

*Use of sera for testing the presence of extraneous viruses in batches of virus fluid*

Sera with titres 16 and above by Method 2 were used. It was assumed that the sera contained an arbitrary number of antibody units equal in number to the titre obtained.

The serum was diluted in the maintenance medium to contain 4 units of antibody. To this was added an equal volume of homologous virus fluid under test, mixed well, and transferred to a fresh container with stopper. The mixture was treated as described earlier and inoculated into primary renal monolayer cultures containing 2 units of the same serum in an appropriate volume of the maintenance medium. The cultures were incubated at 37°C. and observed for 14 days. Fluid was changed in the cultures after 7 days incubation using the maintenance medium containing 2 units of the same serum. Appropriate control cultures were used in each experiment.

So far, 36 (8 type 1, 15 type 2 and 13 type 3) batches of virus fluid have been tested using these sera. Virus breakthrough has not been noticed in any of the tests. The undiluted sera do not contain antibody to SV<sub>40</sub> virus.

#### D. Preparation of Live Oral Poliomyelitis Vaccine (Sabin)

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In recent years poliomyelitis in an epidemic form is being reported in increasing frequency in different parts of India. This has resulted in the demand for a reliable prophylactic against the disease in the country. The Government of India decided that live poliomyelitis vaccine (Sabin) would be the most suitable for the purpose and selected the Pasteur Institute, Coonoor, for the preparation of the vaccine.

The imported as well as some of the indigenously available items of equipment required for this work are being provided by the USAID Mission, New Delhi, while other locally available items of equipment, staff and other expenses are met by the Ministry of Health, New Delhi.

The preliminary work already done has been outlined in the Scientific Report for 1964. The work done so far in connection with the preparation of the seed virus is given below.

(i) *Preparation of virus fluid*

Virus fluid containing all the 3 types of attenuated (Sabin) poliomyelitis viruses were prepared from the seed material kindly made available by Dr. Albert B. Sabin, using methods recommended by him. Primary monolayer cultures prepared from kidneys of the locally available genera of *Macaca* were used. Due to ready availability sheep serum was used instead of equine or bovine sera for the preparation of these cultures. The initial dilution of seed virus material inoculated into cultures was in the range of 1 in 1,000 to 2,000 for all the three types of viruses. It was found that the seed material had to be neutralized with SV<sub>40</sub> antiserum in order to ensure the elimination of this agent from the virus fluid.

(ii) *Testing of virus fluid*

Each batch of virus fluid was tested for the absence of extraneous viral and other agents. The test procedures adopted were essentially those recommended in the WHO Technical Report Series No. 237 except that the BSC-1 cell line was used for testing the presence of SV<sub>40</sub> virus.

In order to eliminate the possibility that this cell line might be less susceptible to the virus than the primary *Cercopithecus* renal cultures, arrangements have been made through the courtesy of the WHO to confirm the absence of the virus in each batch of virus fluid at the Medical Research Council Laboratories, London.

Tests for 25 batches of virus fluid have so far been completed,