

*This review was prepared by an international group convened in Stockholm on 21-25 November 1955. It contains the collective views of this group and does not necessarily represent the decisions or the stated policy of the World Health Organization.*

WORLD HEALTH ORGANIZATION  
TECHNICAL REPORT SERIES

No. 101

# POLIOMYELITIS VACCINATION

## A Preliminary Review

|  | Page |
|--|------|
| 1. Introduction . . . . .  | 3    |
| 2. Experience with poliomyelitis vaccination in various countries . . . . .  | 4    |
| 3. Safety testing. . . . .   | 14   |
| 4. Selection of strains for inactivated poliomyelitis vaccine . . . . .  | 21   |
| 5. Antigenicity tests . . . . .  | 22   |
| 6. Theoretical complications of vaccination against poliomyelitis . . . . .  | 23   |
| 7. Public health application of inactivated poliomyelitis vaccine under different epidemiological conditions . . . . . | 25   |
| 8. Live virus vaccines . . . . .   | 28   |
| 9. Design and techniques of serological surveys . . . . .  | 29   |
| 10. Conclusions . . . . .  | 30   |
| Annex 1. Poliomyelitis vaccine antigenicity and potency tests . . . . .  | 33   |
| Annex 2. Selection of strains for inactivated poliomyelitis vaccine . . . . .  | 36   |
| Annex 3. Present status of work on immunization of human beings with living attenuated poliomyelitis virus . . . . .   | 37   |
| Annex 4. Studies of immunization of man against poliomyelitis with living attenuated virus . . . . .                   | 39   |

WORLD HEALTH ORGANIZATION

PALAIS DES NATIONS

GENEVA

FEBRUARY 1956

## POLIOMYELITIS VACCINATION

### A Preliminary Review

Stockholm, 21-25 November 1955

#### Participants :

- Dr Karl Evang, Director-General of Public Health, Oslo, Norway (*Chairman*)
- Dr S. Gard, Professor of Virus Research, School of Medicine, Karolinska Institutet, Stockholm, Sweden (*Vice-Chairman*)
- Dr J. H. S. Gear, Director of Research, Poliomyelitis Research Foundation, South African Institute for Medical Research, Johannesburg, Union of South Africa (*Rapporteur*)
- Dr R. Haas, Professor, University of Freiburg, Freiburg, South Baden, Germany
- Dr A. D. Langmuir, Communicable Disease Center (Public Health Service), Atlanta, Ga., USA
- Dr P. Lépine, Chef du Service des Virus, Institut Pasteur, Paris, France
- Dr H. von Magnus, Statens Seruminstitut, Copenhagen, Denmark
- Dr F. P. Nagler, Department of National Health and Welfare, Ottawa, Canada
- Dr J. R. Paul, Professor of Preventive Medicine, Yale University School of Medicine, New Haven, Conn., USA
- Dr W. L. M. Perry, Director, Department of Biological Standards, National Institute for Medical Research, London, England
- Dr A. B. Sabin, Professor of Research Pediatrics, University of Cincinnati College of Medicine, The Children's Hospital Research Foundation, Cincinnati, Ohio, USA
- Dr E. T. C. Spooner, Professor of Bacteriology and Immunology, London School of Hygiene and Tropical Medicine, London, England

#### Secretariat :

- Dr W. M. Bonne, Director, Division of Communicable Disease Services, WHO
- Dr A. M.-M. Payne, Chief, Section of Endemo-epidemic Diseases, Division of Communicable Disease Services, WHO (*Secretary*)
- Dr J. T. Warren, Science Attaché, American Embassy, Stockholm, Sweden (WHO Consultant)

This review was originally issued in mimeographed form as document WHO/Polio/17, 29 November 1955.

PRINTED IN SWITZERLAND

# POLIOMYELITIS VACCINATION

## A Preliminary Review

### 1. INTRODUCTION

The discovery by Enders, Weller, and Robbins that poliovirus would multiply in tissue cultures of non-nervous human cells has had many applications. Already it has fulfilled much of its early promise, and important advances in our knowledge of poliomyelitis have been made. It was apparent that this technique could be applied to the production of large amounts of poliovirus, which could in turn be used for the preparation of vaccine. The task of developing this vaccine was undertaken by Dr J. E. Salk, aided by the National Foundation for Infantile Paralysis of the USA. He and his associates in a long series of studies have worked out methods of producing suspensions of poliovirus suitable for the preparation of an inactivated vaccine. They have defined conditions for inactivation of the virus suspensions with formaldehyde and have described in detail the control of the process of inactivation. They have also described a neutralization test of value in determining the antibody response in inoculated human beings and experimental animals.

Based on the principles and methods prescribed by Salk a formalin-treated poliomyelitis vaccine has been produced in large quantities in the USA and in several other countries.

A large-scale field trial of this vaccine was carried out in the USA in 1954 under carefully controlled conditions. The results of this trial were carefully and thoroughly evaluated by a team under Dr T. Francis, and the results of their study were announced in early April 1955.<sup>1</sup> In brief, the findings were that the vaccine was safe and effective.

Immediate steps were taken to apply the vaccine in the USA on a very large scale and preparations were also made in a number of other countries. Probably never in the history of medicine has a new public-health measure been applied so rapidly on a mass scale after the painstaking laboratory research which led to its development. It was almost inevitable, therefore, that this transition should be attended by serious difficulties.

In many countries of the world, information concerning the vaccine reached the public through the popular press before detailed scientific

---

<sup>1</sup> Vaccine Evaluation Center, University of Michigan (1955) *Evaluation of 1954 field trial of poliomyelitis vaccine*, Ann Arbor, Mich.

information had reached the scientists. The enthusiasm with which the news was received was a natural reflection of the great importance of the discovery and of the fear of poliomyelitis which exists in so many countries. Many health authorities were therefore at once subjected to heavy pressure of public opinion to apply the new control measure at the earliest possible moment.

News of accidents following use of the vaccine in the USA led to considerable confusion both among the general public and among health authorities, who were not fully aware of the many problems still remaining to be solved. This confusion has to a large extent persisted in many parts of the world and many health authorities are finding it difficult to decide whether or not they should take steps to bring the new control measure into general use and if so what these steps should be.

However, during the late spring, summer, and autumn of 1955 the vaccine was used on a large scale in the USA, Canada, and Denmark and on a smaller scale in Germany and the Union of South Africa. The time is thus opportune to review the experience in the use of this vaccine in each of these countries in turn, as well as that in France and Sweden, where the problems of vaccination have also been under investigation.

## 2. EXPERIENCE WITH POLIOMYELITIS VACCINATION IN VARIOUS COUNTRIES

The best assessment of the safety and efficacy of any vaccine intended for human use is the result of its use in man. The group therefore summarized the experience of the use of poliomyelitis vaccination in man in their respective countries with brief details of the composition and method of preparation of the vaccine used. It was, however, emphasized that much of the information was preliminary in nature and necessarily incomplete, and in no way purported to be a full report of national experience.

