The work of the WHO Consultative Group on Poliomyelitis Vaccines

W. Chas. Cockburn

WHO has played an important role in the development and use of poliovaccines since their introduction. From 1973 it has been directly responsible for the custody and distribution of the Sabin strains of the live oral vaccine and has exercised strict supervision over production laboratories in cooperation with national control authorities. In addition, the WHO Consultative Group on Poliomyelitis Vaccines has coordinated extensive field studies in 13 countries on vaccine-associated cases of paralysis, and been concerned with the preparation and storage of seed viruses which will ensure adequate supplies of vaccine for the next two centuries, the establishment of standard tests for the neurovirulence of vaccine lots, and the assessment of "markers" for the intratypic identity of poliovirus strains.

The safety and efficacy of the live vaccines have been confirmed in all countries in the study, except for one where vaccine-associated cases continue inexplicably to occur in numbers greater than have been observed elsewhere. The findings of the 15-year investigation of vaccine-associated cases confirm those previously reported, namely that type 1 strain is almost never implicated in post-vaccination paralysis, that type 2 is an occasional cause of paralysis in contacts of the vaccinee, and that most of the very small number of cases which do occur are due to type 3. Countries using killed vaccines recorded good protection but breakthroughs, especially due to type 3, have occurred and caused substantial numbers of cases.

The impressive achievements of this WHO collaborative activity are not least due to the contributions made by national laboratories for the control of vaccines and by vaccine producers over many years.

The work of the WHO Consultative Group on Poliomyelitis Vaccines provides an example of the outstanding contribution WHO makes to the safety, reliability and potency of vaccines and other prophylactic agents by organized collaboration with national bodies. WHO took a leading part in developing poliovirus vaccines and in organizing the First and Second International Conferences on Live Poliomyelitis Vaccines in 1959 and 1960 (1, 2). These Conferences not only brought together up-to-date accounts of early work on live poliovaccines but
also identified problems that could arise from the large-scale use of the vaccines, thus, even at that time, alerting the authorities on the need for effective control of vaccine production and testing and for intensive epidemiological surveillance to ensure continuing safety and efficacy. Since that time, WHO’s activities in the field and laboratory have continued. This report describes mainly the period since 1973 when the Consultative Group was active, and also the work on vaccine-associated paralysis which began three years earlier and was continued by the Group.

PARALYSIS ASSOCIATED WITH POLIOVACCINES

In 1969 a group of WHO consultants reviewed the available evidence on the occurrence, among vaccinated persons or their contacts, of paralysis typical of poliomyelitis, which was more often related to poliovirus type 3 vaccine strain than to the type 1 or type 2 strains. They stressed the need for more accurate information from as many different countries as possible and recommended that WHO should initiate a collaborative epidemiological study in interested countries so that, by using similar methods of investigation and recording, meaningful comparisons could be made. They also recommended that a group of laboratories should be invited to study the reliability of the marker tests that were then available for the intratypic differentiation of poliovirus strains of different origins (3).

When approached by WHO, 13 countries (total population, 509 million) agreed to provide detailed information on all cases of spinal paralysis of acute onset which were typical of poliomyelitis and which lasted for more than six weeks. The countries are listed under code numbers in Table 1 with information on their populations (based on 1975 figures) and on the vaccines used. Reports on the first five and the first ten years of the study in the period 1970–79 have been published and are summarized here (4, 5).

<table>
<thead>
<tr>
<th>Country</th>
<th>Population (millions, 1975)</th>
<th>Vaccines used</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>14.8</td>
<td>TOPV&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Short campaign&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. 2</td>
<td>5.1</td>
<td>Primary IPV&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>booster, TOPV</td>
</tr>
<tr>
<td>No. 3</td>
<td>49.2</td>
<td>TOPV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Throughout the year&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. 4</td>
<td>10.5</td>
<td>Monovalent&lt;sup&gt;d&lt;/sup&gt; OPV; order of administration type 1, type 3, type 2</td>
</tr>
<tr>
<td>No. 5</td>
<td>110.6</td>
<td>TOPV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Short campaigns</td>
</tr>
<tr>
<td>No. 6</td>
<td>4.0</td>
<td>TOPV</td>
</tr>
<tr>
<td>No. 7</td>
<td>34.0</td>
<td>Monovalent OPV until 1973, then TOPV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Short campaigns until 1974, then throughout the year</td>
</tr>
<tr>
<td>No. 8</td>
<td>21.3</td>
<td>Monovalent OPV type 1, then TOPV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Short campaigns</td>
</tr>
<tr>
<td>No. 9</td>
<td>5.2</td>
<td>TOPV</td>
</tr>
<tr>
<td>No. 10</td>
<td>213.5</td>
<td>TOPV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Throughout the year</td>
</tr>
<tr>
<td>No. 11</td>
<td>22.8</td>
<td>TOPV in some areas; and both IPV and TOPV in others</td>
</tr>
<tr>
<td>No. 12</td>
<td>13.7</td>
<td>IPV (but TOPV in outbreaks)</td>
</tr>
<tr>
<td>No. 13</td>
<td>4.7</td>
<td>IPV</td>
</tr>
</tbody>
</table>

<sup>a</sup> TOPV: trivalent oral poliovaccine (Sabin strain).
<sup>b</sup> Monovalent OPV, single types administered.
<sup>c</sup> IPV: inactivated poliovaccine.
<sup>d</sup> Short campaign: annual campaigns over a short period.

Findings for 1970–79

Two countries using TOPV, No. 1 and No. 6 (populations, 14.8 million and 4.0 million) reported no cases of paralysis. Country No. 2 (population, 5.1 million), where children are primarily immunized with IPV and given reinforcing doses of OPV, reported one case; this was a child given three doses of IPV but no OPV. The strain isolated from stools was identified as type 1—‘‘non-vaccine-like’’.

