ENCEPHALOMYELITIS OF MICE

III. Epidemiology

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Since the discovery of the virus of mouse encephalomyelitis in 1933 (1) spontaneously affected animals have been observed every year. The incidence of the disease is exceedingly low, not more than four or five cases being observed each year, although many thousands of mice are used annually in our laboratory. In the original description, the incidence was estimated to be about one or two per thousand. Sabin and Olitsky (2) estimated the incidence in the Rockefeller Institute strain of mice as one to two in one thousand. These estimates are in all probability too high. Olitsky (3), observing a later series of about 4000 mice, found among them no cases of the disease. The low incidence, coupled with the fact that normal mice develop with age relative resistance to an inoculation of the virus, suggested that infection must be very widespread. The resistance which develops with age must be considered specific until shown otherwise. The presence of numerous healthy carriers of the virus would explain to a large extent the epidemiology of the disease.

It had been shown that normal mice in contact with obviously infected animals, either those found paralytic or those artificially infected by intranasal or intracerebral inoculation, do not acquire an infection of the central nervous system, nor do they develop a greater degree of immunity than control mice of the same strain and age kept under the same conditions but not exposed to mice having an infection of the central nervous system (1). It seemed obvious, therefore, that animals showing evidence of involvement of the central nervous system do not act as a source of infection, in spite of the well ascertained fact that such animals are virus carriers, for it has been shown conclusively that virus can be recovered from the central nervous system of mice following intracerebral inoculation for as long as 1 year after inoculation (1). Even in mice which have remained well following an intracerebral inoculation, the virus has been recovered from the spinal cord for as long as 163 days after inoculation.

An obvious hypothesis for explaining the immunity developing with increasing age would be that this immunity is due to a symptomless infection of the central nervous system. There are, however, very few observations in favor of such an hypothesis. Firstly, the virus of mouse encephalomyelitis is very rarely present in the central nervous system of normal mice. Secondly, the immunity produced by an infection of the central nervous system, whether or not the mice are paralyzed, is absolute, whereas the immunity with age is developed slowly, and rarely, if ever, approaches in degree that produced by an infection of the central nervous system.

The important finding by Olitsky (4) that an agent resembling the virus of mouse encephalomyelitis can be recovered from the intestinal tract of normal mice has suggested a new approach to the study of the epidemiology of this disease. Olitsky found that, by the intracerebral inoculation of mice with filtrates prepared from the intestinal contents of normal mice, an infection transmissible in series by intracerebral inoculation could be produced. This infection resembled clinically and pathologically the condition produced by the virus of spontaneous encephalomyelitis of mice. Furthermore, he showed that there was an immunological relationship between the two agents. Mice which had survived the intracerebral inoculation of the agent recovered from the intestine were immune to a subsequent intracerebral inoculation of our virus. The converse was also true.

It seemed reasonable to conclude that the two agents were related and that the intestinal infection was in all probability the hypothetical carrier state assumed to exist to give an explanation of the epidemiology of the disease. For the elucidation of the epidemiology of the disease it seemed important to determine the incidence and duration of this intestinal infection, as well as to locate the source of the virus in the intestinal contents.

Methods

For demonstrating virus in feces or intestinal contents the material to be tested was ground in a mortar with alundum. Broth was added, and the supernatant obtained after centrifugation was passed through a Seitz filter. The filtrate was tested by the intracerebral inoculation of twelve or more Swiss mice, all approximately 6 weeks of age. Usually, inoculated mice were kept under observation for a month when the immunity of the survivors was tested by the intracerebral inoculation of a highly virulent strain of virus. For this purpose strain GD VII was generally used. The characteristics of this strain have been described elsewhere (5). As controls for the immunity test, mice of the same strain and age were inoculated.

EXPERIMENTAL

The observations made by Olitsky on intestinal infection were all made on the Institute strain of mice. The mice used in our laboratory were all of the Swiss strain, being

delivered weekly by six dealers. In a preliminary experiment to determine whether the mice used by us were infected, four normal mice, approximately 6 weeks of age, were killed and the intestinal contents from each collected. The pooled contents were filtered and inoculated intracerebrally into forty-three mice. Of these, twenty-two became paralyzed after an incubation period of 12 to 29 days. The average incubation period was 19.9 days. Of the mice which became paralyzed, eleven died. The average time of death was 25 days. All survivors were given an immunity test by the intracerebral inoculation of the highly virulent strain GD VII. Of the thirty-two mice, sixteen died and sixteen lived. Of the latter, eleven were paralytic as a result of the injection of the intestinal contents.

