STUDIED UPON EPIDEMIC PARALYSIS AMONG GUINEA PIGS

by

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INTRODUCTION

Epidemics occurring among labratory animals are of considerable importance to bacteriologists, because of their direct influence upon the interpretation of experimental work. Guinea pigs are perhaps particularly susceptable to epidemic diseases characterized by paralysis. Romer (1910)[1] reported tha t labratory guinea pigs died of an epidemic paralytic disease which resembled acute anterior poliomyelitis in man. He reported the etiological a gent to be a filterable virus. The disease had an incubation period of nine to twelve days.

Bally, working at the University of Kansas, in 1921 describes a disease which is very similar to that mentioned by Romer. Bally reports that the disease is characterized by a loss of appetite, gradual decrease in weight, slight va riation in temperature, emaciation, diarrhea, general tremor, and indisposition to any movement except when forced from place to place. He states that the average time which elapsed between the onset of any noticeable symptoms and death was four to five days. The initial symptoms were closely followed by flaccid paralysis of the hind limbs. At first when the animal was disturbed would try to move by pulling itself along with the front

feet, but in the advanced stage the animal would fall over on one side, unable to right itself.

Bally describes the lesions found on autopsy to be as follows; lungs, hardened and congested; small bile stained area s throughout the liver; considerable gas in the small intestine. The kidneys, spleen, and suprarenals were usually normal. There were extensive hemorrhagic areas throughout the axillary and inguinal lymph nodes and the adjoining tissues. The presence of the hemorrhagic lymph nodes wa s the most characteristic finding. Sometimes the spleen was enlarged.

Another outbreak of this disease occurred at the University of Kansas in 1927, which has provided the material for the present study. The disease is readily transmitted from one guinea pig to another. Bally reports a guinea pig which had contracted the disease by being e xposed in the same cage with a sick guinea pig for a period of ten minutes.

Bally reports that the disease can be transferred by the injection of the filtrates of emulsions of brain or spinal cord of guinea pigs which have died of the disease. The likeness of the disease to acute anterior poliomyelitis in the human, makes it desirable to study the etiology of the latter disease.

Acute poliomyelitis is an acute, specific, infectious, febrile, disease which occurs in human beings both

sporadically and epidemically. A similar condition may be produced in labratory animals by the injection of the pathogenic virus. Acute poliomyelitis is characterized by widespread lesions in the nervous system, with special localiza tion in the anterior horns of the spinal cord. It is particularly incident in children, although no age is exempt. Heredity and sex are not predisposing factors. Carriers are probably common. The disease was first described in 1840 by von Heine. Badhem in 1835 describes a few cases of paralysis following an acute onset. In 1865 Prevoct and Vulpian studied the lesions in the anterior horn, and attributed them to infantile paralysis.

The incubation period is one to four days. There is a sudden onset characterized by feverishness. An apparently well child will have a fever of 101-106° and have pain in the limbs and joints. In a couple of days the paralysis sets in abruptly, reaching a climax in 24 hours. The paralysis usually localizes in the muscles of one limb, otherwise the child regains health. The mortality rate is 25-37%.

That the organisms causing poliomyelitis are capable of passing through a berkfeld filter, was shown by Landsteiner and Levaditi[2], and Flexner and Lewis[3]. Flexner and Nougouchi[4] report cultivation of the virus

from nervous tissues and from the filtrates of nervous tissue from patients and monkeys dead of the disease. They used sterile rabbit kidney ascetic fluid media. They were unable to obtain good or consistant results with any other media for the first few generations. Heating the media renders it unfit for use. They cultivated the organisms anaerobically for 7-12 days, when there would appear a slight opalescence around the fragments of kidney and nervous tissue in the bottom of the tube. A fragment of brain material was found to give better results than an emulsion. Glycerinated tissues gave less consistant results than did fresh ones. The organisms seem to have no effect on glucose, sucrose, lactose, maltose, dulcite, galactose, levulose, mannite, glycogen, dextrin, inulin, arabinose, or litmus milk. The darkfeild showed globoid bodies .15 to .3 microns in diameter, in chains, pairs, or small masses. They can be stained with wither Gram's or Giemsa's stains. Flexner and Nougouchi describe them a s being Gram variable. The globoid bodies have apparrently fulfilled Koch's postulates. The disease can be transferred to monkeys with the cultures and the organisms obtained again in pure culture. The virulence seems to run down on sucessive transfer in culture. Amoss[5] reports that the cultures will produce the disease as long as thirteen months after isolation and from the twentieth transfer. It will be remembered that the virus

is fully virulent after six years in 50% glycerol. Failure has often resulted in attempts to isolate the globoid bodies, even when using Nougouchi's technic. The globoid bodies have been observed directly in the nervous tissue of subjects of the disease. Cultures ha ve been obtained from the blood stream.

Typical poliomyelitis has been produced in monkeys by repeated injections of the globoid bodies when one injection was insufficient. This is characteristic of several of the filterable virus diseases.

Amoss[5] reports that he was unable to get satisfactory complement fixation using globoid bodies as antigen, and anti-globoid body serum. He occasionally got fixation when monkeys were used. Convalescent serum will not fix complement with golboid bodies, but neither will it fix complement with tissues from poliomyelitis cases. The growing of the globoid bodies in antisera or in convalescent serum causes a clumping of the organisms.