Countries regularly reporting small numbers of cases. Six countries (No. 3, 4, 5, 9, 10 and 11; total population, 403 million), using live vaccine, each reported small numbers of cases fairly regularly and the data were amalgamated for analysis. In these countries 281 cases occurred, of which 122 (43%) were classified as vaccine-associated (52 in vaccinees and 70 in contacts). Trivalent vaccine was administered in five of the countries and from some of the cases in these countries more than one virus was isolated. However, in No. 4, in which monovalent vaccines of each of the 3 types were used during the
whole period, no cases of mixed infections were identified. Of the 122 cases, 10 depended only on clinical diagnosis and 36 had laboratory evidence of more than one infection. In 76 there was evidence of infection with a single type of virus (by isolation of virus from faeces and/or increases between the acute and convalescent levels of antibody). Twenty-seven of these cases were in vaccinees; 17 were associated with type 3, eight with type 2, and two with type 1. Forty-nine were in contacts; 25 were associated with type 3, 21 with type 2, and three with type 1.

Incidence in children 0–3 years of age. As most children complete their immunizations in the first two years of life, the association with vaccine-associated paralysis can be calculated for this susceptible age group. For the six countries mentioned above, the incidence was 0.84 cases per million children, but it varied from 0.5 per million to 3.4 per million—the last being the rate in the country using monovalent vaccine throughout the period.

Two countries with a high incidence. In two countries the risks were much higher; in No. 7 (population, 34 million) there were in the first five years (1970–74) more cases than in any other country in the study. In this period the number varied from 16 to 44 annually (total 139), compared with 0 to 8 annually (total 25) in 1975–79. The cases were mainly associated with poliovirus type 2 in contacts of vaccinees. Three changes occurred at about the time the decrease began. Trivalent vaccine was substituted for sequential monovalent vaccines, and throughout-the-year vaccination was substituted for the previous annual mass campaigns. Also, as each batch of vaccine arrived in the country it was subjected to more rigorous control in the national laboratory than had previously been considered necessary. One or all of these changes may have exerted a favourable influence on the level of risk.

The second country of high risk, No. 8 (population, 21.3 million), prepared its own vaccines. It reported from 8 to 27 cases of typical persistent paralysis annually between 1970 and 1978. Most were infected with type 3 or type 2 polioviruses. In 1979, type 2 and type 3 vaccines were not available for the national mass vaccination campaign and only type 1 vaccine was distributed. No vaccine-associated cases occurred in that year. In 1980, immunization with trivalent vaccine, containing type 2 and type 3 vaccines from the same lots that had been used in previous years, was restarted and the recorded incidence of vaccine-associated cases was again like that for the earlier years. The lots of type 2 and type 3 vaccines prepared in this country and used before and after 1979 underwent both in the national control laboratory and in independent laboratories in other countries the full battery of tests recommended by WHO. The vaccines were found satisfactory in all respects, including a low level of monkey neurovirulence.

Countries using only inactivated vaccines. Country No. 13 (population, 4.7 million) has used only inactivated vaccine since immunization against poliomyelitis began. No cases of paralysis occurred during the 10 years studied (but see next page). Country No. 12 (population, 13.7 million) also relies on inactivated vaccine for routine immunization. Here two outbreaks were reported in 1971 and 1978; both were in communities with religious objections to immunization and both were due to type 1, 'non-vaccine-like' viruses. In the country itself the outbreaks did not spread beyond the communities, but in 1978–79 there were cases in communities with similar religious beliefs in Canada (8 cases) and the USA (11 cases), which were presumed to be associated with contacts from the Netherlands.

Findings for 1980–84

The results from the third five-year period of the study (11) are summarized below.

In the period 1980–84, four countries (No. 1, 2, 6 and 12) had no cases of paralysis. The group of six countries (mentioned earlier) reported 72 cases, of which 61 were vaccine-associated (32 in vaccinees and 29 in contacts). There were 37 cases with evidence of single virus infection (18 in vaccinees and 19 in contacts); the distribution of poliovirus types in these cases is similar to that observed in 1970–79. Among the vaccinees, 11 cases were associated with type 3, six with type 2, and one with type 1. In the contacts, eight were associated with type 3, nine with type 2, and two with type 1. As shown in Table 2, the total number of cases in the group of six countries fell by about half between 1970–74 and 1980–84, but the number of vaccine-associated cases remained about the same.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of cases of APSP</th>
<th>No. of vaccine-associated cases</th>
<th>Vaccine-associated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970–74</td>
<td>153</td>
<td>57</td>
<td>37</td>
</tr>
<tr>
<td>1975–79</td>
<td>128</td>
<td>65</td>
<td>51</td>
</tr>
<tr>
<td>1980–84</td>
<td>72</td>
<td>61</td>
<td>85</td>
</tr>
<tr>
<td>1970–84</td>
<td>353</td>
<td>183</td>
<td>52</td>
</tr>
</tbody>
</table>
Of the two countries with a high incidence previously, No. 7 continued the low rate it had achieved by 1979 and reported only 10 cases during 1980–84, of which one (due to type 3) was in a vaccinee and nine (four type 2, three type 3, and two of unknown type) were in persons with no history of contact with vaccinees. In country No. 8 the high incidence of vaccine-associated cases continued and, in addition, several outbreaks associated with "wild" type 1 and type 2 polioviruses occurred in March–April 1980 and from September 1980 to mid-1982, mainly in unvaccinated or incompletely vaccinated children living in residential homes and institutions.

Country No. 13, where inactivated vaccine has been used since the start of vaccination and where no cases occurred during the first 10 years of the study, reported nine cases (one fatal) in 1984. The cases were scattered throughout the country and were due to type 3 poliovirus, which was isolated from many healthy persons and sewage as well as from the cases. Transmission ceased after the administration of 4.5 million doses of trivalent live vaccine.