The group noted a preliminary report<sup>1</sup> of the field experience with poliomyelitis vaccine in the USA in 1955. Two main problems are considered: the safety of the vaccine, and the evaluation of the effectiveness of the rather extensive vaccinations that were performed. The following is a summary of parts of this report prepared by Dr A. D. Langmuir.

---

<sup>1</sup> Langmuir, A. D., Nathanson, N. & Hall, W. J. (1956) The surveillance of poliomyelitis in the United States in 1955 (to be published in January in the *American Journal of Public Health*)

Both epidemiological and laboratory evidence established that certain lots of vaccine, distributed by one laboratory, contained poliomyelitis virus in infective amounts. A total of 204 vaccine-associated cases occurred. Of these, 79 were among vaccinated children, 105 among family contacts of vaccinated children, and 20 among community contacts. Approximately three-fourths of the cases were paralytic. There were 11 deaths, making a case-fatality rate of 5%.

The onsets of the cases among vaccinated children were concentrated in the interval from 4 to 14 days following inoculation, whereas the onsets of the family contact cases were concentrated in the period from 15 to 28 days, which represents a double incubation period. The small number of community contact cases occurred at intervals somewhat longer than those of the family contact cases. First paralysis began in the inoculated extremity in two-thirds of the paralytic cases among vaccinated children. This relationship is very similar to that reported by Bodian for cynomolgus monkeys injected intramuscularly with Mahoney virus.

Isolation of poliovirus was reported in association with about half of the paralytic cases and one-third of the non-paralytic cases. Type 1 virus was identified in all but two of these instances, and type 2 and type 3 virus were found only once.

Laboratory tests performed on the 17 lots of vaccine distributed by the laboratory in question resulted in isolation of poliovirus from seven lots. Type 1 virus was isolated from six of these lots, type 2 virus was also found in one of these and type 3 virus in two of these six lots. In the seventh positive lot types 2 and 3 virus were found. Attack-rates of vaccine-associated cases by individual lots of vaccine revealed the highest rates for the six lots from which type 1 virus was isolated.

A small number of cases of poliomyelitis was also associated with a single lot of vaccine distributed by another laboratory. The epidemiological relationships of these cases raised strong suspicion of some explanation other than coincidence. However, extensive laboratory tests of this lot have failed to demonstrate poliovirus.

During the period from 12 April to 7 May, approximately 4 million doses of poliomyelitis vaccine manufactured by five different laboratories were administered to children in the USA. Except for the cases associated with several lots of vaccine from one laboratory and the single lot of vaccine from another no other situation involving the possibility of unsafe lots of vaccine was recognized.

Late in May new safety standards were promulgated. Up to 15 November approximately 21 million additional doses had been released for general use throughout the country by both health departments and private

physicians. The actual number of children inoculated is not yet known. A constant nationwide surveillance for the occurrence of vaccine-associated cases has been maintained. No incident has come to light that tends to incriminate any lot of vaccine, from any manufacturer, that has been used since May 1955.

The limitation of vaccine supplies resulted in the restriction of inoculations to first- and second-grade schoolchildren. The great majority of these received only a single injection until mid-September, when schools re-opened after the summer vacation. Thus a unique opportunity for evaluation studies was presented. Many States rapidly developed plans for special studies in collaboration with the Communicable Disease Center of the Public Health Service.

Preliminary reports up to 1 November have been received from 11 States and one city. They reveal attack-rates for paralytic cases from two to more than five times greater among unvaccinated children than among vaccinated children in the same age-groups. Attack-rates for non-paralytic cases showed less marked but generally favourable reductions.

In evaluating these preliminary reports many possible sources of error must be kept in mind, and substantial changes from the preliminary findings can be expected when final reports are completed. However, the consistency of the favourable differences in paralytic rates in all areas is most encouraging.

An independent method of evaluating the effectiveness of the vaccine was found in an analysis of age-specific attack-rates by individual years of age. Since vaccinations were restricted largely to first- and second-grade schoolchildren, a discontinuity in the rates for 7- and 8-year-old children could be anticipated if the vaccines were effective. Preliminary analyses of data submitted from 33 States for the period from 3 July to 14 October reveal that the attack-rates for paralytic cases among the 7- and 8-year-old children were definitely lower than the rates for either younger or older children or adolescents. Thus independent evidence was found indicating the effectiveness of the vaccine as used in the United States in 1955. Also encouraging is the fact that this beneficial effect followed in large degree the administration of a single dose of vaccine.

Dr F. P. Nagler reported that in Canada production of the poliomyelitis vaccine was undertaken by the Connaught Laboratories, Toronto, using the Maitland tissue-culture technique. Rhesus monkey kidney tissues were employed. Formalinization after sintered-glass filtration and neutralization of the formalinized monovalent strain pools were carried out in a fashion similar to that recorded in the *Minimum Requirements*

of the National Institutes of Health, Bethesda, Md., USA.<sup>1</sup> The strains of virus employed were the same as those used in vaccines prepared by the United States manufacturers. Monovalent and trivalent vaccines were tested in tissue cultures and monkeys by the Connaught Laboratories according to the *Minimum Requirements* mentioned above. Twenty-five lots of trivalent vaccine were submitted to the Department of National Health and Welfare, Ottawa, for clearance under the Canadian Food and Drug Act. The Department tested in monkeys and tissue cultures each of these lots along the lines of the *Minimum Requirements*, with the difference that each tissue-culture passage was observed for one week more than is specified by the United States requirements.

The Connaught vaccine was purchased by the Department of National Health and Welfare and the provincial departments of health, which shared the costs equally. It was distributed by the provincial departments of health to their regional medical officers, who organized and supervised the vaccination programme.

Approximately 860 000 children between the ages of 6 and 9 years were injected during the months of April to June 1955. In most of the provinces two doses of vaccine, spaced four weeks apart, were given. Two provinces administered three doses. The great majority of children received the injection of the vaccine subcutaneously, while approximately 100 000 children were given the vaccine intramuscularly. A surveillance team was established in each province and investigated each case of poliomyelitis both in vaccinated children and in unvaccinated children of the same age-groups. Three cases of paralytic poliomyelitis were reported in children within four weeks of vaccination. A thorough investigation of these cases showed that there was only one case in which there might possibly have been a relationship between the vaccination and the disease. Preliminary and incomplete results from some provinces up to the end of October showed a significant reduction in the incidence of paralysis in the vaccinated children compared with unvaccinated children of similar age-groups.