The identity of the etiological factor of poliomyelitis is very much disputed. Rosenow[6], Mathers[7], Nazum and Herzog[8], and others maintain that a pleomorphic streptococci is concerned in the disease. Rosenow reports that he has shown that the specific localizing power of various bacteria is an important factor in the etiology of various diseases, including diseases of the nervous

system. He first reported finding a neurotropic streptoccus in poliomyelitis in 1916. A certain pleomorphic streptococcus was isolated from the tonsils in a large number of cases, and the same streptococcus has been reported to have been found in all of the twelve cases of poliomyelitis in the brain. This organism shows a greenish colony on blood agar with a narrow zone of hemolysis. The filtrates of the streptococci would grow on suitable media. Rosenow and coworkers[9] report that the organism usually loses its pathogenicity on cultivation and upon successive animal passage. They report as evidence the production of paralysis in dogs, rabbits, guinea pigs, cats, and monkeys by the injection of this streptococci.

Sherwood and Downs[10] were able to produce paralysis in rabbits by the injection of a pleomorphic streptococci isolated from normal throats. They also showed these streptococci to be immunologically similar to those claimed by Rosenow to be the cause of poliomyelitis. Bull[11] was unable to get paralysis in animals using pleomorphic streptocci isolated from throats of patients having poliomyelitis. He also reports failure to establish any relationship between these streptococci and the filterable virus of poliomyelitis. He also reports that the sooner that the brain is removed after death, and the greater the

care used in removing it the less chance there is to find streptococci.

Na zum and Herzog[8] also contending that a pleomorphic streptococci was th e etiological factor isolated an organism which resisted glycerin for thirty five days. It was a filter passer. 55° C. for 30 minutes would kill it.

Bulow-Hansen, Harbitz, and Scheel[12], and Giersvold, isolated a Gram positive diplococcus in the cerebrospinal fluid of several cases. Giersvold claims to have produced paralysis and death in animals by the imjection of them. Looft and Dethloft in Norway report the isolation of a meningococcus-like organism from the cerebrospinal fluid of two cases.

Pasteur, Foulerton, and Maccormac reported finding a micrococcus in the cerebrospinal fluid of several cases of poliomyelitis during life, which upon injection into labratory animals would produce typical symptoms and paralysis. Kolmer, Brown, and Freese[13] in culturing the cerebrospinal fluids of many patients reported 15% sterile, no streptococci, 50% diplococci, 20% Gram negative bacillus, 20% diphtheroids, and 20% of their cultures contained Bacillus subtilus as a contamination. All animal innoculations gave negative results.

In summing up the literature on the subject of the etiological factor of poliomyelitis it seems necessary

to state that although the matter is very unsettled, the largest amount of evidence seems to indicate that the organism is a filter-passer, and possibly it is the globoid bodies of Flexner and Nougouchi.

The outstanding points in favor of the globoid bodies are, first, the globoid bodies are found in cultures of poliomyelitic material, second, the injection of the cultures into monkeys gives rise to poliomyelitic-like symptoms, although often no symptoms are obtained, third, they are reported to show chain formation or clumping when grown in convalescent serum, and fourth, the globoid bodies have been reported found directly in poliomyelitic cord.

The main points against the globoid organisms are, first, they have been found only in a small percentage of the cases examined by Flexner and Nougouchi, and in only a few of the many cultures examined, second, the cultures are filterable only with difficulty, while the virus passes the finest filters with comparative ease, third, their cultures have not been found to produce antibodies and give no protection to monkeys while the virus protects the animal, fourth, the human virus through animal passage increases in virulence, which has not been shown to be the case with the culture virus[49], and fifth, the injection of fresh normal tissue of several kinds will give rise to paralysis in animals.

There is enough fresh kidney tissue in the cultures that this possibility must not be overlooked.

Although the globoid bodies have the abundance of experimental evidence in their favor, it is very doubtful if they are related to the disease.

There are several outstanding similarities between poliomyelitis and the epidemic paralysis of guinea pigs. The relation between the etiologies of the two diseases, is of course obvious, both being caused by a filterable virus. Bally reports that when using sterile rabbit kidney rabbit serum media he was able to cultivate the etiological factor of the epidemic paralysis of guinea pigs, and observed in his cultures clumps similar to those reported by Flexner and Mougouchi as globoid bodies. Bally reports the production of the typical disease in guinea pigs following the injection of cultures of the virus, however he did not get paralysis with the guinea pigs so treated.

The two organisms are very resistant to disinfectants. The virus of poliomyelitis is reported to have survived in glycerol for six years[14]. Others[15,16] have also noticed the resistance of the virus. It resists .5% phenol for at least a year, drying over KOH for a month or more, drying over H_2SO_4 for 50 days, freezing for several months, and bile for long periods of time.

Romer, Flexner, and Lewis, Leiner and von Weisner, and Levaditi have noted that the virus heated to 55° C. has no immunizing power. The virus of epidemic paralysis of guinea pigs has been observed to be fully virulent after a year in 50% glycerol at ice box temperature.

The characteristic finding of hemorrhagic lymph nodes in guinea pigs on autopsy reminds us of the work of Flexner and Lewis[17] showing the infection of monkeys with a human mesenteric lymph node.

Since the epidemiology of the two diseases seem to closely resemble each other, it would be well to reveiw what is known of the mode of infection in poliomyelitis.

The organism is probably transferred through direct contact and infection through the nasal passages. It is found easy to innoculate monkeys by application of the virus to the unbroken nasal mucosa of monkeys[18,19]. This method is much more effective than innoculation through the blood stream. It is thought that the virus goes to the brain through the olfactory nerve, since the severing of the nerve just before innoculating the virus on the nasal mucosa prevents the infection. The virus has been found on the nasal mucosa of patients and contacts so it is not thought to be illogical that this is the normal mode of infection.

