A PARALYTIC DISEASE OF GUINEA PIGS DUE TO THE TUBERCLE BACILLUS

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In a recent investigation in which a large number of tuberculous guinea pigs were under observation, a relatively high percentage of the animals became paralyzed early in the disease. These animals had each received subcutaneously 0.1 mg. of a culture of tubercle bacilli designated P. J. Out of 285 animals in the group between 30 and 40 manifested the paralysis. It had been noted previously among our tuberculous guinea pigs, but the cases had been so infrequent and scattered as not to attract serious consideration. In this particular experiment the disease complex made its appearance abruptly when the animals had been infected 2 months and, after new cases had developed for a period of about 10 days, it disappeared equally as abruptly. In considering the nature of the condition the most obvious possibility seemed to be that we were dealing with a tuberculous meningitis. The symptoms observed in our guinea pigs bore a close resemblance to those of experimental tuberculous meningitis of rabbits as described by Austrian (1), Kasahara (2) and Soper and Dworski (3) and of dogs as described by Manwaring (4). However, the occurrence of the malady in such a high proportion of animals, together with its abrupt onset and disappearance, made it seem essential to determine whether we were not dealing perhaps with some superimposed infectious disease of the central nervous system. Conceivably tubercle bacilli might be present and yet not be the essential cause of the paralytic condition. Because of this possibility it seemed advisable to maintain the paralytic disease for study by serial passage in guinea pigs. This was successfully accomplished by the intracerebral injection of emulsions of brain from animals showing the paralysis.

EXPERIMENTAL

There were three evident possibilities to consider in connection with the paralytic disease besides the tubercle bacillus. The first was infection with the filterable virus causing Roemer's (5) guinea pig paralysis; for the disease we were studying exhibited many points of similarity with that malady. A second possibility was that the animals had been infected with a low grade herpes virus at the time of inoculation, since the culture of tubercle bacilli used in this infection had been but quite recently isolated from human sputum. Third, the condition might have been an infection of the central nervous system by some visible organism other than the tubercle bacillus. The investigation was conducted with due reference to the points thus brought up.

The general plan followed in passing the paralytic condition from one animal to another was to remove the brain, under aseptic precautions, from a paralyzed guinea pig and after preparing an approximately 10 per cent emulsion of it in physiological salt solution to inject 0.1 cc. of the emulsion intracerebrally into a trephined normal animal.* The disease was maintained in this way through 9 successive groups of guinea pigs; and the clinical picture remained in all essential details the same throughout. The time elapsing between inoculation and the appearance of the first symptoms of illness varied from 8 to 27 days; and the tendency was for this period to shorten with each successive passage. The incubation period was remarkably constant for the animals of each passage group, however.

The disease as observed in guinea pigs infected experimentally was very uniform in its manifestations. For a period of 12 to 24 hours following the inoculation the animals appeared somewhat listless and disturbed, doubtless because of the procedure to which they had been submitted. Thereafter throughout the incubation period they remained bright, active and normal. The first symptoms noticed were a recurrence ot listlessness and some roughening of the fur. The animal sat quietly in its cage when not disturbed. There was usually some faulty coordination and the guinea pig had difficulty in getting to its feet when placed suddenly on the back. Very soon the incoordination became more marked. It was usually most noticeable in the hind quarters. Other symptoms referable to the central nervous system that were very frequently seen at this time were hyperesthesia, a tendency to move in circles, marked tremor, especially of the head, and a tendency to torticollis. A few animals showed a marked nystagmus. As a rule, the day following the onset of these symptoms a definite weakness of the hind quarters was evident, and there was often loss of sphincter control of both bladder and

* Ether anesthesia was practiced for all intracerebral inoculations.

rectum. This was followed very soon by a definite posterior paralysis, spastic at first and later flaccid. Very occasionally a paralysis of one or both fore legs preceded the posterior paralysis, and again, rarely, the condition resembled hemiplegia more than paraplegia. Following the early period of listlessness the animal appeared bright, and alert even when there was a complete posterior paralysis. One received the impression that the systemic reaction to the infection was very slight. Fig. 1 clearly illustrates the animals' state. As a rule, after 3 to 4 days of paralysis, its general condition suddenly became worse, it lost consciousness, the fur roughened, and, usually within 24 hours, death ensued. In 23 guinea pigs the disease was allowed to progress to a fatal termination and the average survival of these following the onset of the symptoms was 5.7 days, the extremes being 1 and 11 days. We had 6 animals die very suddenly, at the termination of

