Inhibition of Cytopathogenic Effect of Poliomyelitis Viruses in Tissue Culture by Antibiotic M-8450. (20491)

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The chemoprophylactic effect of a mold filtrate designated M-8450 against MM and Semliki Forest viruses in white mice has been described by Powell *et al.*(1). Parenteral administration of M-8450, 24 hours prior to infection protected the mice against approximately 100 LD_{50} of MM virus. Shope(2) has reported similar data obtained from mice infected with Columbia SK encephalomyelitis virus treated with a mold preparation indentified as helenine. Powell and Culbertson(3) reported the inhibition in mice of a type II poliomyelitis virus, MEF1, by antibiotic M-8450.

Since types I and III poliomyelitis viruses do not readily infect mice or other small laboratory animals, an evaluation of M-8450 against these types had to made in other ways. Tissue culture appeared to be one possibility. Demonstration of protection in monkeys, however, would be most desirable. The following is a report of the methods and results of preliminary studies of the inhibitory action of this mold filtrate on examples of the 3 immunological types of poliomyelitis virus grown in tissue culture.

Materials. Tissue cultures. Plasma clot roller tube cultures of the type described previously(4) were employed with testicular tissue from immature cynomolgus or rhesus monkeys. Approximately 6 explants/tube were used and were fed a medium of 30% horse serum (HS), 20% chick embryo extract (EE_{50}), and 50% Earle's salt solution (BSS). 100 units each of penicillin and streptomycin per ml were included in this medium. The cultures were incubated at 37.5°C for 7 to 14 days until sufficient outgrowth had occurred to make them satisfactory for virus growth. At the time of virus inoculation the medium was altered to one of 30% HS, 30% serum ultrafiltrate (OX) (SUF), 5% EE_{50}, or an ultrafiltrate of EE_{50}, and 35% BSS.

Viruses. Two Type 1 viruses labeled Duffy and 787, and a type 3, Leon, were very kindly supplied to us by Dr. John F. Enders, The Children's Hospital, Boston. A cytopathogenic Type 2 strain, YSK, was obtained through the courtesy of Dr. Jerome T. Syverton, University of Minnesota. These viruses have been maintained in our laboratory through tissue culture passages and occasionally retyped with specific antisera to check their immunological type identity. The inocula in all experiments referred to in this paper have been 0.1 ml of the undiluted tissue culture fluid of a previous virus passage. Periodic titrations of these fluids have shown the virus titers to be of the order of 10^{-5} to 10^{-6} ID_{50}'s/0.1 ml of the fluid.

Antibiotic M-8450. This material was in the form of a crude broth which had been harvested aseptically and to which no preservative had been added. No toxicity of the antibiotic was evident at the levels studied. The activity of each lot employed was determined by assay in mice against MM virus.

Methods. The procedure generally followed was as follows: the old culture medium is removed, the M-8450 is applied directly to the cells for a 10 minute period, 1.0 ml of new medium (on occasion the old medium was returned) is added, the cultures are incubated for 24 hours, the medium is again withdrawn, virus inoculation is made directly onto the cells, and the medium is replaced. The M-8450 was also added just 10 minutes before virus and simultaneously with it in some experiments. The dose of M-8450 in these experiments was 0.1 ml of a 1:10 dilution. Another technic of treatment also employed consisted of removing the old medium, saturating the cells with 1.0 ml of the M-8450, incubating for 4 hours on the roller tube apparatus, removing the M-8450, inoculating virus and adding new medium.

Results. In these preliminary tissue culture studies of the antiviral activity of antibiotic M-8450, it has been demonstrated that this

TABLE I. Effect of M-8450 on Virus Growth.

Culture No.	Virus type	Treatment	Results, days after virus inoculation 4th	5th*†	6th	7th	8th*	11th	12th	Results of 5th day subcultures, days
T-42	I	Virus controls	++++	‡						++++ 3
T-43	787		++++	‡						
T-46		24 hr pre-treated	0	0	0	0	0	0	0	
T-47		with 8450	0	0 lost	—	—	—	—	—	++++ 6
T-49			0	0	0	0	0	0	0	
T-16	II	Virus controls	+	+++	+++					++++ 5
T-17	YSK		+	+++	+++					
T-26		24 hr pre-treated	0	0	0	0	0	0	0	
T-29		with 8450	0	0	0	0	0	0	0	0 14
T-33			0	0	0	0	0	0	0	
T-34	III	Virus controls	++++	‡						++++ 4
T-36	Leon		+++	‡						
T-37		24 hr pre-treated	0	0	0	0	0	0	0	
T-40		with 8450	0	0	0	0	0	0	0	++++ 7
T-41			0	0	0	0	0	0	0	