It is found that it takes a much larger amount of the virus to infect by way of the blood stream, than by the nasal mucosa, for the protective influence of the choroid plexus must be overcome following the intravenous injection before the virus can reach the brain. Flexner and Lewis[20] report that if it takes one dose of the virus to produce infection through the nasal mucosa it will take 1,250 doses to produce infection through the blood stream. The incubation period is also lenghtened from six to seventeen days. Aftern entering the brain, the virus affects first the brain, medualla and then the spinal cord.

Flexner, Clark, and Amoss[21] are of the opinion that epidemics occur when a more virulent strain arises and remains virulent for a time. Flexner and Amoss[22] report a strain of poliomyelitis virus which they obtained in 1909. It rose in virulence very quickly in transfer through monkeys, then for several years it was relatively non-virulent, regaining its virulence again after several yea rs, being transferred through monkeys. This resembles the virus III of rabbits reported by Rivers[29]

The reports regarding the passage of acute poliomyelitis to animals other than monkeys vary considerably. Krause and Meinicke reported the passage of a strain through seven generations of rabbits. Lentz and Huntemuller

transferred it through rabbits by various means. They reported that the lesions found in the brains of rabbits were slight compared to those found in man. Marks obtained some striking results by transferring the virus through seven generations of rabbits. He reports that the disease in rabbits cannot be identified with that in man. They died in convulsions but had no paralysis. His animals were from 350-550 grams in weight.

Romer and Joseph, Landsteiner and Levaditi, Leiner and von Weisner, and Flexner all report failure to infect rabbits.

Neustaedter[23] reported the passage of the disease from monkeys to guinea pigs and back to monkeys.

Rosenow reports the passage of a strain through seven generations of rabbits. Young rabbits were much more susceptable. The incubation period for the rabbits was from 2 to 41 days. Rosenow reports two types of the disease in rabbits, a rapid, and a slow progressive type.

Nevin and Rittman[24] also infected rabbits noting the same results as given by Rosenow. Jemma[25] reported the passage of poliomyelitis to rabbits. The rabbits had convulsions, tremors, paralysis, and frequently died.

It can be readily seen by the work already mentioned that more knowledge is needed concerning the etiology and immunology of the guinea pig paralysis.

The objectives in mind when the present work was started a re,

1.More knowledge concerning the specific organism.

2.Attempt to actively immunize guinea pigs against the epidemic paralysis.

3.Attempt to passively immunize guinea pigs.

TECHNIC

1. METHOD OF REMOVING THE BRAINS AND SPINAL CORDS FROM GUINEA PIGS FOR FURTHUR USE.

The animal used was etherized and thonoughly washed with mercuric chloride 1-1,000. The skin was cut on the mid-ventral line and laid back. The internal organs were then removed with sterile instriments. The vertebre were cut longitudinally with sterile scissors and the cord lifted out and placed in a dessicating jar, or in sterile 50% glycerine as desired. The animal was then turned over, the head skinned and the brain removed, observing the same precautions to preserve sterility. The instriments were sterilized in 10% phenol in alchol, and rinsed in alchol before using.

2. METHOD OF TREATING AND PRESERVING THE BRAINS AND CORDS.

One cord was treated with .5% formaldehyde for 24 hours and then dried over KOH for 21 days and placed

in 50% glycerine at ice box temperature. Other cords were placed directly into 50% glycerine. Still other cords were used directly without treatment. The individual method will be given for each cord under Data.

3. TECHNIC OF PREPARING EMULSIONS FOR INJECTION.

The fragments of the nervous material, about an eighthof an inch cube, were ground in sterile saline in a sterile test tube with a sterile glass rod. The nervous material was allowed to settle. Some of the supernatant fluid was tested for sterility by innoculation into broth, blood agar, agar slants, and dextrose broth, and culturing both aerobically and anaerobically. The cultures were always sterile, ruling out any commonly known contamination. The supernatant fluid was then used for the innoculation.

4. TECHNIC OF INJECTIONS.

(a). Intracerebral. The skin on the forehead of the guinea pig was well clipped and washed with alchol. The skin was cut in the mid line and pulled gently to one side. A small hole was bored through the skull with the point of a scalpel. The material was then injected into the region of the ventricles. The skin was replaced and sealed with collodion.

(b). Intraocular. A fine needle was used. The eye was anaesthesized with cocaine. The material was

injected into the aqueous humor by entering at the edge of the iris. The eye would recover within several days so that it would appear normal.

(c). Intradermal. The hair was clipped and the area washed with alchol. The injections were made in small amounts and in many places to spread out the material. The details a re recorded with each guinea pig.

5. TECHNIC OF SECURING TEMPERATURES.

The thermometer was inserted into the rectum to the 95° F. mark and retained in place for 35 to 40 seconds. This was found to give the most consistant results. There wa s very little variation found between these results and those obtained if the thermometer was left in place for a longer time. All temperatures of the animals wore recorded in the fareinheit scale.

DATA AND RESULTS

1. HOURLY VARIATIONS IN A GUINEA PIGS TEMPERATURE.

The temperatures were taken of normal guinea pigs at 2 P.M., 6 P.M., and 8 P.M. The variations were found to be slight, possibly due to changes in ventilation, activity, sunlight, or food. A few typical temperatures are as follows; pig no. 52, 102.3, 102.2, and 102.5; pig no. 50, 102.7, 102.3, and 102.4; pig no. 51, 102.7, 102.7, and 102.6; pig no. 48, 102.3, 102.1, and 102.4; and pig no. 45, 102.8, 102.6, and 102.9. The average temperatures taken for twelve guinea pigs for these three hours wa s the same for all three hours, namely 102.6, 102.6, and 102.6. This shows that the hourly variation in a guinea pigs temperature is usually less than three tenths of a degree, and that the particular hour of the day seems to have little or no constant effect upon it. This conclusion seems to be justified by the results of many guinea pig temperatures taken over long periods of time, upon many guinea pigs from 6 A.M. to 11 P.M.

