Studies on Production of Antipoliomyelitis Serum in Rabbits.* (21303)

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Many investigators(1) have attempted without success to produce poliomyelitis in the rabbit. Furthermore, completely negative results have also been reported(2-4) of attempts to produce neutralizing antibodies in rabbits inoculated repeatedly with virus infected monkey spinal cord suspensions given by various routes of inoculation, including the subcutaneous, intradermal, intraperitoneal and intramuscular. On the other hand, antipoliomyelitis sera have been produced successfully in horses, sheep(5,6), and mice(7).

To our knowledge there have been no reports of the development of neutralizing antibodies in rabbits inoculated with poliomyelitis virus grown in tissue culture. The present report deals with the successful production of type specific neutralizing antibodies by rabbits inoculated with the 3 types of poliomyelitis virus propagated in tissue culture. Both active and ultraviolet irradiated virus antigens were employed.

Material and method. The active and irradiated suspensions used in these studies were prepared from poliomyelitis virus propagated in monkey kidney tissue culture. Each strain of virus was grown individually, titrated, and its identity was checked with type specific antiserum. Table I contains titration data relative to the pools of virus types used for rabbit immunization. Ultraviolet irradiation was carried out with each virus type pool individually as described previously(8). Virus pool titrations and serum neutralization tests were done in test tube cultures of monkey kidney tissue fragments. In all antibody titrations pre and post vaccination serum samples were routinely tested simultaneously. The virus serum mixtures were seeded into tissue culture tubes and contained 100 50% tissue culture infecting doses of virus ($TCID_{50}$) in final mixture with each dilution of serum. Serial dilutions of serum beginning with undiluted (1/1), 1/5, 1/10, etc. were mixed with equal volumes of virus suspension and inoculated into 2 or 3 tubes per serum level. Titers are expressed as the 50% end point and represent the dilution of serum that was added to the virus before inoculation of the tissue culture tubes. New Zealand reds and albino rabbits weighing 3 to 5 lbs were used for immunization. All rabbits were bled by cardiac puncture prior to immunization and at subsequent intervals following immunization. Since we were uncertain of antibody responses, rabbits were immunized repeatedly by the intravenous or intramuscular routes in accordance with the following trial dosage schedules: *Schedule A. Injections.* 5 cc intramuscularly plus 5 cc or 10 cc intravenously on day 1 followed by 10 cc intravenously on days 4, 8, 11, 15, 18 and 22. 1 cc or 10 cc intravenously on day 60. *Bleedings.* Preimmunization specimen day 1, day 28 (R specimen), day 48 (F specimen), day 67 (K specimen). *Schedule B.* 5 cc intramuscularly plus 5 cc intravenously day 1. 10 cc intravenously day 31. 1 cc intravenously day 61. *Bleedings.* Preimmunization specimen day 1, day 38 (R specimen), day 65 (F specimen), day 85 (K specimen). *Schedule C.* 5 cc intra-

TABLE I. Titrations of Pools of Poliomyelitis Virus Used in Production of Rabbit Type Specific Antisera.*

Virus strain	Type	Pool No.	Titer, negative log of 50% end point
Mahoney	I	TC 85	4.0
Brunhilde	I	TC102	3.5
MEF1	II	TC 75	3.0
MEF1	II	TC 88	3.0
Saukett	III	TC 86	5.0

* Titrated in monkey kidney fragment tissue culture with 0.1 cc inoculum per dilution.

* Supported in part by a grant from Armour Laboratories.

† Deceased.

TABLE II. Antibody Response of 15 Rabbits Inoculated with Active or Irradiated Type 1 (Mahoney or Brunhilde Strain) Poliomyelitis Virus by Intravenous or Intramuscular Route.

Rabbit No.	Type	Dosage schedule	Active virus R sample	Active virus F sample	Active virus K sample	Irradiated virus R sample	Irradiated virus F sample	Irradiated virus K sample
1	1 (Mahoney)	A	>1/320	1/160	1/320	—	—	—
2	"	A	"	†		—	—	—
5 S*	"	A	1/320	1/5260	>1/21,140	—	—	—
6 S*	"	A	"	"	" "	—	—	—
6	"	A	—	—	—	1/80	1/20	1/320
8	"	A	—	—	—	1/80	1/20	1/640
7 S*	"	A	—	—	—	1/12	<1/5	<1/12
8 S*	"	A	—	—	—	1/320	1/80	>1/320
10 S	1 (Brunhilde)	A	>1/21,140	1/320	>1/21,140	—	—	—
11 S	"	A	—	—	—	1/108	<1/80	1/108
12 S	"	A	—	—	—	1/108	<1/80	1/320
4	1 (Mahoney)	B	1/160	1/80	1/80	—	—	—
5	"	B	1/640	1/640	<1/320	—	—	—
9	"	B	—	—	—	1/10	†	
10	"	B	—	—	—	1/40	—	—
13 S	"	C	1/1280	>1/320	>1/1280	—	—	—
14 S	"	C	1/108	1/80	1/320	—	—	—
15 S	"	C	—	—	—	1/80	<1/80	>1/320
16 S	"	C	—	—	—	<1/5	—	—

