In a study of the nutritional state of an individual in regard to vitamin C, many factors enter into an evaluation of analyses of the body fluids. Subnormal urinary values may sometimes be occasioned by loss in the sweat,[7] or, in certain manifestations of kidney disease, by retention in the blood.[8] The administration of salicylates[9] has been reported to accelerate the excretion of the vitamin, while the metabolic demands of fever, particularly in pneumonia[10] and tuberculosis,[11] may be responsible for a diminished output. That Pyridium medication may cause erroneously high vitamin C results is a possibility that should be considered.

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## Progression of Different Nasally Instilled Viruses along Different Nervous Pathways in the Same Host.

ALBERT B. SABIN.

*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*

In recent years much evidence has been accumulated to indicate that following nasal *instillation* many neurotropic viruses invade the central nervous system (CNS) by the olfactory pathway, and controversy has centered on whether these viruses progress along the perineural spaces or within the processes of the olfactory neurons themselves. Interruption of the olfactory pathway by surgical means has been shown to be capable of preventing poliomyelitis in monkeys induced by nasal instillation of the virus, in spite of the fact that all the other nerves connected with the nasal mucosa remain intact.[1] Similar interruption of the olfactory pathway in rab-

[7] Lilienfeld, A., Wright, I. S., and MacLenathen, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 184.

[8] Wright, I. S., and MacLenathen, E., PROC. SOC. EXP. BIOL. AND MED., in press.

[9] Daniels, A. L., and Everson, G. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 20.

[10] Bullowa, J. G. M., Rothstein, I. A., Ratish, H. D., and Harde, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 1.

[11] Martin, G. J., and Heise, F. H., *Am. J. Dig. Dis. and Nutr.*, 1937, **4**, 368.

[1] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728; Brodie, M., and Elvidge, A. R., *Science*, 1934, **79**, 235; Lennette, E. H., and Hudson, N. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1444; Howe, H. A., and Ecke, R. S., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 125; Gordon, F. B., and Lennette, E. H., *J. Bact.*, 1938, **35**, 43.

bits did not, however, prevent the encephalitis which follows the nasal instillation of herpetic virus,[2] and tests for virus during the incubationary period of the disease in normal, unoperated rabbits showed that it was present in the Gasserian ganglia but not in the olfactory bulbs, suggesting that invasion occurred along the 5th nerve.[3] It appeared, therefore, that either conditions were dissimilar in the rabbit and monkey or that different viruses behaved differently. To elucidate this question it was necessary either to vary the virus and keep the host constant or to vary the host and keep the virus constant; it proved easier to do the former.

The viruses of vesicular stomatitis (V.S.), eastern equine encephalomyelitis (E.E.E.), and pseudorabies (the last having been shown to belong to the same "generic" group as herpetic and B virus[4]) were therefore studied in 15-day-old mice (Rockefeller Institute strain). Previous experiments on such mice have shown that following nasal instillation, V.S. virus is found in the olfactory bulbs before it can be detected in the Gasserian ganglia or pons.[5] It is necessary to stress that the nasal instillations were carried out without any mechanical injury to the mucosa, the mice being allowed to aspirate small drops of viral suspension placed on their nostrils. It should also be pointed out that with pseudorabic virus (centrifuged 10% suspension of rabbit-brain in broth) only half the number of mice receiving such *instillations* succumbed (first sign being a persistent and violent scratching of the nose and side of the face), while all mice receiving the same or a smaller dose by subcutaneous, intramuscular, or intraocular *injection* succumbed. Mice of this age were thus found to be much less susceptible to nasal instillation with pseudorabic virus than with E.E.E. or V.S. In the present investigation the pathological method already described in a study on V.S. virus[6] could be used to obtain an indication as to whether the other nervous pathways connected with the nasal mucosa are involved. Partial, serial, transverse sections were taken of the entire skull (without the lower jaw) with all the cranial nerve and autonomic ganglia (except the submaxillary) and superior cervical sympathetic ganglia *in situ*, and also of representative regions of the spinal cord. With the V.S. virus (mice succumbing in 4 to 5 days) and E.E.E. virus (2 to 3 days), the lesions which permitted the mapping of viral progression were those of neuronal necrosis, while

[2] Levaditi, C., Hornus, G., and Haber, P., *Ann. Inst. Pasteur*, 1935, **54**, 389.

[3] Levaditi, C., and Haber, P., *Compt. rend. Soc. biol.*, 1935, **119**, 21.

[4] Sabin, A. B., *Brit. J. Exp. Path.*, 1934, **15**, 372.

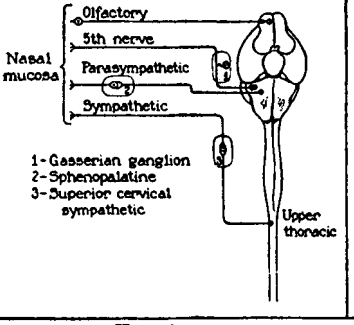
[5] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1937, **66**, 15.

