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Antiviral Action of Helenine on Experimental Poliomyelitis.* (22437)

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Recently crude preparations from 2 species of penicillium, *P. stoloniferum* and *P. funiculosum* have been reported to be effective against certain small neurotropic viruses such as Semliki Forest, MM, and Columbia SK (1-3). The crude filtrate of *P. stoloniferum*, known at M5-8450(4), was also found to be effective against poliomyelitis virus inoculated by peripheral routes in mice(5) and monkeys (6). In view of the suspected similarity between M5-8450 and Helenine, the antiviral agent from *P. funiculosum*(4,7), it was of interest to investigate the effect of Helenine on experimental poliomyelitis in mice and monkeys.

Materials and methods. Young male and female cynomolgus monkeys, weighing between 1.1 and 2.8 kg, and young Swiss albino mice, weighing 12 g or less, were used in these studies. Mice were inoculated intraperitoneally with approximately 10 ID_{50} of the MEF_1 strain of type II poliomyelitis virus adapted to suckling hamsters by Powell and Culbertson(5), while monkeys were inoculated subcutaneously with an estimated 100 ID_{50} of the Mahoney strain of type I poliomyelitis virus. All animals were observed daily for the onset of paralysis throughout an observation period of at least 21 days for mice, and at least 30 days for monkeys. Helenine† was obtained as a 10-fold concentrate of the acetone-precipitated material. For purposes of comparison, one experiment included a group of monkeys treated with M5-8450.† Both materials were kept frozen at −70°C until the day of use.

Results. In preliminary tests the maximum daily dose of Helenine tolerated by mice was found to be 0.5 ml of 2-fold concentrate (8). No toxic effects other than a transitory anorexia were observed in monkeys receiving up to 5 ml of 10-fold concentrated Helenine per kg twice daily for 4 days. More extensive toxicity studies were not conducted because of the difficulty of determining whether observed effects were due to the active principle or to impurities in the available preparation.

Data showing the effect of Helenine-treatment in mice infected with poliomyelitis virus are given in Table I. Mice were given Helenine daily for 4 days beginning the day before virus inoculation. The first, third and fourth doses were injected intraperitoneally while the second dose, given the day of virus inoculation, was injected subcutaneously. In only one experiment with mice did Helenine appear to reduce the incidence of paralysis, but this difference is not significant at the 5% level when the data are analyzed accord-

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TABLE I. Effect of Helenine on Poliomyelitis in Mice.*

Treatment†	Treated		Control	
	Morbidity	Incubation period	Morbidity	Incubation period
Helenine (2×) .5 ml/day, ×2	11/17	8.5	16/19	5.9
" " .5 " ×4	15/20	7.6	16/19	5.9
" (5×) .1 " ×4	18/19	5.0	17/20	3.1

* Inoculated intraperitoneally with approximately 10 ID_{50}'s of MEF_1 poliomyelitis virus.
† Given intraperitoneally beginning day before virus inoculation except on day of virus inoculation when Helenine was given subcutaneously.

ing to the procedure of Mainland and Murray(9). In all cases, however, there appeared to be some prolongation of the incubation period. Using the harmonic mean survival time of the treated and control groups one may derive a survival index similar to that described by Shope(2). When mice were treated with 0.5 ml of 2x Helenine for 2 days the survival index was 1.44, and the indices calculated for the other experiments with mice were 1.29 and 1.47 respectively.

In monkeys, Helenine was injected intraperitoneally as the 10-fold concentrated acetone-precipitate; 2 doses of 5 ml per kg each were given the day before virus, the third dose 4 hours before and the fourth dose 4 hours after virus inoculation, a schedule similar to that previously employed with M5-8450(6). In the present study M5-8450 was re-constituted with sterile distilled water to one-half its original volume and administered on the same schedule. Results of these tests in monkeys are given in Table II. From these data it is seen that treatment with Helenine was effective in protecting monkeys against infection with poliomyelitis. When the data from the monkey experiments are combined it may be seen that the incidence of paralysis was reduced from 100% (11/11) in the control group to 18% (2/11) in the Helenine-treated group. This difference in morbidity between the treated and control groups is significant at a level of less than .01 using the test previously cited(9). Treatment with Helenine, in addition to reducing the incidence of paralysis, also produced a delay in the onset of the disease. The average time to the onset of paralysis in those Helenine-treated monkeys that eventually developed paralysis was 23.5 days, and in the M5-8450-treated monkeys 30.5 days, while the average time of onset of paralysis in the control monkeys was 8.8 days. Survival indices calculated as described above were 18.5 and 12.2 for the Helenine-treated groups and 5.6 for the group receiving M5-8450.

