a single virus, the host factors which are responsible for the protection of the CNS from manifest disease in one species under conditions in which it is constantly involved in animals of another species.

### *Methods*

The technique employed in tracing the progression of the virus in the nervous system and other parts of the body from various sites of inoculation was essentially the same as those already described in the similar studies on mice (1). Necessary variations will be described in the text. The guinea pigs used in the present inves-

* Presented before the American Association of Immunologists and the American Association of Pathologists and Bacteriologists, April 9, 1936, (*Am. J. Path.*, 1936, **12**, 738.)

tigation were of mixed, unknown breed, and the exact ages were known only for the younger animals. Both the New Jersey and Indiana mouse passage strains were employed and tests for virus in guinea pig tissues were made by the intracerebral injection of suitable suspensions in 21 to 30 day old Rockefeller Institute albino mice.

## EXPERIMENTAL

*Comparative Infectivity of the Virus for Mice and Young and Old Guinea Pigs by the Intracerebral Route.*—It is known that guinea pigs develop encephalomyelitis after intracerebral injection of vesicular stomatitis virus (2), but it has not been ascertained whether the minimal amount required to induce manifest CNS disease in them is greater or smaller than that in mice. The purpose of the following experiments, therefore, was to determine in simultaneous tests the comparative susceptibility of the CNS of mice and guinea pigs when the virus was introduced directly into the brain. This information was necessary to indicate whether the absence of manifest CNS disease after peripheral inoculation in guinea pigs was the result of a lesser susceptibility of nervous tissue in general, or of factors related to the mode of virus progression from peripheral sites.

Tests were performed with the Indiana (Ind.) and New Jersey (N. J.) strains, using fresh centrifuged mouse brain suspensions in dilutions from $10^{-1}$ to $10^{-7}$ for intracerebral injection into 3 week old mice, young guinea pigs, 8 to 12 days old, and adult guinea pigs 2 months or more of age. The volume injected was 0.03 cc. in each mouse and 0.15 cc. for each guinea pig. One of the difficulties encountered in these tests was due to the fact that broth,[1] which is used as routine for preparing and diluting the virus suspensions, proved to be highly toxic upon intracerebral injection in guinea pigs, 2 months of age or older, more than 50 per cent of the animals dying within less than 12 hours; young and old mice and the young guinea pigs showed no such effect. Although with physiological salt solution as the suspending medium the titer of the virus is almost always tenfold less than with broth, it, nevertheless, had to be used in the comparative intracerebral tests. 110 guinea pigs and forty-eight mice were required to obtain the data necessary for establishing the relative susceptibilities of the CNS of the two species.

The results of three series of tests for each strain of the virus are shown in Table I. The most significant fact for the present investi-

[1] This broth is the kind usually called hormone broth containing chiefly beef heart infusion and 1 per cent peptone.

gation is that the CNS of young guinea pigs and young mice proved to be equally susceptible to both the N. J. and Ind. strains when the virus was injected directly into the brain, *i.e.* the minimal cerebral lethal dose (M.C.L.D.) for mice also caused a clinically apparent CNS disease in young guinea pigs. At the same time two other facts emerge: (*a*) while young and old mice are equally susceptible to intracerebral injection, old guinea pigs (2 months or older) seem to require on an average about 10 times as much virus as young ones (8 to 12 days) for the production of manifest CNS disease; and (*b*) primary flaccid paralysis of the posterior extremities occurred in twenty-five of twenty-seven guinea pigs which succumbed with the Indiana strain and in only two of thirty-three with the N. J. strain. Only about half the number of guinea pigs paralyzed with the Indiana strain died and the others recovered either completely or more often with marked residual paralysis. Guinea pigs succumbing with the N. J. strain exhibited varied encephalitic signs and all died. With respect to the development of flaccid paralysis of the extremities as the primary and chief nervous sign following intracerebral inoculation, the Indiana strain in guinea pigs thus closely resembles poliomyelitis in *rhesus* monkeys.

*Comparative Infectivity of the Virus for Young Mice and Young and Old Guinea Pigs by the Nasal Route.*—Since there was no record of the effect of nasal instillation of vesicular stomatitis virus in guinea pigs, the present experiment was undertaken to determine it simultaneously in young mice and young and old guinea pigs with a virus suspension whose infectivity by intracerebral inoculation would be established at the same time.

The tests recorded in Table II were carried out simultaneously with those described under Experiment A in Table I, using the same suspensions of virus in broth. 0.075 cc. was instilled in each nostril, using a number of guinea pigs for each of the $10^{-1}$, $10^{-2}$, and $10^{-3}$ dilutions. The largest number of guinea pigs, 12 (7 to 9 days old) and 12 (approximately 90 days old), were given the $10^{-2}$ dilution of the N. J. virus. 15 day old mice were used for control and each received 0.03 cc. of the indicated dilutions.