Production of the vaccine at the Connaught Laboratories has been resumed, employing methods of filtration and control similar to those described in the amended *Minimum Requirements* of the National Institutes of Health. Primary vaccination of first- and second-grade schoolchildren will be resumed early in 1956, and at the same time a booster dose will be given to the children who received their first two injections during the spring of 1955.

---

<sup>1</sup> United States Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health (1955) *Minimum requirements: poliomyelitis vaccine*, Bethesda, Md.

Dr H. von Magnus reported that in Denmark epidemics of poliomyelitis have presented a serious problem for many years, but the epidemic in the autumn of 1952, with a total of 2450 paralytic cases in a population of about 4½ million—a rate of 56.5 per 100 000 population—was the most severe in the history of the country, the range during the previous ten years having been 0.5 in 1951 to 25.5 in 1944. As soon as the results of the large field trial in the USA in 1954 were available, it was therefore decided to start poliomyelitis vaccination immediately. Accordingly, a vaccine prepared at the Statens Seruminstitut, closely following the methods described by Salk and his co-workers, was made available for all children in the first five grades in school (approximately 7-12 years of age). The vaccination programme started on 25 April 1955, and it resulted in the vaccination of approximately 425 000 children during April, May, and June, representing about 98% of the children in these age-groups.

Tissue cultures prepared from trypsinized monkey kidney tissue cells were used for growing poliovirus. The virus strains selected to represent poliovirus of types 1, 2, and 3 were, respectively, the Brunhilde, MEF 1, and Saukett strains. The MEF 1 and Saukett strains were received from Dr Salk's laboratory and are the same as those used by the manufacturers of poliomyelitis vaccine in the USA. The tissue-culture passage of the Brunhilde strain originated from Dr Enders's laboratory and had undergone 12 passages in human tissue culture and, subsequently, about 6 passages in monkey kidney tissue.

Safety tests similar to those outlined in the *Minimum Requirements* of 26 May had been carried out on the vaccine before its release for use.

The vaccine inoculations were given intradermally in two simultaneous injections of 0.1-0.15 ml each, a total of 0.2-0.3 ml per dose. This procedure was repeated after 4-6 weeks, and a third dose will be given 9-12 months after the first. The reactions following the administration of the vaccine by the dermal route were mild and infrequent, consisting mainly of local swelling of the arm on the site of inoculation. A preliminary estimate indicates that this occurred in 0.1%-0.2% of the children.

No cases of paralysis have occurred in any of the vaccinated children in association with the vaccination, and no other serious reactions have been observed in the children.

Blood samples were obtained before vaccination from approximately 2300 children in the second grade of school. A preliminary screening of 2100 of these prevaccination serum samples has been carried out. It was found that 13% of these 8-year-old children had no antibodies to any of the three types of poliovirus. Antibodies to all three types were found in 24% of the sera.

A preliminary study on the antibody response to vaccination in 48 of these children indicates that while the type 2 and type 3 strains seem to have a satisfactory antigenicity under the given circumstances, it would be of advantage if a more antigenic type 1 strain could be incorporated in the vaccine in the future.

Since 1 July a total of approximately 250 000 children in the age-groups 9 months to 7 years have had one or two inoculations of vaccine. No serious reactions have been observed in these children.

The protective effect of the poliomyelitis vaccination carried out in Denmark so far cannot be evaluated, since the incidence of poliomyelitis has been extremely low in 1955, only seven clinical cases of paralytic poliomyelitis having occurred in the country.

Professor P. L epine reported that in France since 1951 the Virus Division of the Institut Pasteur, Paris, has been selecting and studying poliovirus strains of the different types with a view to their application to vaccination. The production of an inactivated vaccine was considered mainly as an interim measure pending consideration of the use of a live attenuated vaccine in the future. Before any attempt at mass production of a vaccine was made, preliminary studies were carried out for over one year on the modification of synthetic media derived from Connaught No. 199 medium to the culture requirements of susceptible cells, such as renal epithelial cells from different African monkey species or fibroblasts of human origin. Since the completion of a specially equipped laboratory for vaccine production, studies have been carried out on the virus yield of cultures under different conditions; on the influence of storage, filtration, temperature, and methods of inactivation on the quality of the final product; and on antigenicity and innocuity tests. Production has gradually been raised to a level of 100 litres per week and some vaccine stock-piled for future use, should the health authorities decide on a vaccination programme.

A group of children 2 to 7 years of age with no poliovirus antibody has been vaccinated by three subcutaneous injections of an inactivated vaccine, and their antibody level has been carefully observed over a period of 14 months to date. At the same time a serological survey of the juvenile population has been carried out and is being extended to the different age-groups and socio-economic levels of the population.

No attempts have been made at mass vaccination in France. A programme for an extended trial vaccination was planned but was withheld pending further information when news of accidents following the use of vaccine in the USA was received.

Professor R. Haas reported that in Germany the production of poliomyelitis vaccine on a large scale has been in progress in the Behringwerke since 1954. So far more than 1500 litres of inactivated virus fluids have been produced. The virus was grown on trypsinized rhesus or cynomolgus kidney cells, using Connaught No. 199 medium diluted with Hank's solution. The strains used were the Mahoney strain for type 1, MEF 1 for type 2, and Saukett for type 3. In September 1955 the Brunhilde strain was substituted for the Mahoney strain. Seitz filtration was used in the production process. Inactivation was performed with formaldehyde. During the first months the vaccine produced was controlled by four different laboratories according to the methods described in the *Minimum Requirements* of the National Institutes of Health and the various amendments thereto, with the difference that larger samples were tested. In March 1955 the Paul Ehrlich Institute, Frankfurt-on-Main, took over the control of the vaccine on behalf of the Government. Control was carried out according to regulations prescribed by the Government of the State of Hessen.

Vaccinations started in November 1954 and were continued until the last week of May 1955. The vaccinations were then stopped following paralysis of two monkeys out of three after intracerebral injection of a special sample of the vaccine. Retests by the Paul Ehrlich Institute on a large scale were entirely negative. But since in the meantime news of the accidents in the USA had been received, the vaccinations were not recommenced and new regulations were formulated in accordance with the *Technical Report on Salk Poliomyelitis Vaccine* of June 1955.<sup>1</sup> It is expected that the new regulations will be put into force at the end of 1955 and that vaccinations will then be recommenced.

