THE SUSCEPTIBILITY OF THE GOLDEN HAMSTER TO THE LANSING STRAIN OF POLIOMYELITIS VIRUS

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THE SUSCEPTIBILITY OF THE GOLDEN HAMSTER TO THE LANSING STRAIN OF POLIOMYELITIS VIRUS

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TABLE OF CONTENTS

INTRODUCTION

Since 1909, when Landsteiner and Popper succeeded in transferring the virus of poliomyelitis to monkeys (1), there have been numerous attempts to adapt this virus to smaller laboratory animals (2). However, various species of monkeys were for many years the only animal suitable for experimental poliomyelitis research. The successful adaption, by Charles Armstrong in 1939, of a monkey-adapted strain to the Eastern cotton-rat and then to the white mouse (3,4), was an important advancement in the history of experimental poliomyelitis research. From then on, many problems of poliomyelitis could be studied experimentally on a large scale, using the Lansing strain of virus and small laboratory animals.

Any further increase in the range of susceptible hosts would, of course, be important to poliomyelitis research. Attempts were made by certain investigators (5,6), to transmit the Lansing strain of poliomyelitis virus to the hamster. However, the reports available are contradictory. It seemed therefore desirable to investigate thoroughly the susceptibility of the Golden hamster to the Lansing strain of poliomyelitis virus.

An adequate supply of Golden hamsters for this study was available in the colony maintained at the quarantine farm by the Michigan Department of Health and through a commercial farm. Eastern cotton-rats and white mice from the quarantine farm have been used as

comparative experimental animals in testing the titer of the Lansing strain of poliomyelitis virus used in the experiments. .

The purpose of this study is to establish whether and under what conditions the Golden hamster can be used as an experimental animal in poliomyelitis research.

I. DESCRIPTION OF THE GOLDEN HAMSTER

The Golden hamster (Cricetus auratus), although only recently introduced into the United States, has attracted considerable attention in many laboratories with respect to its usefulness as a laboratory animal for diversified experimental research.

The Golden hamster is a rodent native to Syria with an apparently restricted geographical distribution. Bruce and Hindle (7), traced the entire laboratory stock of this species in the British Isles to a single litter dug from an 8-foot burrow near Alippo, Syria in 1930. The American laboratory stock of the Golden hamster is probably of the same genetic strain as the British stock. No great difficulty has been encountered in inducing the animal to breed in captivity, but experience has shown that certain precautions are necessary to maintain a successful colony.

The Golden hamster resembles the common hamster of Europe and Northern Asia in that it has relatively large cheek-pouches and a short tail. A full-grown Golden hamster rarely exceeds the length of seven inches, and has a deep golden-brown color, but toward the roots the hairs are dark grey. The fur is short, soft and smooth. The ventral surface is very light grey, with white patches. The eyes are large and black. The cheek-pouches are well developed and can hold a surprisingly large amount of food. In nature, they are presumably

herbivorous, in captivity they are omnivorous and in addition to grain, will feed on roots, nuts, bread, fruits, vegetables, meat, etc. and will carry off and store almost any portable object.

II. REVIEW OF LITERATURE

Investigators have used the Golden hamster in medical research for several years. Dale, Lazansky and Keyes (8) reported that in preliminary studies they found dental caries in Golden hamsters to be grossly and microscopically comparable to the disease in human beings. The type of caries produced in the hamster and the adaptability of the animal to the laboratory seem to render it superior to the rat as a subject for caries investigation.

Stewart, Florio and Mugrage, during the course of experimental work on the transmission of Colorado tick fever to the Golden hamster (9), report complete data concerning the blood elements of this rodent. The susceptibility of hamsters to tularemia (10) approximates that of white mice. They are equally susceptible to subcutaneous and intraperitoneal inoculation as reported by Larson in 1945. Randall and Cooper (11), reported in 1944, that there is evidence that the Golden hamster is the animal of choice for the isolation of Leptospira, especially of the Leptospira canicola type since young guinea pigs and mice are resistant to infection and rats are entirely refractory.

Taylor and Parodi (12) reported the use of the hamster for detection of influenza virus from throat washings. The comparative inoculations which have been made in hamsters and ferrets, indicate that the hamster may be substituted for the ferret in identifying influenza A virus in throat-washings, either through the production of specific neutralizing antibodies to this virus, or as the first step in adapting the virus to mice. Eaton and Van Herrick (13) in a study on experimental immunization with a virus isolated from cases of primary atypical pneumonia utilized both hamsters and cotton-rats. Immunity against this virus was produced by intranasal infection and by intraperitoneal inoculation of active and formalinized virus. After infection the duration of immunity in hamsters was apparently more than five months and in cotton-rats less than three months.

