THE TRANSMISSION OF THE VIRUS OF LYMPHOCYTIC CHORIOMENINGITIS BY TRICHINELLA SPIRALIS

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Little is known about the natural mode by which many viruses survive interepidemic periods to initiate new outbreaks of disease or of the mode by which they penetrate natural barriers to gain access to the tissues of a new host. For this reason, the parasitic nematode, *Trichinella spiralis*, was investigated for its capacity to act as an intermediary for the maintenance and transfer of the virus of lymphocytic choriomeningitis from one host to another. *T. spiralis* and lymphocytic choriomeningitis virus were selected because each is representative of a large class of closely related agents, each has a wide host range that includes man, and each is cosmopolitan in distribution.

It would not be surprising if *T. spiralis* during migration through the tissues of a host ill with lymphocytic choriomeningitis were to acquire the virus, since the virus is widely distributed in the body and regularly present in both acute and inapparent infections. Too, the successful demonstration of the acquisition of the virus by the nematode would not only be of theoretical interest, but would also open a new field for epidemiological investigation. Indeed, there might quite possibly be practical results, since the natural mechanism for the transfer of the virus of lymphocytic choriomeningitis from animal to man is unknown.

In this paper facts will be presented which show that *T. spiralis* can serve as an efficient vehicle for the experimental transmission of lymphocytic choriomeningitis virus from one host to the next.

Lymphocytic choriomeningitis has been recognized as an etiological entity since 1934, when Armstrong and Lillie (1) isolated the causative virus. The disease has been shown to occur in nature in man (1–3), monkeys (4, 5), gray mice (6), dogs (7), and albino mice (8). Experimentally, a high grade of susceptibility to infection has been demonstrated for these same species and for guinea pigs (1), slight susceptibility, as evidenced by asymptomatic infections with the virus temporarily present in the blood stream and the formation of antibodies, for rats (1, 9), ferrets (9), pigs (9), and cats (10); refractoriness to infection (9) by canaries, parakeets, chickens, voles, and hedgehogs.

The natural route for the transfer of lymphocytic choriomeningitis from animals to man is unknown. There is no seasonal incidence. The disease in man, moreover,

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has been characterized by sporadic occurrence—single cases without evidence to support infection by contact.

Infection by contact or feeding was suggested by the demonstration that virus can be present in urine (8) and nasal secretions (8, 11), but in the absence of mechanical interference or trauma there has been little supportive evidence (2, 8, 12, 13). Epidemiological studies, however, have revealed an environmental association between many of the proven human cases and house mice (6, 14–16). This points to mouse excreta as the agent responsible for the transfer of the virus to man.

No vector has been found to date that harbors or transmits the virus of lymphocytic choriomeningitis under natural conditions. Experimentally, it has been shown that mosquitoes (*Aedes aegypti*) (5, 17), ticks (*Dermacentor andersoni*) (12), and bed bugs (*Cimex lectularius*) by fecal contamination (17), can transmit the virus. However, the mosquito and the tick can be excluded, because of their limited seasonal occurrence. It seems improbable that the bed bug can be the sole, or even the principal, vector of the disease.

When the mode of transfer of an infectious agent is not known, it is important to learn the distribution of virus in the host's tissues and to know how long it persists there. In lymphocytic choriomeningitis, the virus appears early in the blood stream, to persist in the body fluids and tissues until the death of the host, or in the non-fatal cases and inapparent infections for weeks or even months.

Materials and Methods

Virus.—The W.E. strain of lymphocytic choriomeningitis virus (2) was used. It was kindly supplied to us in December, 1938, by Dr. T. M. Rivers. This strain of virus is lethal for guinea pigs in from 6 to 40 days following inoculation.

Virus suspensions for inoculation were prepared from the tissues of animals immediately after they died of lymphocytic choriomeningitis or after they had been killed, when already moribund, by illuminating gas, chloroform, or ether. The tissues were triturated with alundum in Locke's solution to yield a 10 per cent suspension. This suspension was centrifuged horizontally at 2500 R.P.M. for 30 minutes and the supernatant fluid was employed as indicated in each protocol.

Animal Host.—Guinea pigs were employed, a species chosen because it is fully susceptible to *T. spiralis* and to lymphocytic choriomeningitis virus, whether the latter be inoculated by the intraperitoneal, subcutaneous, or intracerebral route. The animals were selected within a weight range of from 250 to 350 gm. from a colony of an inbred albino strain known to be free from spontaneous lymphocytic choriomeningitis, or other virus disease. They were utilized for maintenance of the virus by routine passage; as a host for trichinella larvae to acquire virus for intracorporeal transfer; and to test trichinella larvae for the presence of virus. Brain tissue was used as routine to provide virus for passage, and to test for the presence of virus in the host animals that presumably had died from trichinella-transmitted choriomeningitis.