The reliability and use of marker tests for the identification of the origin of polioviruses

Concurrently with the field study of cases of paralysis a collaborative investigation in which laboratories in seven countries took part was made on the marker tests then available. A total of 49 strains of poliovirus of one or other of the three types was obtained from cases in the field studies. The results showed that the rct/40 marker was not a reliable test for differentiating progeny strains from wild strains of the same type, that the McBride, Wecker and aluminium hydroxide gel tests were of some value but did not fully characterize all strains, and that cross-absorbed immune sera were the most dependable. However, the most important outcome was that the study provided a battery of thoroughly tested poliovirus strains for use in assessing the value of new tests as they are introduced.

Comments

This 15-year study confirms that live poliovaccine is safe and effective in routine use and is indispensable for the control of outbreaks. In the group of six countries with a total population of 403 million, the risk of vaccine-associated paralysis is less than one per million children vaccinated. In these countries the total number of cases of acute persistent spinal paralysis typical of poliomyelitis fell from 153 in 1970–74 to 72 in 1980–84, but the number of vaccine-associated cases remained the same (about 60 per quinquennium). More than half the cases in contacts occurred in poorly immunized adults in contact with children who were being vaccinated. To ensure a further reduction in the number of cases, parents should be vaccinated at the same time as their children if their vaccination status is unknown or doubtful. (Some countries recommend the use of killed vaccine for this purpose.)

In country No. 7, where the incidence of vaccine-associated cases was high in 1970–74, the number of cases has now fallen to the levels noted in the group of six countries. However, in country No. 8 the incidence of vaccine-associated cases remains high and in spite of investigations by national and international experts the problem remains unsolved; outbreaks due to "wild" polioviruses belonging to types 1 and 2 since 1980 also cause anxiety about the behaviour of the disease in this country.

Overall the findings confirm those of previous investigators. Type 1 poliovirus is very rarely implicated in vaccine-associated paralysis; type 3 is the most common type in both vaccine recipients and contacts; and type 2 is commoner in contacts than in recipients.

In countries using killed vaccines, good protection has been demonstrated but a serious breakthrough occurred in country No. 13 in 1984 in relation to type 3, after more than 25 years of intensive vaccination with IPV.

HISTORY AND ACTIVITIES OF THE CONSULTATIVE GROUP

Live poliovirus vaccines prepared from the Sabin strains of types 1, 2 and 3 were introduced for large-scale immunization in 1957. From then until 1972, Dr Sabin himself authorized suitable laboratories to produce vaccines from his strains and he controlled the methods of production in collaboration with the control authorities of the countries concerned. Early in 1972 he asked the author, at that time Chief of the Virus Diseases Unit in WHO, if the Organization would take over from him the responsibility for the custody and distribution of his strains and assume his supervisory and advisory role on vaccine production. The Director-General, Dr M. G. Candau, agreed and in May 1973 established a scientific committee to advise him on all matters pertaining to the use of these strains.

This committee, which included Dr Sabin, was called the WHO Consultative Group on Poliomyelitis Vaccines (Sabin Strains); the list of the original members is given in the Annex. Soon it became clear that the group had to deal with all poliovaccines (inactivated as well as live) and in 1980 the name was changed to the Consultative Group on Poliomyelitis
Vaccines. The Group first met in 1974, at which time production laboratories were already in operation in 11 countries. In addition, four other countries were seeking to establish satisfactory production and control laboratories and three succeeded in doing so. Sabin strain vaccine is therefore currently in production in laboratories in 14 countries (Belgium, Canada, China, France, Federal Republic of Germany, Iran, Italy, Japan, Mexico, Romania, United Kingdom, USA, USSR, and Yugoslavia). All these laboratories have been inspected on one or more occasions by members of the Group and the WHO Secretariat who have satisfied themselves that the WHO requirements are being fulfilled.

One of the Group's earliest tasks was to estimate the number of laboratories necessary to ensure adequate supplies of vaccine for the world's needs in the foreseeable future. It was concluded that there were already a sufficient number of laboratories.

Setting up new production and control laboratories is very costly in terms of buildings, equipment, and scientific manpower and there is always the (very remote) risk that a vaccine strain may revert to virulence. Because of these considerations and because of the estimated adequacy of vaccine suppliers, the Group produced a memorandum entitled "WHO conditions for the distribution and use of poliomyelitis vaccine strains (Sabin)" in which the costs and risks were clearly stated. Since 1974, seven countries approached WHO about establishing their own national production laboratories but after considering the information in this memorandum and discussions with members of the Group or the WHO Secretariat, they abandoned their proposals and made other arrangements for the supply of vaccine, e.g., the importation of concentrated bulk vaccine, which is diluted and ampouled in the national laboratory and distributed with labels and instructions in the national language.

**Collaborative studies on tests for the neurovirulence of poliovirus vaccines**

Another subject considered by the Consultative Group at its first and subsequent meetings was the need for standardization of the neurovirulence test in monkeys. At the Group's request information on current and past experience was submitted by national control laboratories and by manufacturers, and discussion meetings were convened under the auspices of the International Association of Biological Standardization and the Consultative Group.

In 1978, the National Control Authorities of Canada, the United Kingdom and the USA authorized their respective staff members concerned with these tests to develop and evaluate a standardized procedure. The project, which began work in 1979 and was named "the tripartite project", made a detailed analysis of the results of tests over the previous 18 years and established a prospective plan for replicate testing of selected monovalent vaccines and candidate reference vaccines. The three laboratories made 19 replicate tests on two candidate reference type 3 vaccines in about 300 cynomolgus monkeys and, in addition, four tests were made on 106 rhesus monkeys.