* Initial and final inoculation 10 cc.
† Rabbit died.

muscularly days 1, 8, 15, and 46. *Bleedings.* Preimmunization specimen day 1, day 22 (R specimen), day 45 (F specimen), day 53 (K specimen).

Results. Immunization with active and irradiated type 1 poliomyelitis virus given intravenously. Comparative results obtained in the immunization of 19 rabbits with Type 1 (Mahoney or Brunhilde strains) with ultraviolet inactivated or active virus, according to dosage Schedule A, are outlined in Table II. None of the rabbits had detectable antibodies for the 3 types of poliomyelitis virus in the prevaccination serum specimens, and, therefore, these negative findings are omitted from all tables. In postvaccination specimens only type specific antibodies were produced and no instances of cross neutralization were observed. There was an excellent specific homotypic antibody response to active Mahoney or Brunhilde Type 1 poliomyelitis virus (Table II). It is also apparent that there is considerable variation in the antibody response of the individual rabbit. The irradiated Type 1 Mahoney virus also produced a significant rise in homotypic antibody titer but not to the same degree as the active virus. Too few rabbits were inoculated with the Brunhilde Type 1 virus to draw definite conclusions, but the superiority of active virus is demonstrated in the only rabbit which survived of the two inoculated. The inactivated virus produced a homotypic antibody response which was definitely inferior to that of the active virus. Rabbits immunized with only 3 injections intravenously of Type 1 Mahoney virus, either active or irradiated, failed to develop antibody levels comparable to those elicited in the rabbits that had received 7 inoculations in the previous experiment (Table II). This result is not unexpected because of the much smaller quantity of antigen injected.

Immunization with Type 1 virus intramuscularly. Four rabbits were inoculated intramuscularly with active or irradiated Type 1 Mahoney strain according to dosage Schedule C. Results obtained are included in Table II. Although only 3 rabbits were available for study of all post-vaccination blood specimens, it is noteworthy that the results approximate those obtained by repeated intravenous inoculations (Table II).

Immunization with Type 2 virus inoculated intravenously. Four rabbits were immunized with one combined intramuscular and intravenous inoculation followed by 6 intravenous inoculations according to dosage Schedule A.

TABLE III. Antibody Response of Rabbits Inoculated Intravenously with Active or Irradiated Type 2 (MEF1 Strain) and Type 3 (Saukett Strain) Poliomyelitis Virus (Dosage Schedule A).

Rabbit No.	Type	R serum sample
	Active virus	
21	2	1/320
22	2	1/80
25	3	1/160
26	3	1/40
	Irradiated virus	
23	2	1/160
24	2	1/160
27	3	1/40
32	3	<1/20

Type 2 MEF1 strain active and irradiated virus suspensions were used. Results obtained are given in Table III. No antibodies were detected in the prevaccination serums and there was a homotypic antibody response to Type 2 virus. The results obtained with the irradiated virus were nearly comparable to those of the active virus.

Immunization with Type 3 virus inoculated intravenously. Four rabbits were immunized with 7 intravenous inoculations according to dosage Schedule A with Type 3 (Saukett) active and irradiated virus. Table III outlines the results obtained. No antibodies were detected in prevaccination serums and there was a homotypic antibody response to Type 3 virus. Although the results obtained represent the inoculation of only 4 rabbits, the active virus appears to be somewhat superior in immunogenicity to the irradiated virus.

Immunization with trivalent irradiated vaccine intravenously. As a matter of interest, 8 rabbits were immunized with 7 intravenous inoculations of trivalent irradiated tissue culture vaccine according to dosage Schedule A. The vaccine was composed of equal parts of Type 1 (Brunhilde strain), Type 2 (MEF1 strain) and Type 3 (Saukett strain). Results obtained with the irradiated vaccine are shown in Table IV. It is evident that the trivalent irradiated vaccine stimulated a significant antibody response to the 3 types of poliomyelitis virus in most of the rabbits that were inoculated.