Invertebrate Host.—Trichinella spiralis (Owen, 1835) Railliet, 1895, was employed as the experimental intermediate host. *T. spiralis* was selected as representative of a large class of invertebrates that parasitize man and other vertebrate hosts. It has a potential host range that includes all carnivorous and omnivorous mammals. Man, hogs, and rats show the highest incidence of natural infection, largely because of their consumption of raw or insufficiently cooked pork. Transfer of the parasite is effected through ingestion by the new host of encysted larvae contained in striated muscle—a vast reservoir—of a previously infected animal.

The larvae are released by the digestive juices to invade intestinal epithelium where they mature, mating occurs within a few days, the males die, and the resulting viviparous females deposit within the intestinal wall migratory larvae which make their way *via* lymph spaces, lymph channels, and the blood stream through the hepatic and pulmonary filters to reach all parts of the body. A single female worm may yield as many as 1000 or more larvae. Migration of larvae extends over a period of about 30 days with the peak of invasion on the 10th day following infection. Transfer to a new host must take place within the encysted larva's period of viability, which ranges from 6 months to 30 years, to terminate in death and calcification.

This consideration of the host-parasite relationship during the life cycle of *T. spiralis* makes it apparent, theoretically at least, that either the adult female worms or the migrating larvae might act as vehicles for the transfer of infectious agents through natural barriers. Virus contained within adult females could readily be liberated in the host's tissues as the worms burrow into the intestinal mucosa to deposit the young larvae of the next generation. After these young larvae migrate to the striated muscles, they increase more than ten times in size to reach the infective stage. During migration and development ample opportunity presumably exists for discharge of virus contained within the body of the larvae. As proof that they transfer virus would be of great theoretical interest and significance, a foremost objective in planning the experiments was to eliminate the possibility that infection might result from external contamination of larvae; *i.e.*, mechanical carry-over. This was accomplished by utilizing the resistance of viable trichinella larvae to hydrochloric acid.

The larvae which were utilized for initiating the present experiments were obtained by the Bachman technique (18) from a normal guinea pig which had been infected 6 weeks earlier.

Preparation of Larvae for Testing their Capacity to Transmit Virus

To learn whether trichinella larvae are capable of transmitting the virus of lymphocytic choriomeningitis, larvae first had to be exposed to virus within infected guinea pigs, separated from host tissues, and treated externally to remove extraneous materials, including the virus.

The method employed was to introduce by rubber tube from 600 to 2400 larvae into the stomach of each guinea pig. From 15 to 21 days later, when larval migration was at its height, 1.0 ml. of a 10 per cent suspension of virus was injected subcutaneously into the animals. The virus regularly proved lethal in from 8 to 11 days. The animal was utilized immediately after it died spontaneously or when discovered to be moribund. The brain was removed aseptically for storage in 50 per cent buffered glycerol until wanted for control purposes to verify the specificity of the infectious agent; the head, skin, feet, and viscera were discarded; the carcass was ground in a meat grinder (Universal No. 1). The ground tissues were digested for 3 hours in a mixture of 0.7 per cent pepsin and 0.6 per cent hydrochloric acid. Following digestion, the pH of the digestive mixture was determined and gross particles of bone and undigested tissue were removed by pouring the mixture through a copper wire sieve (20 mesh per inch) into a large funnel with a clamped rubber tube attached at the bottom. After an hour the clamp was opened and the larvae, which had settled into the neck of the funnel, were drawn off into a 250 cc. beaker. After settling, the supernatant fluid was poured off. The larvae were washed several times in this manner, and finally were separated from the finer particles of bone by pouring the suspension through a copper wire sieve (60 mesh per inch) pervious to trichina larvae. Further treatment of the washed larvae consisted of immersion in hydrochloric acid, 1 per cent, at a pH of 0.86 to 1.1 for from 1 to 21 hours, as indicated in the experimental protocols which follow; washing to remove the hydrochloric acid; treatment for 5 minutes with 1-2000 merthiolate; washing four times with sterile 0.85 per cent saline solution to remove the merthiolate. Larvae so prepared were ready to test for the presence of virus.

Cultures.—A sample of each virus suspension was cultured in Douglas's broth and on blood agar plates and incubated under both aerobic and anaerobic conditions.

