17186. A Virus Isolated from Patients Diagnosed as Non-Paralytic Poliomyelitis or Aseptic Meningitis.*

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It is the purpose of this report to describe the isolation of a filtrable agent from patients with an illness resembling non-paralytic poliomyelitis which occurred during 1948 in southern New England. The virus was sought following the report by Dalldorf and Sickles,[1] and is apparently similar to that which they obtained from the feces of 2 patients diagnosed as paralytic poliomyelitis. Like their agent, the virus infected newborn albino mice and produced in them weakness and paralysis accompanied by diffuse myositis.

The following aspects of work with this virus will be described:
(1) recovery of the virus from the feces of patients and its neutralization by their sera;
(2) description of the experimental disease and some properties of the virus;
(3) inapparent infection of chimpanzees;
(4) accidental infection of man;
(5) occurrence of the virus in "poliomyelitis" patients in Ohio and North Carolina as well as in New England;
(6) occurrence of the virus in extra-human sources (sewage and flies);
(7) immunological types of the virus.

(1) *Isolation of virus from patients diagnosed as having non-paralytic poliomyelitis or aseptic meningitis.* During the summer and fall of 1948 samples of feces were collected from 16 representative patients from Connecticut and Rhode Island, 13 of whom presented clinical features consistent with non-paralytic poliomyelitis (Table I), and 3 of whom had definite muscle weakness and were diagnosed as paralytic poliomyelitis. The fecal samples from these 16 patients were tested in monkeys for poliomyelitis virus and were also examined for the agent infectious for newborn mice.

Fecal samples from 5 (Nos. 1-5) of the 13 patients with non-paralytic illnesses produced disease in newborn mice, but not in monkeys. From one to 4 rhesus monkeys (*Macaca mulatta*) were used to test each of these samples for poliomyelitis virus with negative results. Furthermore the strains recovered in newborn mice were tested in 8 additional monkeys and again the results were negative. The new virus was also found in the feces of 2 patients (No. 18-19) diagnosed as "fever of unknown origin". Lumbar puncture was done in only one of the latter patients and there was no pleocytosis.

The new virus has not been recovered by blind passage of brains of normal newborn mice, nor from separate fecal samples of 29 patients in New Haven with various other infectious and non-infectious diseases.

Fecal samples from two of the patients with non-paralytic disease (Nos. 11-12) yielded poliomyelitis virus when inoculated into monkeys, but did not produce disease in newborn mice. The 2 strains of poliomyelitis virus were typical; they were pathogenic for monkeys but not for cotton rats or mice (3 week old as well as newborn). Samples of feces from the 3 patients with clinical paralytic poliomyelitis (Nos. 13-15) failed to yield an agent when tested in either monkeys or newborn mice.

Tests for neutralization of the new virus with acute and convalescent sera of the patients revealed that:—(i) the sera of 7 patients (Nos. 1-5, 18,19) from whom the new agent was isolated, neutralized the virus (neutralization index from 1,000 to 10,000) in the convalescent stage and to a lesser degree in the acute stage; (ii) the convalescent sera from 5 other patients (Nos. 6-10) with non-paralytic illnesses also neutralized the virus;

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TABLE I.

Isolation of Virus and Appearance of Neutralizing Antibodies in Patients from Southern New England During August-October, 1948.

Patient*	Age	City	Diagnosis	Initial spinal fluid		Results of tests for virus		Neutralization of new virus by patient's convalescent serum
				WBC per cu mm	Ratio P : M	In monkeys (polio virus)	In newborn mice (new virus)	
1	9	New Haven	N.P.P.	31	33:67	—	+	+
2	5	" "	A.M.	36	30:70	—	+	+
3	5	" "	N.P.P.	27	7:93	—	+	+
4	11	Hartford	N.P.P.	125	54:46	—	+	+
5	32	"	N.P.P.	92	32:68	—	+	+
6	12	Providence	A.M.	600	15:85	—	—	+
7	6	New Haven	N.P.P.	129	25:75	—	—	+
8	18	Hartford	N.P.P.	140	74:26	—	—	+
9	6	New Haven	A.M.	42	47:53	—	—	+
10	12	Providence	N.P.P.	90	0:100	N.T.	N.T.	+
11	13	New Haven	A.M.	46	63:37	+	—	—
12	12	Stamford	N.P.P.	130	44:56	+	—	—
13	12	New Haven	P.P.	3	0:100	—	—	—
14	9	" "	P.P.	43	70:30	—	—	— (INC.)
15	13	Providence	P.P.	200	80:20	—	—	—
16	14	"	A.M.	142	49:51	—	—	—
17	15	Hartford	N.P.P.	95		—	—	—
18	14	New Haven	F.U.O.	3	0:100	N.T.	+	+
19	26	" "	F.U.O.	N.T.		N.T.	+	+