As was to be expected, all the mice developed encephalitis and died. None of the twelve young and old guinea pigs instilled with the Indiana virus, however, nor any of the fourteen adult guinea pigs

TABLE I

*Comparative Infectivity of Vesicular Stomatitis Viruses for Mice and Guinea Pigs by the Intracerebral Route*

| Strain of vesicular stomatitis virus | Experiment | Animals used | Age | Dilution of virus | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ |
| Indiana | A Virus in broth | Guinea pigs | 8–12 days | n.t. | n.t. | PP4S*<br>PP4D12† | PP5S<br>PP5S | PP8S<br>PP5D21 | PP6S<br>PP7S | n.t. |
| | | | Over 2 mos. | " | " | D+<br>0 | D+<br>0 | D+<br>D+ | <br>0 | " |
| | | Mice | 21 days | " | " | n.t. | n.t. | 3, 3, 4 | 4, 4, 0‡ | " |
| | B Virus in saline | Guinea pigs | 10 days | n.t. | n.t. | n.t. | PP5S<br>0 | E18D22<br>0 | 0<br>0 | 0<br>0 |
| | | | About 10 wks. | E9D10<br>PP5S<br>PP5R10§<br>0 | PP3D5<br>PP5D10<br>PP5D10<br>PP5D28 | PP6D8<br>PP6S<br>PP7R11<br>0 | PP6S<br>PP6D11<br>D6<br>0 | 0<br>0 | n.t. | n.t. |
| | | Mice | 21 days | n.t. | n.t. | n.t. | n.t. | 4, 4, 0 | 0, 0, 0 | 0, 0, 0 |
| | C Virus in saline | Guinea pigs | 10 days | n.t. | n.t. | n.t. | PP5S<br>0 | 0<br>0 | 0<br>0 | n.t. |
| | | | About 3 mos. | " | " | PP3D5<br>PP7D16 | PP5D7<br>0 | 0<br>0 | n.t. | " |
| | | Mice | 21 days | " | " | n.t. | n.t. | 4, 5, 0 | 0, 0, 0 | 0, 0, 0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| New Jersey | A Virus in broth | Guinea pigs | 10 days | n.t. | n.t. | n.t. | E4D6‖ E6D9 | D+ E6D11 | D4 E6D8 | n.t. |
| | | | About 3 mos. | D+ D+ | E3D4 E4D6 | D+ PP3D4 | D+ PP5D8 | D+ 0 | 0 0 | " |
| | | Mice | 21 days | n.t. | n.t. | n.t. | n.t. | 3, 3, 4 | 3, 4, 5 | " |
| | B Virus in saline | Guinea pigs | 10 days | n.t. | n.t. | n.t. | D2 E3D3 | E3D8 E7D9 | D4 E4D7 | E8D13 0 |
| | | | About 3 mos. | " | E3D4 E3D5 E4D6 | E3D5 E3D5 E4D5 E7D11 | E4D6 E6D10 E7D10 E11D13 | E7D10 E7D10 | D7 0 | n.t. |
| | | Mice | 21 days | " | n.t. | n.t. | n.t. | 3, 4, 4 | 3, 3, 0 | 0, 0, 0 |
| | C Virus in saline | Guinea pigs | 10 days | n.t. | n.t. | n.t. | n.t. | E4D5 E4D7 | 0 0 | 0 0 |
| | | | Over 3 mos. | " | " | " | E4D7 0 | 0 0 | 0 0 | n.t. |
| | | Mice | 21 days | " | " | " | n.t. | 4, 5, 0 | 0, 0, 0 | 0, 0, 0 |

0 = inoculated animal remained well.
n.t. = not tested.
D+ = died within less than 12 hours: broth toxicity.
* PP4S = flaccid paralysis of posterior extremities 4 days after inoculation; survived.
† PP4D12 = flaccid paralysis of posterior extremities 4 days after inoculation; died 12th day.
‡ 4, 4, 0 = three mice inoculated of which two died on the 4th day and one survived.
§ PP5R10 = flaccid paralysis of posterior extremities 5 days after inoculation; complete recovery on 10th day.
‖E4D6 = signs of encephalitis 4th day and died 6th day.

which received the N. J. strain showed any signs of disease. One of the twelve 7 to 9 day old guinea pigs instilled with the 1:100 dilution of N. J. virus developed distinct encephalitic signs (coarse tremors, incoordination, etc.) on the 5th day which lasted for only 3 to 4 days, the animal making a complete recovery. Further experience with nasal instillation of the N. J. strain indicates that guinea pigs over 2 months of age show no signs of disease whatever, while no more and probably less than one of about twenty of the very young ones exhibits some transitory encephalitic signs. When the results for the

TABLE II

*Comparative Infectivity of Vesicular Stomatitis Viruses for Guinea Pigs and Mice by the Nasal Route*

| Strain of virus | Animals used | Age | Dilution of virus | | |
|---|---|---|---|---|---|
| | | | 1:10 | 1:100 | 1:1000 |
| Indiana | Guinea pigs | 8–12 days | 0, 0 | 0, 0 | 0, 0 |
| | | Over 2 mos. | 0, 0 | 0, 0 | 0, 0 |
| | Mice | 15 days | 4, 4, 4 | 4, 5, 5 | 5, 6, 6 |
| New Jersey | Guinea pigs | 7–9 days | 0, E?D6 | 0, 0, 0, 0, 0, 0<br>0, 0, 0, 0, 0, E5 Rec. | n.t. |
| | | About 3 mos. | 0, 0 | 0, 0, 0, 0, 0, 0<br>0, 0, 0, 0, 0, 0 | n.t. |
| | Mice | 15 days | 4, 4, 4 | 4, 4, 5 | n.t. |

E?D6 = dubious signs of encephalitis; died 6th day.
E5 Rec. = signs of encephalitis appeared on 5th day; complete recovery.
Other legends as in Table I.

young animals of the two species are compared it appears that while both are equally susceptible to intracerebral inoculation, nasal instillation of the virus (in adequate amounts) constantly leads to encephalitis in young mice and, with only rare exceptions, not in the young guinea pigs. This in itself suggested that the absence of manifest CNS disease after peripheral inoculation in guinea pigs was not the result of a lower susceptibility of the entire nervous system, but was influenced rather by factors which modified the progression of nasally instilled virus in this species.

