POLIOMYELITIS IN THE EASTERN COTTON RAT

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Walter N. Mack

1940

POLIOMYELITIS IN THE EASTERN COTTON RAT

An Attempt To Propagate A Second Strain Of Poliomyelitis Virus In Sigmodon hispidus hispidus And hispidus littoralis Rats.

by

Walter H. Mack

A THESIS

Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the degree of

Master of Science

Department of Bacteriology

1940

THESIS

POLIOMYELITIS IN THE EASTERN COTTON RAT

An Attempt To Propagate A Second Strain of Poliomyelitis Virus in The Sigmodon hispidus hispidus and hispidus littoralis Rats.

ACKNOWLEDGMENT

This opportunity is taken to acknowledge my indebtedness to Dr. S. D. Kramer and Dr. H. E. Cope of the Michigan State Department of Health for their cooperation in making available the material for this study and to Dr. H. J. Stafseth of Michigan State College for his helpful suggestions.

CONTENTS

INTRODUCTION

Experimental work in poliomyelitis was initiated by Landsteiner and Popper in 1909 (1) who finally found an experimental animal which developed symptoms of this disease. A monkey, inoculated intraperitoneally with a suspension of the spinal cord of a child who had succumbed to the disease, developed symptoms of poliomyelitis. The infection was not carried through subsequent monkey passages as further intraperitoneal inoculations of this monkey's spinal cord failed to produce symptoms. At a later date Flexner and Lewis (2), (3) were able to infect monkeys with this virus by inoculating them intracranially. Serial passages were accomplished by these workers. Strauss (4) in this country also reported similar findings from monkey inoculation. Leiner and von Wiesner (5) in Vienna, and Landsteiner and Levaditi (6) in Paris, all independently succeeded in transferring the disease to an experimental monkey.

Since 1909 a great variety of animals of different species have been inoculated in the hope that a less expensive means of propagation of this virus could be found. All attempts to infect other animals with the virus were unsuccessful (8) until Armstrong (9), (10) in September of 1939 reported that he had successfully transferred a strain of poliomyelitis virus into the Eastern Cotton rat, Sigmodon hispidus hispidus. This strain, recovered from an eighteen year old boy from Lansing, Michigan, had been passed serially through fifteen monkeys with the development of clinical and histological poliomyelitis. The fourth monkey passage cord was

inoculated into the cotton rat, and twenty-five days later the rat developed nervousness and, on the following day, had paralysis in both rear legs. Eleven cotton rats were inoculated with the suspension of this animal's spinal cord and brain and in twenty-nine days one of these rats was paralyzed. The inoculum was a five per cent suspension of the brain and cord in the dosage of 0.06 cc. intracerebrally, 0.06 cc. intranasally, and 0.25 cc. subcutaneously.

The spinal cord and brain from the seventh rat passage was ground to a five per cent suspension and inoculated into mice. One of the five mice developed paralysis (11). This animal's cord and brain, when passed into twenty-four mice, brought down twelve of the animals with paralysis of one or more extremities in from three to twelve days. Successive transfers of the spinal cord and brains of mice have been made, and mouse spinal cord and brains, when inoculated into monkeys, have brought down these animals with typical experimental poliomyelitis.

This study was undertaken in an attempt to determine whether other strains of poliomyelitis virus could be propagated in the cotton rat. Not all the experiments tried will be presented because many different trials were made on the same strain of virus with the same result. Five individual strains will be discussed in detail, and references will be made to tables I to IV which outline the experiment.

METHODS

In the majority of experiments the source of poliomyelitis virus was spinal cords of monkeys taken directly from the monkey, or after storage at three degrees centrigrade in either fifty per cent buffered glycerine or Locke's glycerine. In the experiment with the D.G. strain, (table I), the inoculum used was a pool of fragments of eight monkey spinal cords, all of which had been stored at three degrees centrigrade in buffered glycerine. All cords used were from animals whose clinical symptoms and cord lesions conformed to those of experimental poliomyelitis. Samples of the cord used were weighed and then ground in a mortar with sterile sea sand and saline; in a few cases ten per cent testicular extract (13) was used as suspensoid. The concentration of the spinal cord suspension used was generally ten per cent, but in a few cases a five per cent concentration was made. In one experiment, human faces, from which the virus had previously been obtained in monkeys, was treated with ether, contrated (16) and used as inoculum. Blood plates were seeded with the spinal cord suspensions to determine bacterial contamination. In a few cases, bacteria were found in the spinal cords and brains of mice that were transferred from animal to animal. In the rats, however, no trouble with bacterial contamination was encountered.

The cord suspensions were centrifuged at a moderate rate of speed for a few minutes to throw down the detritus. The supernatant suspension was then poured off and used as inoculum. The virus was kept at three to four degrees centrigrade when not being used, and was never left standing at room temperature unless in the process

of manipulation or use. Virus suspensions more than twenty-four hours old were not used. The injections of the virus were made by various routes i.e., intracerebrally, intracerebrally after trauma (using both needle damage and Sawyer and Lloyd's two per cent starch method (12), intranasally, subcutaneously, and intraperitoneally.