From November 1954 till May 1955 nearly 100 000 vaccinations were performed. The exact figures are not known, because many vaccinations were carried out by general practitioners. About 10 000 individuals have been vaccinated by paediatric clinics of different universities and by the medical service of the Behringwerke and affiliated plants. Questionnaires filled in by the doctors showed that no serious reactions have occurred. It is believed that most vaccinations were performed in children in the age-groups from 1 to 10 years and 10 to 15 years, but a certain number of older individuals have also been vaccinated, including some doctors, nurses, and parents. Only a few children received the full course of three injections, most received two, and an unknown number only one. In most cases the vaccine was administered subcutaneously or intramuscularly. So far no case of paralytic poliomyelitis in a vaccinated

---

<sup>1</sup> United States Department of Health, Education, and Welfare, Public Health Service (1955) *Technical report on Salk poliomyelitis vaccine*, Washington, D. C.

child has been reported. In one instance a vaccinated child was thought to have died from poliomyelitis, but this could not be confirmed by laboratory tests. Samples of blood were taken from several hundred of the vaccinated children ; tests are still under way and the results are not yet available.

Dr J. H. S. Gear reported that in the Union of South Africa poliomyelitis vaccine has been produced on a large scale in the laboratories of the Poliomyelitis Research Foundation since 1954. With minor modifications in the process of formalinization, the methods of production have been similar to those worked out in the USA. Trypsinized kidney cells derived from the common South African vervet monkey, *Cercopithecus aethiops pygerythous*, have been used for the preparation of the tissue cultures for the growth of poliovirus.

The strains of virus used were : type 1, Brunhilde strain ; type 2, Collans strain, isolated by Professor van den Ende in Cape Town from the central nervous system of a fatal adult case ; and type 3, Templeon strain, isolated by Dr N. H. Malhuhe from the faeces of a child with a silent infection, who had been in contact in a nursery school with another infant who died of bulbar poliomyelitis.

The Collans and Templeon strains were chosen originally because they regularly produced high titres of virus in tissue culture and were local South African strains.

The vaccine which has been issued in the Union of South Africa was prepared from these strains, but recently it was decided to substitute relatively non-virulent strains for them. These are, at present, undergoing further virulence and antigenicity tests.

Approximately 1000 litres of virus suspension have been produced but only 200 litres have been processed to prepare vaccine.

A committee consisting of virus and public health experts was appointed to advise the Minister of Health on the issue of this vaccine. When the first reports of cases of paralytic poliomyelitis associated with vaccination in the USA were received it was decided that the issue of vaccine be delayed, and the committee recommended that all the vaccine which had passed the United States *Minimum Requirements* then in force should be retested according to the requirements of the United States Public Health Service of 26 May 1955. This has been done, with negative results.

Vaccine sufficient to inoculate 16 000 children was released in September 1955. A priorities committee considered applications for this

vaccine and decided that children under 6 years old and children up to 16 years old of doctors, nurses, and others whose occupation brought them into more than usually close contact with infections should be given first priority.

No case of paralytic poliomyelitis has been reported among the more than 15 000 children receiving the vaccine. Two cases of illness suspected of having been non-paralytic poliomyelitis were investigated and found to be due to other infections. Two mild skin rashes probably of allergic origin were also reported.

Blood specimens have been collected from a representative sample of those vaccinated for antibody studies but they are not complete and the results are not yet available.

Professor S. Gard reported that in Sweden a formalin-treated polio-virus vaccine has been produced and tested on a small scale.

The virus was grown in human embryonic tissue culture and inactivated with formaldehyde of 0.006 molarity for 11 days at 25°C.

In February and March 1955 a field trial on about 2000 schoolchildren in Stockholm was carried out. The experiment was designed as an antigenicity test to permit a direct comparison with the results obtained in the laboratory. The subcutaneous and intradermal routes of inoculation were also compared and the effect of intervals of three and six weeks between first and second inoculations.

No serious side-reactions of any kind were observed. Neutralization tests were carried out on pre- and post-inoculation sera. Although the numbers of children in each test group were too small to permit definite conclusions, the results appeared encouraging.

Children with demonstrable pre-inoculation immunity to not more than one type were selected for a special study. The results obtained are shown in Table II of Annex 1. The fairly satisfactory correlation between the responses in the subcutaneously inoculated children and the guinea-pig antigenicity test seems worthy of note. (For details of this test see Annex 1.)

A vaccination campaign had been planned for the spring of 1955 pending the publication of the Francis report.<sup>1</sup> However, after the release of the news of accidents following vaccination in the USA and on account of the failure of some batches of Swedish vaccine to pass the safety tests, the vaccination programme was called off.

---

<sup>1</sup> Vaccine Evaluation Center, University of Michigan (1955) *Evaluation of 1954 field trial of poliomyelitis vaccine*, Ann Arbor, Mich.

### Summary

From these reports it is evident that poliomyelitis vaccine has been successfully used on a relatively large scale in a number of countries. In the USA certain batches of vaccine have been responsible for causing paralytic poliomyelitis in a small proportion of the children inoculated with them. In other instances their use has resulted in the introduction of infection into a household where it has become manifest by the occurrence of a paralytic case in an uninoculated sibling or parent. In a few instances there has been further limited spread in the community in which the family or child lived.

Except for these incidents (about 200 altogether) associated with a few lots of vaccine, several million children have been inoculated without mishap in the USA. It has recently become clear that this has been of considerable benefit in reducing the incidence of paralytic poliomyelitis, the rate being significantly lower among the vaccinated children than among the unvaccinated children of a similar age-group.

The preliminary reports available for Canada also show a significant reduction in the incidence of paralysis in the 860 000 vaccinated children compared with unvaccinated children of a similar age-group. This age-group, as in the USA, is predominantly that 7 to 9 years old—the only group in which the effectiveness of the vaccine has so far been demonstrated. Sufficient information is not yet available to draw conclusions for other age-groups.

In Denmark, Germany, and the Union of South Africa poliomyelitis vaccine has been produced on a relatively large scale and approximately 800 000 children have been safely inoculated with no serious untoward reactions. It is still too early to assess the value of the vaccine in these countries in preventing paralytic poliomyelitis.

Only time will tell how long the benefit conferred by vaccination will last. A full appraisal of its value will not be possible until children inoculated in early childhood have grown up and passed through the years of childhood and adolescence, when they are most liable to suffer from paralytic poliomyelitis.

Although it has been clearly demonstrated that a safe and effective formalin-treated vaccine can be produced, it is equally evident that its production is associated with some uncertainty and presents problems which merit consideration.

### 3. SAFETY TESTING

The safety tests at present used throughout the world are all based on those devised in the USA and described in the *Minimum Requirements: Poliomyelitis Vaccine* of the National Institutes of Health. These *Minimum Requirements* have been amended from time to time and those in force at the time of this meeting were the first revision, of 12 April 1955, as amended by Amendment No. 1 (19 April 1955), Amendment No. 2 (26 May 1955), Amendment No. 3 (10 September 1955), and Amendment No. 4 (11 November 1955).

The evidence available to the Technical Committee on Poliomyelitis Vaccine of the United States Public Health Service on which the various amendments were based was not all available to the group. The group therefore wishes to record that its review of the safety tests currently applied to the vaccine in different countries was carried out without a detailed knowledge of the most recent evidence in the USA and that, consequently, apparent criticism may be, in part, the result of this lack of knowledge.