Excellent agreement was achieved between the tests in all the participating control laboratories. Subsequently they collected additional data and established more sophisticated methods of statistical analysis. Other laboratories invited to repeat the tests obtained results similar to those in the tripartite project, both with the reference vaccine and with production lots. A total of 57 tests were made in over 800 cynomolgus monkeys and 12 tests in 240 rhesus monkeys.

Similar tests (but fewer in number because of the relatively greater genetic stability of the viruses) were made on type 1 and type 2 reference vaccines. The test method was incorporated in the WHO revised requirements for OPV vaccine (6) and has been used by vaccine producers and control laboratories since then. The available evidence indicates that it is a satisfactory laboratory check on the acceptability of OPV for vaccinees. However, the Consultative Group stress their frequently repeated recommendations that the surveillance for vaccine-associated cases of paralysis is the principal means of monitoring the safety of OPV and must be continued and, as far as practicable, extended to more countries than the present 13 collaborators in the WHO programme.

Recent research into the molecular basis for the neurovirulence of poliovirus strains has shown that only two mutations are required for the attenuation of the type 3 phenotype whereas many more, scattered throughout the genome, are present in the attenuated type 1 phenotype. It is anticipated that these studies will result in the identification of sequence changes linked to neurovirulence and that ultimately there will be no need to perform the monkey neurovirulence test on each vaccine lot.

**Collaborative study of the titration of poliovirus vaccines**

The WHO requirements for poliomyelitis vaccine (oral) (6) state that the virus concentration in the final vaccine will be determined in cell cultures in terms of infective units per ml. The Consultative Group decided that a collaborative study should be set up to compare assay results between laboratories using a common microtitration technique and also
their own standard procedure, and to determine whether frozen virus suspensions could be designated suitable standards for the assay of potency of monovalent and trivalent vaccines. Laboratories in eight countries took part in the study using Hep-2 cell seed cultures and aliquots of monovalent and trivalent vaccines from common sources. The results have been published (7).

The WHO study method, using microtitration plates inoculated with dilutions of monovalent virus or of trivalent viruses with the appropriate antisera followed by an equal volume of a suspension of Hep-2 cells, presented few problems to the laboratories even when the technique was new to them. The results showed that differences in estimates of infectivity between laboratories was greatest when each laboratory was using its own assay procedures. When the WHO method was used the agreement was good. The combined means for all the laboratories had 95% confidence limits with most minimum and maximum values falling within 0.25 log10 of each other. The reference preparation which had been held at −70 °C for two years had not lost potency.

The National Control Laboratory, London, had prepared 1000 ampoules of the three monovalent viruses and, in view of the results, donated them to WHO for establishment as international reference preparations for virus titres. It is emphasized that these preparations should be used only to demonstrate the sensitivity of the assay system used in the measurement of the virus content of a suspension and not for the estimation of the dilution factor for the preparation of a vaccine from a monovalent bulk.

Killed poliovirus vaccine

Numerous changes have taken place in the production of killed vaccine in recent years, both in the use of substrates, including continuous cell lines, and in techniques for improving the yield, the purification, and the concentration of virus. Vaccine based on the improved methodology is now in production and the WHO requirements for inactivated vaccine have been modified. As the potency of these vaccines may be up to tenfold greater than formerly it is hoped that a two-dose schedule will be sufficient for primary immunization and studies are in progress. The Consultative Group awaits the results. Meanwhile, inactivated vaccine is used for routine immunization in several countries and is recommended in others for certain specific purposes, e.g., for previously unvaccinated adults exposed to high risk.

STOCKS OF SEED VIRUSES

Information made available to the Group by manufacturers and national control laboratories demonstrated that the amount of seed virus available was not sufficient to ensure long-term production. There was also evidence that some strains, especially those belonging to type 3, had undergone more passages than was thought desirable. It was therefore decided to examine Dr Sabin’s original seed viruses (held at the Bureau of Biologics, Bethesda, MD, USA) and to prepare large seed lots from them if they were satisfactory. Because history is embodied in these strains and because of their importance as sources of additional amounts of seed viruses the following details are of interest.

In a letter to the Bureau of Biologics, when he handed over the seeds, Dr Sabin described them as follows:

‘Type 1—LSc 2ab KP2 of 10.10.56. This represents passage 2 in cynomolgus monkey kidney tissue culture from the triply purified plaque that was selected for the vaccine. Approximately 10 ml of this material, that returned by Merck on 9/25/62, is being shipped in the original bottle that was supplied to them.

‘Type 2—P712 Ch2ab-KP2 of 10.10.56. This has the same above history as described for type 1. Approximately 5 ml of the material was returned by Merck on 9/25/62 and is being shipped.

‘Type 3—Leon 12 a1b KP3 of 10.10.56. This represents 3 cynomologus monkey kidney tissue culture passages from the triply purified plaque selected for the vaccine. Approximately 4 to 5 ml of the material in a tissue culture tube is being shipped.’

In the Bureau of Biologics, each type of poliovirus was held in a single container. When the project for the preparation of new seeds was initiated by the Consultative Group the container of each type was opened and the virus distributed in 0.5 ml ampoules which were flame-sealed and stored again at −70 °C or below. This gave a stock of 30 ampoules of type 1 (LSc 2ab KP2), 10 ampoules of type 2 (P712 Ch2ab-KP2), and 9 ampoules of type 3 (Leon 12 a1b KP3). The contents of the ampoules were found to be free from extraneous agents and the virus titres of all 3 types were high.