*Spread of the Virus (N. J. Strain) into Central Nervous Systems of Young and Old Guinea Pigs after Nasal Instillation.*—It will be recalled that in old mice, remaining entirely well after nasal instillation of the virus, the CNS was, nevertheless, invaded along the olfactory pathway and that the progression of the virus appeared to be arrested somewhere in the anterior rhinencephalon (1). The object of the following experiment was to disclose whether or not the resistance of guinea pigs was brought about by a similar mechanism.

TABLE III

*Spread of Vesicular Stomatitis Virus (N. J. Strain) into Central Nervous System of Young and Adult Guinea Pigs after Nasal Instillation*

| Experiment | Time after nasal instillation of virus $0.5 \times 10^5$ M.C.L.D. | Presence of virus in | | | | | |
|---|---|---|---|---|---|---|---|
| | | Young (10–12 days) | | | Adult (10–12 wks.) | | |
| | | Blood | Anterior rhinencephalon | Rest of brain | Blood | Anterior rhinencephalon | Rest of brain |
| | *days* | | | | | | |
| A | 2 | 0, 0, 0 | 3, 3, 0* | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | 4 | 0, 0, 0 | 3, 6, 0 | 3, 3, 3 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | 8 | 0, 0, 0 | 4, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| B | 2 | 0, 0, 0 | 2, 3, 4 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | 4 | 0, 0, 0 | 2, 2, 6 | 3, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | 8 | 0, 0, 0 | 3, 3, 3 | 3, 3, 3 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| C | 6 | n.t. | n.t. | n.t. | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | 10 | " | " | " | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |

* 3, 3, 0 = material injected intracerebrally in three mice of which two died on the 3rd day and one remained well.

Groups of guinea pigs, 10 to 12 days, and 10 to 12 weeks of age, were given nasal instillations of approximately 50,000 M.C.L.D. of the N. J. virus. At the intervals indicated in Table III, different animals were sacrificed and their blood and parts of the brain were tested for virus by intracerebral inoculation in mice. For this series of tests the brain was divided, as in mice, into two parts: the olfactory bulbs and structures ventral to the rhinal fissure up to the optic chiasm constituted one part, referred to as the anterior rhinencephalon, and a number of pieces from representative regions of the rest of the brain constituted the other. The former was ground up in 3 cc. of broth and the latter in 6 cc. of which 0.03 cc. was injected into each of three mice. Great care was taken to establish (by

examination of film preparation, culture, and passage) that mice which succumbed did so as a result of infection with vesicular stomatitis virus; it was in the course of such tests that toxoplasma were isolated on one occasion (3).

The results shown in Table III present a different picture from that obtained in mice. In the adult guinea pigs no virus was detected at any time between the 2nd and 10th days in either the blood or any part of the brain. In the young guinea pigs, on the other hand, virus was regularly found in the brain with none in the blood. In the animals sacrificed on the 2nd day virus was detected only in the anterior rhinencephalon, but in those killed on the 4th and 8th days after nasal instillation it was present there and also in the remainder of the brain. While the fact that virus was detectable only in the anterior rhinencephalon on the 2nd day is to some extent evidence against the widespread dissemination expected of spread in an "open system," it was, nevertheless, clear that it subsequently involved other parts of the brain and was not arrested in the same manner or site as in the resistant mice. It was, therefore, necessary to determine whether this later spread of the virus was diffuse and without relation to the tract connections of the olfactory pathway, (in which case it would be difficult to understand the absence of clinical CNS disease, manifested so constantly in response to the intracerebral injection of the minutest amounts of virus) or whether the progression might still be limited to definite areas, the arrest however, occurring somewhat more posteriorly than in mice, but still in clinically "silent" zones.

This premise was tested in an experiment, recorded in Table IV, in which the rest of the brain was subdivided into several portions. After cutting away the anterior rhinencephalon, the cortex (no separation was then made between the neopallial and olfactory portions) was peeled away from the brain stem and portions of the parietal and occipital regions (including, of course, the piriform lobes and cornu Ammonis) were saved for tests. The diencephalon (including the pars optica hypothalami) and mesencephalon, the pons and medulla, and the cerebellum were the other regions examined for virus. 10 day old guinea pigs, which were given about 500,000 M.C.L.D. of the N. J. virus, were sacrificed at 18 hours, 3, 7, and 10 days after nasal instillation. The blood at all these intervals, and the brain tissue of those killed at 18 hours and 3 days showed no virus. In the guinea pig sacrificed on the 7th day abundant virus was demonstrated in the anterior rhinencephalon, the cortical regions, and the diencephalon and mesencephalon, while none was found in the pons and medulla and cerebellum. On

the 10th day a small amount of virus was still detectable in the anterior rhinencephalon, and diencephalon and mesencephalon, but none in any of the other regions.

These results seemed to indicate that the virus did not spread indiscriminately throughout the CNS, and another series of experiments were undertaken to localize more definitely the affected zones and the site of apparent arrest of progression.

*Fate of Nasally Instilled Virus and Site of Arrest in CNS.*—In the following experiment an attempt was made to ascertain (*a*) the fate

TABLE IV

*Distribution of Nasally Instilled Vesicular Stomatitis Virus in the Central Nervous System of Young Guinea Pigs*

| Time after nasal instillation of virus $0.5\times 10^{-6}$ M.C.L.D. | Presence of virus in | | | | | | |
|---|---|---|---|---|---|---|---|
| | Blood | Anterior rhinencephalon | Parietal cortex | Occipital cortex | Diencephalon and mesencephalon (+ pars optica hypothalami) | Cerebellum | Pons and medulla |
| 18 hrs. | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| 3 days | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| 7 days* | 0, 0, 0 | 3, 5, 0 | 2, 3, 4 | 3, 3, 4 | 3, 3, 3 | 0, 0, 0 | 0, 0, 0 |
| 10 days† | 0, 0, 0 | 6, 0, 0 | 0, 0, 0 | 0, 0, 0 | 5, 6, 0 | 0, 0, 0 | 0, 0, 0 |

* The guinea pig sacrificed on the 7th day had fever on the 6th (104.8°F.) and 7th (105.2°F.) days.

