A VIRUS RECOVERED FROM THE FECES OF "POLIOMYELITIS" PATIENTS PATHOGENIC FOR SUCKLING MICE

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The present report describes the isolation and certain properties of a virus recovered from the feces of children having symptoms similar to those of poliomyelitis. The agent differs from poliomyelitis virus in its host range, being pathogenic for suckling mice and hamsters but not for adult mice or hamsters or for *rhesus* monkeys. The disease in the experimental animal differs from poliomyelitis in that the anatomical response is in the striated muscles rather than the central nervous system (1).

The study was undertaken in 1947 and the original plan was to test fecal suspensions in mice and hamsters from a number of outbreaks. The work was prompted by the report of Milzer and Byrd (2) that autolyzed brain suspension facilitates the isolation of poliomyelitis virus in mice, and by certain unpublished observations of the Battle Hill epidemic of poliomyelitis, which implied that such isolations might be possible. Hamsters were included because of their value in the Battle Hill work (3). After many specimens had been tested without success, it was decided to add an additional group of test animals, suckling mice. This was done because other studies in the Division had suggested that such animals are unusually susceptible, under certain circumstances, to the OT strain of mouse encephalomyelitis virus.

Nature of the Specimens

Poliomyelitis was not epidemic in up-state New York in 1947 and only 925 cases were reported to the Department of Health. Thirty (3.2 per cent) of these died. The non-paralytic cases amounted to 42.6 per cent. Twenty per cent of the patients were more than 20 years of age. The disease did not differ significantly in these respects from that in the 2 preceding years or in 1948.

The specimens were collected by district state health officers from five small outbreaks in widely separated parts of New York State. One or more fecal and blood samples were received from forty-two individuals, of whom fourteen were patients and twenty-eight were contacts.

Methods

The blood was mailed to the central laboratory in the usual fashion. The feces were brought directly to us by messenger or shipped in iced containers. When received, the feces

from the patients were stored at −70°C., those from the contacts at −5°C. This procedure has since been revised and all fecal specimens are now shipped and stored in dry ice. The sera were separated from the clots, distributed in 1 ml. amounts in small tubes, and frozen.

Preparation of Fecal Suspensions.—The feces were ground with sand in a mortar with sterile distilled water to make an approximately 20 per cent suspension. After centrifugation for 10 minutes in a horizontal centrifuge at 3000 R.P.M., ether, approximately 20 per cent by volume, was added to the supernatant fluid which was then stored in the refrigerator. The following day the ether was removed by vacuum and the suspension spun for 1 hour at 3000 R.P.M. The supernatant fluid was aspirated, plated on blood agar, and refrigerated overnight.

Specimens from patients were injected into suckling and 10 to 12 gm. mice of the Albany standard strain, and into adult hamsters. Mice (10 to 12 gm.) and adult hamsters were inoculated as routine with specimens from contacts. Suckling mice also were used for testing the specimens from the contacts of two families in which acute-phase feces from the patients were not received. Inoculations were made intracerebrally and sometimes intraperitoneally as well. The fecal suspension was injected into mice, (10 to 12 gm.), with an equal amount of autolyzed brain tissue prepared after Milzer and Byrd's directions (2). A second group received autolyzed brain tissue with sulfadiazine, since Shaw had noted an apparently more rapid onset of paralysis in mice given intestinal suspensions containing Theiler virus and streptomycin or sulfa compounds (4). The material was given with and without autolyzed tissue in suckling mice. Two adult hamsters were inoculated intracerebrally with each specimen. Animals were observed for 30 days or longer. Brain passages were made from all 10 to 12 gm. animals that appeared abnormal, and, as routine, about the 19th and 33rd day, from pooled brains of two apparently normal mice.

Neutralization Tests.—Baby mice, 2 to 8, preferably 4 or 5 days of age, were inoculated, usually intraperitoneally (0.05 ml.), with equal parts of virus dilution and serum, combined and incubated at room temperature for 1 hour. One amount of virus was used with undiluted serum in the preliminary tests. In the case of the K.H. strain, this represented, for two-thirds of the tests, approximately 100 to 200 median effective doses and between 30 and 1450 M.E.D. for all the tests included in the present report. For the T.T. strain, it represented approximately 300 to 2000 M.E.D. for three-fourths of the tests and from 85 to 2000 M.E.D. for all tests. The amount of serum or of virus was varied in certain later experiments. As a virulence control, three 10-fold dilutions of the agent were given combined with an equal amount of the diluent, physiologic salt solution containing 10 per cent infusion broth. Pooled human serum was also used as a control in each test. Where possible, the median effective dose was estimated by moving-average interpolation (5).

The use of very young mice necessitated an arbitrary evaluation of the animal tests. Mice that died during the 1st and 2nd days following inoculation and mice that were missing at any time were excluded from the calculations. Only those dead, moribund, or paralyzed during the critical period of the test were counted. The test period was usually 12 to 14 days and the mice were sacrificed when paralyzed, except in a few tests in which they were followed to learn whether improvement would occur.

The neutralization tests with one dose of virus were interpreted as follows. When the survivors were fewer than 30 per cent of the test group, the result was considered negative. When the survivors amounted to 30 to 45 per cent, a trace of activity was indicated. Forty-five to sixty per cent survival was given a value of ± to denote moderate neutralization, and more than 60 per cent survival was considered to show that definite neutralization had occurred (+).

Neutralization tests using the Lansing strain of poliomyelitis virus were observed for 21 days and 1st day deaths disregarded.

