Action of an Antiviral Mold Filtrate against MEF1 Poliomyelitis Virus in Mice. (20296)

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We have reported on a penicillium mold filtrate designated serially as M5-8450 (hereinafter called 8450) which has considerable chemoprophylactic action coupled with very slight chemotherapeutic action against MM and Semliki Forest viruses in white mice(1). Protective action against 100 LD_{50} or more of either virus as injected subcutaneously may be obtained by injecting mice intraperitoneally with a single dose of 0.05 ml up to 0.25 ml of 8450 provided filtrate antedates virus by about 24 hours. Other combinations of routes and time of injection of virus and filtrate may be used with varying degrees of effectiveness with the exception that we have obtained negative results whenever (A) 8450 is given orally, or (B) virus is given intracerebrally. We have not observed any refractoriness in mice injected with a series of doses of 8450, also have observed no substantial antiviral immunity in mice once protected from virus by suitable treatment with 8450, nor have we found any indication that this agent neutralizes virus *in vitro*.

We wish to present here some further results of the activity of mold filtrate 8450 in mice infected peripherally with type 2 poliomyelitis virus MEF1.

Materials and methods. The MEF1 strain of poliomyelitis virus was obtained as mouse brain and cord from Dr. William McD. Hammon of the University of Pittsburgh. Typing serum comprising hyperimmune monkey antiserum in the frozen state was obtained through the kindness of Dr. Howard A. Howe of the Johns Hopkins University. Young cotton rats of 40 to 50 g weight and subsequently 7-day-old hamsters were used to passage serially a special line of the MEF1 virus preliminary to its use in chemotherapy tests in mice. The mice utilized were albino Swiss of about 12 g in weight each. 8450 mold filtrate has been referred to(1).

Procedure and results. The MEF1 virus was kept in stock as Swiss mouse brain material removed at autopsy from mice showing various degrees of paralysis following intracerebral injection of seed virus. This stock mouse brain virus was passaged 6 times intracerebrally through 40 to 50 g cotton rats. Sixth cotton rat passage virus in addition to producing paralysis and CNS lesions was neutralized by type 2 antiserum (lot E-397) referred to above as follows. Three decimal dilutions of virus, as judged from previous virulence tests in mice to contain 1, 10, and 100 LD_{50} of virus in ½ the usual intracerebral dose, were mixed with antiserum diluted 1:5 and along with controls let stand 2 hours at 4°C and 2 hours at room temperature, then injected intracerebrally in doses of 0.03 cc into groups of 8 Swiss mice of 15-17 g weight. The results as shown in Table I indicate satisfactory neutralization of MEF1 stock virus. Sixth cotton rat passage of MEF1 virus was passaged intracerebrally in series through 7-day-old hamsters. Hamster passage 21 was neutralized to approximately the same extent by a portion of the same antiserum lot referred to above and the details similar to those just indicated need not be included here. Intermediate hamster passages 12, 15, 17, and 20 in addition to 21 will be utilized as indicated below.

TABLE I. Neutralization Test of MEF1 Virus in Mice with Type 2 Hyperimmune Monkey Serum.

No. LD_{50} inoculated	With antiserum	Controls
100	1/8	8/8
10	0/7*	7/7*
1	0/7†	4/8
.1	—	0/8

* 1 death due to intercurrent disease.
† 1 " " " trauma.
Numerator of fraction = No. dead; denominator = No. used.

Before hamster passage, MEF1 virus either as mouse brain or cotton rat brain material, although virulent peripherally for suckling mice, was practically innocuous peripherally for 12 g Swiss mice. During hamster passage,

TABLE II. Effect of Mold Filtrate 8450 in Mice Infected with Hamster Passaged Poliomyelitis Virus MEF1.

.2 ml MEF1 virus	.25 ml × 4 of 8450	Virus dilution	Day of death or survival (S) for 30 days — Treated mice	Control mice
Hamster passage 12 i.p.	i.p.*	10^{-1}	5, (7), 11, S, S, S, S, S, S, S	3, 3, 5, 6, 6, 8, 14, S, S, S
Hamster passage 15 i.p.	"	"	3, 20, S, S, S, S, S, S, S, S	3, 3, 4, 4, 5, 5, 5, 6, 6, 9
Hamster passage 17 i.p.	oral	"	4, 4, 4, 4, 4, 6, 7, 7, S, S	4, 4, 4, 4, 4, 5, 6, 6, 6, 6
		10^{-2}	8, 15, S, S, S, S, S, S, S, S	6, 7, 7, 8, 13, S, S, S, S, S
	subq.	10^{-1}	3, 3, 4, 6, 6, 6, 6, 6, 14, S	
		10^{-2}	4, 6, 7, 9, 13, S, S, S, S, S	
Pool of hamster passages 17, 20, & 21 i.p.	i.v.	10^{-1}	6, 6, 9, 15, 20, S, S, S, S, S	3, 3, 3, 3, 3, 6, 6, 6, 8, S
		10^{-2}	S, S, S, S, S, S, S, S, S, S	8, 9, 13, S, S, S, S, S, S, S
	i.m.	10^{-1}	3, 3, 6, 6, 6, 6, 6, 6, 8, 17	
		10^{-2}	6, S, S, S, S, S, S, S, S, S	
	i.p.*	10^{-1}	6, 10, 17, S, S, S, S, S, S, S	
		10^{-2}	S, S, S, S, S, S, S, S, S, S	
As above i.m.	"	10^{-1}	6, S, S, S, S, S, S, S	6, 6, 6, 10, 10, S, S, S
		10^{-2}	S, S, S, S, S, S, S, S	S, S, S, S, S, S, S, S
As above i.v.	"	10^{-1}	3, 3, 3, 3, 3, 6, 6, 7, S	3, 3, 3, 6, 6, 6, 6, 6, 7, 9
		10^{-2}	3, 6, 22, S, S, S, S, S, S, S	3, 3, 3, 6, 6, 7, 11, 16, S, S