† The guinea pig sacrificed on the 10th day had fever on the 6th (104.6°F.) and seventh (104.5°F.) days.

Other legends as in preceding tables.

of the virus in the nasal mucosa especially in relation to its subsequent invasion of the CNS, and (*b*) whether or not its localization in the brain was in accord with a progression along the central connections of the olfactory neurons.

The age of the animal and the dose of virus were the controlled variables. The young guinea pigs were 7 to 10 days old and the old ones were at least 6 months of age. One group of each age received a nasal instillation of about 500,000 M.C.L.D. of the N. J. virus and another about 50,000 M.C.L.D. The nasal mucosa and various parts of the brain were tested for virus at 3 hours, 2, 5, and 7 days. The entire nasal mucosa was ground with alundum and 5 cc. of broth; after horizontal centrifugation of the suspension at about 2000 R.P.M. for 45 to 60 minutes,

the supernatant liquid and dilutions prepared from it, were injected intracerebrally in mice. The anterior rhinencephalon was obtained in the usual manner, but the remainder of the brain was dissected somewhat differently. Following along the very sharp and distinct rhinal fissure the cortex was easily separated into the neopallial portion (non-olfactory), and the archi- and paleo-pallial portions containing the piriform lobes, and cornu Ammonis (olfactory zones). The diencephalon (including the pars optica hypothalami) and mesencephalon were tested individually while the pons, medulla, and cerebellum were pooled.

The results shown in Table V may be summarized as follows: The *young guinea pigs* receiving *500,000* M.C.L.D. of virus exhibited: *3 hours later*, virus only in the undiluted suspension of nasal mucosa (*i.e.* about 100–200 M.C.L.D.) and none in any tested part of the brain; *2 days later*, only a trace of virus in the nasal mucosa but a considerable amount in the anterior rhinencephalon with none in any of the other regions of the brain; *5 days later*, small amount of virus in anterior rhinencephalon and none detectable in nasal mucosa or other regions of the brain; *7 days later*, an appreciable amount in the anterior rhinencephalon, and piriform and hippocampal regions with none found elsewhere in the brain or nasal mucosa.

The *young ones* instilled with *50,000* M.C.L.D. of virus exhibited: *3 hours later*, none in the nasal mucosa or brain; *2 days later*, abundant virus in the mucosa (present in the $10^{-2}$ but not in the $10^{-3}$ dilution) as well as in the anterior rhinencephalon; *5 days later* virus absent, or in smaller amount, in nasal mucosa, but present in considerable quantity in anterior rhinencephalon, piriform and hippocampal regions, and some in the diencephalon but not elsewhere in the brain; *7 days later*, none found in the nasal mucosa or any part of the brain with the exception of the diencephalon which contains a considerable amount.

The *old guinea pigs* given *500,000* M.C.L.D. of virus exhibited: *3 hours later*, none in nasal mucosa or brain; *2 days later*, abundant virus in nasal mucosa ($10^{-2}$ dilution positive) and a trace in the anterior rhinencephalon, with none in the other regions of the brain; *5 and 7 days later*, a trace of virus in the nasal mucosa with none in any part of the brain.

The *old guinea pigs* given *50,000* M.C.L.D. of virus exhibited: *3 hours later*, none in nasal mucosa or brain; *2 days later*, none or undetermined small amount in nasal mucosa and none in brain; *5 days later*, trace in nasal mucosa and anterior rhinencephalon with none elsewhere in brain; *7 days later*, none in nasal mucosa or brain.

It appears, therefore, that as in mice (1), almost all the virus instilled into the nose disappears within a very short time, to such an extent that in three of the four guinea pigs tested within 3 hours none was detectable, indicating that of the 50,000 or 500,000 M.C.L.D. of instilled virus less than 100 M.C.L.D. remained fixed or in an infective state. It also seems clear that in both the young and the old guinea pigs there may be quite an appreciable increase in the amount of virus

in the nasal mucosa within the first 2 days which, however, disappears rapidly thereafter. When the results of the present experiment are

TABLE V

*Fate of Nasally Instilled Vesicular Stomatitis Virus (N. J.) and Site of Arrest in Central Nervous System as Influenced by Amount of Virus and Age of Guinea Pigs*