The presence of virus in the intestinal contents suggested that this agent might be demonstrated in the feces in mice. This was found to be so. Filtrates prepared from feces derived from mice supplied by all the six sources were shown to be infective on intracerebral inoculation. It was apparent that all our stock of mice were infected. The strains of virus recovered from the feces of mice were almost all of low virulence, having a relatively long incubation period and a low mortality. Mice which had become paralyzed and had lived following the intracerebral inoculation of fecal strains of virus were invariably immune to strains of virus originating from the central nervous system of mice found spontaneously infected.

Incidence of Intestinal Infection.—

In order to determine the actual incidence and duration of infection, individual mice were placed in clean jars and fed on sterile food and water. To obviate contamination of the food with the mouse's own dejecta, a wire stage was placed in the bottom of the jar. Jars and wire stages were changed daily. In this manner fecal specimens could be collected from individual mice whenever desired.

Fifteen mice, all approximately 6 weeks of age, picked at random from our stock mice and all apparently in perfect health, were placed in individual jars. As seen in Table I, the feces of ten of these were shown to be infective. At intervals feces were collected from these mice in order to determine the duration of infection. The longest time that virus could be recovered after isolation was 53 days. Four mice died during the course of the experiment. An additional four mice were killed at various times in order to determine the site of infection. The remaining seven were killed from 102 to 120 days after isolation and their small intestines tested for the presence of virus. All seven were virus-free. All mice whose feces were shown to be virus-free 1 or 15 days after isolation were likewise negative on subsequent examinations at from 33 to 64 days. In three of these the small intestine was likewise shown to be free of virus 102 and 120 days after isolation at the conclusion of the experiment. Of the ten mice which on the first examination were shown to be infected, four were killed during the course of the experiment, and the remaining six eventually became virus-free. In one of these, although the feces were negative on two occasions, 35 and 37 days after isolation, virus was, nevertheless, shown to be present in the small intestine when the mouse was killed at the latter time.

The results of this experiment clearly show that approximately two-thirds of normal mice 6 weeks of age excrete the virus of mouse encephalomyelitis in their feces and that the infection persists for a considerable time, up to 53 days.

Virus demonstrated in the feces must have originated within the mice as opportunities of ingesting virus were minimized by keeping the animals in isolation and feeding them on autoclaved food and boiled water. Several possibilities presented themselves as to the origin of the virus. Either there was infection of some tissue in the mouse or the virus was actually

TABLE I

Results of Tests for the Presence of Virus in the Feces of Normal Mice Kept in Isolation and Fed on Sterile Food and Water

Mouse No.	Virus demonstrated in feces on days after isolation	Fecal examination negative on days after isolation	Day of isolation on which mouse was killed	Results of examination of small intestine for presence of virus	Remarks
1	1	35, 37	37	Positive	Used for Experiment 3
2	1	35	Died 50	Not tested	
3		1, 52	120	Negative	
4	1, 21		21	Positive	Used for Experiment 2
5	1, 15, 53		53	Positive	Used for Experiment 4
6	1	81	120	Negative	
7		1, 34, 64	102	Negative	
8		1, 34, 64	102	Negative	
9		1, 34, 64	102	Negative	
10	15, 49		49	Positive	
11	15	64	102	Negative	
12	15, 33		Died 39	Not tested	
13	15	1, 64	102	Negative	
14		15, 33, 64	Died 98	Not tested	
15	15	64	Died 114	Not tested	

derived from some element in the gastro-intestinal tract. If the first hypothesis was correct, then the virus in the feces must be looked upon as a secondary phenomenon.

Distribution of Virus in Normal Mice.—Experiments so far had shown that about 65 per cent of all mice 6 weeks of age passed the virus of mouse encephalomyelitis in their feces. In the experiments now to be reported efforts were made to determine the site of the infection in the mice.