2. VARIATIONS IN A GUINEA PIGS TEMPERATURE OVER LONG PERIODS OF TIME.

By averaging the temperatures of guinea pigs over periods of a week or more the variations found to exist

for different hours of the day are lost, and a normal guinea pig is found to have a definite temperature curve. The temperatures of three typical normal guinea pigs are assfollows; pig no. 37, av. temp. 1/8/29-2/5/29 102.1, 2/25/29-3/21/29 102.4, and 3/22/29-4/14/29 102.5; pig no. 47 for three similar periods, 101.6, 103. and 102.8; pig no. 52, 101.9, 103. and 102.7. A normal guinea pig uniformally has a rise in temperature the first week it is received.

There is a sharp contrast between the temperatures of normal guinea pigs and those innoculated with cord taken from guinea pigs having epidemic paralysis, and with the temperatures of animals innoculated with normal nervous material.

Guinea pigs which have contracted the epidemic paralysis, either by contact or innoculation, show a characteristic drop in temperature the last week before death.

Guinea pig no. 5. Injected intracerebrally 3/31/28 with an emulsion of cord from guinea pig no. 101., dead of paralysis. The cord had been dessicated for 14 days over KOH before placing in 50% glycerine. Average temp.,
4/1/28-4/3/28 101.3
4/4/28-4/8/28 100.4 Diarrhea, paralysis, fur ruffled.
4/9/28 Dead. Autopsy revealed hemorrhagic lymph nodes.

Guinea pig no. 6. Injected similarly to no. 5

4/1/28-4/13/28 102.2

4/14/28-4/27/28 100.9 Diarrhea and paralysis.

4/29/28 Dead. Autopsy revealed hemorrhagic lymph nodes.

Guinea pig no. 7. Injected similarly to no.5.

4/2/28-4/13/28 101.6

4/14/28-5/10/28 101.2 Diarrhea and paralysis.

5/11/28 98.6 Very sick. Unable to right itself.

5/12/28 Dead. Autopsy revealed hemorrhagic lymph nodes.

Guinea pig no. 2 Injected intraocularly with an emulsion of cord from guinea pig no. 161. 3/19/28.

3/20/28-4/1/28 101.8

4/2/28-4/17/28 101.4

4/18/28-4/23/28 98.5 Diarrhea and paralysis. The final temperature was 94. Autopsy revealed hemorrhagic lymph nodes.

Guinea pig no. 4. Injected similarly to no. 2.

3/20/28-4/1/28 102.

4/2/28-4/10/28 102.2

4/11/28-4/17/28 101. Diarrhea, fur ruffled, inactive.

4/18/28 Dead. Autopsy revealed hemorrhagic lymph nodes.

Out of fifteen guinea pigs observed with the epidemic paralysis not one had a final temperature higher than the average for the few weeks preceeding. Most showed a characteristic drop of from .1 to 3.3 degrees. One showed

no lowering of tempera ture. One guinea pig which showed recovery had a rise in temperature upon recovery.

Guinea pig no. 1. Injected similarly to no. 2.

3/19/28-4/1/28 101.6

4/2/28-4/19/28 101.3

4/20/28-4/27/28 100.7 Diarrhea, trembles, and was weak.

4/30/28-5/10/28 101.1 Recovery with rise in temperature.

Of the guinea pigs receiving injections of normal cord, ten of thirteen succumbed, showing a rise in temperature the last week of life. This was just as characteristic as the drop in temperature noticed in the paralyzed pigs. A few typical protocols follow.

Guinea pig no. 12. Injected with spinal cord emulsion from normal guinea pig no. A. 4/17/28.

4/18/28-4/22/28 102.7

4/23/28-4/26/28 103.5 Fur ruffled, inactive and complains.

4/27/28 Found dead. Autopsy revealed hemorrhagic lymph nodes. Other organs were normal.

Guinea pig no. 15. Injected with an emulsion of spinal cord from guinea pig no. A. 4/17/28.

4/18/28-4/24/28 101.5

4/25/28-5/9/28 101.8 Fur ruffled, diarrhea, inactive.

5/10/28 Dead. Autopsy showed hemorrhagic lymph nodes.

Guinea pig no. 16. Injected similarly to no. 15.

4/18/28-4/30/28 102.4

5/1/28-5/14/28 102.7 Fur ruffled, diarrhea, inactive. 5/15/28 Dead. Autopsy revealed hemorrhagic lymph nodes. Pale and mottled liver. Red fluid in the pleural cavity.

Guinea pig no. 17. Injected with an emulsion of cord intracerebrally with cord from no. B. 10/8/28.

10/9/28-10/22/28 102.4

10/23/28-10/30/28 102.6 Diarrhea, fur ruffled, inactive.

10/31/28 Dead. Hemorrhagic lymph nodes.

Guinea pig no. 18. Injected similarly to no. 17.

10/9/28-10/22/28 102.4

10/23/28-10/31/28 102.6

11/2/28 Injected .2 cc. emulsion of normal cord as before. Cord also from normal guinea pig no. B.

11/4/28-11/12/28 103.7

11/13/28 Dead. Hemorrhagic lymph nodes. Liver mottled. Bloody fluid in pleural cavity. Heart's blood failed to show growth on blood agar, plain broth, plain agar or dextrose broth within three days at 37° C.