| Age of guinea pigs | Amount of virus instilled | Time after nasal instillation | Guinea pig No.* | Presence of virus in: Nasal mucosa in 5 cc. Undiluted | 1:10 | 1:100 | 1:1000 | Anterior rhinencephalon | Piriform and hippocampus | Diencephalon + pars optica hypothalami | Mesencephalon | Pons, medulla, and cerebellum | Neopallium |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7–10 days | 500,000 M.C.L.D. | 3 hrs. | 1 | 3, 7† | 0, 0 | n.t. | n.t. | 0, 0 | n.t. | 0, 0 | 0, 0 | 0, 0 | n.t. |
| | | 2 days | 2 | 4, 0 | 0, 0 | 0, 0 | 0, 0 | 3, 3 | 0, 0 | 0, 0 | 0, 0 | 0, 0 | " |
| | | 5 " | 3 | 0, 0 | 0, 0 | 0, 0 | 0, 0 | 3, 0 | 0, 0 | 0, 0 | 0, 0 | 0, 0 | 0, 0 |
| | | 7 " | 4 | 0, 0 | 0, 0 | 0, 0 | n.t. | 3, 3 | 3, 4 | 0, 0 | 0, 0 | 0, 0 | 0, 0 |
| | 50,000 M.C.L.D. | 3 hrs. | 5 | 0, 0 | 0, 0 | n.t. | n.t. | 0, 0 | n.t. | 0, 0 | 0, 0 | 0, 0 | n.t. |
| | | 2 days | 6 | 2, 2 | 2, 2 | 2, 3 | 0, 0 | 2, 2 | —‡ | — | — | — | — |
| | | 5 " | 7 | — | 0, 0 | 0, 0 | 0, 0 | 2, 3 | 2, 3 | 2, 0 | 0, 0 | 0, 0 | 0, 0 |
| | | 7 " | 8 | 0, 0 | 0, 0 | 0, 0 | n.t. | 0, 0 | 0, 0 | 2, 2 | 0, 0 | 0, 0 | 0, 0 |
| Over 6 mos. | 500,000 M.C.L.D. | 3 hrs. | 9 | 0, 0 | 0, 0 | n.t. | n.t. | 0, 0 | n.t. | 0, 0 | 0, 0 | 0, 0 | n.t. |
| | | 2 days | 10 | 2, 2 | 2, 2 | 2, 3 | " | 3, 0 | " | 0, 0 | 0, 0 | 0, 0 | " |
| | | 5 " | 11 | 5, 0 | 0, 0 | 0, 0 | " | 0, 0 | " | 0, 0 | 0, 0 | 0, 0 | " |
| | | 7 " | 12 | (4), 0 | 0, 0 | 0, 0 | " | 0, 0 | " | 0, 0 | 0, 0 | 0, 0 | " |
| | 50,000 M.C.L.D. | 3 hrs. | 13 | 0, 0 | 0, 0 | n.t. | " | 0, 0 | " | 0, 0 | 0, 0 | 0, 0 | " |
| | | 2 days | 14 | — | 0, 0 | 0, 0 | " | 0, 0 | " | 0, 0 | 0, 0 | 0, 0 | " |
| | | 5 " | 15 | 0, 0 | 6, 6 | 0, 0 | " | 2, 0 | " | 0, 0 | 0, 0 | 0, 0 | " |
| | | 7 " | 16 | 0, 0 | 0, 0 | 0, 0 | " | 0, 0 | " | 0, 0 | 0, 0 | 0, 0 | " |

* None of the guinea pigs sacrificed for these tests exhibited any signs of disease.

† The brain of at least one sick or dead mouse in each group of two was cultured and subinoculated in animals in order to establish that the illness and death were due to virus.

‡ Dashes indicate that material was contaminated with pathogenic bacteria.

(4) = mouse died on 4th day but material was unsuitable for establishing cause of death.

combined with those of Table III, it is to be noted that among 16 old guinea pigs, a trace of virus was found only in the brains of two (lim-

ited to the anterior rhinencephalon), indicating that in spite of the multiplication of virus which may occur in the nasal mucosa it cannot as a rule invade the brain and that when it does get into the brain on occasions, it can apparently neither multiply nor progress beyond the anterior olfactory region. In the young guinea pigs, however, there is evidence of constant invasion of the brain, the virus becoming detectable in the anterior rhinencephalon on the 2nd day at which time the other regions of the brain show none. It then spreads posteriorly apparently in accord with a definite order localizing in the piriform and hippocampal regions but not in the neopallial regions of the cortex, and occasionally in the diencephalon (or only the pars optica hypothalami) but apparently not beyond. The finding of virus only in the diencephalon in the brain of one guinea pig on the 7th day merely suggests the possibility that it may disappear last from the areas which are last to be involved. This type of localization, however, is completely in accord with progression of the virus within the neurons of the olfactory pathway and not at all in agreement with a spread of the virus in an open system. It can also be inferred, therefore, that the absence of apparent CNS disease in nasally instilled guinea pigs may be effected by the same mechanism which determined the resistance of old mice. The difference between the two species is in (*a*) the sites at which the progression of nasally instilled virus is arrested, and (*b*) in the fact that 7 to 10 day old guinea pigs appear to possess "barriers" which are acquired by mice only at a much later age.

*Spread of Virus after Intramuscular or Pad Inoculation.*—Although in past investigations by others, large numbers of guinea pigs have been injected into the pads with the vesicular contents from horses and cattle, or with the pad passage or more recent brain passage strains, there is no record of any paralysis or other signs of CNS disease occurring in these animals. When the present investigation was begun, twelve guinea pigs, approximately 1 month old, were injected intracutaneously and subcutaneously in the pad of one posterior extremity with mouse brain virus and one developed typical flaccid paralysis of the inoculated posterior extremity on the 5th day followed by paralysis of the opposite leg, and ascending paralysis resulting in death on the 9th day after inoculation. It is regrettable

that no search for virus was made, although no other cause for these signs was found. In view of this unexpected finding many other guinea pigs, and particularly very young ones, were inoculated in the same manner without our ever again observing any evidence of CNS disease.

