problems in biology, to which he has suggested mathematical solutions and, having done that, he dismisses the whole matter in the hope that some eminent mathematician will be inspired to take advantage of the opportunity to make of biology a true science.

UNIVERSITY OF PENNSYLVANIA C. E. McCLUNG

SPECIAL ARTICLES

PATHWAY OF INVASION IN A CYNOMOLGUS MONKEY AFTER ORAL APPLICATION OF POLIOMYELITIS VIRUS[1]

DESPITE a great deal of work on both human and experimental material, the portal or portals of entry of poliomyelitis through the body surfaces has not as yet been precisely determined. Present evidence shows that the olfactory system is not as a rule primarily implicated, and that invasion probably occurs in most cases through the alimentary tract, but it is not yet known whether the upper portion—the mouth and pharynx—or the lower portion—the stomach and intestines—is the more vulnerable to penetration by virus.

In studying this problem we have encountered certain technical difficulties which have apparently prevented others also from obtaining a clear answer. Among these may be mentioned the difficulty of confining the application of virus in the experimental animal to a particular region of the alimentary tract; and the difficulty, in cases of fully developed infection, of determining the portal of entry from the distribution of virus or of lesions in the central nervous system. The latter procedure appears to be better adapted to the exclusion of a given portal than to its positive determination, because, once virus has become implanted and the animal has developed typical symptoms of the disease its spread is remarkably rapid and extensive, even before paralysis has occurred.

It has become clear to us that the experimental animal must be sacrificed at the earliest possible moment of manifest infection or even before this, in order to obtain plain evidence of the primary localization. However, the adoption of routine and systematic examination of the peripheral nervous ganglia—suggested by McClure's recent work[2]—has proved to be helpful, a method the value and significance of which has been surprisingly late in gaining recognition. These ganglia contain the nerve cells whose axons supply the mucous surfaces through which the virus presumably first gains access to the interior of the body and it is highly probable that they are the primary site of multiplication of this strictly neuronotropic virus. Since most of the ganglia (with the exception of those of the vagus) supply a fairly limited portion of the mucous surfaces, the distribution of lesions or of virus in them should afford valuable clues to the portal of entry, provided as we have stated that the examination is made early in the disease. Particularly significant ganglia are the Gasserian, the geniculate and the petrosal, which supply the mucous membrances of the mouth and nasopharynx, and the celiac, which supplies the stomach and intestine. The sympathetic and spinal ganglia also have some localizing value, but the vagus ganglia (nodose and jugular) have such wide-spread connections in the entire alimentary and respiratory tracts as to give but little localizing information.

Using cynomolgus monkeys and Sabin's *Per* strain of poliomyelitis virus, we have applied virus to various parts of the alimentary tract and have killed animals shortly after the onset of fever or, in some cases, without any signs of infection. Tissues of the central and peripheral nervous system have been systematically examined for lesions, including the olfactory bulbs, brain stem and spinal cord; the ganglia of the V, VII (geniculate), IX, X cranial nerves; the sympathetic ganglia of the ganglionated cord at all levels; the spinal ganglia at all levels, and the celiac plexus. The results of the study as a whole will be reported later, but at this time we wish to present the data in one monkey as being of some special interest.

Cynomolgus 9. Capsules, covered with a digestible fat, containing dried virus amounting to about one third of a cynomolgus cord, were inserted into the esophagus on April 5, 1941, in such a manner as to avoid contamination of the mouth. On May 20 and again on September 15, 1941, after zinc sulfate olfactory blockade, the tongue was gently swabbed with a minute amount of 15 per cent. virus suspension. On January 22, 1942, a high enema of 5 cc of 20 per cent. virus suspension was administered. No symptoms and no fever occurred after any of these treatments. The olfactory mucosa was again treated with zinc sulfate on March 14, 1942, and on March 25 and on each of the 3 following days, the mouth was sprayed from an atomizer with 5 cc of a 10 per cent. suspension supernate. On March 30, 5 days after the first spraying, fever, slight weakness of the arms and mild head tremors were noted at 5 P.M. (none of these had been present that morning). It is highly probable that this animal would have become paralyzed. It was sacrificed about 15 minutes later, following our routine

[1] From the Department of Pediatrics, Stanford University School of Medicine, San Francisco, Calif. Sponsored by the National Foundation for Infantile Paralysis, Inc.

[2] G. Y. McClure, SCIENCE, 94: 307, 1941.

procedure of etherization, exsanguination, perfusion with physiological saline solution and 10 per cent. formalin. The nervous tissues were stained with gallocyanin and eosin according to Einarson's method.

