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Detection of a Healthy Carrier of Virus of Poliomyelitis Without History of Contact.*

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We present evidence that healthy carriers of the virus of poliomyelitis without history of contact and in the absence of an outbreak of the disease do exist and to discuss briefly the significance of this finding. The problem was undertaken in an effort to explain the widespread immunity in the normal population to this rare disease. A healthy carrier rate for diphtheria has been established, sufficient in extent to account for the widespread immunity to that disease. As extensive an immunity in the normal population is found in the case of poliomyelitis, a disease one hundred times as rare as diphtheria. It has seemed reasonable to assume that the mechanism involved in such widespread immunity was the same in the two diseases. Specific experimental evidence of the existence of such healthy carriers without history of contact has thus far been lacking.

The virus was recovered from the tonsils and adenoids of recovered cases,[1] and from the nasal washings of contacts and of mild illnesses occurring in close proximity to cases.[2-5] It has also been shown[6] that individuals exposed to the virus of poliomyelitis may develop immunity to the disease without evidence of illness. However individual contacts with cases fail to explain the widespread immunity that is found in the normal population to this disease.

Tonsils and adenoids were selected as the source of the virus, because it has been shown[1] that these portions of the mucous membrane can harbor the virus and because they were readily obtainable in large numbers.

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Through the coöperation of the nose and throat department of the Long Island College Hospital, the tonsils and adenoids removed in the weekly routine operations were collected within an hour following removal. Each set of tonsils and adenoids was placed in a separate container and identified by name of patient and hospital number, then set in the freezing compartment of a refrigerator and kept there until used; (seldom more than 12 to 24 hours). Part of each of the tonsils and adenoids was removed and the fragments, of 2 to 5 patients, were pooled, ground with sterile sand and made up in 10% suspension. The remainder of each of the tonsils and adenoids was placed in individual bottles containing 50% sterile glycerol, identified by name of patient and lot number of pooled tissue and stored in the ice chest. Part of the pooled suspension (about 5 cc.) was set aside for intranasal instillation. The remainder of the material was filtered through a Seitz pressure filter. This procedure usually yielded 20 to 50 cc. of a slightly red tinged sterile liquid.

Recognizing the difficulties in obtaining a primary "take" from this material, we utilized as many routes of inoculation as possible. Healthy monkeys were selected, bled for preliminary testing for the presence of neutralizing substance, and inoculated: (1) 1 to 2 cc. of the unfiltered material instilled into each nostril. (2) 2 cc. of the filtered material inoculated intracerebrally. (3) The remainder of the filtered material inoculated intraperitoneally. The animals were isolated, temperatures taken and observations of their behavior made daily.

The first animals were inoculated March 1934 and nothing unusual was observed in any of the animals until June 21st when monkey 107, inoculated with Lot 16, pooled from 5 individuals, showed elevated temperature to 105°F. On June 25th the temperature was 106°F. The following day the temperature dropped to 102°F., which was essentially the normal temperature of the animal. The animal was observed for other symptoms and except for a disinclination to climb, nothing unusual was observed. The animal was not used again and was observed for further symptoms. In the course of the next few months, the animal did not climb as well or as quickly as the other animals. A neutralization test October 6th, showed a prolonged incubation period. The control animal was prostrated in 8 days whereas the animal used for testing the serum of monkey 107 came down with a mild illness 16 days after inoculation. At this time the muscles of the right thigh were atrophied with beginning contracture of the leg on the thigh (the

right thigh was 2 cm. less in circumference than the left.) The animal was exsanguinated, quickly sacrificed with chloroform, the cord and brain exposed and sections taken from different levels for histologic study.

Attempts to Identify Source of the Virus. The remainder of the material from Lot 16 was ground separately with sand under sterile precautions in 10 to 50% suspensions (depending upon the amount of material available), centrifuged and inoculated unfiltered, intracerebrally, into each of 5 monkeys on November 22.