TABLE VI

*Spread of Vesicular Stomatitis Virus (N. J.) after Intramuscular or Pad Inoculation*

| Site of inoculation | Age and average weight of guinea pigs | Time after inoculation | Presence of virus in | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Blood | Spleen | Regional lymph nodes | Right sciatic nerve | Right sacral and lumbar ganglia | Spinal cord | Left sacral and lumbar ganglia | Left sciatic nerve |
| | | *days* | | | | | | | | |
| Intracutaneously and subcutaneously in right pad (about $10^6$ M.C.L.D.) | About 1 mo.; 300 gm. | 3 | 0, 0, 0 | n.t. | n.t. | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | | 6 | 0, 0, 0 | " | " | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | | 10 | 0, 0, 0 | " | " | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | About 1 mo.; 300 gm. | 6 | 0, 0, 0 | " | " | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | | 10 | 0, 0, 0 | " | " | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| Intramuscularly in right calf muscles (about $10^6$ M.C.L.D.) | 10 days; 167 gm. | 2 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | | 4 | 0, 0, 0 | 0, 0, 0 | 2, 3, 3 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | | 8 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | Over 3 mos.; 580 gm. | 2 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | | 4 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | | 8 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |

The following tests were undertaken to determine whether or not the virus injected intramuscularly or into the pads, was capable of invading the CNS along the nerves supplying the inoculated sites.

One group of 1 month old guinea pigs was given about $10^6$ M.C.L.D. of mouse brain virus (N. J.) intracutaneously and subcutaneously in the right pad. Two animals were sacrificed at 3, 6, and 10 day intervals and the structures, indicated

in Table VI, were pooled and injected intracerebrally in mice. Three other groups of guinea pigs (young and adult) were given approximately the same amount of virus intramuscularly in the right posterior extremity, and various tissues were tested at 2, 4, 6, 8, and 10 day intervals.

The results shown in Table VI indicated that in none of these animals was virus found in the blood, peripheral nerves, spinal ganglia, or spinal cord. Virus was detected but once and that was in the regional lymph nodes of a 10 day old guinea pig, 4 days after intramuscular injection. It was clear, therefore, that in young and old guinea pigs, as in old mice, absence of manifest CNS disease following this form of peripheral inoculation was associated with an inability of the virus to invade the nervous system. In old mice it will be recalled, this was associated with inability of the virus to multiply at the site of inoculation. Although vesicular stomatitis virus is known to produce definite vesicular lesions in the pads of guinea pigs, and, therefore, presumably to be capable of local multiplication, it was nevertheless desirable to establish this fact beyond question particularly for the virus used in these experiments, which has undergone over 100 brain to brain passages in mice.

Twelve 8 to 10 day old guinea pigs and an equal number of old animals weighing on the average 1000 gm. each were given about $10^6$ M.C.L.D. of the N. J. virus into the pad of one posterior extremity, part by "tunneling" and the remainder subcutaneously, and a similar volume of plain broth in the other for control. Only the pads inoculated with virus developed lesions; these were distinct on the 3rd day and were generally less marked in the young than in the old. Histological sections of some of the pads revealed the characteristic picture including intranuclear inclusions and necrosis of epithelial cells. Animals of each group were sacrificed at 2, 22, and 72 hours after inoculation. The pads inoculated with virus were washed with alcohol and ether, dissected away, and ground up with alundum and broth. The pad of an old guinea pig, weighing usually 0.3 gm., was ground up with 6 cc. of broth, that of a young one, weighing 0.1 gm., with 5 cc. of broth. The suspensions were centrifuged at about 2000 R.P.M. for 45 to 60 minutes, and the clear supernatant liquids (designated in the old as the 1:20 dilution and for the young as the 1:50) and further dilutions of them were injected intracerebrally in mice.

The results of this experiment (Table VII) leave no doubt as to the local multiplication of the virus. It is clear, therefore, that local multiplication does not in itself determine the capacity of this virus to invade the peripheral nerves.

*Effect of Intrasciatic Injection of Virus.*—Inoculation of the virus directly into the sciatic nerve of resistant mice resulted in a fatal ascending myelitis in six of eleven animals, indicating that virus progression along peripheral nerve fibers was possible and that the chief barrier to invasion of the CNS was in some structure or structures at the site of intramuscular or pad inoculation (1).

To determine whether or not the same was true for guinea pigs, twelve of them were given a 10 per cent suspension of N. J. virus into the right sciatic nerves. An equal number of guinea pigs which, for control, were injected intramuscularly, in the neighborhood of the same region of the sciatic nerve, remained well.

TABLE VII

*Local Multiplication of Vesicular Stomatitis Virus (N. J.) in Pads of Young and Old Guinea Pigs*

| Age and average weight of guinea pigs | Time after inoculation of about $10^6$ M.C.L.D. | Dilution of pad suspension | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1:20 | 1:50 | 1:100 | 1:1000 | 1:10,000 | 1:100,000 | 1:1,000,000 |
| | *hrs.* | | | | | | | |
| 8–10 days; 121 gm. | 2 | n.t. | 3, 5 | n.t. | 0, 0 | 0, 0 | n.t. | n.t. |
| | 22 | " | 2, 2 | " | 3, 5 | 8, 9 | " | " |
| | 72 | " | 4, 5 | " | 5, 10 | 0, 0 | 0, 0 | 0, 0 |
| Over 3 mos.; 1000 gm. | 2 | 0, 0 | n.t. | 0, 0 | 0, 0 | 0, 0 | n.t. | n.t. |
| | 22 | 2, 3 | " | 2, 3 | 3, 4 | 2, 5 | " | " |
| | 72 | 4, 4 | " | 0, 0 | 5, 7 | 0, 0 | 0, 0 | 0, 0 |

Four of the twelve guinea pigs (Table VIII) receiving the virus in the sciatic nerve, developed typical flaccid paralysis of the posterior extremities, which ascended and caused death in three instances, the fourth animal having been sacrificed for virus tests. Some of the guinea pigs without nervous signs exhibited fever (104–106°F.) but no spread of virus was demonstrable in association with it. Tests for virus in a guinea pig dying with paralysis revealed its presence in the lumbar and cervical portions of the cord, the medulla, and brain, but not in either one of the sciatic nerves. The failure to detect virus in the inoculated sciatic nerve at the time of paralysis and its presence in the lumbar cord, observed also in another guinea pig, duplicates the experience with this virus in old mice and its probable significance has already been discussed (1).

