TECHNICAL PAPERS

An Unidentified, Filtrable Agent Isolated From the Feces of Children With Paralysis

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Several of last summer's small epidemics of poliomyelitis in upstate New York have been studied for evidence of mouse-adaptable viruses. An agent has been isolated from the acute-phase fecal specimens of two children from one outbreak which induces paralysis in suckling mice and hamsters. An additional unusual feature is that paralysis is associated with destructive lesions of the skeletal muscles, the central nervous system being unaffected. A brief preliminary statement is being made at this time, since others may wish to search for the agent this summer.

Patient T.T., a 9-year-old boy, was first examined by a physician on August 23, 1947. His symptoms were of 24 hours' duration and consisted of nausea, violent headache, and pains in both legs. His temperature was 104.0° F. No nuchal rigidity was found, but both legs were weak. Weakness was more marked in the trunk muscles and less so in the back. The boy was admitted to the Catskill Hospital, where cerebrospinal fluid was drawn and examined. The fluid was slightly cloudy and colorless, and contained 250 red blood cells and 64 leucocytes (kind not stated, other than that all types were present); the sugar was 50 mg/100 ml, and the globulin slightly increased. Weakness of the trunk muscles persisted. Seven months later the patient was still unable to sit up from a recumbent position, although his other muscles recovered.

The second patient, K.H., also a boy, was 3½ years old. His illness began on August 16, the initial symptoms being sore throat and lethargy. Two days later the adductor muscles of his left thigh were found to be very weak, but there was no nuchal rigidity. He was also hospitalized. His cerebrospinal fluid contained 10 erythrocytes, 2 polymorphonuclear and 2 mononuclear leucocytes/cu ml, 59 mg of sugar/100 ml, and normal amounts of protein. Six weeks later his adductors were still weak, and his left foot inverted when walking; 8 months later recovery was complete.

Isolation of the agent. Twenty per cent fecal suspensions, prepared by ether treatment and centrifugation, were inoculated intracerebrally into albino mice of the laboratory strain. Suckling mice, 3–7 days of age, became paralyzed, while mice 10–12 gm in weight did not. The isolations were repeated several times. Paralysis has not been induced in suckling mice more than 12 days of age. Numerous attempts to adapt the agent to weaned mice have failed. Similarly, only suckling hamsters are susceptible. After the initial isolation, paralysis in mice usually appears in 3–5 days and occasionally as late as 8 days. Paralysis also follows intraperitoneal or intramuscular injection. Thus far, paralysis has not been obtained in rhesus monkeys.

Mouse-brain suspension filtered through a Mandler candle failed to initiate growth in casein hydrolysate semisolid agar containing sodium thioglycollate at 35° C but induced paralysis in suckling mice and hamsters.

Neutralization tests have been made in suckling mice using one family for each dose of test material. Equal amounts of serum and infected mouse-brain suspension were injected intraperitoneally (0.05 ml) after standing 1 hr at room temperature. Neutralization was obtained with two pools of human serum and with human concentrated globulin without preservative. The acute-phase serum of patient K.H. had no neutralizing activity for either strain, while the convalescent-phase serum taken 24 days later neutralized both. The active-phase serum of the older patient had neutralizing activity which had increased approximately 10-fold in the specimen collected 23 days later and returned to its original value in a specimen collected in the 9th month.

The lesions in mice and hamsters consist of a severe and widespread degeneration of the skeletal muscles. The muscle cells lose their striations and become strongly acidophilic and fragmented. Intense proliferation of young muscle cells occurs and, with endothelial cell phagocytosis, gives the lesions a very cellular appearance. Particularly in late deaths the lesions are evident grossly as opaque, whitish streaks. Muscles of the limbs, the spinal groups, intercostal, masseter, and scalp muscles are among those affected; smooth muscle and the myocardium have been spared. Lesions have not been found in the central nervous system or large peripheral nerves.

The failure of the agent to propagate except in suckling mice or hamsters differentiates it from the Theiler viruses, MM virus, the virus of encephalomyocarditis, and the Lansing-like mouse-adapted poliomyelitis strains. Serologically, it failed to be neutralized by adult normal mouse serum or normal rabbit serum. Pooled human serum, human concentrated globulin, and, to some extent, normal monkey serum had a neutralizing effect. No neutralization was obtained with FA mouse encephalomyelitis mouse or rabbit serum, Lansing poliomyelitis mouse serum, MM virus rabbit serum, lymphocytic choriomeningitis monkey serum, or Aycock poliomyelitis monkey serum.