On November 27, monkey 234 inoculated with material from a child, V. R. 2 years old, showed an elevation of temperature to 106.2°F. The following day the temperature was 104.2°F. Cistern tap on that day showed 140 cells and a positive Pandy. The animal was sacrificed with chloroform. The brain and cord were moderately injected. No brain abscess was found. Sections were taken from the cord for histologic study, and the remainder was glycerolated. A portion of the cord was ground in 10% suspension and inoculated into 6 animals.

Monkey 371 inoculated on 12/20/34 with the cord suspension from monkey 234 showed an elevation of temperature on the 26th. The animal was sacrificed with chloroform; sections were taken for histologic study and part of the cord ground in 10% suspension and inoculated into 4 animals. The remainder of the cord was glycerolated and stored.

Monkey 391 inoculated 1/11/35 with suspension of cord 371, showed no marked elevation of temperature but on the 21st, definite weakness of the left shoulder was observed. This animal developed a severe diarrhea, which may account for absence of temperature response. Monkeys 234 and 371 appeared ill with the rise in temperature but no definite paralysis. Monkey 391 was the first animal to show paralysis although not extensive. The animal was sacrificed under chloroform anaesthesia. There was an unusual amount of injection of both cord and brain. Sections were taken for histologic study; part of the cord was ground into suspension for further passage and the remainder was placed in 50% sterile glycerine and stored.

Monkey 409 inoculated on January 21, with material from monkey 391 succumbed to frank poliomyelitis February 1, showing involvement of the left shoulder and partial paralysis of both lower extremities. The animal was sacrificed; sections removed for histologic study; part of the cord ground up for further passage and the remainder placed in 50% sterile glycerine and stored. At post-

mortem none of these animals showed any evidence of other illnesses.

Histologic Examination. Monkey 107. The following description is from an H & E stain of a section taken from the lower lumbar and sacral region. There is no meningitis. The pia is moderately hyperplastic. In the gray matter the following changes are observed: (a) A disproportion in the number of neurons in the anterior horns of both sides. (Figs. 1 and 2). (b) In the horns con-

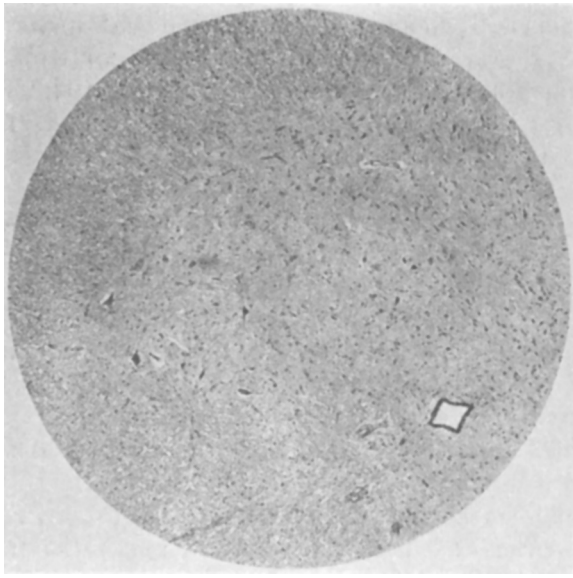

FIG. 1.

Monkey No. 107 H and E. High power of the anterior horn of the atrophied area, showing reduced number of neurons and evidence of degeneration in many of the remaining neurons.

taining the larger number of neurons there is abnormal staining with hemotoxylin. Some of the nuclei are pycnotic and in some cells the nuclei are absent. Some of the neurons show satalitosis and karyolysis. There are cell bodies in various stages of degeneration, almost to the stage of total disappearance. There is a moderate increase in the number of microglia and oligodendroglia cells. (c) In the opposite horn, the same changes occur but more of the neurons have undergone degeneration, to total disappearance. Remnants of cells devoid of nuclei and barely recognizable are seen throughout. The changes are therefore similar but more extreme than those observed in the opposite horn and fewer neurons have survived. (d) There are only moderate generalized infiltrations with lymphocytes,