EXPERIMENTAL

The nematodes were permitted to pass through their natural developmental cycle in guinea pigs ill with lymphocytic choriomeningitis and the resulting larvae were tested for their ability to transmit the virus to new hosts. Eleven experiments were performed. These experiments fall into three groups: (1) those which gave positive results (Experiments 1, 3, and 5), (2) negative experiments (Experiments 9, 10, and 11), those which failed to yield any evidence for either acquisition or transmission of virus by trichinella larvae, and (3) control experiments (Experiments 2, 4, 6, 7, and 8), so planned as to establish unequivocally the results found in the positive transmission experiments.

Experiment 1.—Six guinea pigs received by stomach tube from 625 to 1250 trichinella larvae. Sixteen days later each received by subcutaneous route 0.5 ml. of a 10 per cent suspension of lymphocytic choriomeningitis virus. When all died 8 days later (23 days after infection with trichinella) digestion of each carcass yielded from 11,300 to 65,000 mature larvae. Treatment of these larvae was limited to exposure for 5 minutes to 1-2000 merthiolate and washing to get rid of the merthiolate. The larvae from each pig were separated into approximately equal parts: half of them were given by mouth to one normal test animal and the other half were triturated without alundum and injected subcutaneously into a second animal.

The findings of Experiment 1 are summarized in Table I. From the data, it can be concluded that trichinella larvae were the means for transfer from 5 of 6 guinea pigs dead from lymphocytic choriomeningitis of virus in amounts lethal to 7 of 12 normal test animals.

The results of Experiment 1 showed that lymphocytic choriomeningitis virus which has become associated with trichinella larvae can survive peptic digestion, washing, and treatment with merthiolate and that washed larvae, whether alive when fed by stomach tube or dead when injected subcutaneously, transmit the virus. It should be pointed out, however, that the virus may merely have been carried on the external surface of the larvae.

It was recognized that the low yield of larvae (10,000 to 75,000) from each host animal probably resulted from the small infective dose of larvae and from termination of the developmental cycle on the 24th day as the result of lethal virus infection. Only mature larvae were recovered from the infected animals because digestion destroys immature forms.

Experiment 2 was planned as a control for Experiment 1. Its purpose was to learn whether virus present in the dead guinea pig but not associated with worms could elicit infection after peptic digestion. The supernatant fluid of the digest from each carcass was tested therefore for the presence of virus.

Experiment 2.—A sample of supernatant fluid was removed from the digestion mixture prepared from the carcass of each of the 6 guinea pigs that were employed for Experiment 1. The pH of each sample was adjusted to 7.0 by the addition of sodium hydroxide, after which 1.0 ml. was injected subcutaneously into a guinea pig.

Of the 6 recipients of the fluid from the digestion mixture, only one died (guinea pig 21). The brain of this animal, which succumbed 21 days after inoculation, yielded lymphocytic choriomeningitis virus.

The results of Experiment 2 showed that some of the virus was not inactivated by drastic peptic digestion. The infection of but a single animal

TABLE I

Results of Experiment 1

Transfer by Trichinella Larvae of the Virus of Lymphocytic Choriomeningitis

Host animals					Test animals for detection of virus in trichinella				Interpretation	
Guinea pig No.	Amounts administered of		Virus injected	Death	Guinea pig No.	No. of larvae given	Route of inoculation	Interval to death	Transmission of virus by trichinella	
	Larvae	Virus								
		ml.	*day*	*day*				*days*		
1	625	0.5	16	24	7	2,200	*Per os*	21	Yes	1/2*
					8	6,700	Subcutaneous	S‡	No	
2	"	"	"	"	9	3,200	*Per os*	17	Yes	2/2
					10	13,000	Subcutaneous	25	"	
3	"	"	"	"	11	7,300	*Per os*	S	No	0/2
					12	29,000	Subcutaneous	S	"	
4	"	"	"	"	13	6,300	*Per os*	11	Yes	2/2
					14	57,000	Subcutaneous	10	"	
5	1250	"	"	"	15	4,200	*Per os*	S	No	1/2
					16	17,000	Subcutaneous	11	Yes	
6	"	"	"	"	17	12,000	*Per os*	9	"	1/2
					18	49,000	Subcutaneous	S	No	

* In all tables the numerator indicates the number of guinea pigs that succumbed to lymphocytic choriomeningitis infection; the denominator, the number of animals used in the test.