Immune Sera.—Immune sera were prepared with the T.T. strain in large mice and adult hamsters. Mouse or hamster brain suspension (10 per cent) containing the formalinized or living agent was injected intraperitoneally. The mice received increasing amounts from 0.1 to 0.5 ml. in three weekly series of three daily doses. The hamsters were given four weekly doses of 1.0 ml.

Bleedings were taken from 7 to 13 days after the last injection. The serum, without preservative, was stored in the frozen state.

Histologic examination of selected animals has been practiced throughout. The specimens, in the case of suckling mice the entire animal, were fixed in Zenker's fluid plus 5 per cent glacial acetic acid and sectioned at several levels. The preparations were stained with hematoxylin and eosin and with Giemsa's solution.

Results of the Animal Tests

Acute-phase fecal specimens were available from ten of the fourteen patients and were tested by all four methods; that is, with autolyzed brain, with autolyzed brain plus sulfadiazine, in suckling mice, and in adult hamsters. Two samples, T.T. and K.H., yielded a transmissible agent in suckling mice. All the other tests failed.

Specimens taken during the acute phase of the patient's illness from sixteen of the contacts were tested in 10 to 12 gm. mice, using autolyzed brain tissue suspensions with and without sulfadiazine. All sixteen failed to yield an infectious agent, as did the four that were also tested in suckling mice and adult hamsters. In one instance, weakness of the extremities was noted in suckling mice inoculated with a suspension of feces from a contact and in one or more mice of the third, fourth, and fifth subsequent transfers. The inoculum was found to contain Gram-positive cocci, and a bacteria-free filtrate did not induce paralysis. A second test from the original fecal specimen was also negative.

The two fecal specimens from which an agent was isolated had been collected 5 and 12 days after the onset of symptoms. Of the eight specimens from patients that failed to infect suckling mice, three had been collected on the 5th day, one on the 7th, one on the 10th, and one on the 12th day. The date of collection of the other two was not determined, but they were received on the 5th and 14th days. Thus there was no relation between the time of collection of the specimens and the success or failure in isolating an agent. In several cases, the feces had been held at refrigerator temperature for some days before being sent to the laboratory.

Table I summarizes all the 1947 animal tests, including those made of the two positive cases and their contacts. It will be noted that three separate isolations were made from the acute-phase fecal specimen of K.H.; and from T.T. two isolations were made from one acute-phase specimen and one isolation from a second acute-phase specimen. The suckling mice were paralyzed in the case of T.T. on the 9th, 6th, and 5th days; in the case of K.H., on the 7th, 9th, and 11th days. Since eight other specimens did not induce paraly-

TABLE I

Animal Tests of Patients' and Contacts' Feces Collected in 1947

Place	Status	Age	Mice	Suckling mice	Adult hamsters
		yrs.			
Cortland	Patient	5	0	0	0
	Contact	2	—	—	—
	"	3	—	—	—
	"	30	—	—	—
Jamestown	Patient	4	0	0	0
	"	8	—	—	—
	"	9	—	—	—
Ithaca	Patient	6	—	—	—
	"	7½	—	—	—
	"	10	—	—	—
	"	23	0	0	0
	"	34	—	—	—
Binghamton	Patient	6	0	0	0
	"	10	—	—	—
	"	32	—	—	—
	Contact	3½	—	—	—
Coxsackie	Patient	9	—	+	—
				+	
				+	
	Contact	2	—		
	"	11	—		
	"	15	—		
	"	32	—		
	"	37	0		
	"	66	—		
	Patient	3½	—	+	—
				+	
				+	
	Contact	6	—		
	"	6½	—		
	"	12	—		
	"	31	—		
	"	37	—		
	"	38	—		
	"	41	—		

+ indicates that the agent pathogenic for suckling mice was isolated.

— indicates virus not isolated.

0 indicates not tested.

sis and these two did in each of six trials, we may assume that the agent was present in the patients' feces and not in the test animals.

Twenty-six serial transfers of normal suckling mouse brain suspensions at 4 to 5 day intervals have been made without evidence that our colony harbors a latent infection.

Inoculation of Monkeys.—Young *rhesus* monkeys (*Macaccus mulatta*) were inoculated with the two fecal specimens that yielded the agent, and also with three mouse brain suspensions from subsequent generations.

The suspensions were prepared as before except that ether was not added to the fecal suspensions given intranasally. The monkeys were inoculated intracerebrally (0.2 ml.), intraperitoneally (0.3 and 0.5 ml.), and intranasally (0.5 ml.). Three were given 0.5 ml. intranasally on 4 successive days. Some of the intraperitoneal inoculum was infiltrated into the abdominal muscles. Temperatures were taken daily.

Monkey 4-24 was inoculated with a 10 per cent suspension of T.T. feces. No fever or other signs of illness were noted. The same suspension paralyzed nine of ten suckling mice between the 5th and 8th days.

Monkey 4-20 was inoculated with a 10 per cent suspension of the feces of K.H. No response was seen. The same suspension caused paralysis of seven of twelve suckling mice between the 7th and 11th days.

The tenth mouse generation of the K.H. strain and the second and sixteenth generations of the T.T. strain were similarly tested in monkeys. The second generation preparation, which had been stored in a dry-ice box for 2 months, was inactive in mice and one monkey. The others caused prompt paralysis of suckling mice but no response on the part of the monkeys.

