Antiviral Action of a Mold Filtrate on Experimental Poliomyelitis in Cynomolgus Monkeys.* (20799)

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Recent investigations have indicated that the crude filtrate of a penicillium mold, referred to as M-8450, has antiviral action against certain neurotropic viruses such as MM, Semliki Forest, and Type 2 poliomyelitis in mice(1,2) and against all 3 types of poliomyelitis in tissue culture(3). No activity was seen in mice against Lansing strain of poliomyelitis virus given intracerebrally, fixed rabies virus, or against a variety of non-neurotropic viruses(1). In view of the effectiveness of M-8450 against poliomyelitis in mice inoculated intraperitoneally and in tissue culture it was of interest to determine the effectiveness of this substance in monkeys. This report describes the effect of M-8450 in cynomolgus monkeys infected subcutaneously with Type 1 poliomyelitis virus.

Materials and methods. Young male and female cynomolgus monkeys, weighing between 1.2 and 2.5 kg, were used in this study. They were infected with approximately 10 PD_{50}'s of the Mahoney strain of Type 1 poliomyelitis virus and observed daily for the onset of paralysis for periods of at least 30 days. In previous tests(1,2) of the antiviral activity of M-8450 it was demonstrated that the mold filtrate was most effective in suppressing the experimental disease when both that virus and the filtrate were injected peripherally. Therefore, in tests with monkeys the Mahoney strain was employed because it is capable of inducing paralysis following subcutaneous inoculation. The M-8450† was obtained as the frozen-dried crude broth and stored at −70°C. It was rehydrated to original volume with sterile distilled water and administered intraperitoneally. In 3 of the 4 experiments, treated monkeys were given 100 ml of the rehydrated crude filtrate in 4 divided doses, 2 doses being given on the day before virus, the third 4 hours before, and the fourth 4 hours after virus inoculation. No signs of toxicity were observed in any of the mold-filtrate treated monkeys. In view of the large volumes of fluid involved, virus control monkeys in the first and third experiments were injected intraperitoneally with 100 ml of sterile salt solution according to the same schedule. In addition, since the crude mold filtrate contained 1:10000 merthiolate as a preservative and since LoGrippo *et al.*(4) have shown that certain mercury compounds affect the excretion of a mouse encephalomyelitis virus, controls in one experiment were treated with 1:10000 merthiolate in sterile saline. In both *in vivo*(2) and *in vitro*(3) tests M-8450 appeared to exert maximum antiviral effect when given before virus. Therefore in the fourth experiment the total dose of M-8450 was reduced to 75 ml, divided into 3 equal quantities which were administered either before or after virus inoculation. One group of treated monkeys received 2 doses the day before and the third dose 4 hours before virus inoculation, while the other group received the first dose 4 hours after virus inoculation and the remaining 2 doses the following day.

Results. Results of all the tests are given in Table I. These data indicate that M-8450 exerts a distinct antiviral action in monkeys infected with Type 1 poliomyelitis. In the first 2 experiments only 2 out of 14 treated monkeys developed poliomyelitis while 8 of 15 control monkeys became paralyzed. In addition, the incubation period in the treated animals was longer. In the third test half of the treated monkeys received an additional dose of M-8450 14 days after virus inoculation to determine if the paralysis seen in the first experiment could be prevented. This treatment did not appear to have any bene-

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TABLE I. Effect of M-8450 on Poliomyelitis in Cynomolgus Monkeys Infected Subcutaneously with 10 PD_{50}'s of Poliomyelitis Virus.

Exp.	Treatment	Day of onset of paralysis: Treated	Day of onset of paralysis: Control
1	25 ml, ×3 before, ×1 after virus	24, 30, S, S, S, S, S, S, –*	11, 13, 18, 21, 32, S, S, S, S, S†
2	Same as 1	S, S, S, S, S	10, 20, 24, S, S
3	" " "	42, S, S, S, S	7, 10, 13, 13, S‡
	" " " + 25 ml ×2, 14 days after virus	28, S, S, S, S	12, 29, S, S, S†
4	25 ml, ×3 before virus	22, 22, 23, S, S	8, 8, 11, 14, S
	25 " " after "	12, 15, S, S, S	

* Died 6th day due to bacterial infection. † Controls treated with saline. ‡ Controls treated with merthiolate.

ficial effect. That merthiolate *per se* does not contribute to the antiviral action of the M-8450 is shown by the fact that 4 of 5 merthiolate-treated controls developed paralysis as compared to 2 of the 5 saline controls. Administration of a smaller amount of M-8450, *i.e.* 75 ml either before or after virus inoculation, appeared to be less effective as 3 of 5 and 2 of 5 of the monkeys in the treated groups developed poliomyelitis compared to 4 of 5 in the control group. Although the differences in morbidity between the pre-inoculation and post-inoculation treatment groups are not marked, the somewhat longer interval to the onset of paralysis in the group of monkeys receiving M-8450 before virus inoculation suggests that pre-treatment might prove to be the more effective. Combining the results of all experiments in which monkeys received 100 ml or more of M-8450, only 4 of 24 treated monkeys developed poliomyelitis, the day of onset of paralysis ranging from 24 to 42 days, while 14 of 25 virus control monkeys became paralyzed in 7 to 32 days.

