10498

Relation of Concentration of Virus to the Pathogenesis of Poliomyelitis.*

E. W. SCHULTZ AND L. P. GEBHARDT.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

To determine the route by which the virus spreads from the portal of entry to the medulla and cord, previous investigators have examined different regions of the central nervous system at varying intervals of time during the preparalytic period for the presence of virus. The results of these and related studies have established that poliomyelitis virus spreads axonally,[1] probably as an intraneural infection.

The work we wish to report represents an extension of these earlier studies in which the presence or absence of virus was merely determined and the amount present was not actually measured. Our investigations deal with the concentrations which are reached by the virus during the preparalytic period. They were prompted in part by certain observations on the kinetics of bacteriophagy which indicate that the lytic agent is at first produced in the absence of recognizable bacterial lysis and that actual lysis is not initiated until a certain critical concentration of the agent has been reached.[2] The mechanism of this prelytic formation of bacteriophage is not yet known and need not concern us here. In the studies here reported it was our purpose merely to determine the concentration of poliomyelitis virus which is reached in different regions of the central nervous system and to relate this, if possible, to the extent of neuronal damage sustained in the particular regions, the guiding hypothesis being that the production of virus and of nerve cell damage do not necessarily parallel each other and that certain neurons, although supplying a pabulum for the production of virus may, nevertheless, be relatively resistant to its action.

Part of our observations was made on intracranially inoculated animals; part on intranasally inoculated animals. All were sacri-

* Studies supported by Mary Hooper Somers Fund for Filtrable Virus Research.

1 Hurst, E. W., *J. Path. and Bact.*, 1929, **32**, 457; Fairbrother, R. W., and Hurst, E. W., *Ibid.*, 1930, **33**, 17; Hurst, *Ibid.*, 1133; Faber, H. K., and Gebhardt, L. P., *J. Exp. Med.*, 1933, **12**, 83; *J. Pediat.*, 1938, **13**, 1938; Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1938, **68**, 39.

2 Krueger, A. P., and Northrop, J. H., *J. Gen. Physiol.*, 1930, **14**, 223; Clifton, C. E., and Morrow, G., *J. Bact.*, 1936, **31**, 441.

ficed at varying intervals of time after the onset of the initial febrile temperature rise[3] and before the appearance of the usual recognizable symptoms of the disease. After exsanguinating the animal, individual specimens were removed with fresh sets of sterile instruments. Immediately after removal these were weighed and stored in 50% glycerine until the time of titration. In carrying out the titrations, the previously weighed specimens were ground thoroughly in a motorized grinder for one hour and made up to a 10% suspension in physiological saline. From this stock suspension further dilutions were made. The results presented below are expressed in terms of the *final dilution* of nervous material which proved infectious when injected intracerebrally into rhesus monkeys in 1 cc amounts. For the sake of orientation, we may say that in routine work pools of 5 or more glycerinated entire cords and medullæ from recently paralyzed monkeys and weighed after glycerination (glycerination for one month adds about 25% to the weight), generally prove infectious in dilutions as high as 1:4,000, less often in dilutions above 1:5,000 when injected intracerebrally in 1 cc amounts.

Our first observations were made on 2 intracerebrally inoculated monkeys. Each was inoculated with about 20 MID of virus. The first animal (C184) was sacrificed immediately after the onset of the febrile temperature rise, which was 4 days following inoculation. In this animal the thalamus proved infectious in a final dilution of 1:100, while dilutions of 1:10 of the olfactory mucosa, olfactory bulbs, medulla, cervical cord and the thoracic sympathetic ganglia failed to infect. Histological sections from these regions showed no deviation from the normal (olfactory bulbs were not examined). The second monkey (C183) was sacrificed 1 day following the onset of the febrile temperature rise, or 6 days after inoculation. In this animal the thalamus proved infectious in a dilution of 1:4,000; the medulla in a dilution of 1:3,000; the cervical enlargement of the cord in a dilution of 1:10, while 1:10 dilutions of the hippocampus, the lumbar enlargement and the abdominal and thoracic sympathetic ganglia failed to infect. Although the animal was inoculated intracranially, the bulbs proved infectious in a dilution of 1:10 (higher dilutions were not tested). Histological sections showed early perivascular round cell infiltration in the hippocampus, thalamus and medulla, with slight acidophilia of the nucleoplasm of some nerve cells in the medulla and cervical cord, but other than this there were no apparent changes.

[3] Kramer, S. D., Hendrie, K. H., and Aycock, W. L., *J. Exp. Med.*, 1930, **51**, 933.

Two of the animals on which our observations were made were inoculated intranasally. Both received 3 instillations of 10% pooled virus, all on the same day, each instillation being preceded by an acid phosphate lavage (pH 5). One of these animals (D457) was sacrificed immediately after the onset of the febrile temperature rise or on the third day following inoculation. In this animal the olfactory bulbs proved infectious in a dilution of 1:2,000 (the end point was not determined), the thalamus and uncinate gyrus in final dilutions of 1:100, the anterior perforated space and medulla in dilutions of 1:10, while 1:10 dilutions of the motor cortex and of the cervical enlargement of the cord failed to infect. Nasal washings, a suspension of finely ground olfactory mucosa and a suspension of the tonsils also proved non-infectious.† The tissue showed a beginning perivascular infiltration in the region of the anterior perforated space, hypothalamus, pons, medulla and cervical cord. Scattered nerve cells in these regions showed slight acidophilia of the nucleoplasm, but no retrograde changes associated with satellitosis or neuronophagia were observed. In the hypothalamus the cytoplasm as well as the nucleoplasm of a few nerve cells showed one or more small strongly eosinophilic bodies, resembling type B inclusions (Lentz A stain). Other nerve changes were not evident.