The cotton rats used were shipped from Florida and South Carolina. Sigmodon hispidus hispidus and a closely related subspecies, Sigmodon hispidus littoralis, were used. It was found in our laboratory that the littoralis rat was also susceptible to poliomyelitis, Lansing strain. The animals were kept in stock for a few days before being placed on test. After inoculation, each animal was observed daily, and any animal that did not appear normal was placed under special observation and watched throughout the day. When an animal developed a generalized weakness, a peculiar gait, excitability or inactivity, or a weakness in any extremity, it was either watched for exacerbation or sacrificed for passage of the virus. The animals found dead in their cages at any time other than the day following the inoculation had their cerebro-spinal axis removed, ground to a five per cent suspension, and inoculated into new animals. If the nervous tissue appeared soft from postmortem changes or bacterial invasion, the material was filtered through a Seitz filter and the filtrate inoculated into new rats.

The method used to transfer the virus was as follows: the animals were sacrificed with chloroform anaesthesia and, with sterile precautions, their brains and spinal cords were removed. A

sagittal section of each brain and spinal cord was removed and placed in a ten per cent formalin solution for histological study. The remaining spinal cord and brain, or both, were then weighed and a five or ten per cent suspension made by grinding the weighed material in a mortar and then slowly adding the required amount of sterile saline. The ground suspension was then placed in a sterile test tube and centrifuged at 2,000 r.p.m. for five minutes. After centrifugation the supernatant fluid was inoculated into new animals.

Six to twelve mice were inoculated intracerebrally with 0.03 cc. of the virus for each group of new rats used. The mice were used as controls to indicate the presence of choriomeningitis virus (14), (15) which might have been present in the monkey or rat cords. In a few instances guinea pigs were used, receiving 0.1 cc. of the material intracerebrally.

The amount of inoculum used in the rats varied according to the size of the animal and amount on hand. From 0.03 to 0.4 cc. were given intracerebrally, averaging about 0.1 cc. For intranasal infection, usually one drop per nostril was administered. The amount of the intraperitoneal inoculum ranged from 0.4 cc. to 1.0 cc.

The material for histological study was imbedded in paraffin, sectioned, and stained with haematoxyline and eosin or with Mallory's phyloxive methylene blue.

EXPERIMENT I
Pooled Monkey Cords - D. G. Strain

The most potent strain of poliomyelitis virus, D.G., isolated during the 1939 Detroit, Michigan, epidemic by Kramer, et.al., (16), was used as inoculum. A total of 103 rats, forty-eight mice and ten guinea pigs were used in this series of experiments.

Two rats were inoculated with a pool of fragments of five monkey cords (numbers 22, 28, 61. 67, and 102). At the end of the third day, one of the two animals was sacrificed, the spinal cord and brain removed, ground to a ten per cent suspension, and inoculated into two new rats. This method of rapid passage was repeated four times. One of the animals of the fourth rapid passage series, after a ten day incubation period, was found to have flaccid paralysis of both rear extremities. The animal was sacrificed, the central nervous system removed and inoculated in a ten per cent suspension into one monkey, four rats, two guinea pigs, and eight white mice. A careful dissection of the involved extremity, failed to reveal any local cause for the loss of function. No significant symptoms developed in the new animals. The microscopic examination of the brain exhibited scattered areas of necrosis and infiltration with macrophages. The cord was edemic, the motor cells took the stain irregularly and appeared degenerated.

In the next attempt, fragments of eight monkey spinal cords, all from experimental poliomyelitis confirmed by examination of sections, were pooled, and ground and made into a ten per cent emulsion, and inoculated into eight cotton rats, four hispidus and four littoralis. Two animals, (see table I) numbers 4 and

8 (one of each sub-species), were sacrificed at the end of three days, their central nervous systems removed, and inoculated into new animals. These animals did not have symptoms of poliomyelitis but were sacrificed after three days for rapid passage of the virus. A ten per cent suspension of the spinal cord and brain from rat number 8 was inoculated into two rats, numbers 14 and 15. In turn, rat number 15 was found to be unstable in the rear extremities at the end of three days and was sacrificed. Rats, numbers 34 and 35 were inoculated with a suspension of the spinal cord and brain from this animal. On the twenty-fifth day, rat number 34 developed a weakness in the right rear leg. It was sacrificed and the spinal cord and brain was inoculated into four animals, numbers 72 to 75 inclusive. Of these four animals none developed any abnormality and so they were discarded after the lapse of the usual observation period of thirty days. Some of these animals received one, and others, two reinoculations of freshly ground cord suspensions as indicated in table I.

The spinal cord and brain of rat number 4 of the previous group were inoculated into three animals, numbers 16, 17, and 18. Rat number 17 received intracerebrally, in addition to the virus, 0.06 cc. of a two per cent starch solution (12). This rapid passage on every third day was carried out until four sets of three rats each were inoculated. One of the fourth passage group of rats, rat number 31, developed a weakness in the left rear leg after two reinoculations and twenty-nine days of observation. When examined at autopsy, no abnormalities of the limbs could be found; however, the intestine contained tape worms. The central

nervous system in a ten per cent emulsion was inoculated into rats numbers 85, 86, and 87. The histopathology of rat number 31 presented a hemorrhage in the ventricles of the brain, and some vascular congestion and edema. Of the three rats receiving the spinal cord and brain of rat number 31, rat number 85 developed a tremor on the third day and a definite weakness of the right front leg and foot. The animal fell to the right side, although there was not flaccid paralysis of the limb. The viscera were normal in this animal when examined at autopsy. Upon sectioning, the cord exhibited no cellular reaction, but the motor cells appeared to take the stain poorly. Both the brain and spinal cord were congested and edemic. Since no abnormalities were evident at autopsy and in view of the suggestive symptoms, the central nervous system was ground and made into a ten per cent saline suspension and inoculated into twelve rats, numbers 95 to 106 inclusive. Each animal was given 0.2 cc. intracerebrally, 0.5 cc. intraperitoneally, and 0.03 cc. intranasally. On the fourth day, rats numbers 99, 101, and 103 developed similar symptoms, consisting of tremor and weakness of one or more extremities. Rat number 99 was sacrificed and passed into three new rats, two guinea pigs and six mice. All of this group remained well except rat number 118, which on the fourth day appeared ill and weak, and was sacrificed and inoculated into three rats, numbers 165 to 167, none of which developed any symptoms.