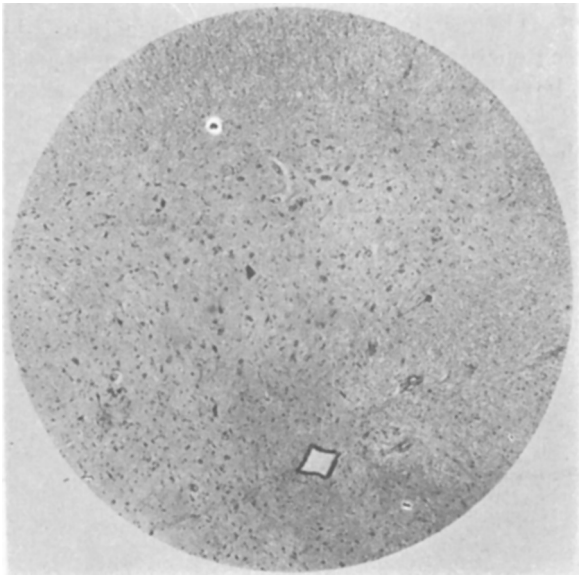

FIG. 2.

Monkey No. 107 H and E. High power of the opposite horn, showing numerous anterior horn neurons, many of which are in various stages of degeneration.

mononuclears and oligodendroglia cells. These changes are compatible with poliomyelitis.

Monkey 234. There is definite infiltration of the gray matter with polymorphonuclear leucocytes and lymphocytes in moderate numbers. Definite neuronophagia. Many of the neurons show chromatolysis, cytolysis and satalitosis. The entire gray matter appears oedematous. There is an increase in microglia and oligodendroglia cells. The pia is hyperplastic and there is a subarachnoid exudation of albuminous fluid and moderate numbers of polymorphonuclear leucocytes and lymphocytes, chiefly polys. There are no marked vascular changes.

Monkey 371. The pia shows local leucocytosis chiefly with polymorphonuclear leucocytes. There is a cellular increase throughout the gray matter chiefly with microglia and oligodendroglia cells. There are some polymorphonuclear leucocytes. The ganglion cells show moderate degenerative changes; chromatolysis, eccentric arrangement of the nucleii, cytolysis, satalitosis and neuronophagia. There are no extensive vascular changes.

Monkey 391. The pia shows intensive inflammatory reaction. There is extensive infiltration with lymphocytes, plasma cells and polymorphonuclear leucocytes. The gray matter shows extensive

infiltration. There is a marked microglial reaction. The ganglion cells show extensive cloudy swelling and neuronophagia. Many of these cells have been replaced with mononuclears. The blood vessels show extensive inflammatory reaction. There is infiltration with lymphocytes and plasma cells. (Fig. 3).

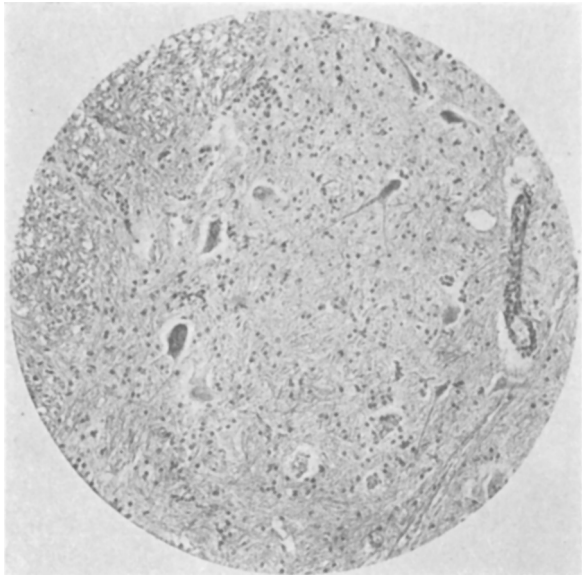

FIG. 3.
Monkey No. 391 H and E. Illustrating generalized infiltration, perivascular infiltration, and various stages of degeneration of anterior horn neurons.

Monkey 409. Presents essentially the same picture as Monkey 391. The last 4 animals give histologic evidence compatible with poliomyelitis, although the changes noted in the latter 2 are more progressive and definitely identified as poliomyelitis.