#### 3.1 Control of inactivation process

The control of the inactivation of the virus by formalin is defined in the *Minimum Requirements* as follows:

##### *Filtration* (2.4)<sup>[1]</sup>

“Within 72 hours preceding the beginning of inactivation, the virus suspensions shall be filtered through a series of filters of efficiency equivalent to that of an S1 Seitz type filter pad or two sequential ‘ultra-fine’ porcelain or fritted glass candles.

##### *Virus titer* (2.5)

“The titer of the virus after filtration is  $10^{-6}$  or greater as confirmed by comparison in a simultaneous test using 10 tubes at 1 log steps or 5 tubes at 0.5 log steps with a reference virus distributed by the National Institutes of Health. Acceptable titrations of the reference virus do not vary more than  $\pm 1$  log from its labeled titer using 0.5 ml. inoculum in tissue culture.

---

[1] The numbers in parentheses refer to the sections in the *Minimum Requirements*. Only those sections considered of importance for this review are reproduced here by kind permission of the United States Department of Health, Education, and Welfare. For full details reference should be made to the original *Minimum Requirements* (see footnote on page 7) and to the amendments noted above.

*Inactivation of virus (2.6)*

“All virus infectivity is destroyed with certainty by the use of an agent or method which has been demonstrated by the laboratory using the method to be consistently effective and reliable in inactivating a series of lots of poliomyelitis virus. If formaldehyde is used for inactivation, it is added to the virus suspension to a final concentration of USP solution of formaldehyde of 1:4000. Due consideration is given also to (a) the temperature of inactivation; (b) the pH of the mixture; and (c) the concentration of virus and other proteins. Tissue culture tests for rate of inactivation of the virus are made on each container of virus being inactivated. Three or more suitably spaced virus titers are determined during inactivation. A graphic representation of the results is submitted as part of the protocol for each lot. Filtration equivalent to that described in Section 2.4 shall be performed after the estimated base line time, but prior to sampling for the first single strain tissue culture test required in Section 3.2. The virus pool is inactivated allowing an adequate margin of safety for complete inactivation of the virus.

*Additional processing (2.7)*

“Single strain or trivalent pools that have failed to pass required safety tests may be treated as follows:

1. Filtration through a series of filters of efficiency equivalent to that of an S1 Seitz type filter pad or two sequential ‘ultra-fine’ porcelain or fritted glass candles.
2. Two negative tests performed as described in Sections 3.2 and 3.3 must be obtained on samples separated by three days while the material is being subjected to further treatment with 1:4000 formaldehyde and heat at 36°-37°C. In the case of single strain pools the volume tested for each tissue culture safety test shall be 500 ml. and in the case of trivalent pools, 1500 ml.
3. Pools which are positive after such processing shall not be included in a vaccine.

*Supplemental inactivation (2.8)*

“Supplemental inactivation in the primary process employing a method capable of reducing the titer of a similarly produced virus suspension by a factor of 1,000,000 may be applied at any point after the filtration as described in Section 2.6 and prior to the taking of the first sample.

*Single strain vaccine tissue culture tests (3.2)*

“ During inactivation each monovalent bulk strain pool is tested for infectious virus by tissue culture methods before pooling to make the final poliomyelitis vaccine. Two tests are made, separated by an interval of at least three days during inactivation at 37°C.

“ The sample for each test consists of at least 500 ml. The sample is inoculated into five or more tissue culture bottles of a suitable capacity, the ratio of the vaccine to the nutrient fluid being approximately 1 : 1 to 1 : 3, and the area of the surface growth of cells being approximately 3 sq. cm. per ml. of vaccine. The tissue culture bottles are observed for at least 14 days.

“ A first subculture is made by planting at least 2% of the volume from each original bottle into suitable tissue culture containers at the end of seven days followed by refeeding.

“ A second subculture is made from each original bottle in the same manner at the end of 14 days.

“ The first and second subcultures are each observed for at least seven days.

“ If cytopathogenic effect occurs at any time, or if cellular degeneration appears before the seventh day or before degeneration occurs in uninoculated control cultures, the pool is considered infectious if active poliomyelitis virus can be identified, and the pool is not acceptable for the preparation of the final vaccine.”

Thus tissue-culture tests are performed not only at the end of the inactivation period, but also on a sample taken out three days prior to this time ; and it is required that no virus be detectable in either of these samples. It has been estimated<sup>1</sup> that if a sample of 500 ml is examined on each occasion, negative findings ensure a reasonable margin of safety ; these estimations were based on the expected clinical administration of a 1-ml dose of the vaccine to each individual.

The tissue-culture tests are carried out on grown-out cells known to be sensitive to small amounts of poliovirus. Cultures of rhesus kidney cells are most commonly used, but other types of cell (kidney cells from other monkey species, human embryonic cells) may be used if a high sensitivity to poliovirus is ensured.

It is essential that the culture cells be maintained in optimal conditions during the test period. Since media containing animal serum in low

---

<sup>1</sup> United States Department of Health, Education, and Welfare, Public Health Service (1955) *Technical report on Salk poliomyelitis vaccine*, Washington, D. C., Chapter 10

dilutions are used in many laboratories in order to ensure a satisfactory condition of the culture, it should be emphasized that it is essential that such a serum should not contain even minimal amounts of antibody or inhibitor to any of the three types of poliovirus. In some laboratories this difficulty is overcome by using bovine amniotic fluid without the addition of serum as the tissue-culture medium for safety tests.

Another factor which may reduce the sensitivity of the cells is the presence of a latent or slowly growing monkey virus, the "foamy agent" in some cultures of monkey kidney tissue. The risk of such reduced sensitivity may be minimized by distributing the vaccine under test into cultures from at least two different pools of monkey kidney cells.

The consistency with which manufacturers obtain negative results in tissue-culture safety tests on single-strain pools is a major factor in determining safety. However, many instances in different countries are known where consistency has been interrupted for no known reason by the detection, after inactivation, of live virus in several consecutive batches. This has been a very distressing feature of the history of vaccine production. The failure consistently to inactivate is almost certainly related to the shape of the inactivation curve, which, it is now generally agreed, is not always linear. The deviations from linearity are systematic in that the rate of inactivation, when not constant, invariably decreases as the time of treatment is extended. The current view of the Technical Committee on Poliomyelitis Vaccine in the USA<sup>1</sup> is that these deviations are due to the occurrence of protected virus particles in aggregates, and the most recent amendments to the *Minimum Requirements* (Amendment No. 4, 11 November 1955) are designed primarily to remove these aggregates during the inactivation procedure. This view may be the correct one. However, the detailed evidence upon which it is based was not available to the group; and, under the circumstances, it was impossible to exclude other explanations of the phenomenon. In certain laboratory studies of the kinetics of inactivation have indicated that reduction in the reaction rate continues throughout the period of inactivation, a phenomenon observed also in the inactivation of certain other viruses by other inactivating agents. The experiments therefore suggested that the laws governing reactions between simple molecules may not be directly applicable to the inactivation of viruses, and that this may be the fundamental cause of the non-linearity of the inactivation curve.