Experiment 1.—Three apparently normal mice 6 weeks of age were killed, and various organs were removed and pooled. 10 per cent suspensions in broth were made by grinding with alundum in mortars. These were centrifuged, and the supernatant fluid was inoculated into groups of five to twenty-four mice. Organs tested in this manner

were brain, spinal cord, liver, spleen, mesentery, stomach, small intestine, cecum, and large intestine. The suspensions of the gastro-intestinal tract were filtered through Seitz filters before inoculation. The mesenteries were included as it was desired to determine whether the mesenteric lymph glands were infected. To obtain these, the entire mesentery was dissected out after the removal of the gastro-intestinal tract. In removing the latter, care was taken to prevent the contamination of the mesentery with the gastro-intestinal contents. In making suspensions of the gastro-intestinal tract, the contents were not removed before grinding. The results are shown in Table II.

Virus was definitely shown to be present in all four parts of the gastro-intestinal tract, stomach, small intestine, cecum, and large intestine. The central nervous system and the thoracic and abdominal viscera were

TABLE II

Results of Infectivity Test with Suspensions Prepared from Organs of Normal Mice

Organ tested	No. of mice inoculated	Results		Results of immunity test 4 wks. after original inoculation			
		No. developing paralysis	No. died	Total used in immunity test	No. died	No. of survivals paralyzed	No. well
Brain	5	0	0	5	5	0	0
Spinal cord	10	0	0	10	10	0	0
Liver	12	0	0	12	12	0	0
Lungs	11	0	0	11	10	1	0
Spleen	6	0	0	6	6	0	0
Mesentery	18	0	1	17	13	4	0
Stomach	18	9	0	18	5	7	6
Small intestine	24	13	2	22	5	7	10
Cecum	20	6	0	20	6	6	8
Large intestine	19	7	1	18	7	7	4

negative. Four weeks after the original inoculation the immunity of all the surviving mice was tested by the intracerebral inoculation of the highly virulent GD VII strain of virus. The results of this immunity test confirmed previous findings. The majority of mice originally inoculated with suspensions prepared from the four divisions of the gastro-intestinal tract lived, indicating that they had become immunized. No evidence of any immunity was found in mice inoculated with suspensions of brain, spinal cord, lungs, liver, or spleen. Suggestive evidence was obtained that a small amount of virus was present in the pool of the three mesenteries. Though no mice developed paralysis after the original inoculation, four of seventeen lived following the immunity test.

The conclusion seems warranted that the virus of mouse encephalomyelitis was shown to be present throughout the entire gastro-intestinal

tract in 6-week-old normal Swiss mice picked at random from the stock. However, as these mice had not been kept in isolation and fed on sterile food, the virus shown to be present in the gastro-intestinal tract could have been derived from the ingestion of contaminated food. This experiment was, however, not valueless in that it supplied definite evidence that the virus could remain active throughout the entire gastro-intestinal tract and also that this virus shown to be present was immunologically definitely related to the virulent strain GD VII originally isolated from the central nervous system of a mouse with a case of spontaneous encephalomyelitis.

Experiment 2.—In order to determine whether the virus shown to be present in the gastro-intestinal tract actually came from some source within the mouse and not from contaminated food, mouse 4 in Experiment 1 (Table I) was killed. This mouse, approximately 9 weeks of age, had been kept in isolation and fed on sterile food and boiled water for 21 days. Daily the jar and its wire stage had been changed. In this manner contamination of the mouse's food with its own feces was reduced to a minimum. Feces collected from mouse 4 one day after isolation had been shown to contain virus. The infectivity of the stomach, small intestine, and large intestine was tested as in the first experiment. The infectivity of other organs was not tested. The inoculated mice were kept under observation for 6 weeks, when their immunity was tested by the intracerebral inoculation of the FA strain of virus. The results are shown in Table III.

TABLE III

Tests for Presence of the Virus of Mouse Encephalomyelitis in the Gastro-Intestinal Tract of Mouse 4 Kept in Isolation for 21 Days

Organ tested	No. of mice inoculated	Result	Results of immunity test with FA virus 6 wks. after original inoculation		
		No. developing paralysis	Total used in immunity test	No. of survivals paralyzed	No. well
Stomach	18	5	9	8	1
Small intestine	18	3	11	5	6
Large intestine	18	5	8	6	2
Feces	12	1	3	2	1

As in the previous experiment, virus was shown to be present in the stomach, small intestine, and large intestine. The results were confirmed by the immunity test which likewise furnished evidence of the immunological relationship between the virus derived from the gastro-intestinal tract and the highly encephalitogenic FA strain of virus. Since mouse 4 had been kept in isolation for 3 weeks and fed on sterile food, the virus shown to be present within the animal must have been derived from the mouse itself and not some outside source. No conclusion, however, was warranted

as to the source of the virus within the animal. Any virus present in the gastro-intestinal tract might quite reasonably have come from some source above the stomach such as the salivary glands, for example. Consequently in the next experiment this hypothesis was tested.