Preparation of master seeds

As these original viruses were still eminently satisfactory it was decided that they should be used to generate stocks at the SO + 1 (Sabin Original plus one passage) level and that these stocks would be the “master seeds”. Behringwerke AG, Marburg/Lahn, Federal Republic of Germany, generously agreed to produce the seeds for WHO, free of charge. The new seeds were produced directly from the originals in tissue cultures of kidneys of cynomolgus monkey
fetuses obtained by caesarean section within a week before their expected birth. After incubation for 48 to 65 hours (depending on the virus type) the volumes obtained were 4.9 litres of type 1, 4.9 litres of type 2, and 5.8 litres of type 3 with titres per ml (in cerec- pithecus cell cultures) of 8.67 log_{10}, 8.67 log_{10} and 8.62 log_{10}, respectively. The amount of each master seed is considered to be sufficient to meet the world needs of vaccine for the next 200 years.

Tests on the master seeds

The seeds were fully tested in accordance with the WHO requirements for poliomyelitis vaccine (oral) (6) in the Behringwerke laboratories at Marburg/ Lahn and in the national control laboratories in Italy and the United Kingdom. All tests, except the neurovirulence tests, were also made in the Bureau of Biologics, Canada. Extra tests for the presence of extraneous agents were included.

For types 1 and 2 all tests in all four laboratories were satisfactory. All tests for type 3 were also satisfactory except one intraspinal neurovirulence test in the United Kingdom control laboratory. In this test the seed was shown to be active in one of the four parameters used for assessment. The intraspinal test was satisfactory in the Italian laboratory and the intrathalamic test was satisfactory in both laboratories, but a second intraspinal test in the Italian laboratory gave results at the limit of acceptability. Again therefore type 3 virus caused concern. A subgroup reviewed the results and considered three possible courses of action: (a) to retest the seeds; (b) to prepare a new seed lot from the original ampoules; (c) to test extensively the working seed (SO+2), which had just been prepared (see below).

It was concluded that there would always be a doubt about the two borderline tests on the master seed even if the next two tests were satisfactory. Recourse to the original seed would solve the problem if the tests were acceptable but it would mean that the very small stock of the original seed now remaining would be exhausted. The subgroup therefore decided to have the working seed (SO+2) exhaustively tested in a number of different laboratories. This was done in theBehringwerke laboratory and in four others—the national control laboratories in the United Kingdom and Italy and the production laboratories in Iran (Razi Institute) and England (Wellcome Research Laboratories). All the tests were satisfactory and the Group were satisfied that the working seed lot (SO+2) prepared at Behringwerke from the WHO master seed type 3 fulfilled the WHO requirements for acceptable neurovirulence tests. Two lots of vaccine (SO+3) prepared from this working seed were also found to be satisfactory. Approximately 1000 x 1 ml ampoules of each type (SO+2) were sent for storage to each of three national control laboratories (in Italy, the United Kingdom, and the USA).

In the meantime some producers, who had found it difficult to meet the neurovirulence requirements of vaccines made from their own already established working seeds, began to use a Sabin type 3 seed provided by Pfizer Ltd from a plaque prepared from viral RNA-extracted material. This seed has inhibition of growth properties at 40°C (rec/40) similar to other properly attenuated strains. It gives more satisfactory results in the intraspinal test for neurovirulence. The seed is at the fifth passage level (SO+5) and is now identified as RS01. The working seed passage level is therefore RS02 and the vaccine RS03—a level of passage higher than has been used as vaccine hitherto. It has already been used safely and effectively in trivalent vaccines for several years in national immunization programmes involving millions of children.

The WHO master seeds are now available for distribution to national control laboratories and recognized producers of vaccine. The following letter is sent out with the seeds.

"The WHO poliomyelitis virus seed materials prepared from the Sabin original viruses (SO) are being provided to you together with the summary protocols of their production and testing in order that you may prepare your own seed lot, from which vaccine should be made. These seeds must not be used directly for the production of vaccine and your production seed lot shall not be more than one passage from the WHO seeds provided to you. Although WHO has taken all precautions to ensure that the WHO seeds meet the WHO requirements for poliomyelitis vaccine (oral) made from the Sabin strains it should be emphasized that your National Health Authority must accept responsibility for the quality of the vaccine produced from your seeds and used in your country."

Since 1976, about 420 ampoules of seeds of each type have been issued to the production and control laboratories.

Working seeds

As stated above, the master seeds are not to be used for direct vaccine production. The Group therefore decided that a WHO working seed should be prepared from each type so that a supply would be immediately available—for example, if there was an accident which destroyed a manufacturer's own working seeds, or a new manufacturer was required to produce some batches of vaccine quickly, or some other unforeseen urgent need arose.

From the master seed, therefore, the Behringwerke laboratory again agreed to prepare working seeds which were also put at the disposal of WHO free of
cost. Fifteen litres of type 1, 6 litres of type 2, and 11 litres of type 3 were prepared, ampouled, and suitably labelled. All tests on all three types were satisfactory. Approximately 500 x 1 ml ampoules of each type are stored in the Bureau of Biologics, USA, and the National Control Laboratory, United Kingdom, and one litre of type 1 is held in the National Control Laboratory, Italy. The rest is stored at Behringwerke. So far, about 1200 ml of types 1 and 2 and 5000 ml of type 3 have been issued to 19 production and control laboratories.

Thus, within 2-3 years of being established, the Group had succeeded in inspecting the already established laboratories, in authorizing three new laboratories, and in restraining seven others from proceeding with their proposals. It had also established a comprehensive quantity of thoroughly tested master seeds and a bank of working seed—the production and the testing of all of which had been carried out by the manufacturers and national control laboratories on a basis of close collaboration and without cost to WHO, apart from limited travel costs for occasional meetings and for visits of inspection to the laboratories.

OTHER STRAINS OF POLIOVIRUSES FOR VACCINES

Because of the genetic instability of the type 3 vaccine strain and doubts about the duration of the immunity it provided, alternative strains have been investigated by various workers.