V. R., the 2-year-old child, whose tonsils and adenoids had been inoculated into Monkey 234, was investigated. She was a healthy girl whose tonsils had been ordered removed by the family physician because of frequent upper respiratory infections. The hospital record diagnosis was hypertrophied tonsils and adenoids. There was no history of contact with a case of poliomyelitis. 1934 was the year of lowest incidence on record for Brooklyn.

The finding of a healthy carrier, without any history of contact, in the normal population has, in our opinion, considerable significance. It perhaps points the way toward establishing the epidemiology of poliomyelitis on as firm a basis as the better known and more common disease, diphtheria.

The assumption of the existence of healthy carriers to explain the widespread immunity in the normal population to so rare a disease has been emphasized repeatedly. The finding of such a carrier strongly suggests that the mechanism involved in immunizing the general population is similar to that accepted for diphtheria. In the latter disease, to which about nine-tenths of normal urban adults are immune, it has been calculated that the number of healthy carriers of the diphtheria organism (variously estimated between ½ and 2%) is sufficient to saturate the population over a period of 20 years.

An equally large incidence of immunity is found in the adult urban population to poliomyelitis. In view of the relative rareness of poliomyelitis, offering fewer opportunities for contact with cases. it is more difficult to explain such extensive immunity than in the case of diphtheria. The presence of healthy carriers of the virus of poliomyelitis explains adequately the widespread immunity to this disease.

The frequency of such carriers cannot be stated. The difficulties involved in establishing a human strain of virus in the monkey are well known. It frequently requires 3, 4 or more inoculations to effect a primary "take" in the monkey with human material. In view of these difficulties, the single finding here reported, takes on added numerical significance. The determination of the actual carrier rate must take these factors into consideration and the final figure will probably be greater than the positive finding of the one in 156 or .64% of the normal individuals included in this report, although even this figure would be sufficient to fulfill the mathematical requirements to account for the widespread distribution of the virus, as well as immunity, in an urban population.

Summary. 1. Tonsil and adenoid tissue obtained from a healthy child 2 years old, without any history of contact, produced the experimental disease when inoculated into 2 animals. 2. One of the animals, Monkey 107, recovered with atrophy and contractures involving the right lower extremity. 3. The other animal sacrificed in the acute stages of the disease showed histologic evidence of poliomyelitis and the material from the cord of this monkey has produced the frank recognizable disease with paralysis in the third and fourth passages (Monkeys 391 and 409).

This investigation is being continued on a more extensive scale with the collaboration of Dr. M. Schaeffer of the Department of Bacteriology, New York University, with material obtained from the Jewish Hospital of Brooklyn. It is hoped that by including

other sections of the community, a more exact estimate of the carrier rate and the geographic and seasonal distributions can be made.

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Immunological Relationships of Strains of Filtrable Virus Recovered from Cases of Human Influenza.

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Smith, Andrewes and Laidlaw[1] isolated a filtrable virus from the nasopharyngeal washings of influenza patients following the inoculation of these materials into ferrets. Two strains of virus isolated by them in England during successive winters were found to be immunologically identical.[2] These same workers reported that the virus of swine influenza isolated by Shope[3] was antigenically related to the human strains.

During the Autumn of 1934, at the Hospital of the Rockefeller Institute, we were successful in infecting ferrets and mice with strains of a filtrable virus obtained from the sputum of cases of epidemic influenza in Puerto Rico.[4] These 2 strains have been called P. R. 5 and P. R. 8. Additional strains of virus have been isolated from cases of influenza in New York and Philadelphia. During the course of these experiments, Andrewes, Laidlaw and Smith[2] reported independently that they had successfully infected mice with the viruses of both swine and human influenza. They also reported that the serum of a hyperimmune horse, or of hyperimmune ferrets, neutralized the infectivity of the respective strains of virus.

The infection in mice, following the intranasal inoculation of the virus is characterized by the development of pulmonary lesions, but death of the animals is somewhat irregular. The serum of ferrets recovered from infection, when mixed with suspensions of the homologous strain of virus and instilled into the nasal passages of mice, has been found to prevent the development of these pulmonary lesions. The serum of normal ferrets, however, has no neutralizing

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