Schabel, Miller et al. (14) reported the susceptibility of the hamster to St. Louis and Japanese encephalitis viruses by feeding. The report describes the extent of the infection in hamsters following feeding with St. Louis (Hubbard strain) and with Japanese (Nakayama strain) encephalitis viruses, as determined by subinoculation of mice with various tissues.

Schlesinger, Morgan et al. (15) reported the transmission of the Lansing type of poliomyelitis virus originating in the Middle East to hamsters, but passage

from these hamsters into others was unsuccessful. Dallorf and Whitney (16) in demonstrating the "sparing effect" or "interference phenomen" in poliomyelitis used young hamsters and a strain of rodent paralyzing virus, recovered from a fatal human case by hamster passage. An interesting report by Sanders (17) on a poliomyelitis-like agent in hamsters inoculated with Lansing strain of virus, showed successful passage of the virus through 24 serial passages in the brains of adult hamsters. The titer of the virus remained the same during the 24 passages in adult hamsters but on transfer to suckling hamsters the titer promptly rose and remained at a significantly higher level during serial passage in infant hamsters. The exact identity of the hamster virus was uncertain. Anti-Lansing virus monkey sera gave inconsistent results in neutralization tests with the adult and infant hamster viruses. Moreover, the hamster viruses were unaffected by four separate pools of normal human sera all of which neutralized the Lansing strain of poliomyelitis virus. It appeared that the hamster virus represented a poliomyelitis-like agent which was infectious for hamsters.

Armstrong (18) reported the transmission of the Lansing strain virus through 2 or 3 passages in hamsters. Plotz et al. (6) were successful in the transmission of the mouse-adapted strain through sixteen Syrian hamster passages. Milzer and Byrd (5)

failed to produce infection in hamsters inoculated repeatedly with the mouse-adapted Lansing strain of virus suspended in buffered saline, pH-4.0, or in normal mouse serum. However, they stated that in using the technique of mixing the virus suspensions with suspensions of autolyzed brain tissue of normal mice, they were successful in transmitting the Lansing strain of poliomyelitis to hamsters.

III. EXPERIMENTAL METHODS

A. Methods of Inoculation

The first step in investigating the susceptibility of the Golden hamster to the Lansing strain of poliomyelitis virus was to find by what method the hamster could consistently be infected with the virus. Three different routes of inoculation were chosen: intracerebral, intranasal and intraperitoneal. The mouse-adapted Lansing strain of poliomyelitis virus was used as the initial source of virus. Spinal cords and medullae of paralyzed mice were ground in a mortar with sterile alundum to a 10 per cent suspension in physiological saline (pH-7.2).

The same standard inocula as employed for infecting cotton-rats were used for inoculating the hamsters. Separate groups of four to eight weeks old hamsters were inoculated with a 10 per cent suspension of infective mouse cords and medullae as follows:

0.06 ml. intracerebrally, 0.3 ml. intraperitoneally and 0.1 ml. intranasally. Comparable numbers of cotton-rats were inoculated by the same methods to check the infectivity of the mouse-adapted virus, and titrations of the virus in white mice were included in certain experiments.

The first three preliminary experiments showed that the Golden hamster could be infected with the Lansing strain of poliomyelitis virus by the intracerebral method of inoculation. At least 50 per cent of the hamsters inoculated intracerebrally became paralyzed after an average incubation period of from two to eight days.

The failure to produce infection in hamsters by either the intranasal or intraperitoneal routes proved to be of value in later experiments when vaccination of the hamsters by the intraperitoneal route was effective in producing an immune response to the Lansing strain of virus.

B. Clinical Symptoms

The signs of illness in hamsters have been found to be similar to those in mice and cotton-rats infected with the Lansing strain of poliomyelitis virus. Poliomyelitis in the Golden hamster runs the following course. A wide variation in the length of the incubation period was noted, with paralysis

occurring in some cases on the second day after infection to as long as 21 days after inoculation. The first symptoms were usually irritability and malaise followed by a swelling of the eyelids, so that they were nearly closed. Often an ocular exuadate was noted and some ruffling of the fur was common. Paralysis usually occurred from 1 to 2 days after the appearance of the first symptoms. The extent of paralysis varied, from slight irregularities in gait, paralysis of only a few toes on one foot to complete paralysis of one or more extremities. In the animals that survived, there was a permanent paralysis followed by atrophy and contractures. Of particular note was the fact that in some cases in which the animal survived, a decided improvement was observed in the extremities after the initial paralysis. In some cases an almost complete recovery took place with little evidence of paralysis remaining a week to ten days after the appearance of the first signs of paralysis.