USOL-D-bac strain

One alternative was the strain designated USOL-D. Information on its origin, and on the preliminary trials made with it, has been published (8) and is summarized below.

This strain was developed as a candidate for vaccine production in Czechoslovakia in 1962 and was extensively studied in the laboratory there. It was freed of SV40 by the Institute of Poliomyelitis, Moscow, where a batch of vaccine for use in field studies was prepared on green-monkey kidney cells. The seed virus and the vaccine were tested for neurovirulence by intrathalamic and intraspinal tests in laboratories in Moscow, Munich, Milan, London and Bucharest and all the tests confirmed the finding in Czechoslovakia that the degree of residual neurotropism was very low—in most tests less than that of the Leon (Sabin) strain. In Czechoslovakia the vaccine was fed to small groups of children, first those with a history of previous vaccination and then to previously unimmunized children. At the same time, for comparison, similar groups were fed with Leon strain vaccine. The two vaccines gave similar results and such differences as there were favoured the USOL-D strain—the antibody levels with USOL-D tended to be higher and the incidence of reversion of viruses isolated from the vaccinees tended to be less than with the Leon strain (as measured by the rct/40 test), and the neurovirulence of the viruses isolated from vaccinees was never greater and was sometimes less than those of the Leon strain. In the light of these findings, 22,000 persons in one region of Czechoslovakia were fed with a mixed type 2 and type 3 (USOL-D) vaccine. Just under half the vaccinees were unimmunized children 2-14 months of age and a similar number were previously vaccinated children 15-72 months of age. The remainder (about 1700) were adults. No untoward reactions occurred and the serological responses were good. In Moscow and in Berne, Switzerland, small-scale trials also gave satisfactory results.

The work was brought to the attention of WHO by Dr K. Raska, previously Director of the Institute of Hygiene and Epidemiology, Prague, Czechoslovakia, and at the time Director of the Division of Communicable Diseases in WHO. He indicated that the Czechoslovak government would wish to hand over the strain to WHO as an alternative to the Leon strain. The data were considered at meetings of the Directors of WHO Virus Reference Centres in 1965 and 1966 and at a meeting of internationally recognized experts convened in October 1966 to advise the Director-General on the future activities of the virus disease programme of the Organization. Recommendations were made by each of these groups that the strain should be further investigated in collaborative studies under WHO auspices.

Accordingly, interested field and laboratory workers were asked to collaborate in small-scale studies and groups in seven countries agreed to do so and to follow a common plan.

A batch of vaccine was prepared in a recognized production laboratory in Milan, Italy, from an ampoule of the batch of vaccine prepared in the Institute of Poliomyelitis, Moscow, mentioned above. Exhaustive tests in the production laboratory and in the National Institute for Medical Research, London, were satisfactory and confirmed in particular the low level of neurovirulence. In the seven countries between September 1967 and March 1968 a total of 163 children, 3-26 months of age (mostly in residential nurseries) were fed the USOL-D vaccine and 112 of similar ages in similar environments were fed the Leon vaccine. After immunization both the vaccinees and their contacts were kept under surveillance. From these trials, the USOL-D vaccine appeared superior to the Leon strain in respect of implantation in the gut,
immunogenicity, and genetic stability as determined by the rct/40 marker test. In five countries no untoward results were noted, but in two, Romania and the USSR, paralytic cases occurred in contacts of children immunized with USOL-D. In both countries the children were in residential institutions. In Moscow one definite case occurred but the etiology was not clear-cut because the child excreted both type 2 and type 3 poliovirus (a routine immunization programme using trivalent vaccine was in progress in the area at the time of the USOL-D trial). In Romania four paralytic cases occurred in contacts of children immunized with USOL-D and only one recovered completely. All four excreted type 3 and had antibody rises to this type. Two months before the trial a trivalent vaccine was given to schoolchildren in the same area in a routine programme. No cases occurred in association with the Leon strain vaccine in any of the trials. Though the evidence from the studies in the USSR and Romania was not entirely conclusive, it was considered sufficient to abandon further investigation of the strain (8).

In Poland, one of the other countries in the studies, children were fed USOL-D vaccine in two residential nurseries, the first group (8 children) in Poznan in November 1967 and the second (9 children) in Slupsk city in February 1968. Also, in November 1967, 10 children were fed with Leon strain vaccine in a nursery in Bialystok. No untoward events were observed in the three nurseries. However, in Poznan city on 14 March 1968, a four-month-old infant developed typical paralytic poliomyelitis in mid-April and early May two other cases occurred, one in a one-year-old child and one in an adult woman.

Stools from the three cases yielded type 3 virus. In May six more cases occurred in the same area and gradually the outbreak spread through the country though over half the total number were in Poznan city and province. In all, 464 cases were reported, of which at least 455 were classified as definitely or probably due to type 3—the virus was isolated from 341. In the paralytic cases, paralysis of the extremities occurred in 72%, bulbar paralysis in 17%, and other paralytic or meningeal forms in 10%; 40% were left with marked disability and 26% with slight disability. Seventeen patients (4%) died. The highest incidence was in children up to two years of age. It was less in the 2–4-year age group and rose again in the 5–8-year age group. This distribution may have been determined by the previous immunization programme in Poland in which killed type 3 vaccine was used from 1961 to 1968 (live type 1 and type 2 vaccines were used at the same time). The killed vaccine may have been reasonably effective for 2–3 years after immunization but its effectiveness may have begun to decrease significantly after 3–4 years (9).