Repeated recovery of the agent from the fecal specimens and the immunologic response of the patients suggest that the agent is capable of infecting man. That it induces paralysis in man is unproven. The patients we have studied may possibly have been coincidentally infected with the new agent and classical poliomyelitis virus, although isolations were not successful in the rhesus

monkey. It his hoped that others will search for the agent and that the muscles of fatal cases of paralytic disease in the young will be thoroughly examined histologically.

Human Saliva as a Germination Inhibitor

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Dold and Weigman demonstrated in 1935–36 that human saliva contains a factor bacteriostatic and bacteriocidal to pathogenic bacteria.

Since various authors have shown that many antibiotics act as germination inhibitors on plant seeds (see *2*), we wished to find out if this is true also for human saliva.

Saliva was obtained from a number of persons of normal health not less than 1–3 hrs after eating and was used immediately after collection.

TABLE 1

INHIBITION CAUSED BY UNDILUTED HUMAN SALIVA

Age	Cases	Germination index
54	1	0 (0, 0)
"	1	0 (0, 0)
"	1	0 (0, 50)
"	1	0 (0, 62)
53	2	12 (7, 80)
"	2	14 (6, 50)
"	2	0 (0, 0)
"	2	6 (8, 60)
"	2	58 (15, 40)
"	2	0 (0, 0)
33	20	0 (0, 0)
6	23	0 (0, 0)
22	7	5 (12, 33)
22	24	16 (21, 14)
15	8	50 (50, 87)
"	8	56 (37, 50)
48	18	52 (40, 85)
16	25	71 (50,100)
36	11	60 (48, 44)
28	3	66 (30,100)
"	3	76 (30, 52)
25	12	68 (50, 52)
20	6	65 (52,100)
"	6	83 (24, 73)
"	6	80 (65, 50)
"	6	82 (50, 70)
26	10	66 (26, 50)
45	13	80 (71, 80)
21	4	80 (29, 77)
44	5	84 (50,100)
20	14	72 (33, 70)
15	15	72 (30, 50)
68	16	64 (33, 60)

The test method was the same as that described by Konis (*3*). We used 50 wheat seeds/Petri dish and 7–8 ml of saliva. The countings were made 48 hrs after the beginning of each experiment.

The numbers given in our tables are germination indices, *i.e.* % of germinated seeds related to the germination percentage of the water controls = 100. The first number in parentheses gives the length of the radicle related to the radicle length of the water controls = 100; the second, the corresponding data for the coleoptiles.

TABLE 2

INHIBITION AND DILUTION

Case	Concentrations (%)	Germination index
1	100	0 (0, 0)
	50	0 (0, 8)
	25	62 (21,100)
2	100	12 (6, 89)
	50	94 (63,100)
	25	98 (63,100)
3	100	64 (33,100)
	50	70 (50,100)
	25	92 (70,100)
4	100	82 (29,100)
	50	90 (55,100)
	25	94 (77,100)
5	100	84 (51,100)
	50	96 (64,100)
6	100	84 (28, 73)
	50	88 (66,100)
	25	96 (77,115)
7	100	5 (13, 33)
	50	90 (19, 66)
	25	88 (59, 72)
8	100	48 (50, 88)
	50	40 (33,100)
	25	66 (76,100)
9	50	82 (35, 75)
10	100	66 (46, 57)
	50	82 (50, 51)
	25	98 (74, 96)
11	100	90 (50, 57)
	50	60 (53, 81)
	25	100 (70, 80)
12	100	68 (54, 54)
	50	92 (83, 61)
	25	96 (85, 70)
13	100	80 (71, 80)
	50	96 (84,100)
14	100	72 (33, 76)
	50	100 (63, 76)
15	100	72 (30, 50)
16	100	64 (30, 60)
17	100	82 (30, 60)
	50	88 (58, 80)
	25	84 (80, 80)
18	100	46 (39, 85)
19	100	90 (60, 71)
	75	88 (75, 93)
20	100	0 (0, 33)
21	70	22 (16, 0)
22	20	76 (180,155)
23	75	0 (0, 0)

The conclusions drawn from the results given in Table 1 are:

(1) In all cases the saliva exerted a germination-inhibiting influence.

(2) Wherever there was a germination of the saliva-