C. Pathology of the Infected Hamster

After the infected hamster was shown to develop clinical symptoms of poliomyelitis, a histologic study of the central nervous system of paralyzed hamsters was desirable. From ten hamsters, showing typical symptoms of paralysis in one or more extremities, sections

of the brain, medulla and spinal cord were examined for lesions.

Grossly the brain and spinal cord exhibited no abnormalities. Microscopically, sections of the brain did not show any evidence of abnormality or lesions. In the medulla and spinal cord at various levels typical lesions, caused by the virus of poliomyelitis, were evident. A well marked perivascular round cell infiltration with degeneration and a decrease in the number of motor cells was prevalent in the medulla and various levels of the spinal cord. The anterior horns of the spinal cord exhibited infiltration by round and wandering cells, neuronophagia and loss of motor cells.

The microscopic lesions observed are similar to those found in other animals (19) infected with the Lansing strain of poliomyelitis virus.

IV. EXPERIMENTAL RESULTS

A. Serial Passages

The results of serial passage of the Lansing strain of poliomyelitis virus made in two different strains of Golden hamsters are shown in Table I. The two strains of hamsters used in this study are those raised at the colony maintained at the Quarantine farm of the Michigan Department of Health and those bought from a commercial firm known as the Tumblebrook farm at Brant Lake, New York. In further references to the two

strains of hamsters they will be designated as hamsters from the Quarantine farm and hamsters from the Tumblebrook farm.

The first series of hamster to hamster passage of the poliomyelitis virus was carried out in hamsters from the Quarantine farm. Mouse-passage Lansing strain virus was used to initiate these passages. An inoculum of 0.06 ml. of a 10 per cent suspension of mouse cords and medullae was injected intracerebrally into five hamsters 6 to 8 weeks old. After 4 days two hamsters became paralyzed. The cords and medullae from these hamsters were then ground and made into a 10 per cent suspension in saline and passed serially into five other hamsters.

Table I, part A shows the result of six successive hamster to hamster passages of the Lansing strain of virus using hamsters from the Quarantine farm. Of the 28 inoculated hamsters 10 or 35.7 per cent developed paralysis. The incubation period varied from 3 to 11 days, the average period of incubation being 5.7 days. Of particular interest was the fact, that on successive passage in hamsters from the Quarantine farm, the virulence of the virus varied until on the sixth passage the tissue suspension was no longer infective for hamsters.

The second series of passages of the Lansing strain of virus was carried out in hamsters from the Tumblebrook farm. The same technique was used to init-

iate this series of passages as was employed with the other strain of hamsters. Table I, part B shows the results of successive hamster to hamster passage of the virus using hamsters from the Tumblebrook farm. Of the 24 inoculated hamsters 13 or 54.2 per cent became paralyzed. The incubation period varied from 2 to 18 days, the average period of incubation being 5.1 days. The CNS tissue material was still infective for hamsters on the sixth passage with one hamster becoming paralyzed on the sixth day after inoculation.

In comparing the susceptibility of the two strains of hamsters on the basis of serial passages, there was an indication that hamsters from the Tumblebrook farm were more susceptible to the Lansing strain of poliomyelitis virus. As shown in Table I, a greater percentage of Tumblebrook hamsters were paralyzed, and the average incubation period was shorter. More information on this point was obtained from later experiments.

B. Comparative Titrations

After the Lansing strain of poliomyelitis was successfully transmitted to the Golden hamster, it was possible to compare the susceptibility of the hamster to that of other animals. The virus was titrated as described below, in hamsters, mice and cotton-rats, and the titers compared. By means of repeated titrations it was hoped to establish whether the titer of the virus in

Table I

Results of Serial Hamster-Passage

Part A - Hamsters from Quarantine Farm

Passage No.	No. of Animals inoculated	No. of Animals paralyzed	Per cent paralyzed	Day after inoculation paralysis occurred
1	4	2	50%	3-4
2	5	2	40%	3-11
3	5	1	20%	3
4	5	4	80%	4-4-8-9
5	4	1	25%	8
6	5	0	0%	0
Totals:	28	10		
	Average:		35.7%	5.7 days

Part B - Hamsters from Tumblebrook Farm

Passage No.	No. of Animals inoculated	No. of Animals paralyzed	Per cent paralyzed	Day after inoculation paralysis occurred
1	4	4	100%	2-3-3-5
2	4	3	75%	3-3-18
3	4	2	50%	4-4
4	4	1	25%	4
5	4	2	50%	5-16
6	4	1	25%	6
Totals:	24	13		
	Average:		54.2%	5.1 days

hamsters is as consistent as in white mice and cotton-rats. It was also interesting to determine whether there was any difference between the susceptibility of the hamster to hamster-passage, to mouse-passage and to cotton-rat passage virus.