For the WHO Collaborative Programme the urgent question was whether the USOL-D vaccine was the original source of the epidemic strain. Intensive field investigations failed to reveal direct or indirect contact between the cases in the epidemic and the children in the two vaccinated nurseries, or their contacts. However, because of the association in time between the introduction of the USOL-D strain and the outbreak, a detailed study of strains isolated from cases was made in four laboratories. Samples of faeces were obtained under code from children vaccinated with Leon 12 a,b strain and with USOL-D and from cases in the epidemic. Virus isolations were made in one laboratory and distributed to the other three laboratories. ‘Wild’ strains isolated before vaccines were in use were included in the tests. Altogether 27 strains were submitted to the antigenic and elution marker tests and the rct/40 test. The antigenic and elution markers clearly differentiated the patients’ strains from the Leon 12 a,b strain, but were incapable of detecting whether they were related to the USOL-D strain or to ‘wild’ control strains. The rct/40 marker test was of no help in determining the relation of the strains from patients to either vaccine strain. It was concluded that none of the available marker tests provided conclusive information on the origin of the epidemic strains (10).

Type 3 vaccine from seed pool 6

Another strain investigated was designated ‘seed pool 6’. It was made available to WHO by a manufacturer as a possible WHO seed virus stock for distribution. The seed at the SO + 1 level was prepared on monkey kidney from the Sabin strain. Vaccines (SO + 2) from this seed, also prepared on monkey kidney, had been fed to millions of children and were found safe and effective.

It was considered that the seed could be issued to manufacturers to make working seed at SO + 2 and vaccine at SO + 3, i.e., one passage beyond that of the original seed and vaccine, but since at the time there was a move to substitute human diploid cells for monkey kidney cells as substrate, it was decided that a working seed lot (SO2) should be prepared from seed pool 6 on human diploid cells and a vaccine (SO3) prepared, also on human diploid cells (i.e., the vaccine would have had a double passage on human diploid cells). And that this vaccine should be tested in the laboratory and in small numbers of human beings before the seed and the method of production were given general approval. Accordingly, a batch of vaccine (SO3) was prepared. The laboratory tests were satisfactory and field studies were organized. The vaccine was first given to about 250 children in a residential home without any untoward reaction.
Later, about 3300 children were fed vaccine in a community programme, again without untoward results and with evidence of good serological responses. Finally the vaccine was used in a countrywide campaign in which children were fed sequentially type 1, type 3, and type 2. About 500 000 children, of whom about 200 000 were primary vaccinees, were fed with each type. In the three weeks after the feeding of type 3, three cases of paralysis typical of poliomyelitis occurred 11, 22 and 33 days after immunization. All three cases excreted strains of type 3 virus identified as "vaccine-like" by laboratory tests, and so satisfied the criteria for classification as vaccine-associated cases.

The level of risk was not significantly greater than that observed in the same country with batches of type 3 vaccines of different origin and although it could not be determined from the evidence whether the use of human diploid cell cultures instead of monkey kidney cultures had been important in this episode, the WHO Consultative Group decided not to recommend seed pool 6 for vaccine production on any substrate.

**The Chung type III strain**

This strain is used in the mass immunization campaigns in China. Since 1979, an intensive surveillance has been carried on and three possible vaccine-associated cases due to type 3 have been identified after the distribution of about 60 million doses of vaccine, which is evidence of a high degree of safety. The attenuated vaccine strain was developed from virus isolated from a case of paralytic poliomyelitis. After it was submitted to the Consultative Group it was tested for neurovirulence in an independent laboratory by the intrathalamic and intraspinal tests and was found to be less neurovirulent than the reference virus. Details of the origin and attenuation of the strain by the producer of vaccine in Kunming and of its use in vaccinees in China have been provided by the National Control Laboratory, Beijing. A working pool has been prepared in a production laboratory in Europe and is being held for further study. The strain has some resemblance, by oligonucleotide mapping, to the Sabin type 3 strain, of which it may therefore be a remote derivative.

**Comments**

Of the three strains investigated as possible replacements for the type 3 (Sabin) strain, the Chung strain has not yet been fully tested under the auspices of the Consultative Group. Although the evidence against the seed pool 6 and the USOL-D strains might not be considered conclusive on strictly scientific grounds, the Group decided that the level of doubt about their safety in human beings prevented any further field studies being made. The studies underline the principle that manufacturing processes shown by experience to have an acceptable safety level must be adhered to unless there are overwhelming reasons against them. If it becomes essential to make even an apparently minor change, the new process must be submitted to all the laboratory tests available and to intensive field studies until its safety and efficacy are clearly demonstrated. There must also be continuous laboratory monitoring and field surveillance during routine use.

**CONCLUSIONS**

This article describing some of the work on the control of poliomyelitis, which was carried out under WHO auspices in the past twenty years, shows the necessity for and value of international cooperation in the development of acceptable standards and agreed requirements for the production and control of vaccines and other substances that are needed for the prevention or treatment of diseases, and for the continuous surveillance of supplies and efficacy of the product. This excellent cooperative effort by national control laboratories and poliomyelitis vaccine producers in many countries demonstrates the willingness of governments and commercial bodies to contribute to international activities which have been well planned and coordinated by WHO. Besides the work carried out by members of the Consultative Group there were important practical contributions from a variety of sources. For example, the master seeds and working seeds to meet possible needs over the next 200 years were prepared by a single commercial producer, and exhaustive tests were carried out in the laboratory of production and in six other government and commercial laboratories which provided (free of all costs) the staff, the monkeys and other animals, as well as laboratory materials and space. Similar cooperation was provided for the other laboratory studies undertaken—the testing of new candidate strains of type 3 virus, the investigation of the reproducibility and reliability of marker tests and, particularly, the tripartite project on standardization of the neurovirulence test, which required not only the work of experienced and highly skilled scientists in 13 laboratories but also the provision of over 1000 monkeys for the tests, as well as laboratory equipment and supplies.