[6] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1938, **67**, 201.

with pseudorabic virus (about 54 hours), there was no neuronal necrosis but striking and unmistakable acidophilic, intranuclear inclusions served to indicate the path of the virus.

It is necessary to recall here the chief nervous pathways connected with the nasal mucosa and the situation of the cell-bodies of the neurons of the first and second orders (Fig. 1): (1) the olfactory pathway with the cell-body of the primary neuron in the olfactory mucosa and its *axon* coming into relation with the dendrites of the secondary neuron in the bulbs; (2) the fibers of the 5th nerve whose cell-bodies in the Gasserian ganglia synapse with the neurons of the sensory nucleus of the trigeminus in the medulla; (3) the sympathetic fibers whose cell-bodies in the superior cervical sympathetic ganglia are in synaptic relation with the neurons in the lateral horn of the lower cervical or upper thoracic region of the spinal cord; and (4) the bulbar autonomic ("parasympathetic") fibers whose cell-bodies in the sphenopalatine ganglia synapse with neurons situated in the region of the 7th nerve nuclei in the medulla.

Diagrammatic presentation of nerve supply of nasal mucosa and situation of neurons of first and second order in the nervous system of the mouse.




Nervous pathways selected by three different viruses in invading the central nervous system after nasal instillation in the mouse.

| Nervous pathway | Virus | | |
|---|---|---|---|
| | Vesicular stomatitis | Equine encephalomyelitis | Pseudo-rabies |
| Olfactory | + | + | − |
| 5th nerve | − | − | + |
| Sympathetic | − | − | + |
| Bulbar autonomic (Para-sympathetic) | − | − | + |

\+ Lesions present along pathway
− " absent " "

FIG. 1.

It has already been reported that following nasal instillation in mice, the viruses of V.S.[6] and E.E.E.[7] invade the CNS along the olfactory pathway. In the present study there was no evidence of progression (neither neuronal necrosis nor inclusions) along the 5th nerve, sympathetic, or parasympathetic routes. With pseudorabic virus, however, the reverse was true; specific viral lesions, *i. e.*, characteristic intranuclear inclusions, were abundant in the Gasserian ganglia and sensory nuclei of the 5th nerve, in the spheno-

[7] Sabin, A. B., and Olitsky, P. K., *Am. J. Path.*, 1937, **13**, 615 (Abstract).

palatine ganglia and region of the 7th nerve nuclei in the medulla, in the superior cervical sympathetic ganglia and the lateral horn of the lower cervical or upper thoracic regions of the spinal cord, but nowhere along the course of the olfactory pathway. In a trial test on an animal sacrificed at the onset of nervous signs (which is about 6 hours before death) virus was found (by subinoculation in rabbits) in the Gasserian ganglia and medulla but not in the olfactory bulbs, indicating that in this case the distribution of virus coincided with the observed pathological changes. The following observations support the assumption that the pseudorabic lesions in these structures are the result of the neural progression of virus from the nose and not of any special affinity which virus in the blood might have for them: (a) after injection into the leg muscles there is abundant virus in the blood but no lesions in any of these structures; (b) after nasal instillation of the virus the lesions have been unilateral (in the mice studied thus far), on the same side in all the involved pathways, and none was found in ganglia having no connection with the nasal mucosa. A more detailed description of the localization of lesions in mice after injection of pseudorabic virus by various routes will be given in a future communication, in which it will also be shown that after intraocular (vitreous) injection this virus utilizes pathways (including sympathetic) not employed by the viruses of V.S. and E.E.E. Perhaps the first evidence of the centripetal progression of a virus along the cranial sympathetic system has been obtained in the present study.

It appears clear, therefore, that the facility with which certain neurotropic viruses (*i. e.*, viruses which attack nerve-cells and may or may not be able to attack other types of tissue as well) invade the CNS after nasal instillation is dependent neither upon any supposed connection between the exterior of the nasal mucosa and the subarachnoid space through the olfactory perineural spaces, nor apparently even upon the special position of the cell-bodies of the primary olfactory neurons with their capacity to take up certain instilled substances,[8] but rather upon the special affinity between certain viruses and certain types of cells. In this connection an explanation is required of some recent observations on the fate of a number of viruses instilled into the nose.[8] It was stated that the viruses of St. Louis encephalitis, rabies and louping-ill could not be detected in the "olfactory area" of the brain in less than 24 hours after nasal instillation in mice (the same was found to be true of V.S. virus[5]), while E.E.E. virus was demonstrated within 2 to 5

[8] Rake, G., *J. Exp. Med.*, 1937, **65**, 303.