Thus, four preparations infectious for suckling mice, including both of the acute-phase fecal specimens, failed to induce signs of disease in *rhesus* monkeys. While the tests are not numerous, they suggest that monkey-pathogenic poliomyelitis virus was not present in the patients' feces and that the agent recovered from the feces is not pathogenic for monkeys. Sera collected from two of these monkeys 1 month following inoculation failed to neutralize the agent.

Two attempts have so far been made to infect newborn guinea pigs. Three guinea pigs approximately 5 hours old were inoculated by the intracerebral route with a mouse brain suspension of the K.H. strain highly infectious for mice. No signs of disease were detected. Six subsequent intraperitoneal injections failed to produce paralysis or to stimulate the production of antibodies. Three guinea pigs less than 1 day old appeared normal after intracerebral inoculation of a mouse-virulent suspension of T.T. Their sera were not tested.

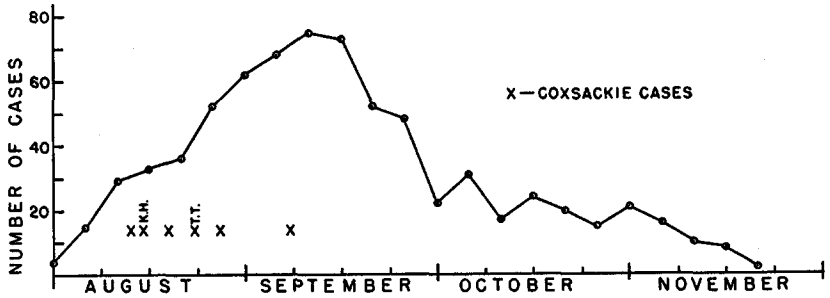
Nature of the Clinical Disease

The original isolations were made from two children who lived in a Hudson River Valley village (population, 2300). Six similar illnesses which occurred within the village during August and early September, 1947, were diagnosed as poliomyelitis. Their occurrence coincided with the rising incidence of polio-

myelitis throughout the state (Fig. 1). All the patients were children and three were paralyzed.

T.T. was a 9 year old boy who complained of headache, nausea, and pain in his legs on Aug. 22. The following day he was febrile (104.0°F.); there was appreciable weakness in both legs, but no nuchal rigidity. His trunk and back muscles became weak and he was hospitalized. His cerebrospinal fluid was slightly cloudy, colorless, contained 250 red blood cells and 64 leucocytes per c. mm., 50 mg./100 ml. of sugar, and slightly increased globulin. During his hospital stay, the child required catheterization once. Weakness of the back muscles was a prominent finding and this persisted throughout the fall and winter. The patient was transferred to the New York State Reconstruction Home. Seven months later he was still unable to raise himself from a recumbent position. A year later he was able to discard his back brace except while playing out of doors and was no longer aware of any disability.

K.H. was a 3½ year old boy whose illness began on Aug. 14 with sore throat and lethargy. Two days later the adductor muscles of his left thigh were found to be very weak. The



TEXT-FIG. 1. Reported cases of poliomyelitis in New York State, exclusive of New York City, 1947.

history does not mention nuchal rigidity. His cerebrospinal fluid contained 10 red blood cells, 2 polymorphonuclear and 2 mononuclear leucocytes. Globulin was not increased. The sugar was estimated to be 50 mg./100 ml. The patient was later transferred to the New York State Reconstruction Home with weakness of the adductors of the left thigh and inversion of the left foot on walking. Paralysis was not recognizable 8 months later.

These two patients lived within a short distance of one another but did not become acquainted until they were hospitalized. The third child known to have been severely paralyzed lived on the outskirts of the village.

The sanitation in the village is satisfactory. The area is one in which poultry and dairy farming form the major enterprises. One large poultry farm is near the home of T.T. The children had not left the village and no record of exposure to a known case of poliomyelitis could be secured. The local physicians recalled other children who had minor complaints during the summer, including headache, nausea, fever, and leg pains but they recovered rapidly and without sequellae.

Relationship of the Agent to the Patients

The recovery of a virus does not constitute proof that it has been responsible for the patient's disease. It is usually necessary that an immune response to the agent be demonstrable. Accordingly, neutralization tests have been per-

formed using the acute- and convalescent-phase sera from the patients and the agents recovered from their feces.

The results of a representative experiment are summarized in Table II. It is evident that the acute-phase serum from K.H. had no neutralizing activity for either strain under the conditions of the test, while the specimen collected 24 days later neutralized both. The acute-phase sample from T.T. had neutralizing activity which increased tenfold within 23 days and diminished in the following months. The median effective dose of acute-phase serum against approximately 125 M.E.D. of T.T. virus was 1:26. Twenty-three days later it had risen to 1:260 and 8 months later had fallen to 1:10. It can be assumed, therefore, that both patients were infected with the agents isolated from their feces at the time that they were ill.

Inhibitory Effect of Feces Collected during Convalescence

Convalescent fecal specimens of T.T. and K.H. collected 28 and 35 days, respectively, after onset of symptoms were also tested for antiviral activity. This was undertaken because other work in this laboratory has shown an inhibitory principle in feces of mice infected with mouse encephalomyelitis virus (6) and the report of similar activity in the feces of monkeys convalescent from poliomyelitis (7).

