

Mouse Tests of Poliomyelitis Vaccine

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During the past, as a substitute for costly monkey tests, we have tested various lots of inactivated polio vaccine in mice for antigenicity. This has been done by injecting groups of mice each with a single dose of vaccine intraperitoneally. Three days later some of these mice are challenged intravenously with Type 2 virus, and four days later other groups are challenged intraspinally with Type 1 and 3 virus respectively. Proper doses of particular strains of virus injected by the routes indicated are necessary for early challenge (1,2). Control mice included suitable groups injected with a standard reference vaccine, and other groups to check virulence of the challenge viruses. Optimum dose of each challenge virus was an amount that would paralyze eight to ten of ten normal control mice and approximately two to four of ten additional controls given one tenth of such challenge. Generally three, ten-fold dilutions of vaccine have been tested, and groups of twenty mice used. These mice have been of about twelve to fourteen grams weight since the challenge viruses we used were best adapted to the smaller mice. Significant antibody titers are not present in these mice at the time of challenge. In his original work with Type 2 components tests, Krech (3) used CFW mice; however, we obtained these mice only occasionally, and generally used various other breeder's mice. These mouse tests are terminated and final results read seven days after challenge.

In general, it has appeared that through use of mouse tests we could distinguish three grades of vaccines, namely (a) good, (b) fair, and (c) ineffective. Only those vaccines purposely heated at elevated temperatures (2) and those more or less drastically treated (4) have been devoid of potency in our mouse tests. Since we used ordinary stock mice, it appeared of little use to try to determine earlier if the test could be rendered more quantitative as Krech has indicated for the Type 2 component.

Many experiments have been done varying the number of dilutions of vaccine used, the number of mice used per dilution of vaccine, and also the kind of mice including local stock mice and small numbers of CFW mice. As a temporary standard reference vaccine we used lot 649332. This lot and the three challenge viruses have already been described (1,2). Vaccine 2769 is a representative trivalent lot made of Mahoney, MEF 1, and Saukette strains currently used in the Salk Vaccine. Comparative tests were first done using these vaccine each in dilutions of 1:5, 1:50, 1:500 and twenty CFW mice per dilution of vaccine for each of the three challenge types. Previous tests mainly utilized five, five-fold dilutions of vaccine and ten mice per dilution per type.

The results shown in Table 1 indicated that ED_{50} values of vaccine 2769 of 24; 61; 24; (Types 1, 2, and 3, respectively) are comparable typewise to ED_{50} values of vaccine 649332 of 71; 83; and 26. These

TABLE 1
 Polio Vaccine Mouse Tests Using Three Dilutions of Vaccine and Twenty CFW Mice
 Per Dilution. Mice Living and OK at Seven Days/Mice Used.

Vaccine Lot	0.5 ml. Vaccine Dilution i.p.	Type 1 Intraspinal			Type 2 Intravenously			Type 3 Intraspinal		
		OK/Used	ED ₅₀	%S	OK/Used	ED ₅₀	%S	OK/Used	ED ₅₀	%S
2769	1:5	11/20			13/20			11/20		
	1:50	9/20			11/20			9/20		
	1:500	4/20	24	40	7/20	61	51	4/20	24	40
	1:5	12/20			12/20			12/20		
649332	1:50	13/20			13/20			7/20		
	1:500	6/20	71	51	8/20	83	55	7/80	26	43
1.0 Challenge dose virus		0/10			0/10			1/10		
0.1 Challenge dose virus		4/10			7/10			7/10		

Legend: Under ED₅₀ is recorded the reciprocal of the vaccine dilution completed by the method of Reed and Muench⁽⁵⁾ to save half the mice. %S indicates the per cent surviving and OK of all mice used.

figures indicate that while 2769 is lower than 649332 in the Type 1 component, the two are about equal in potency of Type 2 and 3 components. Corresponding per cent of total mouse survival of 2769 of 40; 51; 40 may be compared to 649332 showings of 51; 55; 43. Both ED_{50} values and total mouse survival percentages indicate that vaccine 2769 may be classed as fairly good. Mouse survival percentages seem to indicate closer similarities in potency than do ED_{50} values. It appears that the use of three, ten-fold dilutions of vaccine (1:5, 1:50, 1:500) and twenty mice per dilution per type may be preferable to five, five-fold dilutions and ten mice per dilution per type as previously used.

From data in such comparative potency tests using control vaccine, one may assign as potency factors of the three component types of a given batch of vaccine the figures obtained by dividing the ED_{50} values of the test vaccine by the corresponding ED_{50} values of the control vaccine. Table 2 shows, in the first horizontal line, the potency factors of vaccine 2769 as derived from the data in Table 1 in which

TABLE 2
Potency of Polio Vaccine 2769 in Relation to
Standard Vaccine 649332

Vaccine 2769	Potency Against Types:		
	(1)	(2)	(3)
In CFW Mice	0.33	0.73	0.92
In Harlan Mice	0.64	0.41	0.5

Potency is ED_{50} of test vaccine/ ED_{50} of Standard Vaccine.

CFW mice were used. In the second horizontal line of Table 2 are shown potency factors of vaccine 2769 derived from a similar test in which local Harlan mice were used, and details of which need not be included here. The variation in response as shown in Table 2 is about two to one. This appears within expectation for biological tests of this order, and corresponds to a "one tube" variation in certain serological tests. It may be that the three components of the vaccine have independent and variable antigenicity in different strains of mice. Whether any natural or latent infection of mice conditions them to react better or worse in a type selective way to polio vaccine is not known. In most mouse tests we have noted better antigenicity of the Type 2 component than Type 1 which in turn is better than Type 3. The Type 2 component is more stable to heat than the Type 1 component as based on mouse tests (2) and may be a better antigen for this or some other reason.

Further work is being done as time allows to determine the degree of accuracy of the mouse test when different coded vaccines are used as unknowns. These results may be compared to monkey potency test results which are based on antibody titers instead of challenge with live virus.

Mouse tests of polio vaccine may be done in eleven days, are economical, and necessitate practically no interpretation. Immunity is indicated by freedom from paralysis, in addition to survival of mice

against live virus challenge. Vaccines may be graded roughly as "good", "fair", or "ineffective". Mouse tests done under these conditions are not adapted to measurement of virus neutralizing antibody which at this time is likely sessile.

Literature Cited

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