Of the thirteen guinea pigs innoculated with emulsions of glycerinated normal guinea pig cord, three did not die, but lived an apparrently normal life. These three were all injected with a third cord from guinea pig no. C. This guinea pig had no connection with the other guinea pigs which were sacrificed for normal cords. This guinea

pig, no. C, also provided the normal cord for the injection of the three animals which were innoculated with fresh unglycerinated normal cord.

The protocols of the temperatures of the guinea pigs innoculated with emulsions of glycerinated normal cord, and which did not die are given here. All were injected with cord from guinea pig no. C.

Guinea pig no. 45.

2/25/29-3/11/29 101.7

3/11/29 Injected with .15 cc. glycerinated normal cord emulsion intracerebrally from guinea pig no. C.

3/12/29-4/3/29 102.5

4/4/29-4/14/29 102.6 No clinical symptoms were observed. There was no loss of weight.

Guinea pig no. 46.

2/25/29-3/11/29 101.4

3/11/29 Injected similarly to no. 45.

3/12/29-4/3/29 102.6

4/4/29-4/14/29 102.9 No clinical symptoms of disease.

Guinea pig no. 49.

2/25/29-3/11/29 101.7

3/11/29 Injected like no. 45.

3/12/29-4/3/29 103.

4/4/29-4/14/29 102.3 No symptoms of disease.

We notice that this series not only failed to succumb to the injections, but failed to have as noticeable or characteristic rise in temperature as those which were injected with the other two normal cords.

The three guinea pigs innoculated intracerebrally with fresh normal guinea pig cord from pig no. C. gave much the same results. Their protocols follow.

Guinea pig no. 42. Injected with an emulsion of fresh unglycerinated normal cord in saline. 3/3/29.

2/25/29-3/12/29	102.
3/13/29-4/ 3/29	103.1
4/4/29-4/14/29	103.2

Guinea pig no. 43. Injected similarly to no. 42.

2/25/29-3/12/29	101.5
3/13/29-4/3/29	103.2
4/4/29-4/14/29	103.6

Guinea pig no. 48. Injected similarly to no. 42.

2/25/29-3/12/29	101.9
3/13/29-4/3/29	102.5
4/4/29-4/14/29	102.3

None of these guinea pigs showed any clinical symptoms of disease.

Three guinea pigs were innoculated intracerebrally with a sloution of glycerine in saline as a control, to

see the effect if any of glycerine on guinea pigs when innoculated in this manner. Their protocols follow.

Guinea pig no. 41.

1/8/29-3/3/29 102.4

3/3/29 Injected with a solution of glycerine and saline.

3/4/29-3/21/29 102.2

3/24/29-4/14/29 102.3

Guinea pig no. 50. Injected with glycerine 3/3/29.

2/25/29-3/12/29 102.2

3/13/29-4/3/29 102.8

4/4/29-4/14/29 102.4

Guinea pig no.51. Injected similarly to no. 50.

2/25/29-3/12/29 101.9

3/13/29-4/3/29 102.9

4/4/29-4/14/29 102.5

While those guinea pigs which were innoculated with normal cord showed a rise in temperature whether they died or not, those innoculated with glycerine and saline showed no such rise. This would indicate that the clinical symptoms and temperature changes were due to the nervous material and not to the glycerine, or to the effect of the glycerine on the cord.

3. HOURLY VARIATIONS IN A GUINEA PIGS WEIGHT.

Weights were taken for guinea pigs at three different hours of the day, before and after feeding, to determine the difference in weight if present. The weight was found to vary only a few grams and not to be sufficient to cause an error in conclusions. Guinea pigs seem to eat intermittantly at short intervals.

4. VARIATIONS IN A GUINEA PIGS WEIGHT OVER PERIODS OF TIME.

The weights of guinea pigs tell little except as an index of the general health of the pig. If the weight keeps increasing, there is little danger of the animals dying. All guinea pigs which died from any cause lost weight contionously for some time before death. Several hundred grams would be lost in the course of several weeks. Since there is nothing characteristic about the loss, the protocols of the weight will not be given. The hourly variation wa s so slight as not to materially a ffect the amount lost or gained in a day.

5. CLINICAL SYMPTOMS OF CONTROLS.

Those guinea pigs which died following the injection of glycerinated normal cord showed clinical symptoms the same as those which had contracted the epidemic paralysis, except for the difference in temperatures. The guinea pigs

with the epidemic paralysis usually showed marked paralysis during the 24 hours preceeding death. The guinea pigs innoculated with the glycerinated normal cord sometimes were very inactive and occassionally paralyzed. Ruffled fur, diarrhea, caxchexia, emaciation, and loss of appetite were constant findings in all of these sick guinea pigs.

6. AUTOPSY FINDINGS IN GUINEA PIGS INJECTED WITH NORMAL CORD.

These guinea pigs showed hemorrhagic axillary and inguinal lymph nodes. Sometimes the hemorrhagic areas were not prominent in the axillary region. The internal organs were usually normal although sometimes the liver was mottled. There was occasionally red fluid in the pleural cavity. The lungs were usually normal.

7. INJECTIONS INTO THE ANTERIOR CHAMBER OF THE EYE IN AN ATTEMPT TO ACTIVELY IMMUNIZE.

Four guinea pigs were injected in the anterior chamber of the eye with the virus of epidemic paralysis in hopes that in this manner the virus might be more slowly absorbed, and thereby give rise to immunity without giving infection. The first guinea pig recovered. Its protocol showing recovery is given on page 23. The other three died in from 30 to 36 days after the injection.