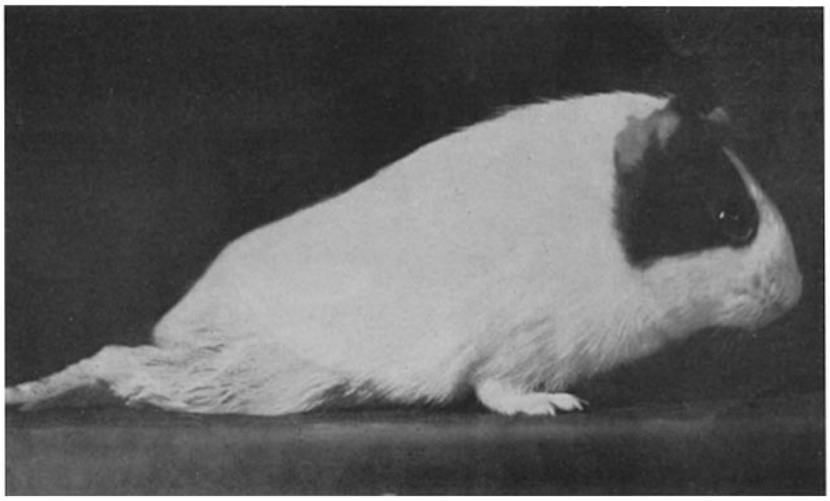

FIG. 1. Guinea Pig Y of the 7th serial passage showing complete posterior paralysis.

what corresponded to the incubation period in other animals of the same inoculation group, without having shown any symptoms whatsoever. No animal in our series that was inoculated with a fresh brain emulsion from either a "spontaneous" or experimental case was immune to infection. In animals living longer than 5 days after onset of the paralysis much loss of weight occurred. It took place, however, to some extent, in all of the animals, beginning late in the period of incubation. In a few, the onset of symptoms referable to the central nervous system was preceded by a very evident loss of weight. At no time was there any febrile reaction to the infection, although when the animal became moribund, the temperature occasionally became subnormal.

Pathology.—In animals in which the condition had been induced by the intracerebral injection of brain emulsion from a previous case a few macroscopic changes were consistently found at autopsy. On opening the calvarium an excess of cerebrospinal fluid was always encountered. This was usually slightly turbid. Nothing abnormal was evident in the brain itself, nor was there anything characteristic along the needle track. Rarely the meninges were adherent to the skull at the trephine region. They were usually somewhat thickened over the area of the mid-brain, pons and medulla at the base of the skull. Examination of the cord revealed no gross lesions.

Outside the central nervous system the only evidence of pathological alteration was found in the spleen. This was usually somewhat larger than normal and was studded with hyperplastic follicles.

Microscopic examination of paraffin sections of brain stained by the usual methods showed a great abundance of acid-fast bacilli in the thickened pia mater but none elsewhere. The condition was found to be a true meningitis with some extension into the encephalon along the lines of the larger blood vessels but elsewhere the encephalon was singularly free from infiltration or other lesions. The pia mater, especially at the base of the brain, was definitely, but not extremely, thickened. The exudate consisted of mononuclear cells, some of them lymphocytes, but large mononuclears chiefly. Necroses were lacking and giant cells and definite tubercle formation were not seen.

Bacteriology.—The brain emulsions as prepared for inoculation were examined for tubercle bacilli and other organisms. In the first of these emulsions to be studied tubercle bacilli were easily demonstrated by the Gabbet method, but in the material similarly prepared from later passages this was no longer possible. Many of the examinations were casual but some were prolonged and thorough.

Cultures of these brain emulsions were made on various media. No growth was obtained in any case on plain or blood agar. On Dorset's egg medium growth of typical tubercle bacilli was quite regularly obtained and these even from specimens in which no tubercle bacilli had been demonstrable in stained smear preparations. Cultures of brain emulsion in a modified Noguchi Leptospira medium (guinea pig serum was substituted for rabbit serum) showed an opalescent clouding of the upper portion of the medium after incubation at 37.5°C. for 10 days to 2 weeks. Later a granular sediment appeared. The upper growth was found on examination to be a pleomorphic Gram negative bacillus. It was non-acid fast by the ordinary Ziehl-Neelsen technique, but when decolorized after the method of Gabbet showed some acid-fast forms. The granular sediment was entirely non-acid-fast. Such cultures were recovered from nine different guinea pigs,—all that were so cultured. After 3 passages on media, guinea pigs were inoculated intracerebrally, some with the upper, diffuse growth and others with the sedimentary growth from one of these cultures. All developed the typical posterior paralysis with its accompanying symptoms. Animals inoculated subcutaneously with the upper portion of the culture developed within 4 days nodules the size of a