The spinal cord and brain of rat number 101, prepared into a ten per cent suspension, was inoculated into three rats, numbers 120, 121, and 122, two guinea pigs and six mice. Rat number 122

of this group developed tremor and weakness in the rear legs. At postmortem examination a nutmeg liver and atrophy of the spleen was found. The spinal cord and brain were inoculated into three rats, numbers 137, 138, and 139. Rats numbers 137 and 138 both developed tremor and weakness in their extremities and were sacrificed on the third day. Sections from the spinal cord and brain of these two animals were similar. Both had a small amount of hemorrhage in the gray matter of the spinal cord. An occasional round and plasma cell could be seen infiltrating the gray matter. Throughout the brain substance, scattered hemorrhages were seen. The meninges covering the cerebrum and the subarachnoid spaces were filled with blood. The cords of these two animals were pooled, a ten per cent suspension made and inoculated into six rats, numbers 140 to 145 inclusive, two guinea pigs and six mice. Some passages were made from this group of animals but did not appear to produce particularly significant results.

Rat number 103 developed symptoms similar to rats numbers 99 and 101 of the above group and was sacrificed on the fourth day. The stained sections of the spinal cord from this animal were very suggestive of poliomyelitis. The viscera were found to be normal with the exception of a nutmeg liver. A ten per cent suspension was made of the central nervous system of this animal and inoculated into rats numbers 123 to 125, two guinea pigs, and six mice. None of these animals developed any paralysis.

In this experiment other passages were made, as can be seen from table I, but they proved to be of no significance. Reinoculations were performed once or twice on some of the animals as

indicated. Starch was used to traumitize the brain tissue in certain animals as indicated in the table. It is interesting to note that rat number 184 was able to withstand 0.2 cc. of the virus and 0.2 cc. of the starch solution intracerebrally.