The fecal suspensions were prepared as before with omission of the treatment with ether. One portion of 20 per cent suspension was filtered through a Mandler candle and a second portion was treated with streptomycin (100,000 units per ml. of suspension) and sodium penicillin (4,000 units per ml.). Both preparations were tested by mixing equal parts of fecal suspension and mouse brain virus preparations and incubating for 3 hours at room temperature before inoculating suckling mice. The test animals were injected intraperitoneally. An unrelated fecal suspension previously found to be non-infectious for suckling mice was used as control.

The results are summarized in Table III. They suggest that the late specimen from T.T. had a measurable antiviral effect.

Nature of the Agent

The agent has so far been tested in mice, hamsters, guinea pigs, monkeys, and fertile hens' eggs, and of these only the first two have shown signs of disease. Eighty-four unsuccessful attempts have been made to induce paralysis in 10 to 12 gm. mice of the Albany standard strain by intracerebral inoculation of virus from the first to the fortieth generation. The 12th day of life is apparently near the end of the period of mouse susceptibility. Mice 11 and 12 days old became paralyzed but the incubation period was somewhat longer than usual. Among three families of mice 14, 15, and 16 days of age, comprising twenty-seven animals, there was only one mouse with doubtful paralysis (15 day old, 7th day).

TABLE II

Immunologic Response of Patients T.T. and K.H. to the T.T. Strain of Virus

Serum	Date of bleeding	Dilution	Virus suspension dilutions			
			10^{-1}	10^{-2}	10^{-3}	10^{-4}
Broth salt			-----	**4,4,4,4,4,4,4**	**4,4,4,5,5,5, 5,6,6,8**	**4,6,8**,S,S,S, S
Normal human serum pool		Undiluted	S,S,S,S,S,S, S,S,S,S,S			
" "		1:5	**4,5,5,5,11,** S,S,S			
" "		1:50	3,3,3,**3,3,3,3**			
K.H.	8/26/47	Undiluted	**3,3,3,3,4,4,4**			
"	9/19/47	Undiluted	S,S,S,S,S,S			
"	"	1:5	**5,6**,S,S,S,S, S,S,S			
"	"	1:50	4*,4*,4*,**4,** **4,4,4**,5,S			
T.T.	8/26/47	Undiluted	**5,5**,S,S,S,S, S,S,S,S			
"	"	1:5	**8**,S,S,S,S,S, S,S			
"	"	1:50	4*,**4,4,5,5,5**			
"	9/18/47	Undiluted	S,S,S,S,S,S, S,S,S			
"	"	1:5	1,2,S,S,S,S, S,S,S,S			
"	"	1:50	**7**,S,S,S,S,S, S,S			
"	5/14/48	Undiluted	S,S,S,S,S			
"	"	1:5	6*,6*,S,S,S, S,S			
"	"	1:50	4*,**4,4,4,4,4,** **5,5,5,5**			

Survivors are shown by "S." The numbers indicate the day on which the animal was found paralyzed, dead, or missing. Paralyzed animals are indicated by bold-faced numbers. Asterisks signify missing mice.

The agent is stable in mouse brain suspension at −70°C. for at least several months and the fecal specimens were infectious 9½ months after collection. It

TABLE III

Tests of Convalescent Patients' Feces for Virus Inhibition

Patient	Dilutions of K.H. Virus			Dilutions of T.T. virus		
	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
Broth salt				**3, 3, 3,** 3* 3*, 3*, 3*	**3, 3, 3, 3,** 3	**4, 5,** 5, S, S
Broth-salt + antibiotics	**4, 4, 4, 5, 5, 5, 5**	**5, 5, 5, 6, 9**	**4,** 5, **6, 6,** S S, S, S, S	**3, 3, 3, 3, 3,** 3, 3*	**3, 3,** 3*, 3*, **4, 4,** 4, 4, 4, 4, 4*	3*, 3,* 3*, 3*, 4, 4, 4*, 5*, S, S
T.T. + antibiotics	**5, 8, 12,** S, S, S, S, S, S, S, S	**4,** S, S, S, S, S		**5, 5, 5, 5,** S	9, S, S, S, S, S, S	
T.T., filtered				**4, 4, 4, 5,** 5*, S, S, S, S	S, S, S, S, S, S, S, S	
K.H. + antibiotics	3, 3, 3, 4, 4, 4*	**3, 3, 3,** 3, **4,** S, S		3*, 3*, 3*, 3*, 3*, 3	**3, 3, 3,** 3, 3, 3, 3, 3, 3	
K.H., filtered				**3,** 3, 3, 3*, 3*, 3*	**3, 3,** 3, 3, 3*, 3*	
K.C. + antibiotics (control)	**3, 3, 3, 3, 3, 3**	**4,** 4, **5, 5, 5,** 5, S				

The convalescent patients' fecal speciemens were collected 28 days (T.T.) and 35 days (K.H.) after the onset of disease.

Survivors are shown by "S." The numbers indicate the day on which the animal was found paralyzed, dead, or missing. Paralyzed animals are indicated by bold-faced type. Asterisks signify missing mice.

is also stable in 50 per cent glycerol for at least 5 months. It is inactivated by 0.25 per cent formalin at room temperature.

Cultural Tests.—Infected mouse brain suspension,K. H. and T.T., filtered through Mandler candles, induced paralysis in suckling mice and hamsters but failed to initiate growth at 35°C. in casein hydrolysate semisolid agar containing sodium thioglycollate. No significant growth was obtained aerobically from unfiltered infected brain suspension on beef extract agar, horse blood agar, or potato infusion–sheep blood agar plates.