Serial sections were made of the olfactory bulbs and peripheral ganglia. Sections of the brain stem were taken at intervals of 0.6 mm and of the spinal cord from upper, middle and lower portions of the cervical, thoracic and lumbar regions (3–4 successive sections, 20 micra thick from each). Lesions[3] in the ganglia consisted of small cell infiltrations, chromatolysis, neuronal necrosis and neuronophagia; in the brain stem, mainly of perivascular infiltrations with occasional parenchymal infiltration. Heavy lesions were found in both Gasserian ganglia and both nodose (X nerve) ganglia. Moderately severe lesions were found in both petrosal (IX nerve) ganglia, in 3 of 6 cervical sympathetic ganglia and 2 of 10 upper thoracic ganglia. Small lesions, few in number, were found in one geniculate (VII nerve) ganglion; in 1 lumbar sympathetic ganglion and in 2 of 14 thoracic spinal ganglia. In the medulla a few typical parenchymal and perivascular infiltrations, without definite cell necrosis, were found in and near the nucleus of the tractus solitarius but nowhere else. No lesions were found elsewhere in the brain stem, in the olfactory bulbs, the lower thoracic sympathetics, the celiac, the cervical spinal or the lumbar spinal ganglia or in the spinal cord.

With the exception of the few very slight lesions in one lumbar sympathetic ganglion (which may have resulted from inapparent infection from the earlier virus enema), it would appear that the lesions found can all be explained on the basis of nerve-borne infection entering through the mouth, mainly through the fibers of the fifth, ninth and tenth nerves (probably including the gustatory), and to a lesser extent through the sympathetics; and it is evident that infection had just begun to invade the central nervous system from its primary neuronal site of multiplication through the central connections of the IX and X cranial nerves in the nucleus of the tractus solitarius in the medulla. It is interesting to note that although the Gasserian ganglia were heavily involved, their central connections contained no lesions. The dorsal motor nucleus of the X nerve likewise was uninvolved.

The experiment is of special interest because it demonstrates entry of virus through the mouth and pharynx after oral administration, and also the mode of progression of infection from the exterior mucous surfaces to the local peripheral nervous system into the central nervous system. It would appear that the mouth and pharynx are readily vulnerable to penetration by this virus. We are far from wishing to use this experiment or others like it as evidence in exclusion of other possible portals, such as the lower alimentary tract. Indeed, we have some positive evidence that infection can also enter via the latter. However, it may be pertinent to note that the very frequent occurrence of headache, vomiting, nuchal pain and other symptoms in the preparalytic stage of the human disease strongly suggest early involvement of the brainstem, particularly the medulla, which is better accounted for by entry from the oropharyngeal passages than from the more distant intestines. It has too generally been assumed that bulbar paralysis is the sole criterion of primary bulbar poliomyelitis. Primary involvement of the afferent centers is, in our opinion, of at least equal importance and possibly more common. The reason why bulbar motor involvement is frequent after adenotonsillectomy is probably that infection is traumatically introduced into the motor nerves of the pharynx and so conducted directly to their motor nuclei. In the ordinary case, in which deep trauma is not a factor, infection entering through the oropharyngeal membranes would rather invade the afferent nerves into the peripheral ganglia and thence into the sensory bulbar centers, whence it could progress to other centers but without necessarily involving the motor nuclei of the medulla. Experimentally, it has been thoroughly proven that, following olfactory and intracerebral inoculation, poliomyelitic infection can pass freely down through the medulla into the spinal cord and produce spinal paralysis without accompanying bulbar paralysis.

Since poliomyelitis is probably acquired as a rule by the alimentary route, the oropharyngeal mucosa is obviously the first site of contact with virus in contaminated food and drink and on fingers, which children so often put in the mouth. Here the contacts of the virus with the mucous surfaces are immediate and the virus is at its maximum concentration. In the stomach and upper intestine, dilution by secretions and destruction by acid and proteolytic enzymes may to some extent protect the body against infection. In the present experiment the failure of large amounts

[3] In one control cynomolgus monkey fairly numerous infiltrative lesions were found in several of the peripheral ganglia. This animal had been kept for over 10 months in the same animal room with others that had been freely exposed to virus by enema, etc. The lesions resembled in all respects those found in animals treated with poliomyelitis virus. While it is possible and perhaps probable that this was an instance of "spontaneous" poliomyelitis infection, one must be guarded in concluding that lesions in the ganglia are necessarily due to poliomyelitis and not to some other neurotropic virus. In the present instance the prompt sequence of infection after mouth exposure, to poliomyelitis virus, the localizations of the lesions and the typical early lesions in the medulla are believed to make the diagnosis of poliomyelitis reasonably secure. McClure (personal communication) has also found lesions in the peripheral ganglia in rhesus monkeys not directly treated with poliomyelitic material.

of virus to infect the same animal when previously administered by stomach without mouth contamination gives some support to such a concept.