In some countries the period of inactivation is determined from a study of the shape of the inactivation curve, and the group expressed

---

<sup>1</sup> United States Department of Health, Education, and Welfare, Public Health Service (1955) *Interim report of the Public Health Service's Technical Committee on Poliomyelitis Vaccine*, Washington, D. C.

concern that the interpolation of a filtration step during the process of inactivation, as suggested in the fourth amendment to the *Minimum Requirements*, might make the interpretation of the shape of such curves impracticable.

Should the current view of the Technical Committee on Poliomyelitis Vaccine of the US Public Health Service be confirmed, a much higher degree of consistency in inactivation of virus might be expected, and this would represent a major achievement in securing the safety of the vaccine.

Nevertheless, even if a clinically safe product were consistently obtained, it must be recognized that, owing to the nature of the inactivation process itself, no assurance could ever be given that the last trace of active virus has been removed. The existing tissue-culture safety tests have been estimated<sup>1</sup> to ensure, with a probability of 0.99999, the absence of more than five infective units per litre of vaccine. To ensure certainty of freedom from all live virus particles is completely impracticable. Whether the residuum of active particles is of clinical significance is still unknown. The provisional results of the large-scale vaccination campaign in the USA indicate that it did not appear to be of significance in children aged 7-9 years ; the relative sensitivity of younger children, among whom poliomyelitis represents the major problem in many countries, is unknown. It would therefore appear that, although it is important to improve the sensitivity of the methods of test, more reliance should be placed on the incorporation in the vaccine of strains of virus attenuated as far as is consistent with the maintenance of adequate antigenicity (see section 4). If this were done, the danger from traces of residual active virus would be minimized.

### 3.2 Final tissue-culture safety test

The final tissue-culture safety test applied to the vaccine is defined in the *Minimum Requirements* as follows :

#### *Final vaccine tissue culture test (3.3)*

“ Each lot of final vaccine is prepared by pooling approximately equal parts of monovalent bulk strain pools which have passed all tests described in Section 3.2.

“ A sample consisting of at least 1500 ml. of the final vaccine is tested by tissue culture methods and its acceptability determined as described in Section 3.2.

“ Furthermore, a lot which has yielded a positive tissue culture test in the final pool is not acceptable for release unless it passes two

---

<sup>1</sup> United States Department of Health, Education, and Welfare, Public Health Service (1955) *Technical report on Salk poliomyelitis vaccine*, Washington, D. C., p. 69

consecutive negative tissue culture tests with separate samples of at least 1500 ml. each. The final pool may not be reheated."

Thus from each lot of final vaccine a 1500-ml sample is tested by tissue-culture methods identical to those used in the control of the inactivation process. The acceptability of the final vaccine is dependent on this test.

If a lot has yielded a positive tissue-culture test in the final pool, it may be acceptable for release—since accidental contamination of a tissue culture can occur—if it subsequently passes at least two consecutive negative tissue-culture tests with separate samples of at least 1500 ml each.

### 3.3 Monkey safety test

The monkey safety test applied to the vaccine is defined in the *Minimum Requirements* as follows :

#### *Final vaccine test for active virus in monkeys (3.5)*

"Vaccine from a sufficient number of final containers selected at random from each filling of each lot is pooled to provide a test sample of at least 100 ml representing the filling. The test sample is inoculated into a group of five or more monkeys, at least four of which must survive the test period, without weight loss exceeding 10 per cent. of original weight. Animals which fail to survive the first 48 hours after injection may be discarded and replaced by an equal number. If less than four animals in a test survive the observation period, two may be replaced if three survive, but the test must be repeated if less than three survive.

"If the number of fillings of the lot is less than four, at least twenty monkeys are used.

"The vaccine is injected by combined intracerebral, intraspinal, and intramuscular routes into healthy rhesus or cynomolgus monkeys under deep barbiturate anesthesia. The intracerebral injection consists of 0.5 ml. into the thalamic region of each hemisphere. The intraspinal injection consists of 0.5 ml. into the lumbar spinal cord enlargement. The intramuscular injection consists of 1.0 ml. injected into the right leg muscles. At the same time an injection of 200 mgms of cortisone acetate is given into the left leg muscles, and 1 ml. of procaine penicillin (300,000 units) into the right arm muscles. The monkeys are observed for 18 days and symptoms suggestive of poliomyelitis are recorded.

"Samples of nervous tissue at the end of the observation period are taken for virus recovery and identification. Histological sections are prepared from both spinal cord enlargements and examined.

(A pre-injection serum sample must contain no neutralizing antibody against the three poliomyelitis virus types in a dilution of 1:4 when tested against not more than 1,000 TCID<sub>50</sub> doses of virus.)

(3.51) "The monkeys inoculated as described in Section 4.61 are sacrificed at the time of collection of the final serum samples described in Section 4.62.<sup>[1]</sup> Histological sections are prepared from both spinal cord enlargements and examined.

(3.52) "Doubtful histopathological findings necessitate (1) examination of a sample of sections from several regions of the brain in question and (2) attempts at virus recovery from the nervous tissues previously removed from the animal. The test is considered negative if the histological and other studies leave no doubt that poliomyelitis infection did not occur."

The group noted the relevant changes made in the *Minimum Requirements* in Amendment No. 4, 11 November 1955; but in the absence of detailed information about the reasons for introducing them, it did not feel competent to review them fully. Concern was expressed about the effect of injecting 0.5 ml of vaccine intraspinally in monkeys, since experience in several countries indicated that this relatively large volume of fluid often causes traumatic paralysis with lesions which could, however, be distinguished by experienced histologists from the effects of poliomyelitis infection. Furthermore, since cortisone is known to increase susceptibility to other infections more efficiently when given not as a single dose but in divided doses, it was considered that certain countries might wish to investigate a modification of this kind.

Concern was also expressed about the possible interactions that might occur from the simultaneous administration of vaccine by the intracerebral, intraspinal, and intramuscular routes. Possibilities of interference were created, and, in particular, it was felt that antibody formation resulting from intramuscular dosage might prevent the full development of the infection resulting from the intracerebral inoculation, especially since the period of observation had been shortened to 18 days. Experience in several countries suggested that the intramuscular dose in these monkeys might well be abandoned, and that a further separate test of the intramuscular injection of a much larger volume in cynomolgus monkeys might be re-introduced.