Pooled Monkey Cords D.G. Strain*

Table III

Inoculated 1-30-40

- RAT 1 (HH) — Reinoc, 2-15-40; Reinoc, 2-27-40; Inoc, Armstrong Polio - 5 days.
- RAT 2 (HH) — Reinoc., 2-15-40; Reinoc, 2-27-40
- RAT 3 (HH) — Reinoc., 2-15-40; Reinoc., 2-27-40
- RAT 4 (HH) — R.P - 3 days; Post: Liver Cysts
  - Inoculated 2-2-40
    - RAT 16 (HH) — Irritable; Sacrificed 8-days
      - Inoculated 2-10-40
        - RAT 36 (HH) — Reinoc 2-15-40; Weak and Ill; Sacrificed - 11 days.
          - Inoculated 3-9-40
            - RAT 90 (HH) — Discarded 5-21-40
            - RAT 91 (HH) — Inactive HH DII; Spares right front; Sacrificed 5-days
              - Inoculated 3-15-40
                - RAT 115 (HH) — Tremor, Weak Left rear Sacr. 3-days Broken leg Splenic edemic
                - RAT 116 (HH) — Irritable Weak Left rear Necrotic Left rear. 5-3 days
        - RAT 37 (HL) — Reinoc., 2-15-40; Reinoc., 2-28-40
    - RAT 17 (HH) — Reinoc., 2-15-40; Reinoc., 2-28-40; .06cc Starch
      - Inoculated 2-5-40
        - RAT 19 (HH) — R.P. - 3 days
          - Inoculated 2-8-40
            - RAT 31 (HH) — Reinoc., 2-15-40; Reinoc., 2-28-40; Spares Left rear Tapeworms.
            - RAT 32 (HH) — Reinoc., 2-15-40; Reinoc., 2-28-40; Died 5-8-40 Tapeworms.
            - RAT 33 (HH) — Reinoc., 2-15-40; Reinoc., 2-28-40; Died 4-6-40 injury.
              - Inoculated 3-8-40
                - RAT 85 (HH) — Weak right front Tremor Sacrificed 3 days.
                - RAT 86 (HH) — General Weakness. Cysts Sacrificed 8 days
                - RAT 87 (HH) — Died 5-7-40 No Symptoms
                  - Inoculated 4-2-40
                    - RAT 161 (HL)
                    - RAT 162 (HL)
                    - RAT 163 (HL)
                    - Discarded 5-26-40
        - RAT 20 (HH) — Reinoc, 2-15-40; Reinoc, 2-28-40; Armstrong Polio-Polio 5-days
        - RAT 21 (HH) — Reinoc., 2-15-40; Reinoc., 2-28-40; 6-young 5-1-40; 0.06cc Starch
    - RAT 18 (HH) — R.P. 3 days
      - Inoculated 3-11-40
        - RAT 95 (HH)
        - RAT 96 (HH)
        - RAT 97 (HH)
        - RAT 98 (HH)
        - RAT 99 (HH) — Tremor weak in rears 5-4 days Anemic liver
          - Inoculated 3-15-40
            - 6 Mice
            - G. Pig — Discarded 4-15-40
            - G. Pig
            - RAT 117 (HH)
            - RAT 118 (HH) — Sick Weak 5-4 days. Worms
            - RAT 119 (HH) — Discarded 5-26-40
              - Inoculated 4-9-40
                - RAT 165 — Disc. 5-26-40
                - RAT 166 — Died 4-5-40
                - RAT 167 — Disc. 5-26-40
        - RAT 100 (HH)
        - RAT 101 (HH) — Weak legs Ill. 5-4 days Liver Cysts
          - 6 Mice
          - G. Pig
          - G. Pig
          - RAT 120 — Found Dead 4-5-40
          - RAT 121
          - RAT 122 — Tremor Weak left leg 5-6 days nutmeg liver
            - Inoculated 3-21-40
              - RAT 139 (HH) — Disc. 5-26-40
              - 6 Mice — Discarded
              - 6 Mice — 4-3-40
              - 6 Mice
              - G. Pig (2)
              - RAT 137 (HH) — Tremor weak legs 5-3 days
              - RAT 138 (HH) — Weak rears 5-3 days
                - Pool
                - Inoculated 3-25-40
                  - 6 Mice
                  - G. Pig (2) — Discarded No Symptoms
                  - RAT 140
                  - RAT 141
                  - RAT 142 — Died 3-25-40
                  - RAT 143
                  - RAT 144 — Sacrificed 7-day Brain Clot
                  - RAT 145
                    - Pool
                    - Inoculated 4-16-40
                      - RAT 178 — Discarded 6-2-40
                      - RAT 179
                      - RAT 180
        - RAT 102 (HH)
        - RAT 103 (HH) — Tremor, Weak. Tremor Ill. 5-4 days Nutmeg liver
          - Inoculated 3-18-40
            - RAT 123 (HH) — Discarded 5-26-40
            - RAT 124 (HH) — weak coat rough 5-18-days
            - RAT 125 (HH) — Died 1-day
            - 6 Mice
            - G. Pig — Discarded 4-16-40
            - G. Pig
        - RAT 104 (HH) — Ill, weak 5-7 days
        - RAT 105 (HH)
        - RAT 106 (HH) — Died 5-7-40
- RAT 5 (HL) — Reinoc., 2-15-40; Reinoc., 2-27-40
- RAT 6 (HL) — Died - 1 day; Cerebral hemorrhage
- RAT 7 (HL) — Reinoc., 2-15-40; Reinoc., 2-27-40; Died 4-6-40 Discarded.
- RAT 8 (HL) — R.P. 3-days
  - Inoculated 2-2-40
    - RAT 15 (HL) — Sacrificed R.P. 3-days
      - Inoculated 2-5-40
        - RAT 22 (HL) — No Paralysis Ill - No lesions Nutmeg liver R.P. 3-days
          - Inoculated 2-8-40
            - RAT 34 (HL) — Reinoc., 2-15-40; Reinoc., 2-28-40; Right rear Weak 5-25 days - Worms
              - Inoculated 3-4-40
                - RAT 72 (HH) — Discarded 5-8-40
                - RAT 73 (HH)
                - RAT 74 (HH)
                - RAT 75 (HH) — Discarded 5-8-40
            - RAT 35 (HL) — Reinoc., 2-15-40; Reinoc., 2-28-40
        - RAT 23 (HL) — Reinoc., 2-15-40
    - RAT 14 (HL) — Reinoc., 2-15-40; Reinoc., 2-27-40

Reinoc - reinoculated : R.P. - rapid passage : s- Sacr. - sacrificed.
Inoc - Inoculated : HH - hispidus hispidus : HL: hispidus litteralis.
Disc - discarded.

*Number of monkey cord pooled - 22-28-61-67-102-108-109-88

Reinoculation cord numbers pooled - 22-28-61-67-102-108-109-88-1

EXPERIMENT II
The use of virus from Fecal Specimen S.M.

The source of the virus in this experiment was the stool of a child who had had contact with the Poliomyelitis virus, as reported by Kramer, et. al., (16). This stool specimen had been stored in the ice chest at four degrees centrigrade for six months and eleven days (reference in press).

In this experiment twenty-nine rats and seven mice were inoculated with this specimen.

The stool was treated with ether and concentrated and inoculated into monkey number 123 (see table II) and four rats, numbers 48 to 51 inclusive. On the sixteenth day monkey number 123 developed flaccid paralysis of both rear extremities and was sacrificed. Sectioning of the spinal cord confirmed the diagnosis of experimental poliomyelitis. The cord was ground and made into a ten per cent suspension in saline and inoculated directly into five rats, numbers 109 to 113 inclusive. Rats numbers 112, 113 and control rat number 114 were placed in a cage in a refrigerator at four degrees centrigrade. It was hoped that the virus would propagate in living tissue at that temperature. Experience had demonstrated that it could be so stored without loss of virulence. Rats numbers 109, 110, and 111 (see table II) did not show symptoms while rat number 113 on the third day was found on its side in the cage with all extremities spastic. This animal was sacrificed and the autopsy presented a nutmeg liver and atrophy of both kidneys. The pathological picture presented fatty degeneration of the liver. The brain contained an abscess and the kidneys were degenerated and infiltrated with macrophages. The anterior horn cells of the cord were poorly

stained while those of the posterior horn took the stain well. The cord and brain were ground and made into a ten per cent saline suspension and inoculated into five rats. One of these animals, rat number 148, developed symptoms and was sacrificed; lung worms were found in the pleural cavity as well as small hemorrhages in the lungs. The remaining animals of this group were discarded without symptoms after one month's observation.