Table II, part A shows the results of the first comparative titrations using mice, cotton-rats and hamsters from the quarantine farm. The outcome of the experiments with mouse-passage, rat-passage and hamster-passage virus are summarized. Five-fold dilutions of the virus in physiological saline (pH-7.2) were used with the initial dilution for hamsters being 5 per cent and for mice and cotton-rats 0.5 per cent. The virus titer has been expressed as the LD_{50} (Fifty percent end-point), calculated by the method of Reed and Muench (20), for those titrations in which a clear-cut end-point was reached. For accurate calculation of the LD_{50}, %, at least either 100% or 0% mortality end-point should be reached, preferably both.

The source of virus for these titrations was a fresh stock supply of spinal cords and medullae from paralytic hamsters, mice and cotton-rats. The virus material was stored in a "Deep-Freeze" at -28.0°C. until used. The cords and medullae were thawed at room temperature, ground in a mortar with sterile alundum to a 10 per cent suspension in saline. From the 10 per cent suspension the final virus dilutions were prepared for

the titrations. The inoculum for hamsters and cotton-rats was 0.06 ml. intracerebrally and for mice 0.03 ml. intracerebrally. Except for the first titration, four hamsters and four cotton-rats and six mice were inoculated for each dilution. The observation period after inoculation was 30 days, and the animals were checked daily.

The first comparative titrations carried out with mouse-passage and rat-passage virus gave a very low titer in hamsters with a relatively high titer in cotton-rats and mice. The hamster-passage virus gave an exceedingly low titer in hamsters, mice and cotton-rats. The initial dilution for hamsters was taken as 10 per cent and that for mice and cotton-rats as 5 per cent in this titration.

A second series of comparative titrations in mice, cotton-rats and hamsters from the Tumblebrook farm was carried out. With the mouse-passage virus a relatively high titer was obtained in hamsters, mice and cotton-rats. An LD_{50} titer of 0.015 per cent was obtained in hamsters and a titer of 0.0016 per cent in mice. The titration of mouse-passage virus in cotton-rats did not give a clear-cut endpoint as the 0 per cent mortality was not reached with a virus dilution of 0.0008 per cent.

The rat-passage and hamster-passage virus gave a relatively good titer in mice, cotton-rats and hamsters. The titers obtained with hamster-passage virus should be

noted in comparison to the first comparative titration using hamster-passage virus. An LD_{50} titer of 0.60 per cent was obtained in hamsters, 0.018 per cent in cotton-rats and 0.054 per cent in mice. The outcome of the second series of comparative titrations suggested that the strain of hamsters from the Tumblebrook farm were more susceptible to the Lansing strain of poliomyelitis virus than those bred at the Quarantine farm.

In order to check the difference in susceptibility between the two strains of hamsters, a comparative titration was carried out using mouse-passage virus as the infective virus material. Because the 100% or 0% mortality end-points were not reached, the titration was not suitable for calculating the LD_{50} titer. However, a marked difference in susceptibility between the two strains of hamsters was again indicated. As this titration is not included in Table II a word of explanation is necessary. Of 20 hamsters inoculated for each strain, using five-fold dilutions of the virus, 10 hamsters or 50 per cent from the Tumblebrook farm became paralyzed, as compared to 6 hamsters or 30 per cent from the Quarantine farm. The most significant difference was shown by the fact that hamsters from the Tumblebrook farm became paralyzed when given a dilution as low as 0.008 per cent while the lowest infective dilution for hamsters from the Quarantine farm was 1 per cent.

Table II

Part A - First Comparative Titrations

Date	Material Inoculated	Animal Used	Dilutions					
			5%	1%	0.2%	0.04%	0.008%	LD_{50}, %
8-4-47	Mouse-passage virus	Farm hamsters	2/3*	0/2	0/3	1/3	0/3	- - -
			0.5%	0.1%	0.02%	0.004%	0.0008%	
		Cotton-rats	2/2*	3/3	2/2	1/2	0/2	0.004%
		White mice	2/2	3/3	3/3	1/2	3/3	- - -
			5%	1%	0.2%	0.04%	0.008%	
8-15-47	Rat-passage virus	Farm hamsters	1/4	0/4	0/4	0/4	0.4	- - -
			0.5%	0.1%	0.02%	0.004%	0.0008%	
		Cotton-rats	3/4	3/4	2/4	0/4	0.4	0.029%
		White mice	4/5	6/6	2/5	0/6	0/5	0.028%
			10%	2%	0.4%	0.08%	0.016%	
9-5-47	Hamster-passage virus	Farm hamsters	1/4	0/4	0/4	0/4	0/4	- - -
			5%	1%	0.2%	0.04%	0.008%	
		Cotton-rats	2/4	0/4	0/4	0/4	0/4	- - -
		White mice	3/4	2/5	0/5	0/5	0/6	1.67%

