

# Use of White Rats for the Measurement of Immunogenic Potency of Inactivated Poliomyelitis Vaccine

V. D. SOLOVIEV<sup>1</sup> & U. Z. GENDON<sup>1</sup>

*There are many advantages in the use of small laboratory animals rather than primates for tests to determine the immunogenic potency of inactivated poliomyelitis vaccine. The white rat, in particular, is cheap, easy to keep and use in sufficiently large numbers to ensure the statistical validity of results, and highly resistant to intercurrent diseases. The experiments reported show that this animal can be successfully used for evaluating immunogenic potency when a single intraperitoneal inoculation of inactivated poliomyelitis vaccine is administered, with results as accurate as those obtained with the more expensive guinea-pig.*

In recent years, more and more use has been made of small laboratory animals, primarily guinea-pigs and chicks, for evaluating the immunogenic potency of inactivated poliomyelitis vaccine. The use of small laboratory animals has numerous advantages. Their comparatively low cost and the ease with which they are kept make it possible to include a sufficiently large number of animals in each experiment—thus ensuring the statistical validity of the results obtained—and to evaluate the immunogenic potency of the vaccine by computing the extinction limit. Moreover, it is possible to make a direct comparison of each series of vaccine with the standard antigen, for which purpose, bearing in mind the resistance of small laboratory animals to poliomyelitis, live active poliovirus may be used.

The purpose of this communication is to describe our experience in using white rats for measuring the immunogenic potency of inactivated poliomyelitis vaccine. As is well known, these animals are highly resistant to intercurrent diseases and easy to breed and keep. So far as cost is concerned, under our conditions, white rats are approximately six times cheaper than guinea-pigs.

In earlier investigations<sup>2</sup> it was shown that white rats respond well to the injection of live poliovirus

by forming antibodies. It was noted that the best methods of injection of the antigen were the intravenous and the intraperitoneal, and preference was given to the intraperitoneal method as it was the simpler. This work sets forth the results of the use of white rats for evaluating the immunogenic potency of inactivated poliomyelitis vaccine when a single immunization is given.

## INVESTIGATION METHODS

The immunogenic potency of inactivated poliomyelitis vaccine in white rats on single vaccination was measured as follows. White rats weighing approximately 200 g were inoculated intraperitoneally with 1 ml of inactivated poliomyelitis vaccine taken in three dilutions, up to 10<sup>-2</sup>, ten rats to each dilution. Blood was taken after 4 × 24 hours (the thoracic cavity was opened under deep ether narcosis, the lower vena cava at the entrance to the heart was incised, and the emergent blood was drawn out by means of a Pasteur pipette). The presence of antibodies was determined in the blood sera (diluted 1:4) by means of the colour test;<sup>3</sup> then, using the Reed & Muench method, the immunogenic potency of the preparation was determined and the extinction limit calculated—that is, the limit dilution of the vaccine causing the formation of antibodies in 50%

<sup>1</sup> Institute of Poliomyelitis Prophylactics, Moscow, USSR

<sup>2</sup> Gendon, U. & Marchenko, A. (1959) *Acta virol. (Bratislava)*, 3, No. 2, 89; 3, No. 4, 250 (English edition)

<sup>3</sup> Melnick, J. L. & Opton, E. M. (1956) *Bull. Wld Hlth Org.*, 14, 129

of the vaccinated animals. As a comparative standard antigen, use was made of a 1:10 diluted mixture of equal amounts of three types of poliomyelitis—type 1, Brunenders strain; type 2, MEF 1 strain; type 3, Saukett strain—with the same initial titre,  $10^{-5.6}$  TCID<sub>50</sub> per ml, and stored at a temperature of  $-60^{\circ}\text{C}$ .

## RESULTS OF INVESTIGATION

The dynamics of antibody formation after a single injection of poliomyelitis antigens were studied in the first experiments. It appeared that when the rats were injected with live virus there was a rapid

TABLE 1  
EVALUATION OF IMMUNOGENIC POTENCY OF  
POLIOMYELITIS VACCINE ON WHITE RATS  
AFTER SINGLE IMMUNIZATION

Vaccine series	Extinction limit ( $\log_{10}$ )		
	type 1	type 2	type 3
Trivaccine A-5	0.76	1.03	1.99
A-7	0.48	1.59	2.00
A-10	1.20	1.55	2.10
V-2	0.88	1.04	1.86
B-8	1.84	1.24	1.64
V-10	0.48	0.79	2.13
D-1	0.76	0.88	1.56
D-2	0.73	0.67	1.86
D-3	0.77	1.54	1.59
D-5	0.73	1.74	>2.50
D-6	0.88	1.46	1.87
D-7	0.72	0.94	1.82
D-9	1.96	1.52	1.61
S-10	0.17	0.20	0.49
K-68-2	0.24	0.93	0.69
K-78-2	0.98	1.21	0.79
Bivaccine, types 1+3	0.66	<0.00	0.81
Monovaccine, type 1	0.84	<0.00	<0.00
type 2	<0.00	1.36	<0.00
type 3	<0.00	<0.00	1.82
Media 199 (control)	<0.00	<0.00	<0.00
Standard antigen (live virus)	0.90	0.64	0.81