The autopsies on these guinea pigs showed hemorrhagic lymph nodes with the other organs normal, suggesting that infection was produced by the virus.

8. INTRADERMAL INJECTIONS OF CORD FROM CASES OF EPIDEMIC PARALYSIS IN AN ATTEMPT TO ACTIVELY IMMUNIZE.

Four guinea pigs were innoculated intracutaneously with from .25 cc. to .4 cc. of an emulsion of the diseased cord in from 6 to 8 intradermal injections. This cord had been treated with .5% formaldehyde for 24 hours, and then dessicated over KOH for 21 days and placed in 50% glycerine. It came from guinea pig no. 102. Two of the four guinea pigs died on the 5th and 6th days respectively. The internal organs were found normal on autopsy. The cause of the death was undetermined. The other two guinea pigs died on the 32nd and 35th days respectively. Paralysis preceeded death by several days. The autopsy revealed typical condition of the internal organs as found in epidemic paralysis.

9. INTRAOCULAR INNOCULATION OF YOUNG RABBITS IN AN ATTEMPT TO PRODUCE EPIDEMIC PARALYSIS EXPERIMENTALLY.

Five young rabbits, 238-351 grams in weight, were innoculated in the anterior chamber of the eye with 1/20 cc. of an emulsion of cord from guinea pig no. 101. The rabbits all gained weight and showed no change from

normal, which was a temperature of about 103°. One of the rabbits on the 36th day showed a drop in temperature of 2 degrees and had diarrhea. The animal promptly recovered, showing no other signs of disease. Two of the rabbits, nos. 3 and 5, were innoculated intracerebrally on the 19th day with an emulsion of the diseased cord from guinea pig no. 101 to see if the rabbits were susceptable. One of the animals, no. 3, died from the effects of the injection. The other showed a steady gain in weight and no symptoms of disease. This rabbit had been bled two days before this injection so that its serum might be tested for antibodies. The four rabbits lived for several more months and were then released from the experiment.

10. TESTING THE PROTECTIVE POWER OF SERUM FROM THE RABBITS INNOCULATED INTRAOCULARLY, BY GUINEA PIG INJECTION.

Guinea pig no. 5 was innoculated intracardially with one cc. of rabbit serum from rabbit no. 5 mentioned in the preceeding experiment. A few minutes later the pig was innoculated intracerebrally with an emulsion of cord from guinea pig no. 101, which had died of epidemic paralysis. The guinea pig died in ten days from diarrhea, loss of weight, and decrease in temperature. The autopsy revealed hemorrhagic lymph nodes in the axillary and inguinal

regions. The rabbit serum failed to protect.

Guinea pig no. 6 was injected intracerebrally with an emulsion of cord from guinea pig no. 101., emulsified in 1 cc. of serum from rabbit no. 5. The emulsion was incubated at 37° C. for two hours preceeding the innoculation. The protocol has already been given on page 21. The guinea pig died from typical epidemic paralysis.

Guinea pig no. 7 was innoculated intracerebrally with an emulsion of cord from guinea pig no. 101., being the same cord as wa s used with guinea pigs nos. 5 and 6. This protocol is also given on page 22 showing typical epidemic paralysis.

11. TESTS TO RULE OUT AN ORGANISM OF THE HEMORRHAGIC SEPTICEMEA GROUP AS AN ETIOLOGICAL FACTOR.

Guinea pig no. 24 was innoculated with normal cord from guinea pig No. B. Fourteen days later the guinea pig was very sick and was bled for 2 cc. of blood. This was immediately injected into guinea pig no. 29. The guinea pig receiving the injection showed no change in weight, nor temperature, remaining normal for several months. When the heart's blood was tested soon after death, no organisms were ever obtained which would show growth on simple media at 37° C.

DISCUSSION

There are a few definite symptoms in epidemic paralysis which can always be depended upon. There is loss of appetite, gradual decrease in weight, slight variation in temperature, which gradually decreases as the disea se progresses, emaciation, diarrhea, general tremor, ruffled fur, and finally paralysis. At the present time it seems that the fall in temperature during the progression of the disease is the most characteristic symptom, differentiating the malady from closely related one.

The disease is undoubtedly produced by a filterable virus, by which we mean the virus will pass through a berkfeld filter. It will be noted that the disease has the characteristics of a filterable virus disease,,such as the difficulty in producing immunity without producing infection. Romer and Bally both reported the filtration of the virus. The discovery that innoculation with normal nervous material will produce paralysis puts doubt on the filtration experiments of Romer for he reports nothing to differentiate the disease produced by normal virus from that produced by the virus of epidemic paralysis. It is altogether possible that the filtrates of normal

nervous tissue might produce a disease similar to epidemic paralysis.

Bally reports that he cultivated the organisms and produced the experimental disease from the cultures. His technic, a s recorded by himself, was to remove the growth from around the fragments of nerve and kidney tissue in his kidney tissue rabbit serum media. With this technic it is quite possible that sufficient nervous material was innoculated to produce disease and paralysis irrespective of the virus of epidemic paralysis. There is also the possibility that the virus did not reproduce, but remained in sufficient numbers to still cause the disease when taken from the test tube. It will be remembered that the virus of poliomyelitis is very resistant to chemical and physical agents.

Plantureux[26] in 1926 found that the injection of normal cord into dogs did not produce paralysis, but did cause loss of weight and caxchexia. Stuart and Krikorian[27] showed that the neurotropic accidents following antirabic treatment are due to repeated innoculations of the brain tissue carrying the virus and not to the virus itself, and that similar results may be obtained with the injection of normal tissue. Rivers reports similar results following

the innoculation of homogenous and heterogenous nervous material.