Microscopic and cultural examination of unfiltered mouse brain suspension infected with the T.T. strain failed to detect the presence of microorganisms of the pleuropneumonia group or of any bacteria. The media used were beef heart infusion broth containing 30 per cent ascitic fluid and broth with 30 per cent normal horse serum. Beef heart infusion agar with 30 per cent ascitic fluid and agar containing 30 per cent normal horse serum were also included. Seven serial transfers were made in each fluid medium at 2 to 4 day intervals; 0.2 ml. amounts were tested on the surface of the solid media in plates at the same time. All ascitic fluid and serum agar plates were incubated for 7 days or longer. Darkfield and Gram-stained preparations were made of the original material and several of the fluid media.

TABLE IV

Neutralizing Activity of Sera of Patients and Household Contacts

Individual	Status	Age	Test virus					
			T.T.		K.H.		Lansing	
			Acute	Convalescent	Acute	Convalescent	Acute	Convalescent
K.H.	Patient	3½	−	+	−	+	?	?
K.C.	Cousin	6			−	−	+	+
B.C.	"	12			−		+	
R.H.	Mother	31			+	+	+	+
B.C.	Aunt	37			−	±	+	+
K.C.	Uncle	38			+	+	+	+
C.H.	Father	40			−	−	+	±
T.T.	Patient	9	+	+	+	+	?	?
E.T.	Brother	11	−	−			+	+
W.T.	"	15	+	+			+	+
G.T.	Mother	32	+	±			+	+
N.T.	Father	37	−	−			+	+
R.T.	Grandmother	66	−	−			+	+

Size.—Preliminary tests indicate that the virus is small. A 10 per cent suspension of mouse brains infected with the K.H. strain was centrifuged for 30 minutes in an air-driven Beams and Pickels type ultracentrifuge at 350, 450, and 550 R.P.S., approximately 50,000, 75,000, and 100,000 times gravity. The undiluted supernatant fluids from all three runs proved to be virulent for suckling mice. A 10 per cent suspension of mouse brains infected with the T.T. strain, which has a higher titer than the K.H. strain, was ultracentrifuged at 550 R.P.S. for 30 minutes. The supernatant fluid, diluted to 10^{-2}, was infective for 100 per cent of the mice. Greater dilutions were not made in this experiment. Preliminary measurements based on Elford membranes and examination with the electron microscope confirm these results and suggest that the virus is less than 40 mμ in diameter and approximately spherical.

Tests of the Sera of Household Contacts

The sera of the patients' household contacts have been tested for the presence of neutralizing activity for the T.T. and K.H. strains and also for the Lansing

strain of poliomyelitis virus. The results are summarized in Table IV. It will be seen that of the ten from whom convalescent-phase serum samples had been secured, five had neutralizing activity for the new agent. All the samples neutralized the Lansing virus. The sera of all but the very young in New York usually neutralize Lansing virus. The different behavior of these sera with the new agent indicates either that the maturation effect which has been postulated as an explanation of the reaction of adult sera with poliomyelitis virus (8) does not apply, or that infection with the new virus is not prevalent or has not occurred frequently in the past. The persistent or repeated prevalence of virus is an alternative explanation of the ubiquitousness of antipoliomyelitis serum activity. Taken at face value, the tests suggest that one brother and the mother of T.T. had been infected, and also that three adult members of the household of K.H. had been infected with the new agent.

Sera from other poliomyelitis patients in New York have also been tested by the same methods. Neutralizing activity was found in acute- and convalescent-phase sera of two of nine patients, both adults. Twelve pairs of sera have been received from Dr. Thomas Francis, Jr. They were collected during the summer of 1947 from Michigan children with indefinite symptoms. Both sera of two patients reacted strongly with the K.H. strain and two others gave weak reactions.

Nature of the Disease in Mice

The incubation period of the T.T. and K.H. strains is usually 3 days. After the ninth generation, approximately 70 per cent of passage mice inoculated intracerebrally with brain suspension 10^{-1} showed paralysis or other signs of infection on the 3rd day. A few were paralyzed on the 2nd day and approximately 17 per cent on the 4th day. The small number of animals showing signs of disease on the 5th, 6th, 7th, 8th, and 9th days were mainly from families 9, 11, and 12 days old.

The prodromal signs of disease in the baby mice are lethargy and generalized weakness of the body and extremities, delayed response to touch, a tendency to move in circles suggestive of unilateral leg weakness, and at times stunted growth and poor nourishment. Paralysis or death follows by a day. One or more extremities may be paralyzed. The legs may remain flexed or extended, while the toes fail to spread and pressure on them produces no reaction. Some mice develop labored breathing. Spasms and convulsions have not been observed. In the few mice that have lived for more than the day following the prodromal signs, the paralysis has become progressively more severe and generalized. Some have developed swelling and induration of the muscles and others have shown apparent swelling of the joints. Such mice are conspicuously stunted, probably in part because they are unable to suckle.

Mice that have been paralyzed for a short period show no gross lesions. Animals paralyzed for a day or more show opaque, whitish muscles, especially in the pectoral group, the longissimus dorsi, and in paralyzed extremities. Severely paralyzed muscles are firm to

touch and very white. Gross lesions have not been observed in the viscera or organs of the central nervous system.