TABLE 2  
COMPARATIVE DATA ON THE IMMUNOGENIC POTENCY  
OF POLIOMYELITIS VACCINE ON GUINEA-PIGS  
AND WHITE RATS

Vaccine series	Guinea-pigs (double vaccination)			White rats (single vaccination)		
	Extinction limit			Extinction limit		
	1	2	3	1	2	3
A-4	<0.00	1.47	<0.00	0.09	0.93	<0.00
A-7	0.59	1.50	1.37	0.48	1.59	2.00
B-8	1.04	1.82	2.34	1.84	1.24	1.64
V-10	0.77	1.48	2.30	0.48	0.79	2.13
D-2	0.87	1.00	1.48	0.73	0.67	1.86
D-3	0.53	1.60	1.82	0.77	1.54	1.59
D-5	1.08	1.95	2.10	0.73	1.74	>2.50
D-9	1.11	1.67	1.38	1.96	1.52	1.61
K-68-2	1.50	1.65	1.37	0.24	0.93	0.69
S-01	0.49	0.00	0.00	0.17	0.20	0.49
Standard virus	1.01	1.65	1.01	0.90	[0.64	0.81

increase in the antibodies, reaching its maximum after 4-5 days, and a subsequent sharp fall in the titre. When the rats were injected with poliomyelitis vaccine there was also a rapid growth of antibodies, reaching the maximum for types 2 and 3 after 4-5 days and for type 1 after 3-4 days, again with a subsequent fall in the titres.

Repeated experiments on more than 1000 white rats showed that in all cases these animals form neutralizing antibodies in response to the injection of poliomyelitis vaccine. In this connexion the weight and age of the animals is of great importance. Thus it was found that young rats weighing less than 150 g react badly to the injection of poliomyelitis vaccine. Sexually mature rats weighing 200 g and more respond best of all to the injection of the vaccine. No substantial difference was discovered in the immunogenic response of males and females.

Table 1 shows the measurements of immunogenic potency of 20 series of poliomyelitis vaccines when white rats are given a single immunization.

As may be seen from this table, the method of single vaccination of white rats may be successfully used for measuring the immunogenic potency of poliomyelitis antigen when computing the extinction

limits. The high specificity of the immunogenic response of rats when given a single injection of poliomyelitis vaccine is confirmed by experiments in which when given injections of monovaccines the rats formed antibodies only to the type that was used for the vaccination.

It should be noted that inhibitors to poliomyelitis virus were not discovered in the blood sera of rats, taken before the vaccination.

In specially set up comparative experiments, the results of which are set forth in Table 2, the immuno-

genic potency of poliomyelitis vaccine in simultaneous investigations on guinea-pigs and on white rats using the method described (Gard method) were studied, and it was found that the results agree well.

The unimportant loss of white rats in the course of the experiment (0.9%), the short duration of the experiments, the simplicity of keeping the rats and the possibility of using active poliovirus as a standard antigen makes the method of measuring the immunogenic potency of poliomyelitis vaccine by means of a single vaccination of white rats a very suitable one.

## RÉSUMÉ

L'utilisation des petits animaux de laboratoire, de préférence aux singes, pour évaluer l'activité du vaccin antipoliomyélique inactivé, tend à se généraliser. Le cobaye et le poulet sont fréquemment employés.

Les auteurs ont procédé à des essais avec le rat blanc, beaucoup moins coûteux que le cobaye, facile à élever en lignée pure et à maintenir en laboratoire, très résistant aux maladies intercurrentes et à la poliomyélite, de sorte que l'on peut utiliser du vaccin vivant comme antigène de référence.

Dans les recherches qui font l'objet de cet article, les

auteurs ont évalué l'activité du vaccin antipoliomyélique inactivé, administré au rat blanc en une seule injection, par voie intrapéritonéale. L'évaluation de l'activité a été faite par la méthode de l'extinction antigénique. La réponse-anticorps des rats est hautement spécifique: l'injection de monovaccin donne lieu à la formation exclusive d'anticorps correspondant au type de l'antigène. Aucun inhibiteur non spécifique n'a été trouvé dans le sérum des rats. Les résultats de la méthode appliquée d'une part au cobaye, d'autre part au rat, sont concordants.