### 3.4 Other safety tests

The vaccine, besides being tested for the absence of detectable live poliovirus, is also subjected to tests to ensure the absence of other agents

---

[1] These sections are not reproduced here. They concern potency tests in monkeys.

pathogenic for man. In addition to routine sterility tests, these tests include examination of the vaccine for the presence of B virus, lymphocytic-choriomeningitis virus, and *Mycobacterium tuberculosis*. None of these tests, devised in the USA, has presented serious technical problems. The tests are defined in the *Minimum Requirements* as follows :

*The virus pool (3.1)*

“Prior to inactivation each virus pool is tested for the presence of B virus and of *Mycobacterium tuberculosis*. For the test for B virus two or more rabbits are each inoculated with 1.0 ml. intracutaneously into multiple sites and 9 ml. or more subcutaneously. Alternate routes of inoculation may be used if these are shown to be of equal sensitivity. For the test for *M. tuberculosis* four or more guinea pigs are inoculated intraperitoneally. The rabbits are observed daily for at least 21 days and at least two rabbits must survive for this period. The guinea pigs are observed for 42 days and at least two guinea pigs must survive for this period, at which time they are necropsied. Animals which sicken or die after the first day of the test period are necropsied and studied by culture or animal passage as indicated, to determine the infecting agent. Each virus pool is also cultured directly for the presence of *M. tuberculosis*. The virus pool is discarded if there is evidence of any infectious agent pathogenic for man.

*Final vaccine lymphocytic-choriomeningitis test (3.4)*

“For the test for lymphocytic-choriomeningitis virus ten or more mice are inoculated intracerebrally. The mice are observed daily for at least 21 days and at least eight mice must survive for this period. Mice which sicken or die after the first day of the test period are necropsied and studied by culture or animal passage as indicated, to determine the infecting agent. The vaccine pool is discarded if there is evidence of any infectious agent pathogenic for man.”

#### 4. SELECTION OF STRAINS FOR INACTIVATED POLIOMYELITIS VACCINE

Minute amounts of virus which may escape the process of inactivation can be dangerous only if the strains that are used are of such a character that minimal amounts can multiply extensively after intramuscular, subcutaneous, or intracutaneous injection, and if the multiplied virus possesses sufficiently high neurotropic activity to cause paralysis in man.

The group discussed the basis upon which a valid selection of attenuated strains could be made (see Annex 2) and agreed that the following criteria

could be used in selecting the best strains of each type for inclusion in an inactivated virus vaccine :

1. *Maximum antigenicity after inactivation*, as measured by a quantitative test in guinea-pigs, rabbits, monkeys, or other suitable animals ;
2. *Least neurotropic activity*, as measured by intraspinal titration in cynomolgus monkeys ;
3. *Least capacity for multiplication after intramuscular, subcutaneous, or intracutaneous injection*, as measured by a titration in monkeys designed to test the capacity of small amounts of virus inoculated by these routes to produce antibody.

The selection of a strain of each type possessing the optimum available combination of the above three characteristics can now be made from among several strains which have already been shown to possess a very low neurotropic activity in monkeys.

The group considered the possibility that vaccine made from strains isolated in the geographical area in which the vaccine was to be used might be more effective than vaccine made from strains isolated elsewhere, but it agreed that there was no reason for believing this to be likely. The group also discussed the effect of incorporating attenuated strains in the vaccine on the protective power of the resulting material. The group did not consider that there was any *a priori* reason for suspecting that the substitution of a strain selected in the manner outlined above would alter the protective power of the vaccine and consequently did not consider that a field trial, although highly desirable, was an essential preliminary to its use.

## 5. ANTIGENICITY TESTS

The group considered the tests in current use to determine the potency of vaccine. Some of these tests aim at assaying the capacity of a given vaccine to produce antibodies at high titre levels ; others, at determining the minimal amounts of vaccine necessary to elicit a demonstrable immune response. Most laboratories have used monkeys for these tests. In practice, however, it has been found that the antibody response of monkeys is variable and often poor, even with vaccines of known good antigenicity. The use of rabbits and guinea-pigs has yielded more consistent results. The group was particularly impressed by a test in guinea-pigs currently in use in Sweden (see Annex 1).

As poliovirus does not multiply in the tissues of the rabbit or guinea-pig as it does in the monkey and as it may do in the mouse, the use of the former animals has the added advantage that such tests determine the true

antigenic value of an inactivated vaccine irrespective of whether it contains residual live virus particles or not.

The group also noted that the antigenicity of poliomyelitis vaccine could be determined by measuring its antibody-combining capacity. In this test<sup>1</sup> the effective antigen combines with antibody; and by titrating virus in the presence of the serum-vaccine mixture, the amount of effective antigen is reflected by the antibody that is combined.

The group emphasized that, in assessing the antigenicity of any vaccine, a comparison should be made with the results given by a reference vaccine,<sup>2</sup> which should, if possible, have been standardized previously in children and should have been proved to be stable.

## 6. THEORETICAL COMPLICATIONS OF VACCINATION AGAINST POLIOMYELITIS

In the long history of vaccination against various infections there have been several minor incidents and some major disasters following the use of vaccines. It is, therefore, wise to consider the theoretical dangers inherent in the use of poliovirus vaccines other than those associated with the presence of live virus. These may be classified as follows: (1) sensitization to one or more of the constituents of the vaccine;<sup>3</sup> (2) allergic reactions; (3) toxic reactions; (4) neurological sequelae; and (5) paralytic poliomyelitis, resulting from the provocative effect of the inoculation (see section 7).

These possibilities may now be considered in turn.

### 6.1 Sensitization

#### *Antibiotics*

Sensitization may occur to any of the constituents of the vaccine but appear to be of most importance in relation to the antibiotics included in the vaccine. It was considered that the amounts of penicillin and streptomycin in poliomyelitis vaccine were so small that the danger of sensitization to these antibiotics as a result of vaccination was very slight.

---

<sup>1</sup> Salk, J. E. (1955) *Outline of procedure for performing test for antigenic potency of poliomyelitis vaccine by measuring its antibody-combining capacity* (Unpublished working document WHO/Poliomyelitis/16). See also Krech, U. (1955) *J. exp. Med.*, **101**, 331.

<sup>2</sup> In the USA, Dr Salk has used Reference Vaccine A and has offered to make this available to those undertaking vaccine production.

<sup>3</sup> For an outline of the constitution of poliomyelitis vaccine, see section 2 of the *Minimum Requirements: Poliomyelitis Vaccine*. The nutrient fluid generally used is Connaught No. 199 medium with the addition of animal serum during the growing-out phase of the culture.

Nevertheless, the results of sensitization of even a very few individuals might be so serious that the problem must be kept in mind. Consequently, the desirability of employing an alternative, less widely used, antibiotic in the production of poliomyelitis vaccine should be considered.