‡ Survived.

and its death after a prolonged incubation period suggest, however, that but little virus withstood the procedure. It is of interest that this virus came from the carcass of the only guinea pig (No. 3) that failed to yield virus-infected larvae.

Experiment 3 was designed to learn whether washed living larvae, kept at a pH of 1 or less for 90 minutes, could transfer virus.

Experiment 3.—Each of 2 guinea pigs (Nos. 25 and 26) was given by stomach tube 2400 larvae. 19 days later, 1.0 ml. of a Berkefeld N filtrate of virus suspension was injected into

each subcutaneously. These animals died 28 days and 29 days, respectively, after infection with the nematode. 210,000 larvae were harvested from the carcass of guinea pig 25, while guinea pig 26 yielded 450,000. They were exposed for 90 minutes to 1 per cent hydrochloric acid, and then utilized for passage to normal guinea pigs.

The results of Experiment 3 are presented in Table II. Eight of 9 recipients died of lymphocytic choriomeningitis. The ninth probably died of trichinosis after 53 days.

The results of Experiment 3 provided further evidence that trichinella larvae can transmit lymphocytic choriomeningitis virus. Moreover, this experiment, by demonstrating that transmission of the disease took place after

TABLE II

Results of Experiment 3

Transfer by Trichinella Larvae of the Virus of Lymphocytic Choriomeningitis

Host animals						Test animals for detection of virus in trichinella				Interpretation	
Guinea pig No.	Amounts administered of		Virus injected	Death	No. of larvae recovered	Guinea pig No.	No. of larvae given	Route of inoculation	Interval to death	Transmission of virus by trichinella	
	Larvae	Virus									
		ml.	*day*	*day*					*days*		
25	2400	1.0	19	29	210,000	27	3,500	*Per os*	17	Yes	5/5
						28	7,000	" "	18	"	
						29	60,000	Subcutaneous	27	"	
						30	"	"	18	"	
						31	"	"	11	"	
26	"	"	"	28	450,000	32	7,000	*Per os*	15	"	3/4
						33	3,500	" "	S*	No	
						34	200,000	Subcutaneous	35	Yes	
						35	"	"	27	"	

* S = survived to die of trichinosis.

exposure of the larvae to a pH of less than 1, made it improbable that virus was carried only on their external surface.

After proof was at hand that the guinea pig of Experiment 3 which died 43 days after having received 3500 larvae by the intragastric route had succumbed to lymphocytic choriomeningitis, a test was planned to reveal the relation of lethal titer to survival time in the case of free virus.

Experiment 4.—Brain tissue from a guinea pig (No. 36) moribund from lymphocytic choriomeningitis was triturated with alundum in Locke's solution and a 10 per cent suspension was made. This was centrifuged horizontally at 2500 R.P.M. for 30 minutes and the supernatant fluid undiluted, or as a successive decimal dilution, was employed in a 1.0 ml. dose for injection subcutaneously into each of 2 guinea pigs.

The findings of Experiment 4 are presented in Table III. The time of death ranged from 6 to 9 days for the guinea pigs that received the 10^1 and 10^2 dilutions to 39 days and survival for animals that were given the 10^7 and 10^8 dilutions.

This experiment made it apparent that after a minute dose of virus was given the guinea pigs lived for 39 days, or survived.

TABLE III

Results of Experiment 4

Titration of Lymphocytic Choriomeningitis Virus in Guinea Pigs

	Dilution*							
	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8
Time from inoculation to death, *days*.....	8,7	9,6	11,9	11,11	20,22	12,29	39,S‡	S,39

* 2 guinea pigs inoculated subcutaneously with 1.0 ml. of each virus suspension.
‡ S = survival.

TABLE IV

Results of Experiment 5

Transfer by Trichinella Larvae of the Virus of Lymphocytic Choriomeningitis

Host animals						Test animals for detection of virus in trichinella				Interpretation	
Guinea pig No.	Amounts administered of		Virus injected	Death	No. of larvae recovered	Guinea pig No.	No. of larvae given	Route of inoculation	Interval to death	Transmission of virus by trichinella	
	Larvae	Virus									
		ml.	*day*	*day*					*days*		
53	2400	1.0	19	27	260,000	56	4,000	*Per os*	43	Yes	2/2
						57	250,000	Subcutaneous	22	"	
54	"	"	"	26	450,000	60	200,000	"	8	"	2/2
						61	"	"	30	"	
55	"	"	17	"	170,000	62	7,000	*Per os*	52	No	2/4
						63	3,500	" "	59	"	
						64	75,000	Subcutaneous	22	Yes	
						65	"	"	25	"	

The purpose of Experiment 5 was to find out whether infective larvae, after immersion in 1 per cent hydrochloric acid for 1 hour at 37°C. and for 20 hours at 4°C., could transmit the virus.