Twenty-five suckling mice and two suckling hamsters have been extensively examined histologically. None of the animals inoculated with either strain has had lesions of the central nervous system but nearly all have shown widespread changes in the skeletal muscles. The lesions begin as a hyaline degeneration of the muscle fibers, followed by complete destruction. The fibers are transformed into amorphous masses, the fragments being quickly absorbed and phagocytosed (Figs. 1 to 3). Regeneration is evident from the first, and large masses of young, actively multiplying muscle cells give the lesion a very cellular appearance. In one mouse, examined 7 days after the initial symptoms, repair was so extreme that the lesion resembled a rhabdomyosarcoma (Figs. 4 to 6). The lesion resembles Zenker's degeneration as it occurs in a variety of apparently unrelated conditions. We have observed similar lesions in the spinal muscles in hamsters infected with MM virus and in mice infected with other neurotropic agents. A comparison of these lesions is being made at present. Study of the terminal nerve structures is incomplete. Little has been found in the other organs. Myocarditis, said to occur in EMC virus infection (9, 10) and which we have once observed, as well, in a mouse infected with mouse encephalomyelitis (OT) virus, has not been found.

Further Isolations of the Agent

Agents that induce similar signs and lesions in suckling mice have been isolated from other outbreaks and isolated cases of apparent poliomyelitis. Nine isolations were made during 1948 from patients ill during that summer in New York. Only one had been frankly paralyzed although another, an adult woman, had questionable weakness of the leg. Three of the strains differ from the T.T. and K.H. strains. They induce lesions of the central nervous system as well as less severe changes in the muscles. They may be distinguished by the behavior of infected mice.

Five isolations have been made from fecal suspensions of patients from the 1947 Wilmington, Delaware, epidemic. The material was supplied by Dr. Robert Ward, New York University, College of Medicine, and by the Communicable Disease Center, United States Public Health Service. Three of these patients were described as having had weakness of certain muscles. Since numerous attempts by others to recover monkey-paralyzing virus from the Wilmington outbreak had failed, and because the disease was unusual epidemiologically and clinically (11, 12), a comprehensive study of the materials at hand is being made.

It seems evident that the agent is at present disseminated rather widely, whatever its importance may be as a cause of disease. The newer strains reveal apparent antigenic differences and also other variations that are still being investigated.

Relationship to Other Viruses

Other viruses that induce similar lesions in the striated muscles—mouse encephalomyelitis, Columbia SK, MM, and EMC viruses—do not selectively paralyze suckling mice and do induce lesions of the central nervous system.

Columbia SK, MM, and EMC viruses are serologically related (13, 14). Serum produced with one of them, MM virus, did not neutralize the new agent nor did rabbit antiserum or mouse antiserum of FA mouse encephalomyelitis virus.

Immune animal sera for Newcastle disease, lymphocytic choriomeningitis, and the Aycock and Lansing strains of poliomyelitis have also failed to neutralize the agent. Adult normal mouse and normal rabbit sera were inactive under the conditions of the test but the serum of one normal monkey of the several tested had a slight neutralizing effect.

DISCUSSION

The isolations to date have all been from patients diagnosed as having had abortive or paralytic poliomyelitis, but, since no effort has yet been made to recover virus from patients with other diseases or from healthy individuals, no conclusions based on the association seem warranted. Sufficient specimens have been examined to indicate that carriage of the agent is not commonplace.

The deficiencies in our information that have seemed most important to us are due to the lack of human material. It would obviously be most valuable to be able to examine the tissues of an individual infected with the agent. Indeed it has seemed to be premature to suggest a name for the virus or the disease with which it has been associated in the absence of knowledge of the kind of anatomic response it induces. Similarly, recovery of virus from human organs rather than from feces would be a matter of importance, especially if the presence of the virus and a lesion were associated. No such specimens have been available. We have examined a limited number of specimens of nervous tissue from recent fatal cases of poliomyelitis without success, but in none of these had the suckling mouse virus been demonstrated in the feces. Muscle biopsies would obviously be valuable specimens both as material for histologic examination and for animal tests. Fifty-four specimens of spinal fluid have been tested in suckling mice with negative results.

It should perhaps be pointed out that, while we have not found lesions in the brains or spinal cords of our mice, we regularly use these tissues for the transfer of virus since the infectivity titer is high and the brain is a uniform and easily harvested sample.

The muscle lesions in the immature mice may be similar to those described in monkey and human poliomyelitis by Carey and his colleagues (15, 16) and in man by Dublin, Bede, and Brown (17). These authors paid particular attention to the terminal nerve structures but Carey *et al.* described degeneration of the muscle fibers, including loss of striations and hyalin changes, and Dublin reported atrophy of the muscle fibers ending in dark, pyknotic masses. The material examined by Dublin and his associates was from children 35 to 48 days following the onset of poliomyelitis. Carey had examined earlier stages of the

process and found changes in the myoneural junction within 36 hours of infection.

Muscle fiber degeneration of the kind seen in our suckling mice occurs to a limited extent during MM virus infection in the hamster. Similar lesions were found by Rustigian and Pappenheimer (18) in the muscles, at the site of injection of mouse encephalomyelitis viruses and SK (Columbia) virus. They distinguished between these lesions and the inflammatory infiltrate that follows the intramuscular injection of lymphocytic choriomeningitis virus and the non-specific reaction in muscles to certain other neurotropic virus inocula. The last, they believe, is a reaction to brain tissue rather than to virus. Thus, the presence of myositis of a striking kind has recently assumed considerable significance in the pathogenesis of poliomyelitis and poliomyelitis-like diseases and has, in certain instances, been found to resemble the lesions that occur in suckling mice and hamsters.