On the ninth day after inoculation, rat number 48, referred to above, favored the left rear leg and was sacrificed. At autopsy the liver contained echinococcus cysts and the intestine was filled with tape worms. The spinal cord upon sectioning was found to be normal except for a small amount of edema. A ten per cent suspension of the spinal cord and brain was inoculated into two rats, numbers 76 and 77, which did not show symptoms of poliomyelitis and were discarded after the observation period.

On the third day after inoculation, rat number 49 developed a generalized weakness and favored the left side. On the fourth day the animal was sacrificed. The autopsy presented an enlarged spleen and the brain appeared congested. The tissue when sectioned, showed vascular congestion with some edema. The spinal cord and brain from this animal were ground and made into a ten per cent saline suspension and 0.2 cc. intracerebrally, 0.5 cc. intraperitoneally, and 0.03 cc. intranasally were inoculated into each of six new animals, rats numbers 61 to 66.

Of these six animals, rat number 62 and 66 developed symptoms. The remaining rats were discarded without showing symptoms after the usual observation period. On the third day, rat number 62 was

was inactive and seemed to spare the right rear extremity; on the fourth day this animal was ill and very inactive and was sacrificed. When the animal was autopsied, a large unorganized clot of blood was found in the extremity involved; at the base of the clot an echinococcus cyst was found. The liver also contained echinococcus cysts and the spleen was moderately enlarged. Sections of the brain showed edema and moderate vascular congestion with localized areas of hemorrhage. A ten per cent saline suspension of the spinal cord and brain of this animal was inoculated into two rats which after a month's observation presented no symptoms and were discarded.

The second rat of this group, rat number 66, did not develop a paralysis, but was irritable on the fourth, fifth, and sixth days. On the seventh day the right rear leg appeared to tire easily and the animal was sacrificed for passage. With the exception of the gall bladder, which was filled with stones, the viscera were negative. Microscopically, the spinal cord exhibited in one horn, motor cells staining poorly, otherwise, the spinal cord and brain appeared normal. A ten per cent saline suspension of the spinal cord and brain of this rat was inoculated into three rats, numbers 78 to 80, and seven mice.

Rat number 80 received 0.2 cc. intracerebrally, 0.03 cc. intranasally, and 0.7 cc. intraperitoneally. On the thirteenth day after inoculation, the animal was sacrificed because of weakness and irritability. The spinal cord and brain were sectioned, and scattered hemorrhages were found in the brain tissue as well as in the ventricle spaces. The cells of the choroid plexus were

hyperplastic. The spinal cord was negative. This material was not inoculated into new animals, but was stored in glycerine. The other two rats, numbers 78 and 79 remained free of symptoms. Of the seven mice, number M-81, also receiving the specimen from rat number 66, one mouse was found to have weak legs on the day after the operation. It was sacrificed, and the cord and brain, after being suspended in saline, were inoculated into six mice, cage number M-168 (see table II). All these animals were discarded without showing symptoms of any kind after the usual observation period. The six remaining mice, cage number M-81, were also discarded without showing symptoms.

Fecal Specimen* S. M. Table II

*RATS 112-113-114 Were kept at 4°C. HH= hispidus hispidus.

NOTE - Disc.= Discarded: Inoc.= Inoculated: Reinoc = Reinoculated.

TITLE * Etherized and concentrated.

EXPERIMENT III
Kessel Strains

The infectivity for the cotton rat of three human strains of poliomyelitis, isolated in California by Kessel, were studied. The spinal cord of monkeys stored in glycerine were the source of the virus, designated as the Kahn, McCall, and Schultz strains. Thirty rats and five mice were used in this experiment.

The Kahn strain, in a ten per cent suspension, was inoculated into four cotton rats numbers 41, 42, 46, and 47, and five white mice. Each rat received 0.1 cc. intracerebrally, 0.7 cc. intraperitoneally, and 0.03 cc. intranasally. Rat number 41, in an observation period of three months, was at first slow and inactive, but later irritable. After this animal was discarded from the test, it was given 0.1 cc. intracerebrally of the stock Armstrong Lansing strain of virus, and developed typical paralysis and poliomyelitis in seven days. On the fourth day rat number 46 had some weakness in the left rear leg with an eversion of the foot. On the following day the symptoms were about the same. The left side of this animal was definitely weak on the sixth day, and the animal was sacrificed; the spinal cord and brain were made up into a ten per cent suspension and inoculated into nine rats, numbers 52 to 60 inclusive. Two of the nine rats, numbers 54 and 55, became inactive, and on the eighth day after the operation, both rats were sacrificed. The spinal cords and brains of these animals were pooled in a ten per cent suspension and inoculated into three new rats, numbers 82, 83, and 84. The viscera and

extremities of rats numbers 54 and 55 were normal when examined for gross pathology. The stained sections of the spinal cord and brains, however, showed some edema and vascular congestion. No cellular degeneration of the motor cells could be found.

The three animals receiving a pool of two spinal cords and brains of rats numbers 54 and 55 all developed symptoms. Rat number 82 did not present paralysis, but on the third day had a peculiar gait. When it was sacrificed, the liver was anemic and contained echinococcus cysts. When the brain was sectioned, it exhibited edema, vascular congestion, and small scattered hemorrhages. The cord exhibited only slight congestion, and small scattered hemorrhages. Rat number 84 also developed a transient tremor on the third day. The organs were normal upon examination and sections of the spinal cord and brain presented only slight edema and some vascular congestion. The spinal cords and brains of these two animals, numbers 82 and 84 were pooled and inoculated into three animals numbers 92, 93, and 94, of which none presented symptoms of the disease.