Discussion. The present studies reveal a significant antibody response of the rabbit to intravenous or intramuscular inoculations of the three types of poliomyelitis virus grown in tissue culture. In no instance were antibodies detected in the preimmunization serums of the inoculated rabbits. Both active and ultraviolet irradiated virus were effective in stimulating antibody formation in the rabbit, although the former was definitely superior in most experiments. The antibody response obtained in rabbits inoculated with active or irradiated tissue culture virus was surprisingly good in view of the fact that these early experimental preparations used were not optimal and represent initial efforts at both virus growth and irradiation. Antibody response was proportional to the amount of antigen and number of inoculations given as shown in

TABLE IV. Antibody Response of Rabbits Inoculated Intravenously with Trivalent Irradiated Vaccine (Dosage Schedule A).

Rabbit No.	R serum sample type 1	2	3
47	1/8	1/64	1/32
48	1/8	1/16	1/1
49	1/8	>1/32	1/16
50	>1/32	>1/32	1/16
51	1/8	1/1	1/1
52	1/1	1/8	1/4
53	1/4	>1/32	<1/1
54	1/8	1/64	1/1

Table V. Three inoculations of 5cc intramuscularly at weekly intervals were approximately equivalent to seven 10cc inoculations given twice weekly by the intravenous route. The antibody response to each virus type was homotypic. There were inadequate numbers of albino and pigmented rabbits to afford a comparison of antibody responses in these two varieties.

TABLE V. Antibody Response of Rabbits Inoculated Intramuscularly with Varying Amounts of Active Type 1 (Brunhilde Strain) Virus (Dosage Schedule C).

Rabbit No.	Amount inj., cc	R serum sample Type 1
17 S	.05	>1:1280
18 S	.05	1:320
20 S	.5	1:1280
21 S	.5	1:80
22 S	.5	1:80
23 S	5.0	>1:5260
24 S	5.0	1:1280
25 S	5.0	1:5260

Although an occasional rabbit died of intercurrent infection, there was no "clinical" evidence that any of the 3 types of tissue culture virus was pathogenic for the rabbit, thus confirming the results of earlier workers(1). The variability in antibody responses of individual rabbits emphasizes the need of using adequate numbers in each test—possibly 5 animals per specimen. The response of the rabbit to the trivalent irradiated vaccine suggests the possible use of a rabbit test for assay of the immunogenic potency of different batches of vaccine for the selection of more antigenic strains of virus for vaccine production, and for determining the antigenicity of vaccines inactivated by various chemical or physical methods. Finally, storage tests of different types of poliomyelitis vaccine may be carried out.

Summary. Type specific neutralizing antibodies were produced in rabbits inoculated with the 3 types of poliomyelitis virus grown in tissue culture. Rabbits were successfully immunized by repeated intravenous or intramuscular inoculations of active or ultraviolet irradiated virus suspensions. The possibilities of a rabbit test for the assay of poliomyelitis vaccine are discussed.

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Endogenous Infection in Mice Irradiated with Fast Neutrons or Gamma Rays.* (21304)

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The development of generalized infection has been shown to play a significant role in the death of mice exposed to moderate doses (450-600 r) of total body x-irradiation in a single exposure(1,2). Such infections, which were always caused by bacteria of enteric origin, were demonstrated by blood and spleen cultures of mice killed at daily intervals after x-irradiation, and in a more recent study, by serial blood cultures(3). Bacteremia occurred most frequently during the second week following x-irradiation. In mice exposed to an equivalent dose of fast neutrons, death has been found to occur much earlier—between the 4th and 8th day(4), *i.e.*, before bacteremia becomes an important factor as a cause of death following x-irradiation. Field studies utilizing nuclear test devices in 1951 and 1952 had demonstrated this difference in mortality time between neutron- and γ-irradiated mice(5). This shorter survival time of neutron-irradiated mice raised the question whether generalized infection has time to develop and contribute to the lethal effect as it does in x-irradiated mice. The following study was undertaken to determine the incidence of bacteremia in mice irradiated with fast neutrons and, for comparison, mice irradiated with gamma rays from Cobalt 60.

Methods and materials. Female CF No. 1 mice, 5-6 weeks old, were shipped† to the laboratory by air express and placed in isolation for 3 weeks. They were maintained on a diet of Rockland mouse pellets and water

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† From Carworth Farms, New City, N. Y.