For a time it was thought that some of the animals used in these experiments might have died of malnutrition, and not from the effects of the material innoculated into them. The outstanding evidence against this possibility is the characteristic differences in temperatures between the guinea pigs innoculated with infected cord and those innoculated with normal cord, showing that there was something in the cord which caused a reaction in the animals.

Cole and Kuttner demonstrated that 85% of normal guinea pigs have in their salivary glands a filterable virus which is capable of producing paralysis when injected intracerebrally.

Miller, Andrewes, and Swift[28] report the finding of a filterable virus in normal rabbit tissue. Testicular material was taken from a rabbit and emulsified, the emulsion being innoculated intratesticularly into another rabbit. At the end of four days and emulsion was made of the testicular material of this rabbit and innoculated into a third rabbit, This series was then carried on indefinitely. Out of three such series a virus was obtained which would produce acute orchitis in rabbits. The virus could be propogated indefinitely. Intradermal innoculations

produced erythema in three to six days. Intrathoracic innoculations gave pericarditis and myocarditis. Nuclear inclusion bodies were found in the testis, skin, pericardium and heart muscle. Rabbits were refractory and would resist the virus after two weeks.

Rivers[29] working separately at the same time, in a similar series of experiments, obtained the virus in five out of eleven series. The virus would appear between the fourth and the eighth passage. He named it virus III of rabbits. Immunization experiments show that different strains of the virus obtained in this manner are immunologically identical. Intracerebral injection would produce encephalitis.

Carrel[30] reports that by treating with tar, arsenic, and indol, cultures of spleen taken from embryionic chicks he produces a substance which upon injection into chickens subcutaneously produces sarcoma. This sarcoma can be transferred by the berkfeld filtrates of the tumorous material, resembling in many respects a typical filterable virus. It can apparrently be cultured on suitable media and again produce the disease upon injection. The sarcoma produced with tar will kill chickens in from

four to five days while Rou's Sarcoma filtrates will kill chickens in two weeks.

The question arises as to whether or not the etiological agent is a virus which causes the epidemic paralysis. It might be an enzyme or a phage, on the nature of bacteriophage. The term virus suggests a formed element, while enzyme suggests fluid. Whether phage is a fluid or has a structure is very much debated. Certainly there is no evidence that these forms have a definite structure like bacteria. Injection of sublethal doses of an enzyme is supposed to cause the animal to produce antienzyme. This is not the case in epidemic paralysis, for one innoculation seemed to cause the animal to be more susceptable so that upon a second injection of a similar amount the animal contracted the disease. This is a characteristic of some other diseases attributed to filterable viruses, eg. poliomyelitis. It is certainly not a factor occurring in normal guinea pigs, for there is a distinct difference in the clinical picture of guinea pigs innoculated with normal cord and those innoculated with cord from epidemic paralysis. The difference between a phage, as bacteriaphage, and the filterable viruses is not yet distinct, and there seems to be no valid grounds for a definite distinction to be made between the two.

Bacteriophage is often classed as a virus disease of bacteria. It is also classed as a disease possibly produced by enzyme action. For these reasons, namely, tha t an enzyme should incite the production of antibodies upon injection, and that the status of a phage is not yet settled, it is thought best to call the etiological factor a virus.

The occurrance of a virus following the injection of apparrently normal material has occurred too often to be considered as contamination. There are perhaps three possibilities a s to the presence of the virus.

1. It is injected with the normal tissue, causing the supposedly normal tissue to be in effect, pathological tissue.

2. It is in the host already, in a resting, latent stage, and is activated by the innoculation of foreign material.

3. It is neither in the host or in the innoculated material before the injection, but is produced by the mixture of the two at the time of innoculation.

The latter theory may seem absurd, but the present considerations of filterable viruses in general are far from satisfactory. It is to be remembered that in all the

previously cited cases, there appeared a filterable virus from apparrently nothing. This idea is not in discord with some previously held beleifs. Beijerinck in 1898 spoke of the etiological agent of tobacco mosaic as being a contagious living fluid. Woods in 1902 asserted that it was an enzyme. Sanfelice in 1914 described one filterable virus as being an inanimate poison. Although it is possible that the last explanation may prove to be correct, in the light of the present evidence, one of the former explanations would seem more plausible.

It will be remembered that out of sixteen guinea pigs innoculated with normal nervous material, ten were innoculated with material from guinea pigs A and B, all of which died from emaciation, diarrhea, and general weakness, with a characteristic rise in temperature. The six which were innoculated with cord from guinea pig C survived. Of this six, some were from one guinea pig breeded and some from another. This indicates that the material which is being innoculated contains the etiological agent, since all the animals innoculated with the same cord either lived or died depending upon which cord was used.

It may be possible that these normally occurring viruses occur epidemically in a similar manner to the

viruses which are called pathogenic. Perhaps these so-ca lled normal viruses do not have the capicity to invade until the resistance of the host is weakened by the innoculation of foreign material. If this is the case, it is altogether possible that the reason those guinea pigs innoculated with the cord from animals A and B died, was because of an epidemic of normally occurring, apparrently non-pathogenic virus among them before being innoculated with the nervous material, or due to an epidemic of weakly virulent virus just following the innoculations. The experiments ca rried out with cord A were completed several months before the work on cord B was begun, ani this work finished Before the work with cord C wa s begun. This would have made it possible for epidemics to have occurred in one series and not to have affected the other series of experiments.

Perhaps the most important conclusion to be drawn from this work is the danger of overlooking in experimental work the possible presence of organisms in apparrently normal animals which may under favorable conlitions produce disease.