Experiment 3.—Mouse 1, approximately 11 weeks of age, kept in isolation and fed on sterile food for 37 days, was killed. Virus had been shown to be present in the feces of this mouse taken 1 day after isolation. Following removal of the brain, the head was severed from the body. The head containing all the salivary glands but not the skin was ground thoroughly in a mortar with alundum, broth was added, and the suspension

TABLE IV

Distribution of the Virus of Mouse Encephalomyelitis in Mouse 1 Kept in Isolation for 37 Days

Organ tested	Results of intracerebral inoculation			Results of immunity test with GD VII 1 mo. after original inoculation			
	No. of mice inoculated	No. developing paralysis	No. died	Total used in immunity test	No. died	No. of survivals paralyzed	No. well
Brain	11	0	0	11	9	2	0
Head	25	0	0	25	24	0	1
Stomach	21	0	0	21	21	0	0
Small intestine	25	3	1	24	18	5	1
Cecum	23	0	0	23	17	4	2
Large intestine	22	0	0	22	13	4	5
Kidney and spleen	23	0	0	23	20	3	0
Liver	22	0	0	22	18	3	1
Lungs and heart	22	0	0	22	21	1	0
Feces	22	0	0	22	17	3	2
Normal uninoculated mice	36	0	0	36	33	3	0

after centrifugation was filtered through a Seitz pad. In addition to the brain and head, the infectivity of the following organs was tested: stomach, small intestine, cecum, large intestine, a pool of kidney and spleen, liver, and lungs and heart. Groups of eleven to twenty-four 6-week-old Swiss mice were given intracerebral inoculation of the organ suspension. After 1 month's observation the immunity of all surviving mice was tested by the intracerebral inoculation of the virulent strain GD VII. The results are shown in Table IV.

The only organ in which virus was shown to be definitely present was the small intestine, and in this it was present in but small amounts as only three out of the twenty-four mice inoculated developed paralysis. The results of the immunity test are not clear-cut, although it furnished suggestive evidence that virus was also present in two other portions of the gastro-intestinal tract, namely the cecum and large bowel, but not the stomach.

No evidence was obtained of any virus in the head, thus ruling out as the source of virus in the small intestine such organs as the salivary glands, mucosa of the mouth or nasopharynx. This is borne out by the fact that no virus was demonstrated in the stomach. It is noteworthy that no virus was definitely demonstrated in two specimens of feces, one collected on the day the mouse was killed and the other 2 days previously, showing that fecal examination is no sure method of determining whether a mouse is infected.

TABLE V

Distribution of the Virus of Mouse Encephalomyelitis in Mouse 5 Kept in Isolation for 53 Days

Organ tested	No. of mice inoculated	Results		Results of immunity test with GD VII 1 mo. after original inoculation			
		No. developing paralysis	No. died	Total used in immunity test	No. died	No. of survivals paralyzed	No. well
Brain	24	0	0	24	22	1	1
Head	48	0	0	48	43	3	2
Esophagus	25	0	0	25	22	1	2
Stomach wall	37	0	0	37	34	3	0
Stomach contents	35	2	2	33	26	5	2
Small intestine wall	35	13	1	34	7	10	17
Small intestine contents	36	9	2	34	13	9	12
Kidney	24	0	0	24	19	5	0
Mesentery	35	1	0	35	32	1	2
Feces	24	7	1	Not tested			
Normal uninoculated	71	0	0	71	68	2	1

Experiment 4.—The results of the previous experiment had shown that the source of virus present in the intestinal tract of mice kept in isolation was probably the small intestine and was not due to a source of infection above that organ. The evidence so far, however, does not warrant the conclusion that the source of the virus is the cells of the intestinal wall and not perhaps some source in the intestinal contents. In this experiment, consequently, the infectivity of the intestinal wall and contents was tested separately. To remove the contents, the small intestine was slit longitudinally and with a spatula the contents were gently removed, care being taken not to damage the mucosa. The intestinal walls were then washed in a strongly flowing stream of tap water for several minutes. Mouse 5, 13½ weeks of age, was killed after 53 days of isolation. The feces of this mouse had been found to contain virus 1 and 15 days after isolation, definitely showing that the animal was infected. As in the previous experiment, the brain and head were tested. In addition to the small intestinal wall and contents, infectivity of the stomach wall, stomach contents, esophagus, kidney, and mesentery was tested. One month after inoculation the immunity of all surviving mice was tested by the intracerebral inoculation of strain GD VII. The results are shown in Table V.