* Number of paralyzed animals over the total number of animals (paralyzed plus survivors)

Table II - continued

Part B - Second Comparative Titrations

Date	Material Inoculated	Animal Used	Dilutions					LD_{50}, %
			5%	1%	0.2%	0.04%	0.008%	
10-3-47	Mouse-passage virus	Tumblebrook hamsters	3/3*	3/4	3/4	4/4	2/4	0.015%
			0.5%	0.1%	0.02%	0.004%	0.0008%	
		Cotton-rats	3/4	2/2	4/4	3/3	3/4	- - -
		White mice	5/5	6/6	6/6	4/5	1/3	0.0016%
			5%	1%	0.2%	0.04%	0.008%	
10-17-47	Rat-passage virus	Tumblebrook hamsters	4/4	3/3	2/4	2/4	0/4	0.09%
			0.5%	0.1%	0.02%	0.004%	0.0008%	
		Cotton-rats	4/4	4/4	4/4	0/4	0/4	0.009%
		White mice	5/5	5/5	5/5	3/5	0/6	0.0015%
			10%	2%	0.4%	0.08%	0.016%	
10-10-47	Hamster passage virus	Tumblebrook hamsters	3/4	2/4	2/4	2/4	0/4	0.60%
			5%	1%	0.2%	0.04%	0.008%	
		Cotton-rats	4/4	3/3	4/4	3/3	0/4	0.018%
		White mice	5/5	4/4	2/5	1/4	3/5	0.054%

* Number of paralyzed animals over the total number of animals (paralyzed plus survivors)

C. Evaluation of the Effect of low pH of Inoculum and of the Autolyzed Brain Tissue Techniques

In a report by Hammon and Izumi (21) it was stated that in the mouse, employing the Lansing strain of mouse-passage virus, a definite quantity of virus can be rendered more effective in producing infection by suspending it in a menstruum of low pH. The virus suspension at pH-4.0 appeared to be as effective as 4 to 16 times as much virus at pH-7.0. The value of this technique to increase the susceptibility of the Golden hamster was determined by comparative titrations using the mouse-passage virus at low pH (4.0) and at higher pH (7.0).

The buffered solution of saline at pH-4.0 was prepared by adding definite amounts of N/10 acetic acid and N/10 sodium hydroxide to a liter of unbuffered physiological saline. The solution was sterilized by filtration using a Seitz filter, then the pH was checked and any necessary adjustment was made with sterile reagents. 10 per cent suspensions of infective mouse cords and medullae was prepared by grinding equal portions of cords and medullae in a mortar with sterile alundum using saline solutions at pH-4.0 and at pH-7.2. Five-fold dilutions of the virus were made up using the saline at pH-4.0 and at pH-7.2 respectively. Comparative titrations were carried out in hamsters from the Tumblebrook farm only. The initial dilution of the mouse-passage virus was 5 per cent. The hamsters were inoculated

intracerebrally, employing a dosage of 0.06 ml. The hamsters were observed for a period of 30 days with a daily check of the animals. Three comparative titrations were carried out using mouse-passage virus suspensions at these two pH values. The first comparative titration did not have a definite end-point; however a sufficient number of hamsters were paralyzed to show that the virus was more infective at pH-7.2 than at pH-4.0. The second comparative titration clearly demonstrated this fact when the titration at low pH gave an LD_{50} titer of 0.262 per cent as compared to an LD_{50} titer of 0.058 per cent at pH-7.2. The third comparative titration was inconclusive with approximately the same number of hamsters becoming paralyzed at pH-4.0 as compared to the titration at pH-7.2.

The data from these comparative titrations suggests that an increased acidity of the menstruum does not render the Lansing strain of mouse-passage virus more infective for the Golden Hamster.

Milzer and Byrd (5) reported that autolyzed brain tissue diluent shortens the incubation period and facilitates the transfer of poliomyelitis virus to CFW mice, hamsters, and rhesus monkeys. By means of this technique they were able to isolate several strains of poliomyelitis virus from infected human feces and spinal cord in CFW Swiss mice. They reported the transmission of poliomyelitis virus (Lansing strain) to hamsters by

using the technique of mixing virus suspensions with suspensions of autolyzed brain tissue of normal mice. Therefore, it seemed desirable in this study to establish whether the use of this technique had any effect on the susceptibility of the hamster to mouse-passage virus.