The second intranasally inoculated animal (D586) was sacrificed one day after the onset of the febrile temperature rise, or on the 6th day following inoculation. In this animal the olfactory bulbs were infectious in 9 different dilutions ranging from 1:25 to and including 1:10,000 (the endpoint was not determined); the hypothalamus in a dilution of 1:3,000; the thalamus, medulla and cervical enlargement of the cord in dilutions of 1:1,000; the lumbar enlargement of the cord in a dilution of 1:100, and the uncinate gyrus in a dilution of 1:10. Microscopically there was early perivascular round cell infiltration in the thalamus and this was more pronounced in the hypothalamus and medulla. No definite nerve cell changes were observed in the thalamus and hypothalamus. In the medulla a few nerve cells showed acidophilia of the nucleoplasm, but no definite retrograde changes nor evidence of satellitosis or of neuronophagia were observed. In both the cervical and lumbar enlargements of the cord only beginning perivascular round cell infiltration was observed.

We are unable to say anything about the histology of the olfactory bulbs in these animals since the entire bulbs were ground and used in

† Sabin and Olitsky[4] have recently also reported failure to demonstrate virus in the nasal mucosa of intranasally inoculated monkeys.

[4] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1938, **68**, 39.

the titration. Histological observations have, however, been made on the bulbs of a number of other intranasally infected and extensively paralyzed animals. These agree in the main with those recently reported by Sabin and Olitsky,[5] except that we have not observed extensive mitral cell involvement. At any rate neuronal damage here is less evident than in the anterior horns of the cord despite the high concentration of virus reached in the bulbs.

We have also titrated the cervical and lumbar enlargements of the cord of 2 intranasally infected monkeys (PMD623 and PMD 642A), sacrificed several hours after the onset of paralysis. In one of these (PMD623), which showed paralysis of all 4 limbs, the cervical enlargement infected in a dilution of 1:20,000, while the lumbar cord did so in a top dilution of 1:30,000 (however, 2 monkeys inoculated with the 1:10,000 dilution failed to develop the disease). Microscopic sections from the cervical and lumbar enlargements of the cords showed the usual extensive nerve cell changes and tissue reactions seen in early acute experimental poliomyelitis. In the other animal (PMD642A), which had developed paralysis of the arms, but not yet of the legs, the cervical enlargement of the cord infected in a dilution of 1:15,000, the lumbar in final dilution of 1:30,000. Microscopically, nerve cell changes were widespread in both the lumbar and cervical enlargements of the cord, but these seemed more advanced in the cervical enlargement where satellitosis and neuronophagia though early also seemed more evident.

In addition, we have titrated pools of 10 cervical and 10 lumbar enlargements of the cord; also 10 pairs of olfactory bulbs, all from intranasally infected monkeys, sacrificed within one day after the onset of complete paralysis. The maximum dilution in which the pool of cervical enlargements of the cord infected was 1:8,000; that of the pool of lumbar enlargements was 1:16,000, while the pool of olfactory bulbs infected in a maximum dilution of 1:5,000.

Although the titration figures given above probably do not represent exact endpoints, they give some information as to the approximate concentrations reached in the localities mentioned.

Our observations support the evidence obtained by previous investigators that the virus spreads axonally. In addition, they throw some light on the concentrations attained by the virus during the preparalytic period in those regions through which it passes enroute to the cord and where neuronal damage is much less evident than in the cord.[6] They seem to show, furthermore, that even in the cord the

[5] Sabin, A. B., and Olitsky, P. K., *J. A. M. A.*, 1937, **21**, 108.

[6] Hurst, E. W., *J. Path. and Bact.*, 1929, **32**, 457.

virus may reach rather high concentrations before the usual extensive neuronal damage appears and frank paralysis sets in. The highest concentration seems to be reached at the time of paralysis, which is presumably immediately after the bulk of the anterior horn cells have suddenly crumbled under the action of a certain rather high critical concentration of virus.

Superficially at least, the relationships seem to be analogous to those observed in bacteriophagy in that fairly large amounts of virus may be formed in the absence of apparent nerve cell damage and in that actual cell destruction may be a function of the concentration reached by the virus, as well as of the general level of susceptibility of the cells exposed to it. In other words, virus production and nerve cell damage do not altogether parallel each other. It may be that the amount of damage sustained by the nerve cells in any given region of the nervous system depends rather more on the concentration which a given virus is capable of building up than upon its invasiveness as such.

In the case of bacteriophages it is known that individual bacteriophages on serial passage eventually reach a certain concentration endpoint, either low or high, beyond which they cannot be increased. To us it seems possible that like some strains of bacteriophage, which despite serial passage remain of low titer and low lytic power, there are constantly low titer strains of poliomyelitis virus, which although not lacking in invasiveness may be unable to build up a concentration high enough to damage even the more susceptible nerve cells. Naturally acquired immunity may rest largely on exposure to such low titer strains, which may be quite distinct from those capable of inducing epidemics of the paralytic disease.

10499

Micro Determination of Serum Proteins by Gasometric Carbon Analysis.

CHARLES L. HOAGLAND AND D. JEAN FISCHER. (Introduced by David P. Barr.)

From the Department of Internal Medicine, Washington University School of Medicine, St. Louis.

The successful adaptation of the Van Slyke manometric apparatus to the measurement of carbon in organic compounds by wet combustion is well illustrated by the micro-gasometric measurement of