It is clear from these experiments that vesicular stomatitis virus injected directly into a peripheral nerve like the sciatic can invade

TABLE VIII

*Effect of Intrasciatic Injection of Virus*

| Age and average weight | Guinea pig No. | Result | Presence of virus in | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Right sciatic nerve | Left sciatic nerve | Lumbar cord | Cervical cord | Medulla | Brain | Spleen |
| 10–12 days; 200 gm. | 1 | Fever 5th and 6th days; paralysis of posterior extremities 5th day; dead 7th day | 0, 0 | 0, 0 | 2, 2 | 3, 3 | 3, 3 | 2, 2 | n.t. |
| | 2 | Fever* 6th, 7th, 8th, 10th days; remained well | | | | | | | |
| 10 weeks; 500 gm. | 3 | Fever 5th, 7th, 10th days; paralysis of posterior extremities 5th day; encephalitic signs, 13th day; dead 15th day | | | | | | | |
| | 4 | Fever 10th, 11th, 12th days; remained well | | | | | | | |
| About 6 mos.; 600 gm. | 5 | Fever 7th, 10th, 11th, 12th days; remained well | | | | | | | |
| | 6 | Fever 11th, 12th, 13th days (105–106°F.); no nervous signs; sacrificed 13th day | 0, 0 | 0, 0 | 0, 0 | 0, 0 | 0, 0 | 0, 0 | 0, 0 |
| 6–10 wks.; 350–500 gm. | 7 | No fever; remained well | | | | | | | |
| | 8 | " " " " | | | | | | | |
| | 9 | Fever 3rd, 4th, 6th days; paralysis right posterior extremity 5th day; paralysis both posterior extremities 6th day; dead 7th day | | | | | | | |
| | 10 | No fever; paralysis of both posterior extremities 6th day; no change 7th day; sacrificed 7th day | 0, 0, 0 | 0, 0, 0 | 3, 5, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| | 11 | No fever; remained well | | | | | | | |
| | 12 | Remained well | | | | | | | |

* Fever refers to a distinct rise in temperature of 1.5°F. or more over the preceding level and reaching at least 104°F.

the CNS of guinea pigs which it is incapable of accomplishing after inoculation into a site supplied by this nerve. In view of the fact also that the CNS is highly susceptible and that the virus can multiply in the area of peripheral injection affecting tissue cells supplied by these peripheral nerves, one is forced to consider, by a process of elimination, that the terminal, specialized nerve endings may constitute the real barrier to invasion of the CNS.

## DISCUSSION

A number of observations made in the present investigation indicate that a neurotropic virus to which the CNS of two species is equally susceptible may, after peripheral inoculation, cause encephalitis or myelitis in one but not in the other, the difference being determined by variations in the character of the nervous pathways along which the virus must spread. When vesicular stomatitis virus is introduced directly into the brain, the minimal dose (1 M.C.L.D.) can produce encephalitis in young guinea pigs as readily as in young mice. On the other hand, while 100 to 1000 M.C.L.D. instilled intranasally in young mice, invariably gives rise to a fatal encephalitis, 1000 times that amount is, with very rare exceptions, entirely unassociated with any clinical signs of CNS involvement. Since the nasally instilled virus, nevertheless, regularly invades the CNS of young guinea pigs and spreads through the olfactory regions of the brain along pathways which it has been shown to utilize in susceptible mice, it would appear that some variation in these nervous pathways is responsible for the arrest of its progression in the terminal olfactory areas or diencephalon. After inoculation into tissues supplied by spinal nerves (*e.g.* sciatic) the virus undergoes local multiplication in young guinea pigs as in young mice, but causes myelitis only in the latter while in the former it fails to invade the nervous tissue altogether. The fact that direct intrasciatic injection is frequently followed by a fatal ascending myelitis tends to eliminate the peripheral nerves themselves as the barriers to invasion of the CNS and forces consideration of a variation in structures, such as the myoneural junctions or other specialized nerve endings, through which is effected the intimate relationship between the axis cylinders and the inoculated tissues. The rôle of localized barriers in hosts of different age or species in preventing or

TABLE IX

*Localized Barriers as a Factor in Preventing or Arresting Infection of the Central Nervous System (Vesicular Stomatitis Virus)*

| Host | Route of inoculation | Susceptibility of young and old | Probable site of barrier |
|---|---|---|---|
| Mouse | Intracerebral | Both equally susceptible | 0 |
| | Intramuscular<br>Subcutaneous, pad | Young, regularly myeloencephalitis<br>Old, 100 per cent resistant | Muscle or myoneural junction; epithelium or specialized nerve endings |
| | Intraocular | Young, regularly encephalitis<br>Old, more than 90 per cent resistant | Retina |
| | Intranasal | Young, regularly encephalitis<br>Old, resistance varies from 50 to 90 per cent | In CNS, anterior olfactory region (between 2nd and 3rd olfactory neurons?) |
| Guinea pig | Intracerebral | Young guinea pigs and mice equally susceptible, old guinea pigs somewhat less | 0 |
| | Intramuscular<br>Subcutaneous<br>Intracutaneous | Young and old resistant | Myoneural junction and specialized nerve endings |
| | Intranasal | Young and old resistant | Young, (*a*) between olfactory cortex and remainder of brain<br>(*b*) between diencephalon (or pars optica hypothalami) and remainder of brain<br>Old, generally between nasal mucosa and CNS |

arresting infection of the CNS with vesicular stomatitis virus is summarized in Table IX.