For this study the autolyzed brain tissue was prepared from normal white Swiss mice that were killed by applying sufficient pressure at the base of the brain to sever the spinal column. The sacrificed mice were kept at room temperature (24.0° to 25.0°C.) for a period of about 17 hours to allow the tissue to autolyze. A 10 per cent suspension by weight of autolyzed brain tissue in nutrient broth (Baltimore Biological Laboratory, pH-6.95) was then prepared by grinding the brains in a sterile mortar. The suspension was checked for sterility and stored in a refrigerator over night. Immediately before being used the suspension of autolyzed brain tissue was filtered through sterile gauze. The virus suspension was prepared by grinding the cords and medullae of paralyzed mice in B.B.L. nutrient broth.

Ten hamsters were inoculated with a 4 per cent suspension of mouse-passage virus mixed with an equal amount of a 10 per cent suspension of autolyzed normal mouse brain tissue in B.B.L. nutrient broth. Ten other hamsters were inoculated with a 4 per cent suspension of mouse-passage virus mixed with an equal amount of B.B.L. nutrient broth. An evaluation of this technique was made

on the basis of the number of paralyzed hamsters and the average length of the incubation period. Hamsters from the Tumblebrook farm were used. The results of the first experiment were as follows: of the 10 hamsters inoculated with virus and autolyzed normal mouse brain tissue, 5 became paralyzed after an average incubation period of 4 days, while of the 10 control animals inoculated with virus and B.B.L. broth, 7 became paralyzed after an average incubation period of 3 days.

The second experiment was carried out using Tyrod's solution (pH-7.9) for preparing the autolyzed brain tissue and virus suspension. The results of this experiment are as follows: 4 of 10 hamsters were paralyzed after an average incubation period of 7.5 days for the hamsters inoculated with the virus suspension mixed with an equal amount of autolyzed normal mouse brain tissue in Tyrode's solution; in comparison, 5 of 10 hamsters were paralyzed after an average incubation period of 4 days for those hamsters inoculated with the virus suspension mixed with an equal amount of Tyrode's solution.

The successful transfer by Milzer and Byrd (5) of a monkey-passage strain (Leon) of poliomyelitis virus to white Swiss mice, using autolyzed brain tissue diluent suggested an investigation of this result utilizing hamsters. Ten hamsters were inoculated with a 10 per cent suspension of monkey cord, infected with a human strain

of poliomyelitis virus not adapted to rodents, mixed with an equal amount of 10 per cent suspension of autolyzed normal mouse brain tissue in Tyrode's solution. All the hamsters remained normal during an observation period of thirty days. An investigation to check the susceptibility of the Golden hamster in adapting human or monkey strains of virus to the hamster by this technique should be considered in further experiments.

D. Immune Response

A study has been made to test the immune response of the hamsters using the mouse-passage, rat-passage and hamster-passage virus. The hamsters were vaccinated intraperitoneally with 0.3 ml. of a 10 per cent suspension of active virus. The hamsters were vaccinated twice weekly with a total of seven vaccinations, and bled at the end of two, three and four weeks. The antibody titer of the sera was checked by neutralization tests using white Swiss mice as the test animal. A negative control of normal hamster serum and a positive control of human immune serum globulins were included in each experiment. Both hamsters from the Quarantine farm and hamsters from the Tumblebrook farm were tested for immune response to the Lansing strain of poliomyelitis virus.

The neutralization tests were carried out with constant amount of virus (1 per cent final dilution)

and decreasing amounts of serum. The final serum dilutions were 1:10, 1:20, 1:40, 1:80, 1:160. The serum-virus mixture was allowed to stand at room temperature for a period of two hours, then for each dilution six mice were inoculated intracerebrally with 0.03 ml. of suspension. The mice were checked daily for a period of thirty days. The antibody titer of the serum has been expressed as the LD_{50} (Fifty per cent end-point), calculated by the method of Reed and Muench.

Table III summarizes the results of four experiments carried out to test the immune response in hamsters. As shown by neutralization tests the antibody titer of the serum was greatest at the end of three weeks (seven vaccinations) for those hamsters vaccinated with mouse-passage and hamster-passage virus. The hamsters which were vaccinated with cotton-rat passage virus demonstrated a gradual increase in the antibody titer of the serum over the two to four weeks period. No evidence of any neutralizing antibodies was found in the normal hamster serum. The positive control of human immune serum globulins neutralized the Lansing strain of mouse-passage virus. As shown in Table III, the LD_{50} titers obtained with sera of immunized hamsters were of the same magnitude as those obtained with a preparation of human immune serum globulins.