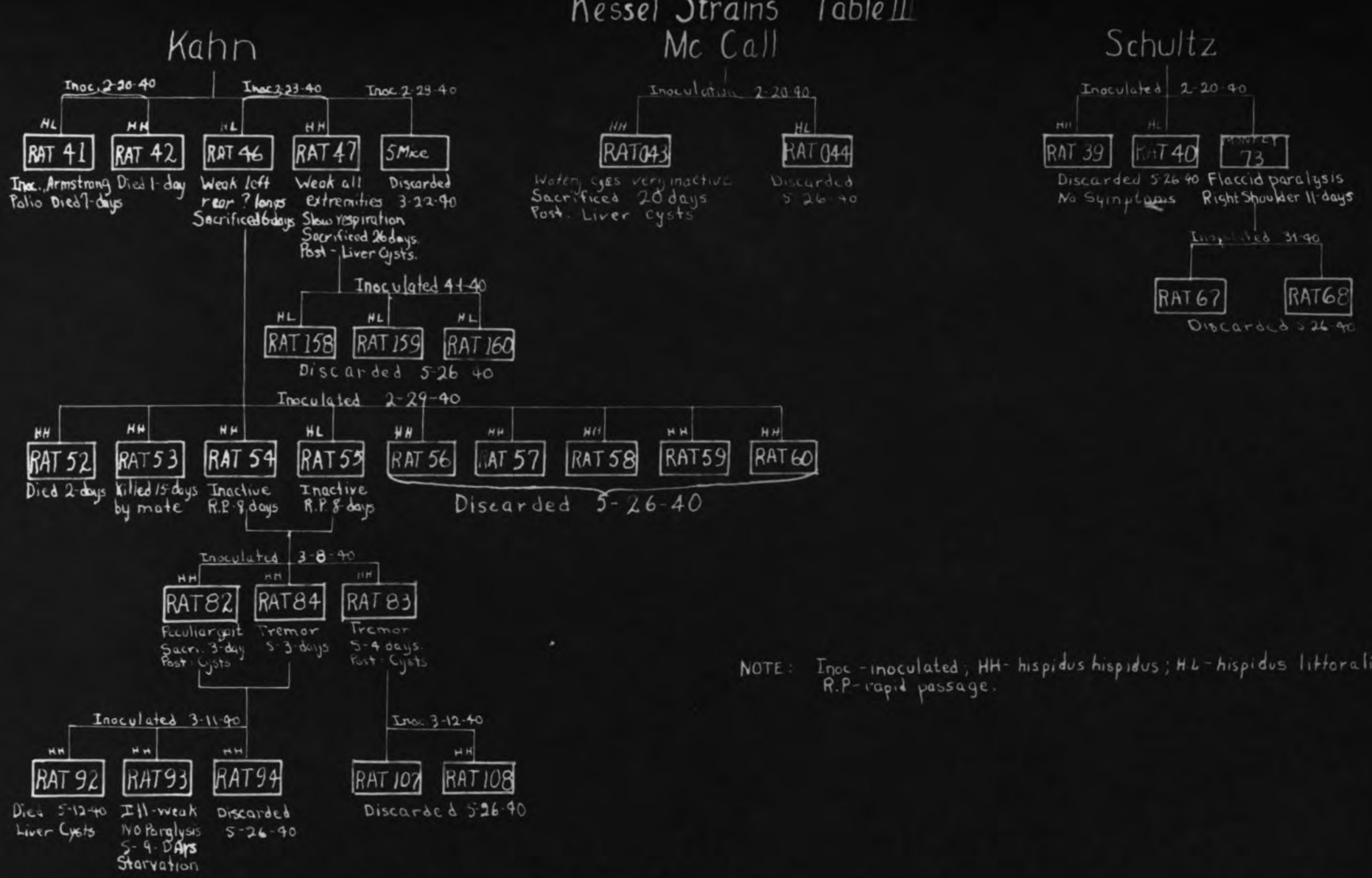
Rat number 83 of the previous group was found on the fourth day to have a fine tremor and was inactive. When sacrificed, liver cysts were present. On microscopic examination, the brain and spinal cord were found to be slightly congested, the former having an occasional small hemorrhage into the brain substance. Transfer of a suspension of this spinal cord and brain into two new animals was made, rats numbers 107 and 108. Neither animal developed symptoms during the month's observation period.

The McCall strain of virus was inoculated into two rats, numbers 043 and 044. Each animal received 0.1 cc. intracerebrally, 0.7 cc. intraperitoneally, and 0.03 cc. intranasally. On the twentieth day, rat number 043 developed a systemic weakness and was sacrificed. The liver of this animal contained echinococcus cysts, and white patches were found on the kidneys. The microscopic pathology of the kidneys was that of an acute hemorrhagic and toxic nephritis. The nervous system of this animal was not inoculated into new animals.

The other animal receiving this strain of virus did not develop sufficient symptoms to warrant an autopsy, and was discarded after three months daily observation.

The third California strain, Schultz, was inoculated into two rats, numbers 39 and 40, and monkey number 73. The rats were discarded without having shown symptoms after the usual observation time. Monkey number 73 on the eleventh day developed flaccid paralysis of the right shoulder and was sacrificed. Examination of sections from the spinal cord of this monkey confirmed the diagnosis of experimental poliomyelitis. Two rats, numbers 67 and 68, were inoculated with 0.15 cc. intracerebrally, 0.7 cc. intraperitoneally, and 0.03 cc. intranasally with a ten per cent suspension of the spinal cord of monkey number 73, but did not develop any symptoms.