P : M—Ratio of Polymorphonuclear to Mononuclear Cells.
N.P.P.—Non-paralytic Poliomyelitis.
A.M.—Aseptic meningitis, cause unknown.
F.U.O.—Fever of Unknown Origin.
P.P.—Paralytic Poliomyelitis.
N.T.—Not Tested.
INC.—Incomplete, acute sample 6 days after onset was negative, convalescent sample not tested.

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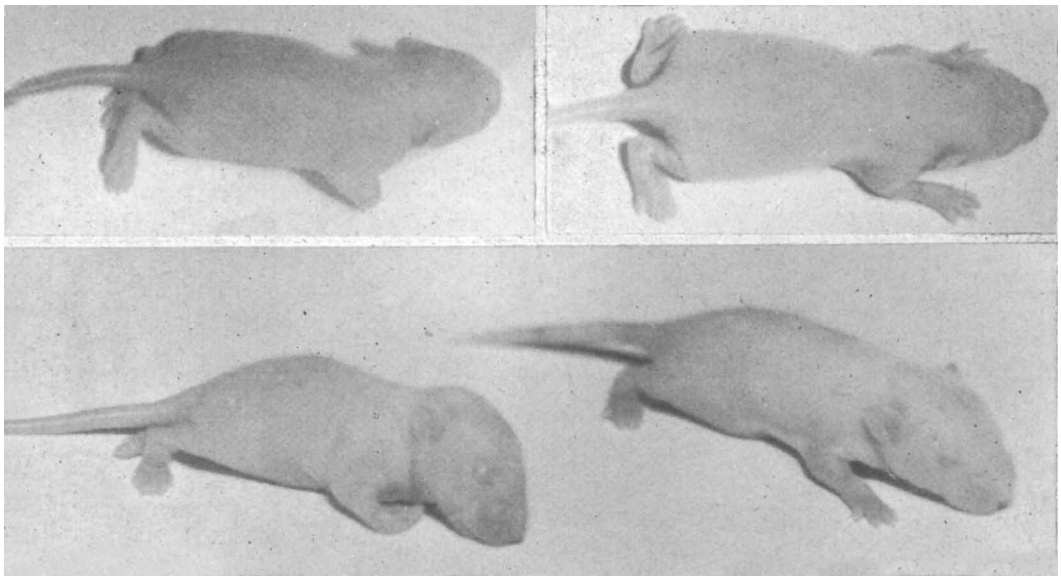

FIG. 1.

Two litter mates, the mouse on the left with paralysis of both legs and right arm (wrist-drop), the one on the right without signs of disease. Virus was inoculated intracerebrally and intraperitoneally on the 3rd day of life. Illness was first noted 6 days later when the photographs were taken.

FIG. 2.

Myositis in paralyzed limb of 8-day-old mouse, sacrificed on the first day of paralysis. This animal was inoculated by the intracerebral and intraäbdominal routes within 24 hours after birth. The photomicrogrph was taken at 155 × magnification.

(iii) the convalescent sera of the 3 patients diagnosed as paralytic poliomyelitis and the serum of the 2 non-paralytic patients from whom poliomyelitis virus was isolated, all failed to neutralize the virus. The results of complement fixation tests with sera from these

patients indicated that mumps virus was not the etiological agent of their illnesses.

(2) *Description of the experimental disease and some properties of the virus.* Swiss mice generally between the 1st and 2nd day of life have been used. Infectivity titers have ranged from 10^{-5} to 10^{-6} with suspensions of brain and slightly higher with suspensions of muscle. The mice have been inoculated intracerebrally and/or intraperitoneally. Signs of disease have appeared within 2 to 10 days, manifested by weakness and paralysis of one or more extremities (Fig. 1) and followed by death generally within 24 hours. In very young mice marked ataxia may be the only sign before death. The outstanding pathological finding has been an extensive myositis in the skeletal muscles especially of the limbs (Fig. 2). In some mice lesions have been noted in the heart muscle and in the brain.