Virus was shown to be definitely present in both the small intestinal wall and contents, as well as in stomach contents. The presence of virus in the stomach contents could quite reasonably be ascribed to regurgitation of the contents of the small intestine since the stomach walls proved to be virus-free and since there was no source of virus above that organ, the head and esophagus being negative. There appeared to be more virus in the small intestinal wall than in the contents of this organ, though no efforts were made to compare the two quantitatively.

One of the mice inoculated with the suspensions of the mesentery developed definite signs of weakness in the fore limbs. The animal lived and was later shown to be immune to the virulent strain GD VII. This is very suggestive evidence that the mouse had been immunized. However, of 71 uninoculated normal mice of the same age and strain used as controls for the immunity test, three survived, one without showing any signs of paralysis.

The results of this experiment clearly indicated that virus could be recovered from the intestinal walls after removal of the contents. It is not claimed that the method used for washing the walls removed every trace of the contents, but it is felt that the mucosa of the intestine was sufficiently clean so that the virus present in the walls could not be due entirely to contamination by means of the contents. It seems far more reasonable to assume that the virus present in the contents is derived from the intestinal walls.

An additional experiment essentially similar to the one just described has been done. Mouse 10, 13 weeks of age and known to be infective, was killed after 49 days of isolation. Virus was shown to be present in both the small intestinal walls and contents. Brain, heart, esophagus, stomach walls, and stomach contents were virus-free. The results of this experiment confirm the fact that the source of virus in normal mice is in all probability the intestinal wall. In this experiment definite evidence of virus in the mesentery was obtained. Two of twenty-three mice inoculated with the suspension of mesentery developed signs of paralysis. These were killed and the brains and spinal cords removed. A group of twelve normal mice were inoculated intracerebrally with a suspension of these organs. Four of these developed paralysis.

DISCUSSION

The findings, to date, of experiments undertaken to determine the incidence and duration of intestinal infection in normal mice with the virus of mouse encephalomyelitis indicate clearly that approximately two-thirds of mice 6 weeks of age are infected and that this infection may persist for a considerable time. The source of virus demonstrable in the feces has been shown to be in all probability the intestinal wall. Olitsky has presented

evidence that young mice acquire intestinal infection shortly after weaning and that almost all are infected by the time they are 30 days of age (3). In mice 6 months of age or over the virus can be recovered but irregularly. Olitsky's observations and our own make it seem probable that all mice become infected early in life and that the infection may persist for long periods of time, up to 6 months of age or over. Infection is universal and as a result mice develop an immunity with age. For some reason, at present entirely unknown, in a small minority of mice the virus invades the central nervous system, producing the well known paralytic condition. Infection of the central nervous system is consequently a rare and accidental event. At present no evidence of any seasonal incidence is available, nor are there any reliable observations of any age incidence, although one has the definite impression that most, if not all, mice found paralytic are young. This statement is based on the observation that all spontaneously paralyzed mice so far found by us have been observed in mice shortly after arrival at the laboratories.

Experimental infection of the central nervous system can be produced by intracerebral, intranasal, and intraperitoneal inoculation. The incubation period in groups of mice inoculated with the same suspension of virus by these three methods varies. Following intracerebral inoculation the incubation period is shortest; it is longest after intranasal instillation of virus. The incubation period following intraperitoneal inoculation lies in length between the other two. That the long incubation period following intranasal inoculation as compared with the other two is not due to the inefficacy of this route of inoculation, is shown by the fact that mice are more susceptible to the intranasal route than to the intraperitoneal route. Both of these routes are, however, far less efficient than the intracerebral. These considerations lead one to speculate on the possible route of invasion of the central nervous system. Obviously the intranasal route cannot be excluded, as all mice must be exposed to this route of inoculation, the virus being so widespread. However, it is difficult to produce an infection of the central nervous system by this route unless very large amounts in terms of M.L.D. intracerebral units are administered. It has only been due to the discovery of the unusually virulent strains of virus GD VII and FA that the conditions necessary for infection by this route have been successfully investigated. Infection by this route in nature must be very rare unless some other at present unknown factor is involved. This factor might be a physiological one. That is, very young mice might be far more susceptible to an intranasal inoculation than mice 6 weeks of age.