Experiment 5.—Trichinella larvae were harvested from 3 animals dead of lymphocytic choriomeningitis. Final treatment of each of these 3 lots of larvae consisted in immersion in 1 per cent hydrochloric acid for 1 hour at 37° C. and for 20 hours at 4° C., washing 12 times to remove the acid, treatment for 5 minutes with 1-2000 merthiolate, and final washing to eliminate the merthiolate. The larvae from each guinea pig were divided into 2 lots for intragastric administration as living larvae and for subcutaneous injection as dead larvae.

The data that relate to the dosages, routes of inoculation, and the results are presented in Table IV. Of the 8 animals, 6 died of lymphocytic choriomeningitis and 2 of trichinosis. These latter lived for 52 and 59 days respectively.

The results of Experiment 5 again proved that trichinella larvae can acquire a lethal dose of lymphocytic choriomeningitis virus from a host with experimental lymphocytic choriomeningitis infection, and that the larvae can transmit the virus either in the natural course of transfer and infection of a new host or as ground and injected organisms. Moreover, the virus associated with nematodes remains lethal after they are exposed to a pH of 0.92 for 21 hours: 1 hour at 37°C. and 20 hours at 40°C.

It seemed reasonable to assume that all of the virus except that protected by the larvae must have been destroyed by the hydrochloric acid but proof in the matter was needed. Another point to be settled was whether normal larvae would on prolonged immersion in a suspension of infected tissue acquire virus for transfer. Accordingly, three experiments were carried out to learn the facts.

Experiment 6.—The brain and carcass from each of 5 guinea pigs, procured immediately after death from lymphocytic choriomeningitis, were ground in a meat grinder and then mixed with from 200,000 to 350,000 normal trichinella larvae. The mixture was subjected to peptic digestion for 3 hours after which the larvae were separated and washed. These washed larvae were then placed in 1 per cent hydrochloric acid for 90 minutes, washed, triturated without abrasive in 3.0 ml. normal saline, and injected subcutaneously into 8 guinea pigs.

Each of the 8 test animals was observed and its temperature recorded daily for 40 days. None gave evidence of illness or fever and none showed resistance after 60 days to a challenge dose of virus.

The findings in Experiment 6 demonstrated that the same environmental conditions employed in Experiment 2 were effective in the elimination of lymphocytic choriomeningitis virus from the surface of trichinella larvae. Moreover, the results support the belief that the virus shown to have been transmitted in Experiments 1 and 2 was acquired from the diseased host of the nematodes, was harbored by the larvae during the processes of peptic digestion, washing, and treatment with hydrochloric acid, and was released from them after infection of a new host. It would appear that the possibility of virus transport on the surface of the larvae was eliminated by this demonstration.

More stringent tests of the effects of washing and exposure to hydrochloric acid on virus mixed with larvae and incubated were made in Experiments 7 and 8.

Experiment 7.—Brain tissue from 2 guinea pigs was made into a 10 per cent suspension. To 40 ml. of this suspension were added 1 million normal larvae immediately after they had been treated with merthiolate, 1-2000, and washed. After this mixture had incubated at 37° C. for 3 hours, the larvae were washed 12 times with warm tap water to remove debris and free virus. They were then incubated at pH 0.98 by keeping them in 1 per cent hydrochloric acid at 37° C. for 3 hours, washed, and used for the injection of normal guinea pigs. Each of the 3 guinea pigs was given 2500 of the living larvae by the gastric route; two more received into the stomach 100,000 larvae killed by repeated freezing at −70°C. in a carbon

dioxide-alcohol mixture and thawing; two each were inoculated subcutaneously with 400,000 larvae which had been killed by trituration.

Three of the guinea pigs died after 35, 60, and 28 days, respectively. All were heavily infected with trichinae, but brain tissue from none yielded lymphocytic choriomeningitis virus. The remaining 4 guinea pigs survived for 60 days, and showed, when killed, no evidence of either trichinosis or lymphocytic choriomeningitis.