In view of the marked effectiveness of Helenine as a prophylactic agent, an attempt was made to detect therapeutic activity. Ten monkeys were infected subcutaneously with the Mahoney strain of poliomyelitis virus. Rectal temperatures were recorded daily and when an animal exhibited a temperature of at least 104°F, or showed evidence of paralysis, treatment was initiated. Alternate animals in the order of onset received Helenine, 5 ml of 10x-concentrate per kg twice daily for not more than 4 days, or equal volumes of buffered saline intraperitoneally. The group of 5 monkeys which was treated with Helenine

TABLE II. Effect of Helenine and M5-8450 in Prevention of Poliomyelitis in Cynomolgus Monkeys.*

Treatment	Treated		Control	
	Day of onset	Morbidity	Day of onset	Morbidity
Helenine (10 ×) 5 ml/kg, IP × 4, twice day before, twice day of virus inoc.	27, S, S, S, S, S	1/6	7, 8, 9, 9, 10, 13	6/6
Helenine (10 ×); same	20, S, S, S, S	1/5	7, 7, 8, 10, 10	5/5
M5-8450 (2 ×), 12.5 ml; same	15, 46, S, S, S	2/5	"	"

* Inoculated subcut. with estimated 100 ID_{50} of Mahoney strain of Type I poliomyelitis virus.

and the 5 control animals each had an average survival time of 2.6 days after the detectable onset of disease. These data indicate that impure preparations of Helenine such as were available had marked prophylactic activity against poliomyelitis in monkeys but no apparent therapeutic activity.

Discussion. Helenine, like M5-8450(6), was found to be an effective prophylactic against poliomyelitis in monkeys. In contrast to its effectiveness in monkeys, Helenine did not alter significantly the incidence of paralysis in mice, although it did appear to prolong the incubation period. A species difference in the response of the host to this material seems unlikely in view of the effectiveness of Helenine against other neurotropic viruses in mice(2,3). One can speculate that the observed effects may have represented type specific differences in susceptibility between types I and II poliomyelitis viruses. Type differences have been encountered in susceptibility to inactivation with merthiolate in the 1954 poliomyelitis vaccine(10) and have been reported to occur *in vitro* with certain chemical agents(11). On the other hand, differences in effectiveness also could have been due to differences in reactivity of the whole host-virus complex; Mahoney virus in the monkey versus MEF_1 poliomyelitis virus in the mouse. A direct comparison of Helenine and M5-8450 is not possible with these data owing to the differences in dosage employed. The data presented do not justify any conclusion as to the relative effectiveness of the two materials, but seem to be a further indication of their apparent similarity(4,7). Additional studies of the antiviral action of such penicillium filtrates are planned to obtain information regarding their activity and mechanism of action.

Summary. Helenine, an antiviral agent derived from *P. funiculosum,* was found to have marked prophylactic activity in cynomolgus monkeys infected with poliomyelitis virus.

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Protection by Sodium Hydrosulfite Against X-Ray-Induced Mitotic Inhibition in Grasshopper Neuroblast.* (22438)

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It is well known that a reduced oxygen tension during irradiation causes a corresponding reduction in sensitivity to ionizing radiations. The literature on this phenomenon at the cellular level has been reviewed by Giles(1) (chromosome breakage), Muller(2) (mutations), and Gray(3). Recovery from X-ray-induced mitotic inhibition in grasshopper neuroblasts as affected by oxygen tension during irradiation of the intact egg was observed by Gaulden, Nix, and Moshman(4). They found that at 64 r the delay of mitosis was detectably decreased when embryos *in vivo* were irradiated *in vacuo*. At lower doses (3.5 and 8 r) the recovery of mitotic activity appeared to be independent of the oxygen tension

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