Table III—Immune Response in Hamsters to the Lansing Strain Virus

Exp't No.	Virus Material for Vaccination	Animal Used	Time Period - No. of Vaccinations	Serum Dilutions 1:10	1:20	1:40	1:80	1:160	LD50
No. I	Mouse-passage virus	Tumblebrook hamsters	Four vaccinations Two weeks	2/5*	4/6	6/6	3/6	4/5	1:20
			Seven vaccinations Three weeks	0/6	4/6	2/6	5/6	3/6	1:47
			No additional vaccinations - Four weeks	1/5	2/5	2/5	3/6	3/6	1:45
			Negative control	6/6	6/6	4/4	6/6	6/6	No neutralization
			Positive control	0/6	0/6	0/5	1/2	1/4	Unable to calculate
No. II	Mouse-passage virus	Farm hamsters	Four vaccinations Two weeks	2/6	2/6	3/4	4/4	4/5	1:24
			Seven vaccinations Three weeks	1/6	1/6	1/5	1/5	3/5	1:96
			No additional vaccinations - Four weeks	1/6	3/5	3/5	1/2	2/3	1:27
			Negative control	3/3	5/5	5/5	3/3	5/5	No neutralization
			Positive control	1/6	0/4	1/5	3/5	3/6	1:92
No. III	Hamster-passage virus	Tumblebrook hamsters	Four vaccinations Two weeks	5/5	4/4	6/6	4/5	5/5	No neutralization
			Seven vaccinations Three weeks	1/5	2/4	4/5	6/6	3/4	1:27
			No additional vaccinations - Four weeks	3/6	4/6	3/6	4/5	5/5	1:18
			Negative control	5/5	6/6	5/5	4/4	5/5	No neutralization
			Positive control	1/6	1/5	0/5	0/4	3/5	1:102
No. IV	Rat-passage virus	Tumblebrook hamsters	Four vaccinations Two weeks	2/6	2/3	3/4	3/3	5/6	1:17
			Seven vaccinations Three weeks	0/5	0/5	1/5	4/5	3/6	1:73
			No additional vaccinations - Four weeks	0/6	0/5	3/6	1/6	2/6	1:126
			Negative control	6/6	3/3	6/6	5/5	6/6	No neutralization
			Positive control	1/5	2/6	2/4	1/5	5/6	1:64

* Ratio of paralyzed mice to total number of mice (paralyzed plus survivors).

V. DISCUSSION

One of the objects of this study on the susceptibility of the Golden hamster to the Lansing strain of poliomyelitis virus was to find whether these animals have any advantage over other rodents as an experimental host for research on experimental poliomyelitis. The experimental work completed in this study indicates that the Golden hamster is susceptible to the Lansing strain virus, the infection produced in the hamster being much like that of other rodents susceptible to this strain. The clinical and pathologic manifestations seen in the hamster are similiar to those observed in other experimental animals.

The failure to maintain the virus through more than five to six serial passages is of importance in considering the value of this rodent as an experimental animal for poliomyelitis research. One of the essential criteria of usefulness of an experimental animal for research is the successful transmission of a particular strain of virus through numerous serial passages. The comparative titrations with hamsters, mice and cotton-rats, using mouse-passage, rat-passage and hamster-passage virus, show clearly the irregularity in the susceptibility of the Golden hamster to Lansing strain of virus. The titers obtained in hamsters as compared to those in mice and cotton-rats seem to preclude the use of hamsters as an experimental animal in work with

poliomyelitis. The evalution of the effectiveness of the low pH of the inoculum and the autolyzed brain tissue techniques indicated that these techniques have no apparent value in shortening the incubation period and increasing the susceptibility of the hamster to the Lansing strain of virus.

Because of the known occurrence of latent neurotropic viruses in various animals, particular attention was paid to the identity of the agent recovered from the Golden hamster. It should be pointed out that Sanders (17) recently reported the isolation from hamsters, inoculated with Lansing strain virus, of a "poliomyelitis-like agent" of uncertain identity. This virus was not neutralized by human sera containing anti-Lansing antibody, and gave inconsistent results with anti-Lansing monkey sera. Furthermore this virus was carried through 24 serial passages while in this study the Lansing strain virus could not be maintained through more than 6 passages.

Since mouse-passage Lansing strain virus was used for the neutralizations tests, and since this mouse-passage virus was neutralized by antibodies produced in hamsters in response to vaccination with mouse-passage, cotton-rat passage and hamster-passage virus, it seems that the hamster-passage virus in this study actually is the Lansing strain and not a virus of unknown identity accidently picked up.