It may be well to mention other similarities between poliomyelitis and the disease under consideration. The clinical similarities have been mentioned. They cannot be satisfactorily analyzed on the evidence we have so far accumulated. The seasonal occurrence of both diseases appears to be the same. Both viruses are unusually small and relatively stable in glycerol. Both occur in the feces.

These similarities are listed not with the thought of suggesting that the two diseases, or viruses, are necessarily closely related but to call attention to the possible difficulties in studying outbreaks of poliomyelitis if such a disease as the present one occurs simultaneously. This may have occurred during the past two summers in New York and very probably did in Delaware in 1947, since Melnick isolated monkey-pathogenic strains of poliomyelitis virus from patients in the suburbs of Wilmington (19) while we recovered the new agent from urban patients. One is reminded of the 1934 epidemic of poliomyelitis in Los Angeles (20–22). It occurred earlier in the summer than is the rule, the number of patients more than 10 years of age was abnormally high, the mortality rate exceptionally low, and there was striking evidence of high communicability. Multiple cases occurred in 12.5 per cent of the households that were afflicted. Poliomyelitis virus was recovered from a number of the fatal cases (23) and from the nasal washings of one mild case (24). It is possible that classical poliomyelitis occurred simultaneously with an outbreak of a second but more benign disease. Under such circumstances the isolation from fatal cases of virus pathogenic for monkeys would be of limited significance since the sample would be drawn from the more virulent infection, while the unknown disease, by its numbers, would account for the peculiarities of the epidemic. Something of this kind may well have occurred in Wilmington and an effort should be made to test as many specimens as possible to determine whether it did happen.

SUMMARY

A virus has been recovered from the feces of two children having symptoms similar to those of poliomyelitis. The virus is pathogenic for suckling mice and hamsters but not for *rhesus* monkeys. It induces striking lesions in the skeletal muscles of the experimental animal but not in the central nervous system. Other viruses inducing similar signs and lesions in suckling mice have been isolated from several other outbreaks of a poliomyelitis-like disease, including one large urban epidemic.

The examinations for microorganisms of the pleuropneumonia group and the electron microscope observations were made by Miss Julia M. Coffey; the ultrafiltration and ultracentrifugation studies, by Mr. James J. Quigley. Infectivity tests in embryonated eggs were made by Dr. Irving Gordon.

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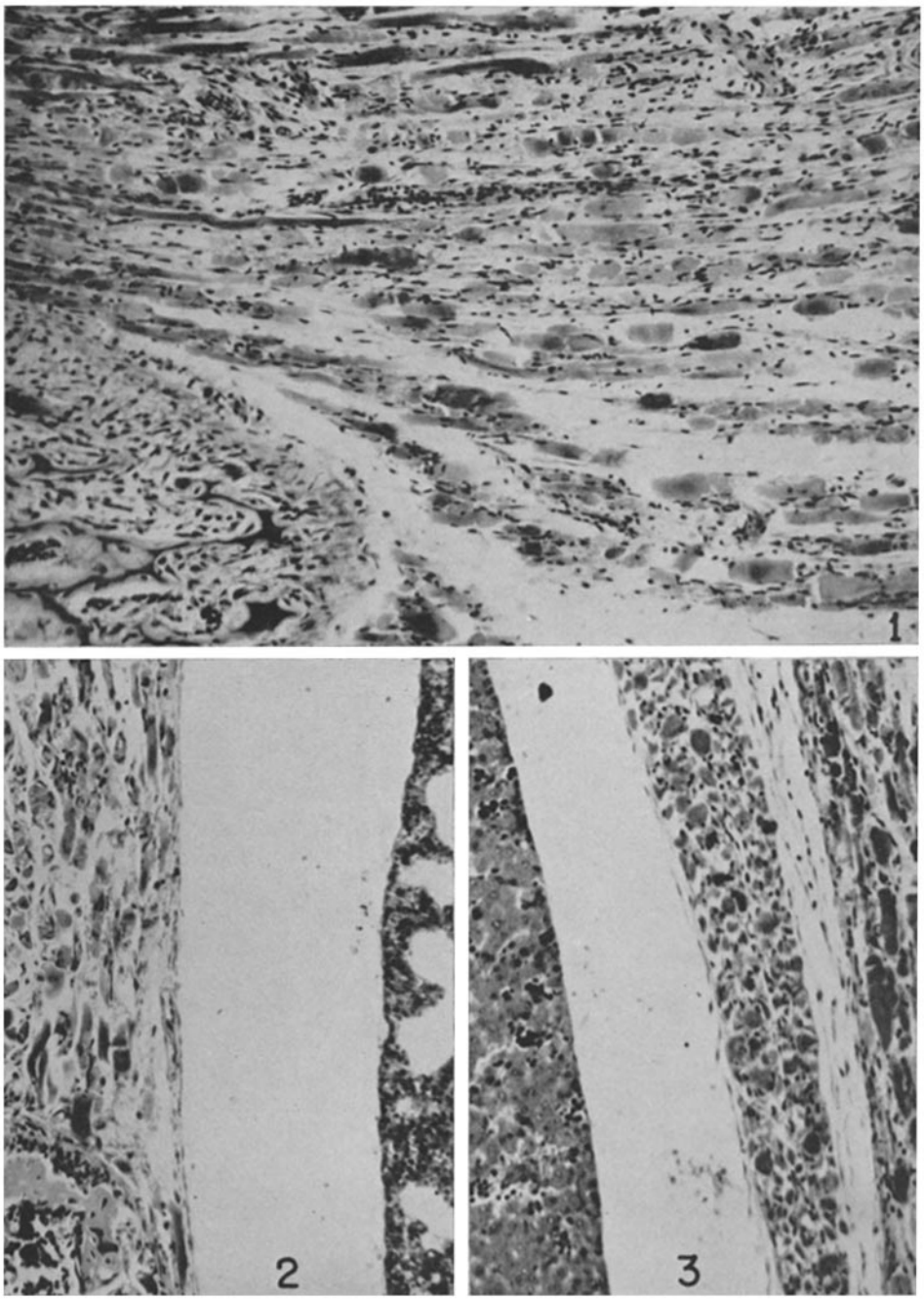
EXPLANATION OF PLATES