split pea at the site of inoculation. This was followed very soon by the appearance of large, shotty, inguinal lymph nodes. The nodules at the site of inoculation were discharging a cheesy pus within 2 weeks, and numerous organisms similar to those that had been grown in Noguchi media were found in this caseous material. Autopsy of these animals 2 months later revealed the presence of lesions typically encountered in guinea pig tuberculosis. The organism isolated and cultured in Noguchi medium was obviously a tubercle bacillus despite its atypical staining reactions.

Susceptibility of Rabbits.—Recourse was had to inoculations into rabbits in order to determine whether we were dealing with a mild form of herpes encephalitis, or with Roemer's virus which is described as specific for guinea pigs. Two rabbits were inoculated intracerebrally with brain emulsion from guinea pigs of the 3rd and 7th experimental serial passages. The animals developed a typical posterior paralysis with loss of bladder and rectal sphincter control, after incubation periods of 25 and 39 days respectively. There was nothing in their disease to suggest herpes encephalitis. Brain emulsions from these animals injected intracerebrally produced the disease in a second series of rabbits.

Non-Filtrability of the Infectious Agent.—Five Berkefeld N filtrates of brain emulsions from experimentally infected guinea pigs have failed to produce the disease when injected intracerebrally into 16 guinea pigs and 1 rabbit. Three of these filtrates were prepared by merely grinding the brain with sterile sand and salt solution in a mortar and then filtering; one was prepared by grinding in the same way, centrifuging the material, and passing it through cotton and paper before filtration; and in one, after the grinding, the preparation was shaken for half an hour with sand and glass beads and filtered first through cotton and paper and then through a Berkefeld N filter.

DISCUSSION AND SUMMARY

The experimental data collected during this study of a transmissible type of paralysis developing in tuberculous guinea pigs indicate the condition to be a true tuberculous meningitis. We have been able to rule out the possibility that it is due to a non-tuberculous infection of the central nervous system caused by Roemer's virus, or by an atypical herpes virus, or by some bacterium other than the tubercle bacillus. Roemer's virus and herpes could be eliminated from considera-

tion when Berkefeld N filtrates of infectious brain emulsions proved incapable of reproducing the disease. Furthermore, rabbits could be infected as they cannot with Roemer's virus, and the disease elicited in rabbits bears no semblance to herpes encephalitis. No organism other than the tubercle bacillus could be obtained on culturing brain or brain emulsions from experimental cases, and no others were seen in examining fresh smear preparations from the central nervous system. In a modified Noguchi medium a tubercle bacillus possessing atypical staining properties was obtained. This organism was capable of producing the typical paralytic disease when injected intracerebrally into guinea pigs, and also generalized tuberculosis in animals inoculated subcutaneously with it.

Typical tubercle bacilli were readily demonstrable in sections of the meninges from animals with the disease, and culture of pieces of brain on Dorset's egg medium usually yielded a growth of tubercle bacilli. Only in the first of the experimental passages, on the other hand, was it possible to demonstrate acid-fast organisms in fresh smear preparations from the central nervous system. This fact and the attributes of the atypically staining organisms encountered in the cultures in Noguchi media will be considered more fully in a subsequent publication.

In view of the much discussed question of the filtrability of the tubercle bacillus our observations concerning the failure of this organism to pass a Berkefeld N filter are of interest. No animal in our series inoculated intracerebrally with brain emulsion from either a "spontaneous" or experimental case of tuberculous meningitis failed to develop meningitis, and that rather acutely, while no animal in our series injected with a Berkefeld filtrate of brain emulsion has developed tuberculous meningitis or any other form of tuberculosis. In connection with this observation it must be recalled that the organism was atypical in respect to its staining qualities at least.

BIBLIOGRAPHY

1. Austrian, C. R., *Johns Hopkins Hosp. Bull.*, 1916, **27**, 237.
2. Kasahara, M., *Amer. J. Dis. Child.*, 1924, **27**, 428.
3. Soper, W. B., and Dworski, M., *Am. Rev. Tuberc.*, 1925, **11**, 200.
4. Manwaring, W. H., *J. Exp. Med.*, 1912, **15**, 1.
5. Roemer, P. H., *Deut. med. Wochschr.*, 1911, **1**, 1209.