In light of the difficulty encountered in attempting to immunize with the filterably viruses, it is not surprising that the attempts failed with epidemic paralysis. Out of the four guinea pigs innoculated in the anterior

chamber of the eye, only one recovered. This one was lost before it was possible to determine whether or not it was immune or whether its serum would neutralize the virus. Out of the four guinea pigs innoculated with the virus intradermally none lived showing that the virus was too potent, although it had been attenuated in a weak solution of formaldehyde (0.5%) for 24 hours and dessicated over KOH for 21 days before use.

Attempts to produce immune serum in rabbits failed. Infection did not seem to occur in rabbits and it has been reported by numerous workers that immunity does not occur in filterable virus diseases without infection.

Flexner and Lewis[31] report that one infection of poliomyelitis in monkeys produces immunity. Romer and Joseph report finding that the serum of such monkeys will neutralize the virus. Similar antibodies, capable of neutralizing the virus have been found in the serum of persons recovering from poliomyelitis. Such serums have a therapeutic value for persons accidentally infected and monkeys experimentally innoculated.

Many cases are on record[32] where convalescent serum has been used to treat cases of poliomyelitis, injections being made intra venously or intraspinally. The artificial

method of introducing the antibodies has several advantages over waiting for the patient to develop natural antibodies. There is the element of time. The natural antibodies appear about the sixth day. The injection of antibodies into the cerebrospinal fluid also has the advantage of overcoming the effect of the choroid plexus, and thereby gets the antibodies in the serum closer to the virus.

Rosenow[33] reports the treatment of many cases with antistreptococcus serum, with wonderful results. His findings have not been checked satisfactorily. Mazum and Willy[34] reported good results injecting antistreptococcus serum, but other observers have reported equal results injecting normal horse serum intraspinally. The beneficial effect seems to be in changing the permeability of the choroid plexus by the injection of foreign material.[35] Wood and coworkers[36,37,38] have very adequately worked out the relation of the choroid plexus to the quantity of the cerebrospinal fluid, and the variation of the pressure of the fluid by the innoculation of hypertonic salt solution.

Smith[39] experimenting with man and Aycock and Amoss[40] experimenting with monkeys have shown the beneficial effects of the use of hypertonic salt solution intravenously, with immune serum intraspinally.

Flexner and Amoss[18,41] have shown that many substances a re sufficiently toxic that if injected intracerebrally will set up an aseptic imflammation of the choroid plexus allowing immune bodies to penetrate it, while otherwise they would not.

McKinley and Larson[42] have shown beneficial effects by treating monkeys having poliomyelitis with sodium ricinoleate. Their work grew out of Larson's work showing the detoxicating action of sodium ricinoleate on va rious bacteria and their products.

Most observers report failure to vaccinate with attenuated viruses. The virus must be sufficiently potent to produce the disease in a mild form. Romer[43] reports successful va ccination with a virus heated to 45° C. for 30 minutes. He occasionally got an infection following the injection.

Aycock and Kagan[44] report fairly sucessful vaccination with injecting the virus intradermally. It was their results which prompted the work reported in this paper on epidemic paralysis.

Flexner and Amoss[45] report having an immunizing strain of poliomyelitis. However the monkeys receiving this stra in have a residual paralysis.

Several observers have noted that in the filterable virus diseases that one injection of the virus, if failing to give rise to infection will make the animal more susceptable ra ther than immune to subsequent injections. This was found in one experiment in the epidemic paralysis of guinea pigs. Guinea pig no. 18 was injected with an emulsion of normal cord. The guinea pig failed to rea ct in any way so after 25 days was again innoculated with an emulsion of the same cord. Twelve days later the guinea pig died with paralysis, emaciation, general weakness, and a rising temperature. The autopsy revealed hemorrhagic lymph nodes in the inguinal and axillary regions. The hearts blood failed to show organisms which would grow on blood agar or plain broth at 37° C. when cultured both anaerobically and aerobically.

CONCLUSIONS

1. Epidemic paralysis among guinea pigs is a specific, epidemic, febrile disease, characterized by loss of weight, diarrhea, ruffled fur, paralysis, hemorrhagic axillary and inguinal lymph nodes, and a gradual decreasing temperature.

2. Inability to produce immunity with intracerebral, intraocular, or intradermal innoculations was because the virus was not sufficiently attenuated. This is characteristic of filterable virus diseases in general.

3. The disease is transmitted to rabbits with much greater difficulty than to guinea pigs.

4. Serum from rabbits innoculated previously with the virus failed to neutralize the virus when injected into guinea pigs.

5. Serum from rabbits innoculated previously with the virus failed to protect guinea pigs from subsequent innoculation.

6. Normal guinea pig cord may give the same effects upon injection that does cord containing the epidemic paralysis virus, except that the injection of the normal cord causes a final rise in temperature.

7. This production of infection by normal cord seems to be a property of the cord injected, as there is a hundred percent correlation between the cord used and whether or not there is infection.

8. Glycerine has no effect upon guinea pigs when innoculated in small amounts intracerebrally.

9. The effect produced by normal cord is seemingly due to a virus. This virus may be from (a) the material injected, its being pathogenic for the guinea pig which received the injection, but not for the guinea pig which had furnished the cord, (b) from the guinea pig receiving the innoculation, having been in a latent stage and being activated by the innoculation of foreign material, or (c) it may have arisen from the union of the two substances, the cord injected and the cells of the host. The former view has the most experimental evidence in its favor.

10. A second injection of normal cord produced an infection while the first one did not in the one case so tried.

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