HAROLD K. FABER
ROSALIE J. SILVERBERG

A VIRUS OBTAINED FROM A PNEUMONIA OF CATS AND ITS POSSIBLE RELATION TO THE CAUSE OF ATYPICAL PNEUMONIA IN MAN

A RESPIRATORY tract infection in cats—variously called nasal catarrh, influenza or distemper—has been observed frequently within the past year or so in the Northeastern United States. The main characteristics of the disease are its highly infectious nature, debilitating effects and long course of about a month. Its respiratory nature is recognized by sneezing and coughing, which is accompanied by a mucopurulent discharge from the eyes and nose. The existence of a pneumonia is not determined by the usual clinical examination unless the animal is markedly affected, but an autopsy reveals grayish, densely consolidated areas in the anterior lobes of the lungs.

Suspensions of lungs from cats showing typical clinical symptoms and pneumonia were inoculated intranasally into mice. The mice became sick in the first passage, and those inoculated with two of the strains died in 3 to 5 days. In another attempt to isolate the agent, the mice appeared sick but survived the inoculation. At autopsy all inoculated mice presented a definite pneumonia with more than half the lung substance consolidated. Serial passage reduced the time interval to a point where death occurred in 2 to 3 days following the intranasal inoculation of a 10 per cent. suspension of infected lungs. Similar serial passages from uninoculated mice from the same source were entirely negative.

The agent was easily transferred to eggs, which had been incubated for 5 days, by inoculation into the yolk sac. The embryos died consistently within 2 to 3 days in serial passage, even when relatively large amounts of infectious material were inoculated.

When suspensions of lungs of inoculated mice or of yolk sac membranes of inoculated eggs were given intranasally to normal kittens the typical disease was produced. From these inoculated cats the disease went by contact to normal kittens.

Cultures from the lungs of naturally infected cats and of infected mice showed few bacteria and were frequently negative. All attempts failed to demonstrate a cultivable agent from infected eggs on blood agar plates and on a variety of special media designed for the culture of anaerobes and pleuropneumonia-like forms. These findings suggest that the agent is a virus, yet attempts to pass the agent through Berkefeld filters gave irregular results. The nature of the agent, however, became apparent when sections of the yolk sac membrane stained with Giemsa, or films from lungs of mice or yolk sac membrane treated by Machiavello's method, revealed numerous elementary bodies similar to those of psittacosis.

Centrifugation of infected mouse lungs and yolk sac suspensions at 10,000 r.p.m. for 30 minutes removed much of the infective agent from the supernatants and concentrated it in the sediments. This is added evidence that the observed elementary bodies are the etiological agent.

A number of instances of contact between sick cats and people who subsequently developed atypical pneumonia have been brought to our attention. For example, Dr. Francis G. Blake (personal communication), of Yale University, observed an atypical pneumonia in a rural family in Connecticut which occurred where cats were sick with a pneumonia. Dr. C. W. Barber, of the New York State Veterinary College, noted the reverse, where a child sick with atypical pneumonia played with a kitten that later became sick. It may be of epidemiological interest that the disease in man and in cats is occurring simultaneously.

Complement fixation tests have been made, using antigens of partially purified and concentrated elementary bodies prepared from infected mouse lungs. Sera obtained from cats before infection and again after they had recovered were tested. All the 6 cat sera obtained before infection failed to fix complement when 0.1 cc or less was mixed with 0.1 cc of the antigens. Using the same amount of antigen and testing at the same time, the convalescent cat sera fixed complement when from 0.02 to 0.0025 cc was used. Five sera drawn from man during the acute and convalescent stages of atypical pneumonia were obtained from Miss Catherine Grenci and Dr. Norman Moore, Cornell Infirmary, Ithaca, New York, and 7 more similar sera from Dr. Frank Horsfall, of the Hospital of the Rockefeller Institute. Eight of these sera drawn during the acute illness fixed complement; and the convalescent sera from 5 of these cases showed a definite increase in this property, while 3 showed a questionable increase and 4 no increase.

Sera from 9 presumably normal individuals were examined for controls. 0.1 cc of 2 specimens failed to fix complement, while the same amount of 4 others fixed more or less completely; 2 specimens fixed with 0.05 cc and 1 with 0.025 cc. As controls in this test, one serum drawn during the acute stage of the disease fixed in an amount of 0.0125 cc, while the convalescent serum fixed in $\frac{1}{4}$ this amount, or 0.0031 cc. Another serum drawn during the acute stage failed to fix when 0.1 cc was used, whereas 0.0125 cc of the convalescent serum fixed.