* Culture medium changed, 10-fold dilution made of original drug and virus.
† All 6 groups subcultured.
‡ Discarded.
++++ = complete cell damage; 0 = no damage.

mold filtrate will protect monkey testicular cells growing in tissue culture from the destructive action of all of the samples of the 3 immunological types of poliomyelitis viruses employed. Like the previous reports by Powell *et al.*, the M-8450 again was found to be most active when the cells were treated with it before the introduction of virus. Incubation of mixtures of M-8450 and virus for 24 hours prior to inoculating cultures caused no inhibition of viral activity in the culture. One-tenth ml of a 1:10 dilution of M-8450 placed on the cells, as described above, 24 hours before inoculation with approximately 100,000 ID_{50} (titered) of a Type 1 virus (Duffy) almost completely protected the cells against virus damage. The untreated controls were completely destroyed after 3 days incubation. When the M-8450 was administered just 10 minutes before virus inoculation, or simultaneously with it, no protection was afforded. Similar observations were made employing Type 2 (YSK), and Type 3 (Leon) viruses, with the exception that complete protection was obtained against these viruses following 24 hour pretreatment. Repetitions of the experiment with Type 1 virus have also demonstrated complete protection of the cells against this type. Pretreatment of cells with the uninoculated mold culture broth produces no such antiviral effect.

The second method of study mentioned above involving saturation of the cells with 1.0 ml of the M-8450 was also found to afford protection against a Type 1 virus (787) in some experiments. This treatment rendered the cells completely refractory to the virus while untreated controls were completely destroyed in 3 days. A similar experiment done at another time employing the Type 3 (Leon) virus failed to demonstrate protection of the cells. This finding with the Leon virus has not been reexamined nor explained at this time, but further testing is planned.

In more recent studies the protective effect of the antibiotic has been demonstrated upon addition of 0.1 ml of a 1:10 dilution to the tissue culture medium 24 hours before virus inoculation. Thus the 10 minute period during which the cells were directly exposed to the M-8450 alone before culture medium was added has been eliminated. Further study of the value of this 10 minute period of direct treatment to the cells has revealed that removing the M-8450 and rinsing the cells with BSS before addition of medium, and 24 hours incubation prior to virus inoculation, does not leave the cells protected against virus

damage. The data reported in this paper, however, were obtained from studies in which the 10 minute period of direct treatment had been employed without subsequent removal of the M-8450 or rinsing of the cells.

Table I illustrates the data obtained from a typical experiment employing all 3 types of virus and the 24 hour pretreatment schedule of M-8450. As can be seen, the 787 and Leon virus controls were destroyed in 4 days, while the M-8450 treated cultures inoculated with these viruses revealed no evidence of virus destruction during the 12 day observation period. Destruction of cells by YSK virus was slower than desired, indicating less virus to be present than in the 787 and Leon controls. The M-8450 treated cultures challenged with the YSK virus again failed to exhibit virus cytopathology throughout the course of the experiment. The subcultures from each of the virus controls were destroyed in the usual time proving the presence of virus in these damaged cultures. The subcultures from the treated cultures in the 787 and Leon series also were destroyed, but the time required was almost twice as long as with their respective controls. The virus demonstrated to be present in the treated and undamaged cultures probably represented a part of the inoculum, as similar observations have been noted when attempts were made to culture poliomyelitis viruses in cell types not suitable for their growth, *e.g.*, chick embryo cells. The failure to recover the YSK virus from the treated cultures in this experiment may have been due to a smaller content of virus in the original inoculum. As mentioned in the section on materials, our virus stocks have frequently been rechecked for their immunological type specificity. Such examinations made with specific antisera before and after these studies have indicated that each strain of virus has maintained its type specificity as labeled in Table I. The observation that YSK infected mice produced typical symptoms provided additional evidence of the identity of this virus, whereas 787, Duffy and Leon viruses failed to infect the mice. The Duffy has produced paralysis in monkeys within 4 days. Neither the 787 nor Leon viruses have been studied in monkeys.