An alternate route of invasion of the virus is from the intestinal tract. Here two routes are available, one *via* the lymphatics and blood, and the

other by neural pathways. In favor of the circulatory system is the fact that virus has definitely been shown to be present in the mesentery of normal mice, conclusive evidence that the virus can penetrate the intestinal wall. However, as it has not yet been proved that the infection of the intestine is the primary site of infection and not the terminal manifestation of a more systemic type, it might be that both the nervous system and the gastro-intestinal system become infected from the same source.

The cardinal symptom of the disease as it occurs spontaneously is a flaccid paralysis of the extremities, usually the posterior ones, suggesting that the virus either has a predilection for the anterior horn cells of the spinal cord, no matter by what route invasion of the central nervous system takes place, or that the anterior horn cells are invaded by neural routes from the periphery and that the symptomatology is conditioned by the level of the cord at which the virus enters.

Invasion by neural routes of the central nervous system from a focus of infection in the intestine would produce infection of the spinal cord and consequently paralysis. Observations made with the FA strain of virus are pertinent to this question. The distinguishing feature of this virus, apart from its great virulence, is its marked encephalitogenic quality. When mice are inoculated either intracerebrally or intranasally, the cardinal symptoms produced are referable to the brain and not the cord. However, when this virus is inoculated intraperitoneally, the disease picture simulates in every way that seen in mice with a spontaneous infection of the central nervous system, that is, the mice apart from a flaccid paralysis, usually of the hind limb, appear perfectly well with an almost complete absence of cerebral symptoms. This suggests that following an intraperitoneal inoculation, invasion of the central nervous system takes place through neural routes entering the spinal cord.

In considering the immunity developing with age, the important points are that this immunity is gradual and does not approach in degree that produced by artificial immunization by a non-fatal infection of the central nervous system. This does not mean that non-fatal infections of the central nervous system do not take place in nature but does indicate that this is not the major type of immunization. We have, in fact, shown that such symptomless infections of the nervous system can be produced by the intracerebral inoculation of avirulent strains of virus. With this observation in mind we have tested the infectivity of the spinal cord of mice which had remained well following the intracerebral inoculation of a highly virulent strain of virus and have on one occasion been able to recover a strain of virus differing in its virulence from that with which the mouse had been inoculated, which is clear evidence that this mouse

had a naturally acquired symptomless infection of the central nervous system.

The increasing resistance to artificial inoculation of virus observed with increasing age might result from the development of antibodies due to the infection of the intestinal tract. Finding of the virus in the mesentery of normal mice shows clearly that at least during one part of the infection the virus is able to invade the animal organism and presumably acts as an antigen for the production of antibodies. The older the animal, the longer the infection, and consequently the greater the immunity. The weakness in this hypothesis is the fact that up to the present no satisfactory neutralization test has yet been found. Evidence has, however, been obtained that neutralizing antibodies are produced with age, though more exact information on this point must await further study. On this hypothesis, namely that the immunity developing with age is due to the production of antibodies as a result of the intestinal infection, the occasional invasion of the nervous system could be ascribed to a deficient antibody-producing mechanism.

How mice acquire the intestinal infection has not been determined. It would seem that the most likely route would be by mouth. As practically all mice 3 weeks of age or older are virus excretors for relatively long periods, the environment in which mice are kept is continuously infected.

SUMMARY

1. In the feces of approximately two-thirds of normal mice 6 weeks of age an agent in all respects similar to the virus of mouse encephalomyelitis can be recovered.

2. In isolated mice, fed on sterile food and water, excretion of virus has been shown to persist up to 53 days after isolation.

3. In normal mice known to be virus carriers virus has been demonstrated in the gastro-intestinal tract but not in the central nervous system, thoracic or abdominal viscera, or any organs of the head.

4. The source of the virus excreted in the feces has been shown to be located in all probability in the intestinal wall.

5. Evidence is presented that the virus can invade the animal organism, as virus has been demonstrated in the mesenteric lymph glands.

BIBLIOGRAPHY

1. Theiler, M., *J. Exp. Med.*, 1937, **65**, 705.
2. Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1938, **67**, 201.
3. Olitsky, P. K., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 296.
4. Olitsky, P. K., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41**, 434.
5. Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, **72**, 49.