There are many other instances in nature where a virus is highly neuroinvasive in one species and apparently not at all (or very rarely) in another. As a classical example one may cite the virus which, while causing only herpes simplex in human beings, will, when transferred to the skin, cornea, or mucous membrane of the rabbit, cause not only local lesions like those in man, but also clinically apparent and fatal disease of the CNS. Pseudorabies (4) and B virus (5, 6) which are pantropic and highly neuroinvasive in the rabbit, will cause encephalitis when injected intracerebrally but not peripherally (skin, muscle) in the *rhesus* monkey; this is associated with a loss of the capacity to produce lesions in non-nervous tissue of the monkey in the case of pseudorabies but not of B virus. That variation in the character of the specialized nerve endings in different species may be involved here, as in the case of vesicular stomatitis virus, receives some support in Hurst's finding that intrasciatic injection of pseudorabies virus in the *rhesus* monkey frequently causes myeloencephalitis (4).

The observations made in this series of studies on vesicular stomatitis virus in different hosts may supply a pattern for at least one type of inapparent infection of the CNS. It is generally assumed, for example, that the majority of the human population are subject to inapparent infection with the virus of poliomyelitis, while only rare individuals exhibit the clinically apparent form of the disease. Faber (7) has thus postulated on theoretical grounds that poliomyelitis virus in man, spreading axonally along the olfactory pathways, might be halted in its progression in silent zones of the CNS in the majority of instances. That such a thing is possible and actually occurs with another neurotropic virus is evident from the demonstrated behavior of vesicular stomatitis in guinea pigs which seems to offer a remarkable parallel for many of the manifestations of poliomyelitis in man. There are the rare, individual guinea pigs which after nasal instillation exhibit clinical signs of CNS disease, while in the majority which appear well there is, nevertheless, transitory multiplication of the virus in the nasal mucosa with involvement of definitely limited zones in the brain; and to accentuate the parallel

even further it may be stated here that antiviral bodies regularly appear in the blood of all guinea pigs regardless of the extent of CNS involvement. (Detailed studies on the immune response to arrested infection with vesicular stomatitis virus will be presented in a future communication.) Other patterns of inapparent infection of the CNS, which have been described recently, must also be considered. Burnet (8), for example, showed that louping ill virus which regularly causes encephalitis in the mouse, is followed by no signs of disease when the virus is injected by intracerebral or peripheral routes in young or old rats. After nasal instillation in rats there appears, nevertheless, to be local multiplication of the virus and an invasion of the brain that is generally limited to the olfactory bulbs. The observations of Webster and Clow (9) on mice with a high inborn resistance to the virus of St. Louis encephalitis demonstrate still another type of clinically inapparent infection of the CNS; intracerebral and peripheral inoculation are equally harmless in these mice, and nasal instillation is followed by as widespread an invasion of the brain as in the susceptible animals. The absence of nervous signs here is correlated with a distinctly lower level of virus multiplication throughout the CNS, rather than with localized barriers to virus progression.

## SUMMARY AND CONCLUSIONS

Peripheral inoculation of vesicular stomatitis virus is constantly followed by myelitis or encephalitis in young mice, but not in young (or old) guinea pigs. The cause of this variation was elucidated by investigating the fate of the virus after inoculation by a number of different routes.

Direct intracerebral injection of minimally infective amounts of virus was found to be equally fatal for young mice and young guinea pigs, indicating that the central nervous system as a whole was as easily injured by the virus in one species as in the other.

The events following nasal instillation of the virus varied in young and old guinea pigs. While there appeared to be a transitory multiplication of virus in the nasal mucosa of both young and old, the central nervous system was regularly invaded only in the young. In these, virus was first found only in the anterior rhinencephalon; later it spread to the piriform and hippocampal (olfactory regions)

but not to the neopallial portions of the cortex, and the only other area to exhibit virus was the diencephalon (including the pars optica hypothalami), where its further progression was apparently arrested.

Absence of central nervous system disease following inoculation into sites supplied by spinal nerves (*e.g.* sciatic) was found to be due to inability of the virus to invade the nerves.

Since direct intrasciatic inoculation frequently led to a fatal ascending myelitis, it was evident that the central nervous system could be invaded along the spinal nerves, and that they did not constitute the main barrier. Furthermore, since multiplication of virus was demonstrated in tissues supplied by the spinal nerves, a process of elimination made it seem possible that the specialized, terminal nerve endings might be the structures which prevent the progression of the virus from the infected tissues to the axons and hence also to the central nervous system.

7 day old guinea pigs (or guinea pigs as a species) were thus found to possess much the same type of barriers to the progression of peripherally inoculated vesicular stomatitis virus as are acquired by mice at a considerably later age.

In a discussion of the present data, they have been correlated with known variations in neuroinvasiveness of other viruses and their bearing on the nature of inapparent or subclinical infections of the central nervous system has been considered.

## BIBLIOGRAPHY

1. Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1937, **66,** 15, 35; 1938, **67,** 201.
2. Cox, H. R., and Olitsky, P. K., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30,** 653.
3. Sabin, A. B., and Olitsky, P. K., *Science,* 1937, **85,** 336.
4. Hurst, E. W., *J. Exp. Med.*, 1936, **63,** 449.
5. Sabin, A. B., *Brit. J. Exp. Path.*, 1934, **15,** 321.
6. Sabin, A. B., and Hurst, E. W., *Brit. J. Exp. Path.*, 1935, **16,** 133.
7. Faber, H. K., *Medicine*, 1933, **12,** 83.
8. Burnet, F. M., *J. Path. and Bact.*, 1936, **42,** 213.
9. Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1936, **63,** 827.