Treatment with 8450 was 1 dose/day for 4 days beginning 1 day before virus inj. In experiments in which both 8450 and virus were given intrap. and marked *, the dose of 8450 was omitted on the day virus was inj. (7) indicates mouse dead of intercurrent infection in 7 days. In 1 exp. we used 8 mice/group and in others 10. Nearly all mice that died were observed to have paralysis in one or more extremities for some time before death.

a considerable degree of peripheral virulence developed for 12 g mice, a fact noted heretofore by others. This appeared to offer a test method superior to tests in suckling mice whereby filtrate 8450 could be tested against peripherally injected poliomyelitis virus before resorting to tests in monkeys.

In Table II are shown the results of several chemoprophylactic tests in 12 g mice of 8450 given in a series of 4 doses, the first dose of which was given the day before MEF1 poliomyelitis virus was injected. (This particular lot of 8450 when injected as a single intraperitoneal dose of 0.25 ml the day before infection gave full chemoprophylaxis against 100 LD_{50} of MM virus injected subcutaneously in tests in mice not shown here.) The virus used in experiments in Table II comprised passage 12, 15, 17, and a pool of 17, 20, and 21, from a series of passages conducted in hamsters of 7 days age.

It may be observed that 8450 given to mice intraperitoneally accomplished demonstrable chemoprophylaxis against MEF1 virus also given intraperitoneally. In tests of this sort in which both filtrate and virus were given intraperitoneally, filtrate dosage to the mice was omitted on the day of virus injection. Administration of filtrate 8450 orally or subcutaneously had practically no effect on mice injected intraperitoneally with MEF1 virus, however, when the filtrate was injected intravenously into mice there was apparent chemoprophylaxis against MEF1 virus injected intraperitoneally. 8450 did not appear to be effective when injected intramuscularly. A trace of effectiveness was observed when virus was given intramuscularly (virulence not high enough) and filtrate was given intraperitoneally, and also when virus was given intravenously and filtrate was given intraperitoneally. It appears that some further tests of this last type should be done since the virus appeared a little more virulent intravenously than anticipated. Also, it remains to be determined whether filtrate lots which are active against MM and Semliki Forest viruses in mice (both activities apparently due to the same principle) are necessarily active to the extent described against MEF1 virus.

Summary. 1. A penicillium mold filtrate designated as 8450, and reported previously to have chemoprophylactic and chemotherapeutic activity against MM and Semliki

Forest virus in mice, has been found to have demonstrable chemoprophylactic effects in 12 g mice infected peripherally with a special hamster passaged line of MEF1 poliomyelitis virus. 2. Experiments are under way to find out (A) if stronger chemoprophylaxis can be obtained, (B) if more prolonged action can be obtained, and (C) if oral activity can be attained by 8450 against poliomyelitis virus.

1. Powell, H. M., Culbertson, C. G., McGuire, J. M., Hoehn, M. M., and Baker, L. A., *Antibiotics and Chemotherapy*, 1952, v2, 432.

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A Quantitative Comparison of Antibody Produced by X-Radiated and Normal Rabbits.* (20297)

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The antibody response can be divided into a brief initial radiosensitive phase and a second radioresistant phase which occupies the remainder of the period of response(1a,b,c,d). Once initiated, antibody production is little affected by large doses of whole body radiation in spite of the fact that lymphatic tissues, bone marrow, and spleen—all thought to be important in the production of antibody—are extremely radiosensitive. Several explanations of this observation have been given: first, the radiosensitive tissues might function only in the brief, initial phase of the antibody response and that the actual synthesis of antibody might take place in radioresistant tissues(1c); second, since even large doses of radiation do not destroy all of the radiosensitive cells in the body it is possible that the small number of these cells remaining might carry on the full antibody response; and third, that there are radioresistant reserve tissues capable of carrying out antibody formation once it has begun in spite of damage to radiosensitive tissues(2).

In view of the above, it seemed worthy to investigate whether the character of antibody molecules might be altered in radiated rabbits. The only readily demonstrable differences between antibodies are immunochemical in nature (3a,b,c,d) and these differences are based on the average behavior and reactivity of all the different kinds of antibody to the same antigen. The present report deals with a comparison of the immunochemical characteristics, as evaluated by the quantitative precipitin reaction and the initial combining ratio of antibody to antigen, of antibodies to bovine gamma globulin§ (BGG) and bovine serum albumin‖ (BSA) formed in X-radiated and normal rabbits.

Materials and methods. Male albino rabbits weighing from 2 to 2.3 kg were immunized and radiated as indicated in Table I. A single intravenous injection of either I^{131} labelled BGG (I*BGG), 30 mg/kg, or I^{131} labelled BSA (I*BSA), 15 mg/kg, was given to each animal. Preparation of the iodinated antigens has been previously described(4a,b). Two hundred and twenty KV whole body X-radiation was given as previously described(1c) in either 400 r (LD_{5-30}) or 800 r (LD_{75-30}) doses. Antigen concentrations in the blood were determined by protein bound radioactivity(4b) and bleedings for antibody analyses were made 3 days after elimination of all antigen at which time serum antibody concentrations were usually at a maximum. In order to have sufficient serum for quantita-

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§ Armour and Co. Fraction II bovine serum.

‖ Armour and Co. crystalline bovine serum albumin.