Experiment 8.—A 10 per cent suspension of brain tissue from 2 guinea pigs dead of lymphocytic choriomeningitis was prepared. 40 ml. of this suspension was mixed with an equal volume of saline solution containing numerous trichinella larvae which had been treated with 1 per cent hydrochloric acid and 1-2000 merthiolate to render them bacteria-free, and the mixture was incubated at 37°C. for 5 hours. To control the infectivity of the virus this time, 2 guinea pigs were injected subcutaneously with 1.0 ml. each of the supernatant fluid. The larvae were then separated, washed 12 times, placed for 5 hours in 1 per cent hydrochloric acid (pH 0.95), washed 4 times in water, exposed to 1-2000 merthiolate for 5 minutes, and washed 4 times with saline solution. The final yield was 360,000 larvae. The larvae were triturated in 3 ml. of saline solution, and the suspension was injected subcutaneously into 2 guinea pigs.

The 2 animals that received supernatant fluid both died 10 days after injection, thereby showing that the virus kept at 37°C. for 5 hours did not lose its pathogenicity. On the other hand, the 2 guinea pigs that received triturated larvae survived for 60 days, but showed when killed no evidence of lymphocytic choriomeningitis or trichinosis. It can, therefore, be concluded that the virus was unable to survive exposure for 5 hours at a pH of 0.95.

The results of Experiments 6, 7, and 8 established three points: (1) that lymphocytic choriomeningitis virus is inactivated by exposure to a pH of less than 1 during a period of from 1.5 to 5 hours, (2) that normal mature larvae suspended in fluid containing a large quantity of the virus do not thereby acquire the virus, and (3) that the virus, when acquired by larvae from guinea pigs with coexistent experimental trichinosis and lymphocytic choriomeningitis, must be harbored intracorporeally, doubtless protected by the intact nematodal integument.

DISCUSSION

Under the experimental conditions described in the present paper, trichinella larvae from animals with coexistent trichinosis and lymphocytic choriomeningitis are efficient agents for transmitting lymphocytic choriomeningitis to new hosts. Thus, it is possible that this nematode, and others, in the natural process of migration through mammalian tissues—a normal activity in the development of successive stages of the life cycle—may acquire the virus from a host ill with a blood-borne disease and later transfer it to a susceptible host. From the available data it is impossible to say whether these findings have any practical implication in the epidemiology of human lymphocytic choriomeningitis. However, lymphocytic choriomeningitis infection of mice under natural conditions has been reported in the United States (6, 8, 14, 16), England (3), France (19), and Japan (20). The virus, therefore, probably has a rodent reservoir of cosmopolitan distribution comparable to that of

trichinella. Even though the virus from naturally infected mice is readily recoverable from the blood, brain, urine, and nasal secretions, transmission (insofar as recognized) is largely limited to contact with secretions and to infection *in utero*. Moreover, the presence for many months of the virus in the blood of chronically infected mice offers a remarkable opportunity through superinfection for trichinella to acquire the infectious agent. It would not be surprising, therefore, were sporadic infections of lymphocytic choriomeningitis in animals, or possibly even in man, traced to trichinella. Nor is it difficult to believe that other viruses might similarly be acquired by trichinella or that other nematodes might function in the direct transmission of a wide variety of blood-borne viruses.

The remarkable rôle that a parasitic nematode can play during interepizootic periods as a reservoir for the maintenance of a virus under both experimental and natural conditions has been disclosed by Shope (21). He found that the swine lungworm, a nematode parasitic in the bronchioles of swine lungs, could act as a reservoir host for swine influenza virus. The lungworm as it passes through successive developmental stages in its intermediate host, the earthworm, and its definitive host, swine, harbors the virus in masked form during the interepizootic phase of swine influenza. Obvious signs of influenza result when a provocative stimulus effects the release of virus in active form from lungworms resident in the swine bronchioles. Thus, the swine lungworm serves to perpetuate the virus of swine influenza and after long latent periods to initiate new infections from which an epidemic may originate. It is of interest in relation to the present study that the virus of swine influenza exists in lungworms as masked virus and that attempts by Shope to demonstrate direct transfer of the viruses of swine influenza and hog cholera were unsuccessful.

SUMMARY

In experiments in which guinea pigs were infected concurrently with the virus of lymphocytic choriomeningitis and the parasitic nematode, *Trichinella spiralis*, proof was obtained that trichinella larvae, after maturation in the muscles, had acquired the virus and were capable of transmitting it to new susceptible hosts. Transmission resulted both when living larvae were fed to normal guinea pigs and when triturated dead larvae were injected subcutaneously.

Control experiments and other tests made plain that transmission of the virus was not due to mere adherence of it to the outer surface of the larvae but that these actually harbored it.

The significance of these experiments in relation to natural transmission of the virus of lymphocytic choriomeningitis remains to be determined.

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