Kessel Strains Table II



NOTE: Inoc - inoculated; HH - hispidus hispidus; HL - hispidus littoralis
R.P - rapid passage.

EXPERIMENT IV
The effect of hyperpyrexia induced by short wave induction therm on the transmissibility of the virus of Poliomyelitis.

Four rats, all littoralis, were subject to a short wave induction current until the body temperature was increased. Two animals were exposed for ten minutes, and a body temperature of 105.4 degrees Fahrenheit obtained. The second two animals were exposed for eighteen minutes, and temperatures of 108.4 and 109.4 degrees Fahrenheit were obtained. The normal temperature of the animals ranged between 101 degrees and 103 degrees Fahrenheit. Immediately after exposure, each animal was given 0.15 cc. intracerebrally, and 0.6 cc. intraperitoneally of a ten per cent emulsion of the spinal cord of monkey number 88. (D. G. strain).

On the sixth day one of these rats developed weakness of the right rear leg. On the ninth day the involved leg was in about the same condition as the previous three days, and the animal was sacrificed. On autopsy the liver was very dark and firm in consistency. The cord and brain were removed and stored in glycerine while sections of this specimen were examined. The motor cells of the spinal cord stained poorly, and both spinal cord and brain showed some vascular congestion.

The remaining three animals recovered from the exposure to the inductive current and operation without symptoms of poliomyelitis for an observation period of two months.

EXPERIMENT V
The susceptibility of immature rats to the virus of poliomyelitis.

Sabin and Olitsky in 1937 and 1938 (24), (25) determined that young mice receiving Vesicular Stomatitis virus succumbed to the disease with much more consistent incubation period and susceptibility. Therefore, six rats, two at the age of one week old, two two weeks old and two three weeks old were given proportionate dosages of freshly triturated spinal cord of monkey number 109 (D.G. strain). Table four gives the results of this experiment.

TABLE IV

No. of Animal	Age	Dosage	Symptoms	Outcome
197	1 wk.	.03 IC 1-dr. IN	Weak 4th day.	Starvation on 5th day.
196	1 wk.	do	Weak 2nd day.	Starvation on 4th day.
195	2 wk.	.06 IC .2 IN	None	Discarded 1 mo.
194	2 wk.	do	None	Discarded 1 mo.
193	3 wk.	.06 IC	None	Discarded 1 mo.
192	3 wk.	do	None	Discarded 1 mo.

Note: IC - Intracerebrally
IN - Intranasally

DISCUSSION

Burnet and Macnamara in 1931 (17) (18) and Weyer in 1932 (19) were able to show immunological differences between strains of poliomyelitis virus using the neutralizing and cross protection tests. In 1933, Paul and Trask (26) and again in 1937, Paul, Trask et. al., (27) found marked differences in some of the strains of virus they studied. All these workers used the monkey as their experimental animal.

With the immunological differences of strains of virus in mind, it was hoped that, first, a second strain of poliomyelitis virus might be found that would infect the cotton rat or second, a closely related immunological strain might be so modified as to propagate in this inexpensive experimental animal.

In experiment number I, the method of rapid passage was carried out. Every third day the central nervous system of an animal was removed, triturated in a mortar, centrifuged, and inoculated into new animals. This method was carried on until four transfers of the virus were obtained. Sawyer's method of traumatizing the brain with an inoculation of a two per cent starch solution was tried in experiment number I along with the rapid passage. Many of the animals were reinoculated two or three times with freshly triturated spinal cord of monkeys. However, no direct results with reinoculations were obtained.

The cotton rat receiving the fourth rapid passage transfer rat number 31, as well as the subsequent animals, appeared to show symptoms of some significance. Although none of the animals developed a flaccid paralysis, weakness appeared between three and

seven days in some of the animals, and was carried through three or four passages with similar symptoms. The pathological lesions were suggestive of those found in the spinal cords and brains of rats which succumbed after inoculation with the Lansing strain of virus isolated by Armstrong (9). However, no definite neuronophagia was found in the experimental animals' spinal cords. Lillie and Armstrong (21) report vascular congestion, polymorphonuclear infiltration, neuronophagia, and cellular necrosis from the Lansing strain of virus in the rats' spinal cords. The symptoms in the experimental animals disappeared after the fourth transfer and could not be duplicated in other attempts.

In experiment number II it was observed that strain S.M. produced flaccid paralysis in a Macacus rhesus monkey. The spinal cord of this monkey when suspended and inoculated into rats, failed to produce the disease.

Rats received positive poliomyelitis feces, treated with ether and concentrated; glycerinized as well as fresh suspension of the spinal cord of monkeys, but did not develop typical symptoms of the disease. Some of the animals were kept at three to four degrees centigrade after inoculation with the virus. Both the symptoms and the pathological picture were negative. The virus stored in glycerine at that temperature has been shown by Rhoads (20) to survive for eight years without detectable deterioration.

Three strains of poliomyelitis virus isolated in California were inoculated in to rats. Whether differences in strains might vary with the geographic location could not determined from exper-

iment number III. The Kahn strain produced enough symptoms in animals to warrant four passages but no paralyzed animals were encountered. The other two strains tested were also negative, however, the Schultz strain infected a monkey.

Results of inoculation with the virus after increasing the animals body temperature with short wave induction current was negative.