Equally outstanding is the work of the epidemiologists and clinicians in the 13 countries, who during 15 years of continuing surveillance provided detailed information about every case of acute persistent spinal paralysis which is known to have occurred.
The results of these efforts are summarized below.

(1) Type 1 live poliovirus vaccine has been confirmed to be as safe and effective as any biological substance can be. Type 2 strain is safe for recipients of the vaccine but on rare occasions can cause paralysis in contacts, so that any contact whose vaccination status is doubtful should be vaccinated at the same time as the original vaccinee. Type 3 strain is much less stable genetically than the other two strains and requires constant monitoring in the laboratory and the field.

(2) There are now available sufficient quantities of thoroughly tested master seed and working seed to supply the global requirements for polio vaccine for the next 200 years.

(3) Agreement has been reached on the techniques for the neurovirulence tests and on the methods for analysing and recording results.

(4) The joint field and laboratory studies of established and new strains demonstrate that, especially in relation to type 3 virus, neurovirulence tests do not necessarily detect all batches of vaccine which are capable of causing occasional cases of paralysis. This important observation underlines the essential need for laboratory tests to be complemented by effective surveillance as a part of every immunization programme.

(5) Methods for identifying virus strains by marker tests have been investigated. The role of current tests has been assessed and this basic information is valuable for the development of tests using new techniques which are expected to provide more accurate and more reproducible means of identifying strains and determining their origin.

(6) In contrast to the above list of positive achievements, the continuing occurrence of vaccine-associated cases due to type 3 and 2 strains in one of the collaborating countries remains an enigma. For this reason, as well as for the surveillance of safety and efficacy of vaccines in general, the Group's work must continue as an integral part of poliomyelitis control activities, especially at the present time when current research suggests that an even more effective vaccine may become available in the future.

(7) IPV has been used in only a few relatively small countries, while OPV has been fed to hundreds of millions of people in every part of the globe. The Group's limited experience with inactivated vaccine indicates that although this vaccine can control the disease for periods as long as two or three decades, there is a risk that a serious breakthrough may occur, particularly with type 3 virus.

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The members of the Consultative Group and the author record with admiration and gratitude the contributions of the late Dr F. Assaad and the late Dr F. T. Perkins, members of the staff of WHO, to the work reported here.

RÉSUMÉ

Le travail du Groupe consultatif OMS sur les vaccins antipoliomyélitiques

L'OMS a largement contribué à l'élaboration de mesures de lutte contre la poliomyélite. Ce rapport expose les activités du Groupe consultatif sur les vaccins antipoliomyélitiques, créé en 1973, et les résultats de l'étude de la paralysie associée à la vaccination, portant sur la période 1970–1984. L'étude des cas de paralysie survenant après administration d'un vaccin oral confirme que ce vaccin est efficace et sans danger lorsqu'il est convenablement surveillé sur le terrain et au laboratoire. Toutefois, la souche vaccinale de type 3 est moins satisfaite que les deux autres et exige une surveillance et un contrôle au laboratoire particulièrement rigoureux. Malheureusement, il n'a pas encore été possible de mettre au point une souche plus satisfaite. On estime que le risque de paralysie post-vaccinale est inférieur à un cas par million d'enfants vaccinés.

La sécurité et l'efficacité des vaccins vivants ont été confirmées dans tous les pays participant à l'étude, sauf pour l'un d'entre eux où les cas de paralysie post-vaccinale continuent de se produire, de façon inexplicable, plus souvent qu'ailleurs. Les résultats de l'enquête conduite sur 15 ans confirment les résultats antérieurs, à savoir que la souche de type 1 n'est pratiquement jamais impliquée dans une paralysie post-vaccinale, que le type 2 est parfois associé à une paralysie chez les contacts du sujet vacciné, et que la majeure partie des cas de paralysie post-vaccinale (qui sont toutefois en très petit nombre) est due au type 3. Les pays utilisant des vaccins tués ont enregistré une bonne protection, mais on a observé un nombre non négligeable d'échecs, en particulier avec le type 3.

Les autres activités du Groupe ont porté sur la préparation et la conservation de virus de sémen, qui devraient
couvrir les besoins en vaccins pour les deux prochains siècles, l'établissement d'épreuves normalisées de neuro-virulence pour les lots de vaccins, la recherche d'épreuves de mise en évidence de "marqueurs" des différentes souches et la recherche (jusqu'à présent infructueuse) de souches de virus de type 3 qui pourraient servir de souches de remplacement pour la production de vaccins.

Ces résultats remarquables sont en partie dus à l'importante contribution des laboratoires nationaux de contrôle et des fabricants de vaccins.

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Annex

Original members of the WHO Consultative Group on Poliomyelitis Vaccines

I. Archetti, Istituto Superiore di Sanita, Rome, Italy
W. C. Cockburn, Lothian Health Board, Edinburgh, Scotland (after 1976)
I. Domingo, National Institute of Hygiene, Budapest, Hungary
S. G. Drozdov, Institute of Poliomyelitis and Viral Encephalitis, Moscow, USSR
J. Furesz, Bureau of Biologics, Drug Directorate, Ottawa, Ontario, Canada
J. Kostrewski, National Institute of Hygiene, Warsaw, Poland
H. M. Meyer, Bureau of Biologics, Food and Drug Administration, Bethesda, MD, USA
I. Tagaya (deceased), National Institute of Health, Tokyo, Japan

WHO Secretariat

W. C. Cockburn, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland (until 1976)
F. T. Perkins (deceased), Biologicals Unit, World Health Organization, Geneva, Switzerland
F. Assaad (deceased), Division of Communicable Diseases, World Health Organization, Geneva, Switzerland
J. L. Melnick, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, TX, USA
A. B. Sabin, Fogarty International Center, National Institutes of Health, Bethesda, MD, USA