Discussion. These studies show that the crude filtrate of a penicillium mold has an antiviral action in monkeys infected subcutaneously with Type 1 poliomyelitis. Previously other investigators had reported its action against Type 2 poliomyelitis in mice(2) and against the 3 types of poliomyelitis in tissue culture(3). Although the relatively large volume of the crude filtrate that was employed contained 1:10000 merthiolate, this did not appear to be responsible for any of the antiviral activity observed with M-8450 and merthiolate treatment alone had no antiviral effect.

Pre-treatment with M-8450 conferred marked protection against poliomyelitis both by reducing the incidence of paralysis in monkeys and by increasing the incubation period in those animals finally developing paralysis. The probability of chance occurrence of this difference in morbidity is less than 5 times in 1000.‡ The prolongation of incubation period is even more marked. Statistical evaluation of this incubation period is complicated by the presence of survivors but may be accomplished by employing the reciprocal of the incubation period and using O as the reciprocal incubation period for a survivor, as suggested by Gaddum(5). Using this procedure the mean reciprocal incubation period of the treated group was 0.0056 day^{-1} and of the control group 0.0403 day^{-1}. Such a difference in mean reciprocal incubation periods would be expected to occur as a chance phenomenon less than one time in 10,000 similar replications, and statistically the incubation period of the treated group was concluded to be significantly greater than that of the control group.

Additional experiments would be required to demonstrate that treatment with 75 ml of M-8450 is much less effective than 100 ml although these experiments suggest that trend. While the numbers of monkeys developing poliomyelitis after treatment with the lesser amount are not significantly different statistically the results tend to be poorer. The

‡ The authors are indebted to Dr. F. M. Hemphill, Department of Public Health Statistics, School of Public Health, University of Michigan, for guidance in the statistical evaluation of these data.

relative prophylactic and therapeutic effectiveness of M-8450 in monkeys also requires additional investigation although it appears that its antiviral activity is greater when administered before virus, in agreement with the findings in mice(2) and in tissue culture(3). In its present state of development M-8450 does not appear to have direct therapeutic application. However, its effectiveness in both preventing infection with and prolonging the incubation period of poliomyelitis in monkeys seems to justify further investigation of its properties and mechanism of action.

Summary. The crude filtrate of a penicillium mold, M-8450, was shown to have antiviral action in cynomolgus monkeys inoculated subcutaneously with the Mahoney strain of Type 1 poliomyelitis. Treatment with at least 100 ml of this crude filtrate reduced the morbidity and increased the incubation period of infected animals. Less intensive treatment appeared to be less effective. The antiviral action of this substance appears to merit further study.

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Presence of 17-Ketosteroids in Adrenal Perfusates.* (20800)

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Studies on the nature and effects of adrenocortical steroids have indicated that the adrenal cortex synthesizes C_{19} as well as C_{21} steroids. Reichstein and coworkers isolated Δ^4-androstene-3,17-dione(1), Δ^4-androstene-3,11,17-trione (adrenosterone)(2), and androstane-3β,11β-diol-17-one(3) from adrenal extracts. Cow adrenal venous blood was shown by Gassner *et al.* to contain an androgenic steroid characterized as an α,β unsaturated, hydroxylated, 17-ketosteroid(4). Bush reported the possible presence of Δ^4-androstene-11β-ol-3,17-dione in the adrenal venous blood of rats, sheep and cats(5), but more recently considers his compound to be 17-hydroxyprogesterone(6). In the systematic investigation of steroidal compounds obtained during adrenal perfusions Hechter and his group found several Zimmermann reactive zones on chromatograms run on the perfusates(7). Recently Δ^4-androstene-11β-ol-3,17-dione has been identified in human adrenal venous blood(8) and urine(9) after ACTH administration. The data presented here represents a preliminary report on the presence of Δ^4-androstene-3,17-dione, Δ^4-androstene-3,11,17-trione, and Δ^4-androstene-11β-ol-3,17-dione in blood perfused through beef adrenals.

Experimental. Six liters of citrated beef blood, to which 120,000 units penicillin and 0.6 g streptomycin had been added, were perfused through 6 beef adrenals for 5 cycles as described by Hechter and coworkers(10). After the first cycle, 25 mg ACTH (Armour) was dissolved in propylene glycol-saline and infused into blood over 3 hours. Acetic acid-1-C^{14} was added to the blood prior to perfusion in one experiment (perfusion no. 5). A total of 5 perfusions was conducted. The perfusates were extracted with 3 portions of twice the volume of redistilled ethyl acetate by gently shaking the blood and solvent together(11). The extracts were evaporated to dryness under

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