PLATE 27

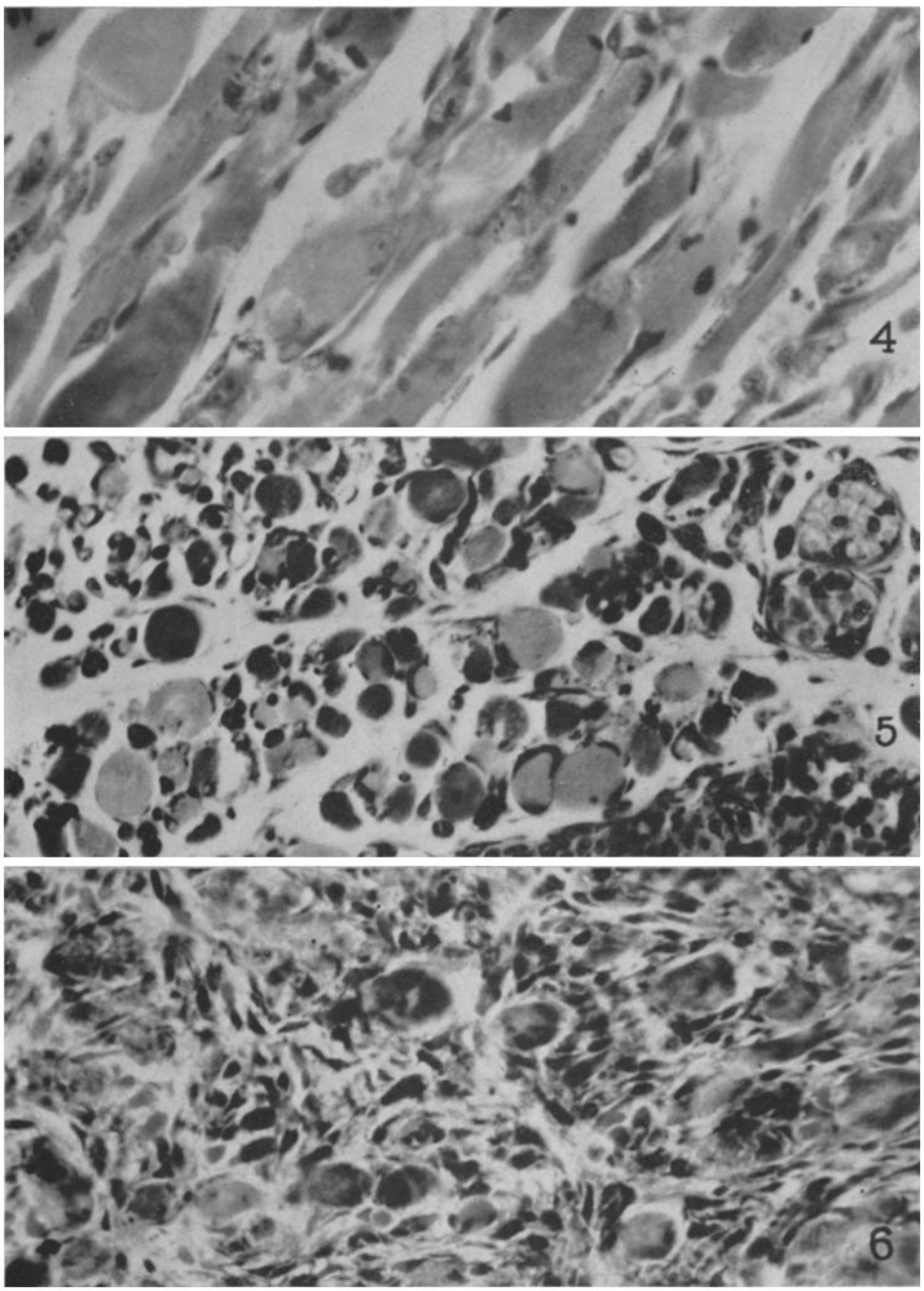
FIGS. 1 to 3. The usual appearance of the muscles in paralyzed suckling mice. Fig. 1 shows muscle of a leg; Fig. 2, the muscle of a thoracic wall and Fig. 3, that of the abdominal wall. × 140.



(Dalldorf *et al.*: Virus from feces of "poliomyelitis" patients)

PLATE 28

FIGS. 4 to 6. The stages in the evolution of the lesion in striated muscle. Fig. 4 represents the early phase in which degeneration of adult muscle fibers is the dominant lesion. In Fig. 5 the process is well advanced and phagocytosis of deteriorated fibers and regeneration of muscle cells are prominent. Fig. 6 illustrates the lesions seen in a mouse which survived 7 days. Note the extreme cellularity of the repair. ×580.



(Dalldorf *et al.*: Virus from feces of "poliomyelitis" patients)