minutes although not at 6, 8, and 10 minutes or at 6 hours. On the basis of these data, it was assumed that the former 3 or 4 viruses which were called "neurotropic" invaded the nervous system differently from the E.E.E. virus which was termed "pantropic". E.E.E. virus, however, is just as neurotropic as the others and its capacity to multiply in non-nervous tissue elements of mice is shared to a certain extent by the St. Louis encephalitis,[9] louping-ill,[10] and V.S. viruses.[11] It appeared probable that this singular result with the E.E.E. virus may have been due to the fact that a given dilution of mouse-brain suspension contains more infective units of this virus than any of the other four; the amount of E.E.E. virus instilled may be estimated at 20 million to 200 million minimal cerebral lethal doses (M.C.L.D.), while the dosage of the other viruses probably ranged between 100,000 and 2 million M.C.L.D. Also it is conceivable that with the larger amount of virus in the nares in the first few minutes (nasally instilled virus has been shown to be rapidly washed away[5, 12]) it might be possible to pull up one or more infective units in taking out the olfactory bulbs. An experiment designed to elucidate this question revealed the following: (a) that when the olfactory bulbs were taken out without precautions to avoid pulling, a trace of virus was found in them (within 2 to 4 minutes) when 10 million M.C.L.D. of E.E.E. virus were instilled but not 1 million; (b) that 200 million M.C.L.D of E.E.E. virus could be instilled without any of it being demonstrable in the olfactory bulbs when they were removed by first being severed from

TABLE I.

Does Equine Encephalomyelitic Virus Invade the Brain Within a Few Minutes After Nasal Instillation?

Mice killed at 2 minutes, exsanguinated and nerve tissue removed from cranial cavity within 4 minutes.

| Method of removing olfactory bulbs | Dilution of virus and No. of M.C.L.D. instilled | Mouse No. | Test for virus No. of mice injected | No. of mice dead |
|---|---|---|---|---|
| Special precautions to prevent pulling | 1:5 (200,000,000) | 1 | 2 | 0 |
| | | 2 | 2 | 0 |
| | | 3 | 2 | 0 |
| | | 4 | 2 | 0 |
| Olfactory bulbs pulled | 1:100 (10,000,000) | 5 | 2 | 1* |
| | | 6 | 2 | 0 |
| | 1:1,000 (1,000,000) | 7 | 2 | 0 |
| | | 8 | 2 | 0 |

[9] Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1936, **63**, 433.

[10] Fite, G. L., and Webster, L. T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 695.

[11] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1937, **66**, 35.

[12] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1938, **67**, 229.

*Virus proved to be cause of death.

the rest of the brain and then from the olfactory nerves without injury to the cribriform plate and lifted out without any pull on structures in the nasal mucosa (Table I). It would appear, therefore, that there is as yet no evidence that nasally instilled viruses invade the CNS by any direct, open space; the available data seem to point rather to progression along nerve cells and their processes.

*Conclusions.* Different neurotropic viruses instilled into the nose of the same host (mouse) can select different nervous pathways for invading the central nervous system. The viruses of vesicular stomatitis and equine encephalomyelitis were shown to use the olfactory pathway but not the trigeminus, sympathetic, or parasympathetic pathways, while pseudorabic virus invaded along the latter three routes and not along the olfactory.

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## Inhibition of Streptococcal Hemolysin by Sulfonamide Compounds.

PAUL GROSS, FRANK B. COOPER AND MARION LEWIS. (Introduced R. R. Mellon.)

*From the Institute of Pathology, the Western Pennsylvania Hospital, Pittsburgh, Pa.*

*In vitro* experiments, in which prontosil I* appeared to inhibit streptococcal hemolysin and leukocidin, have led Levaditi and Vaisman[1] to believe that certain sulfonamide compounds exert an antitoxic action.

The present report concerns the effect of sulfanilamide and other substances upon streptococcal hemolysin, and also the effect of sulfanilamide upon staphylococcal hemolysin.

Hemolysin was prepared by growing beta hemolytic streptococcus strain C 203 in 2% neopeptone beef broth of pH 7.6-7.8 for 16-18 hours and removing most of the bacteria by centrifugation.

In some experiments this hemotoxin was incubated with various amounts of sulfanilamide† (50 mg. per 100 cc. up to saturation) for 30 minutes at 37.5°C. The untreated toxin and the sulfanil-

* Prontosil I (4-sulfonamide-2',4'-diaminoazobenzol) and prontosil II (disodium salt of 4-sulfamidophenyl-2'-azo-7'acetylamino-1'hydroxynaphthalene-3',6' disulfonic acid) kindly supplied by Winthrop Chemical Co., N. Y.

1 Levaditi, C., and Vaisman, A., *Compt. rend. Soc. de biol.*, 1935, **120**, 1077.

† Kindly supplied by E. R. Squibb & Sons, New York.