Aerobic and anaerobic cultures of suspensions of tissues used for passage have shown no bacterial growth on ordinary media. The agent as it occurs in human feces and in mouse brain used for passage, has been passed through a tested, Corning, bacterial, glass-fritted filter. The agent is resistant to the action of ether, penicillin, streptomycin and chloromycetin. It has failed to produce disease in 3 week old mice, cotton rats, snowball rats, albino rats, hamsters as well as in young adult monkeys.

Studies on the distribution of the virus in newborn mice indicate that it is widespread at the time of paralysis; it has been recovered from the blood, brain, muscles, heart, liver, spleen and also from the intestinal contents. That the agent recovered from this latter source was not Theiler's TO virus was indicated by its lack of pathogenicity for 3 week old mice.

Preliminary data on sedimentation suggest that the agent is one of the smaller viruses. Thus, although some of the virus may be thrown down together with the particles which sediment at 18,000 r.p.m. for 30 minutes (6 inch rotor), most of the virus remains in the supernatant fluid. It may be sedimented readily at 36,000 r.p.m. for 60 minutes.

Neutralization tests have been carried out in newborn mice by the intraperitoneal route. It was found that the virus is readily neutralized by homologous antiserum prepared by repeated inoculation of mice or monkeys. It is not neutralized by antiserum from animals hyperimmunized against the following viruses; poliomyelitis (Lansing strain, North Carolina 1948 strain, Texas 1948 strain), Theiler's FA and GDVII strains of mouse encephalomyelitis virus, mumps, herpes, lymphocytic choriomeningitis, encephalomyocarditis, louping ill, Venezuelan equine encephalitis, and Newcastle disease.

(3) *Infection of chimpanzees.* As mentioned above, rhesus monkeys do not appear to be susceptible to infection with this virus when inoculated intracerebrally, intraperitoneally, intramuscularly, intracutaneously or subcutaneously. Two chimpanzees were each given one oral administration of the virus. No clinical signs of illness developed. However, virus was recovered from their feces for 12 days after the feeding and from their throats on the 5th, 6th and 8th days. Neutralizing antibodies, absent before exposure to virus, were present on the 14th and 28th days.

(4) *Accidental infection of man.* One of the physicians engaged in work with this agent developed a vague febrile illness of 8 days duration which was diagnosed as "fever of unknown origin." The only suggestion of involvement of the central nervous system was minimal stiffness of the back; a spinal tap was not done. Virus was recovered from the feces and nasopharyngeal washings during the acute illness. Neutralizing antibodies were not found in serum collected before or during the early acute phase of illness, but appeared in increasing titer during convalescence. The neutralization index on the 43rd day after onset of illness was 10,000.

(5) *Occurrence of the virus in "poliomyelitis" patients in Ohio, 1947 and North Carolina, 1948.* Samples of feces collected from different parts of the country and frozen since collection were also examined for the virus. It was not found in pooled specimens collected during 1944 from patients with paralytic poliomyelitis in New York City, nor from similar specimens collected from patients in

Los Angeles, California in 1948. These pooled samples were proven by monkey inoculation to contain poliomyelitis virus. The new virus, as well as poliomyelitis virus, was isolated from pooled fecal samples of 6 patients who had non-paralytic poliomyelitis in 1947 in Akron, Ohio. Both viruses were also present in pooled fecal samples collected in 1948 from patients with paralytic poliomyelitis in Winston-Salem, N. C. An ultracentrifuged concentrate of the Winston-Salem specimens had titers of 10^{-3} for poliomyelitis virus in monkeys and of $10^{-3.5}$ for the new virus in newborn mice.

(6) *Occurrence of the virus in extra-human sources.* The virus has been sought in samples of the sewage from 6 cities, 3 situated in Connecticut (Hartford, Norwalk, New Haven) and 3 in North Carolina (Greensboro, High Point, and Winston-Salem), and in non-biting flies trapped in 2 of these cities. In the summer of 1948 "mild poliomyelitis" appeared to be prevalent in Connecticut, and a severe epidemic of classical poliomyelitis occurred in North Carolina. As already mentioned, the new virus was isolated from patients in both areas.