VI. SUMMARY

It has been shown that the Golden hamster, (Cricetus auratus), is susceptible to the Lansing strain of poliomyelitis. The typical symptoms of paralysis and the lesions are similar to those of experimental poliomyelitis in other animals. The virus was successfully transmitted through only six passages in two different strains of hamsters. The mouse-adapted Lansing strain virus was neutralized by antibodies produced in hamsters in response to vaccination with mouse-passage, cotton-rat passage and hamster-passage virus.

From the data presented, it appears that the Golden hamster can be used in certain phases of experimental poliomyelitis research. However, the limited number of successful serial passages and the results of the comparative titrations suggest the definite superiority of cotton-rats and white Swiss mice as experimental animals for the Lansing strain of poliomyelitis virus.

VII. BIBLIOGRAPHY

1. Lansteiner, K., and Popper, E., Uebertragung der Poliomyelitis acuta auf Affen, Ztschr. f. Immunitatsforsch. u. exper., Therap. 2; 377, 1909.

2. Toomey, J. A., Experimental poliomyelitis in animals other than the monkey, review of literature, J. Pediat. 16: 519, 1940.

3. Armstrong, C., The experimental transmission of poliomyelitis to the eastern cotton-rat, Pub. Health Rep. 54: 1719, 1939.

4. Armstrong, C., Successful transfer of the Lansing strain of poliomyelitis virus from the cotton-rat to the white mouse, Pub. Health Rep. 54: 2302, 1939.

5. Milzer, A. and Byrd, C. L., Autolyzed brain tissue as a means of facilitating transmission of experimental poliomyelitis, Science, 105: (2716), 70, 1947.

6. Plotz, H., Reagen, R. and Hamilton, H. L., Transmission of the murine strain of poliomyelitis to the Syrain hamster, Proc. Soc. Exptl. Biol. and Med., 51: 124. 1942.

7. Bruce and Hindle, Proc. Zool. Soc. London, Part 1: 361, 1934.

8. Dale, P. P., Lazansky, J. P., and Keyes, P. H., Production and inhibition of the dental caries in Syrian hamster, preliminary observations, J. of Dental Research, 23: 445, 1944.

9. Steward, M. D., Florio, L., Mugrage, E. R., Hematological findings in the Golden hamster, J. of Exp. Med., 80: 189, 1944.

10. Larson, C. L., The susceptibility of the Golden hamster to tularemia, Pub. Health Rep. 60: 839, 1945.

11. Randall, R., and Cooper, H. K., The Golden hamster as a test animal for the diagnosis of leptospirosis, Science 100: (2589), 133. 1944.

12. Taylor, R. M. and Cooper, H. K., Use of hamster (Cricetus auratus) for detection of Influenza virus in throat washings, Proc. Soc. Exptl. Biol and Med., 49: 105, 1942.

13. Eaton, M. D., and Van Herick, W., Experimental immunization with the virus from primary atypical pneumonia, J. Inf. Dis. 81: 116, 1947.

14. Schabel, R. W., Jur., Sherwood, M., Abendroth, M., Gordon, F. B., Susceptibility of the hamster to St. Louis and Japanese encephalitis viruses by feeding, Proc. Soc. Exptl. Biol. and Med., 66: 332, 1947.

15. Schlesinger, R. W., Morgan, I. M., and Olitsky, P. K., Transmission to rodents of Lansing type poliomyelitis virus originating in the Middle east, Science 98: 452, 1943.

16. Dallforf, G., and Whitney, E., A further interference in experimental poliomyelitis, Science 98: 477, 1943.

17. Sanders, F. K., A poliomyelitis-like agent in hamsters inoculated with Lansing virus, Abstract in: Fed. Proc. 7 (1): 309, 1948.

18. Armstrong, C. and Packschanian, unpublished data, referred to in Vanderbilt Lectures, April, 1941.

19. Lillie, R. D., and Armstrong, C., Cerebrospinal pathology of experimental poliomyelitis in the eastern cotton-rat, Sigmodon hispidus hispidus, and in the white mouse, Mus musculus, Pub. Health Rep. 55: 718, 1940.

20. Reed, L. J., and Muench, H., A simple method of estimating Fifty per cent end-points, Am. J. Hyg. 27: 493, 1938.

21. Hammon, W. M., and Izumi, E. M., Armstrong mouse adapted poliomyelitis virus: Effect of pH of inoculum and of "Enteric organism filtrate," Proc. Soc. Exptl. Biol. and Med. 48: 579, 1941.

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