Discussion. The results obtained in these studies show quite clearly that exposure of monkey testicular cells in tissue culture to relatively small amounts of antibiotic M-8450, 24 hours before inoculation with any of the samples of the 3 immunological types of poliomyelitis viruses causes the cells to become refractory to virus infection. This finding agrees very well with the previous observations of *in vivo* tests in mice against various neurotropic viruses. That the antibiotic exerts its effect on the cells, rather than the virus, is indicated both by the fact that pretreatment of the cells is necessary to demonstrate the protection whereas pretreatment of the virus has no such effect, and by the observation that viable virus can be demonstrated in the undamaged, treated cultures as long as 5 days after inoculation. In the mouse, efforts to recover virus (MM) from treated animals have failed, but it is likely that the intact animal is capable of eliminating the non-infecting virus by several routes. In the culture these mechanisms for elimination are not present, and since poliomyelitis viruses are stable, the inoculum will survive even though no reproduction occurs. Thus the M-8450 appears to have no neutralizing effect on the virus itself under the conditions studied in tissue cultures.

The explanation of the mechanism of the action of antibiotic M-8450 is an intriguing question and one which is under consideration. Evidence now available points to the probability that the mode of action may be one of interrupting or preventing some stage of intracellular multiplication of virus. An effect on virus attachment to the cell is not precluded; however, the need for a period of pretreatment decreases the attractiveness of such an explanation. Study of the mechanism of action may lead to an insight into the processes occurring during virus reproduction. Such information might in the long run be of more value to the whole problem of poliomyelitis infection than any immediate significance of antibiotic M-8450 as a possible treatment for this disease.

Summary. Pretreatment of tissue cultured monkey testicular cells with antibiotic M-8450 inhibits the cytopathogenic effect of all 3 immunological types of poliomyelitis virus

tested, but does not destroy or neutralize the viruses.

1. Powell, H. M., *et al., Antibiotic and Chemother.*, 1952, v2, 432.
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Effect of Adrenocorticotropic Hormone on Pneumococcal Infections in Rabbits. (20492)

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Many investigators have reported on the effects of large doses of cortisone and Compound F on the resistance of various animal species to a variety of infections(1-20). Shwartzman(21) has shown that adrenocorticotropic hormone (ACTH) and cortisone in combination, or cortisone alone, produced a marked acceleration of poliomyelitis infection in mice and an enhancement in the susceptibility of hamsters to this infection. He has reported, however, that ACTH alone failed to produce this effect and hence has postulated that the adrenals may elaborate an unknown factor capable of reversing the enhancing effects of cortisone on infection. It seemed important, in view of the possible widespread significance of these observations, to determine if Shwartzman's findings applied to other infections and to other species. The present report is concerned with the effect of ACTH on an experimental pneumococcal infection in rabbits.

Methods. Male albino rabbits, weighing between 2.0 and 2.5 kg each, were infected intradermally with a Type I pneumococcus by injecting into the shaved abdominal skin 0.1 cc of a 2 x 10^{-3} dilution of a 6-hour culture grown in nutrient broth containing 5% defibrinated rabbit blood. The virulence of the culture was such that 0.5 cc of a 10^{-9} dilution (1-3 organisms) was lethal for a mouse. The size of the skin lesion resulting from the injection of the pneumococci was measured by tracing the periphery of the lesion on transparent paper and the area of the skin lesion was subsequently obtained by retracing the paper outline with a planimeter. The blood culture studies were performed by plating 0.1 cc of blood obtained from the marginal ear vein in 5% blood agar. Serial 10-fold dilutions of the blood were plated in order to assure accuracy in determining the number of bacterial cells. The plates were then incubated for 24 hours at 37°C.

A sample of crude ACTH, equivalent to Armour Standard La-1-A, was given in doses of 0.25 mg to 2.0 mg every hour night and day. Treatment was initiated 18 hours before the bacterial injection and then continued for 96 hours or until death of the animal. Similar studies were repeated with a highly purified ACTH containing 200-300 units per mg (22) with essentially the same results.

Results. Survival studies. The data presented in Table I indicate that 76% of the control animals survived the infection. Animals treated with ACTH, in contrast, began to die within 24-48 hours after the bacterial injection, and only 17% of the animals receiving ACTH survived the 10-day observation period. These data indicate that ACTH is capable of decreasing the survival of rabbits

TABLE I. Survival of Animals Treated with ACTH and Infected Intradermally with *D. pneumoniae.*

	Dose ACTH in mg/hr				
	Saline	.25	.5	1.0	2.0
No. of animals	17	6	6	17	6
% survival	76	17	17	18	17