The new virus was found in the sewage of all of the above 6 cities during some part of the summer and fall of 1948 and usually several serial samples from each city were positive. Serial sampling during the winter has for the most part been negative but the data are too few to indicate whether the occurrence of the virus in sewage follows the seasonal pattern of poliomyelitis virus.[2,3]

Tests of flies from the above areas for the presence of the new virus have not yet been completed, but thus far 2 isolations have been obtained, one from flies trapped in August, 1948 in Hartford, Connecticut and another from flies trapped in July, 1948 in High Point, N. C. In addition to studying this material from Connecticut and North Carolina, flies from Texas were also examined. These were trapped serially in 1948 during an epidemic of poliomyelitis in the lower Rio Grande Valley.‡ From some batches of flies, separated according to species, both the new virus and poliomyelitis virus have been isolated. Other batches of flies have yielded either one virus or the other, and many batches have yielded neither virus. The species of flies which have given positive tests for both viruses are (a) *Musca domestica,* (b) *Phaenicia sericata* and *P. pallescens,* and (c) *Sarcophagula* and *Sarcophaga* spp. The two viruses (poliomyelitis and the new virus isolated in mice) even when obtained from the same batch of flies do not appear to be related by the tests we have thus far used (host range, virus neutralization by hyperimmune sera.)

(7) *Immunological types.* By means of cross neutralization tests, it has been found that two strains of the murine infecting virus isolated from patients in New Haven are related to each other as well as to a strain isolated from Hartford sewage. Furthermore a strain from Texas flies was found to be related to a North Carolina sewage strain, but not to the Connecticut strains. Thus each strain was readily neutralized by homologous hyperimmune sera, but antisera against Connecticut strains failed to neutralize the Texas virus, and, in similar fashion, Texas antisera had no effect on the Connecticut virus.

Summary. This paper reports the isolation of a filtrable virus from the feces of patients diagnosed either as non-paralytic poliomyelitis or aseptic meningitis and from 2 patients with "fever of unknown origin." The agent is similar to that reported by Dalldorf and Sickles[1] in producing paralysis with moysitis in newborn mice. The recovery of virus was correlated with the appearance of neutralizing antibodies in the patients' sera. At least two immunological types of the virus exist. The virus was widespread in this country during the summer of 1948 having also been isolated from the sewage of a number of

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‡ The flies in Texas were caught and identified by Dr. Richard P. Dow, Entomologist, and Staff, attached to the Dysentery Control Project, U. S. Public Health Service. They were collected as a part of field studies on epidemic poliomyelitis being carried out by this unit.

cities and from flies collected in widely separated areas. Subclinical infection may be produced in chimpanzees by oral administration of the virus. A laboratory worker has been accidently infected with the virus.

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17187. Effect of Phenergan (N-Dimethylamino-2-Propyl-1-Thiodiphenylamine, 3277 RP) on the Arthus Reaction in Rabbits.

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The so-called antihistaminic compounds have been of great value in that they have made possible the study of various biological phenomena, principally those related to histamine reactions, and the treatment of histamine-like allergies. As yet, none of the antihistaminics have been found to be effective in modifying the necrotizing type of allergic reactions. The lack of effect of pyribenzamine on the Arthus phenomenon and bacterial allergic reactions has been reported.[1] The present study is presented because an unusually potent antihistaminic, possessing, in addition, properties apparently unrelated to its antagonism to histamine, has been found effective in inhibiting the Arthus reaction.

The Fourneau compounds[3,4] and antergan[2] were modified to produce phenergan, N-dimethylamino - 2 propyl - 1-thiodiphenylamine, (3277 RP) which was extensively studied by Halpern.[5,6,7] In addition to being 30 times more effective than antergan in protecting guinea pigs against histamine, phenergan apparently protects capillaries against injury by various noxious stimuli.[8-12] The drug was kindly given for this study by Dr. B. N. Halpern. A quantitative technic for the induction of the Arthus reaction with known quantities of a single antigen and its homologous antibody has been described.[1,13] This method was employed in the present study.

Experimental. Arthus reactions were simultaneously induced in untreated control rabbits and in those treated with varying amounts of phenergan (25 to 100 mg/kg). In preliminary experiments, the Arthus reaction was induced in multiple sites in 9 albino rabbits by the local injection of antibody and of antigen as previously described.[1] It was found that 75 mg/kilo of phenergan in saline intramuscularly prevented the development of a severe Arthus reaction. The technic for producing the Arthus reaction was then modified by the administration of the antigen intraven-

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