In the last effort to propagate the virus, young rats born in the laboratory were inoculated with an emulsion of spinal cord of a monkey. Ages varied from one to three weeks. The rats one week old starved after removal from the mother, the remaining animals recovered from the operation and at the end of one month were well, developing no symptoms.

The cotton rats used in this work were wild rats captured in South Carolina and Florida and shipped to the laboratory. The majority of animals were infested with parasites of one kind or another. This may explain some of the symptoms seen in the rats. However, many of the animals had symptoms and no parasites were found at postmortem examination.

All surviving experimental animals were inoculated with Armstrong's Lansing strain of virus. They uniformly developed expermental poliomyelitis. This indicates that no immunity was developed following the inoculation of this material.

Toomey and Takacs in March, 1940 (22) report negative results in the attempt to infect S. hispidus and littoralis rats with nine strains of poliomyelitis virus in their laboratory. Seventy-nine rats were used by these workers in attempting to propagate the nine

strains of virus.

Parachivesco and Papazoiu in 1940 (23) report failure, using irradiated mice inoculated with monkey passage spinal cord poliomyelitis virus.

It appears that differences in strains of virus of poliomyelitis exist. So far only the Lansing strain of poliomyelitis virus will infect the cotton rat and white mouse. The reason for this variation remains an unsolved problem.

SUMMARY

1. An attempt was made to establish other strains of poliomyelitis virus in the Eastern cotton rats Sigmodon hispidus hispidus and hispidus littoralis.

2. Eight methods known or suspected as contributing to successful infection with a virus disease in animals, were employed without success.

3. Five strains of the virus studied failed to produce typical symptoms or pathological lesions of the disease in the cotton rat.

REFERENCES

(1) Landsteiner, and Popper, E. Uebertragung der Poliomyelitis acuta auf Affen. Ztschr. f. Immunitatsforsch. u. exper. Therap., Orig., 1909, 2, 377.

(2) Flexner, S., and Lewis, P.A.: The transmission of Acute Poliomyelitis to Monkeys: J. A. M. A., 1909, 53, 1639. First note.

(3) Flexner, S. and Lewis, P.A.: Transmission of Epidemic Poliomyelitis to Monkeys; a further note, second note. J.A.M.A., 1909, 53, 1913.

(4) Strauss and Huntoon: N.Y. Med., Jour., 1910, XCI, 64.

(5) Leiner and von Wiesner: Wien., klin., Wchnschr., 1909, XXII, 1698.

(6) Landsteiner and Levaditi: Compt., rend., Soc., de Biol., 1909, IXVII, 592.

(7) Peabody, F.W., and Draper G.: Dochez, A.P.: Monographs of the Rockefeller Institute No. 4, June 24, 1912.

(8) Poliomyelitis, International Committee: Williams and Wilkins Co., Baltimore, 1932, p. 107.

(9) Armstrong, Chas.: Public Health Reports Vol., 54, Sept. 22, 1939.

(10) Armstrong, Chas.: Jour., Bact., 39; 63, Jan. 1940.

(11) Armstrong, Chas.: Public Health Report, Vol., 54, No. 52, Dec. 29, 1939.

(12) Sawyer, W.A., and Lloyd, W.: Jour. Exper., Med: 54, 533, 1931.

(13) Hoffman, D.C., and Duran-Reynals: Science, Nov. 14, 1939, Vol. LXXII, No. 1872, p. 508.

(14) Traub, E.: Science: Vol. 81, p. 298, 1933.

(15) Traub, E.: Jour., Exper., Med., Vol. 63, 1936, p. 533.

(16) Kramer, S.D.: Public Health Reports; Vol. 54, No. 43, Oct. 27, 1939.

(17) Burnet, F.M. and Macnamara, J.: Immunological difference between Strains of Poliomyelitis Virus: Brit. J. Exp. Path., 1931, 12, 57.

(18) Macnamara, J.: Serum Therapy in Acute Poliomyelitis, Canad. Pub. Health. Jour., 1932, 23, 318.

(19) Weyer, E.B., Immunological differences between a Strain of Monkey Virus and Human Poliomyelitis Virus: Proc. Soc. Exp. Bio. and

Med., 1931, 29, 289.

(20) Rhoads, C.P.: Jour. Exp. Med.: April 1, 1929, Vol. XLIX, pp. 701-704.

(21) Lillie, R.D. and Armstrong, Chas.: Public Health Reports, Vol. 55, Jan. 19, 1940, P. 115.

(22) Toomey and Takacs: Proc. Soc. Exp. Bio. and Med., Vol. 43, No. 3, March 1940, P. 536.

(23) Parachivesco, M. (Mme.) and Papazoiu (Mlle.), Compt. rend. Soc. Biol., p. 503-508. 1940.

(24) Sabin, A.B., and Olitsky, P.K.: Influence of Host Factors on Neuroinvasiveness of Vesicular Stomatitis Virus, J. Exp. Med. July 1, 1937, Vol. 66, No. 1, P. 15-57.

(25) Sabin, A.B. and Olitsky, P.K.: Influence of Host Factors on Neuroinvasiveness of Vesicular Stomatitis Virus, J. Exp. Med. February 1, 1938, Vol. 67, No. 2, P. 201-249.

(26) Paul, J.R. and Trask, J.D.: A Comparative Study of Recently Isolated Human Strains and Passage Strains of Poliomyrlitis Virus; J. Exper., Med., 58: P. 513-529, 1933.

(27) Paul, J.R., Trask, J.D: Beebe, A.R., and German, W.J.: Viruses of Poliomyelitis,: Jour. Exper. Med., 65: 687-704: 1937.


WAGENINGEN