#### *Rh sensitization*

Theoretically this is a possibility resulting from the antibodies formed against antigens in the vaccine derived from the components of monkey kidney cells or monkey red cells present in the tissue cultures. Recent work, however, has failed to detect any trace of Rh factor in the vaccine.

#### *Kidney damage*

Anti-kidney antibodies formed in response to the inoculation of poliomyelitis vaccine may theoretically predispose to kidney damage. Such few studies as have been carried out appear to indicate that this theoretical possibility is of little, if any, practical importance. However, it is recommended that data bearing on this problem should be accumulated, so that in time more accurate appreciation of the danger will be possible.

#### *Animal serum*

Animal serum is added to the medium in the primary phase of the tissue-culture growth, but not in the phase of virus multiplication, and therefore the serum originally added is highly diluted. As the amount is so small, sensitization from this source should be rare.

In spite of the apparently negligible dangers of sensitization, the group considered that studies should be continued to find ways of eliminating as much foreign protein as possible. The other constituents of the vaccine, being of a non-protein nature, are not likely to result in sensitization.

### **6.2 Allergic reactions**

It may be anticipated that, in any vaccination campaign against poliomyelitis, examples of allergic reactions against one or other of the constituents of the vaccine will be found; already this anticipation has been realized and a number of such cases have been reported. Fortunately, all of them have so far been of a minor nature.

### **6.3 Toxic reactions**

As the constituents of poliomyelitis vaccine are in themselves relatively non-toxic, there is no reason to think that toxic reactions will be frequent. However, such reactions may occur if there is accidental bacterial, fungal, or other contamination during the preparation; stringent control of the

sterility of the vaccine at all stages of manufacture will guard against their occurrence.

#### 6.4 Neurological sequelae

In the case of poliomyelitis vaccine, neurological sequelae such as polyneuritis, radiculitis, and encephalopathy are possible, but as this vaccine is a much more highly refined product and contains much less protein than other vaccines in common use it is probable that neurological sequelae will be extremely rare.

(The possible provoking effect of injections of poliomyelitis vaccine is considered in section 7.)

### 7. PUBLIC HEALTH APPLICATION OF INACTIVATED POLIOMYELITIS VACCINE UNDER DIFFERENT EPIDEMIOLOGICAL CONDITIONS

There are a number of questions to which the health officer needs an answer before he can decide whether to recommend poliomyelitis vaccination as a general public health measure.

The first question is whether the vaccine is safe for use on a mass scale, and whether commercial production is sufficiently developed to produce consistently a safe product.

These problems have been discussed in previous sections of this report and it should be noted that, apart from the major incident described in section 2 and one relatively minor incident not as yet fully clarified, there has been no evidence that the vaccine caused poliomyelitis among the 10 million or more children who have been inoculated. The latest developments in production techniques and methods of testing should be a further assurance of the safety of the product.

The second question is whether the vaccine is effective. The conclusions of the Francis report<sup>1</sup> and the experience outlined in section 2 show that it has been proved to be effective under certain conditions in school-children between the ages of 6 and 10 years. Adequate information is still lacking as to its effectiveness in other age-groups, and information is especially needed regarding very young children. Nor is it known how effective it will be under very different epidemiological circumstances.

---

<sup>1</sup> Vaccine Evaluation Center, University of Michigan (1955) *Evaluation of 1954 field trial of poliomyelitis vaccine*, Ann Arbor, Mich.

The third question health authorities may ask is whether the vaccine should be applied on a mass scale everywhere, or whether its use should be restricted to specific epidemiological conditions; and whether there are circumstances in which it is inadvisable to apply mass vaccination. The answer to this should be derived from two main considerations, which are to some extent interdependent. On the one hand, there is the seriousness of the problem of poliomyelitis in the country concerned, that is to say, the incidence of paralytic disease. The age incidence must also be considered since paralytic poliomyelitis tends to be more severe in adult life. On the other hand, there are the cost and the practicability of a mass vaccination programme, which must be considered in relation to the funds and facilities available, to the other demands upon the available funds, and to the saving in human suffering and in the cost of hospital and social care.

In many countries it may be difficult to determine these facts. Reliable statistical information may be lacking. Every effort should therefore be made to determine the actual situation in the country before reaching a decision. This information will also be essential for the planning of a mass programme. If such a programme is planned, a decision will have to be taken as to which age-groups to immunize. There are two ways in which this could be done. The first is by a study of the age incidence of paralytic poliomyelitis, preferably accumulated over a number of years. For example, it has been confirmed by serological studies that where environmental sanitation is still inadequate the disease is usually predominantly infantile—90% or more of the cases occurring in the first two or three years of life—and almost everyone over the age of 5 years is immune; thus there is no point in vaccinating them. On the other hand, in countries where as many as one-third of all cases occur over the age of 15 years it may be necessary to vaccinate persons up to the age of 40 or even older.

In many areas, reliable information regarding the age incidence of poliomyelitis is lacking. Where this is so every effort should be made to improve the collection of morbidity statistics as soon as possible. However, as an interim measure, valuable information can be obtained from properly designed serological surveys to determine the immunity status of the population as outlined in section 9. It should, however, be emphasized that where adequate morbidity statistics are available serological surveys need not be considered a prerequisite for a vaccination programme.

In the present state of knowledge there are many questions which cannot be answered. In a country where the disease is practically restricted to early childhood it is not yet known how effective the vaccine will be,

nor is it known what effect vaccination will have on the epidemiological situation. Should it prove that mass vaccination will greatly reduce dissemination of poliomyelitis virus in an area, artificial immunity will not be reinforced by "natural" exposure, and the vaccinated population will be immune only for as long as the protection of the vaccine lasts, unless reinforcing doses are given at regular intervals. There is at present no knowledge of the long-term effects of vaccination. The possibility must be considered that in such areas the favourable balance between infection and immunity which at present exists may be upset.

On the other hand, it must be recognized that in recent years many countries in tropical and sub-tropical areas have noted an increase in the incidence of poliomyelitis, and there is reason to believe that poliomyelitis may become a public health problem of major importance in areas where now it is considered of minor importance. It is therefore emphasized that every effort should be made to acquire a better knowledge of the poliomyelitis problem in all countries as soon as possible by the improvement of statistical information and, if necessary, by suitable serological surveys. It should be noted here that in small islands and other isolated communities serological surveys may be the only way of obtaining a true picture of the situation. Although poliomyelitis virus may only rarely be introduced into such communities, it may cause devastating epidemics when this does happen.

Practical problems which face the public health administration in planning a vaccination campaign include such questions as the dosage; the route of injection; the number of injections and the intervals between them; the duration of immunity and the need for reinforcing doses; the probable incidence of complications and their treatment; the control of vaccine production and of the finished product; and the stability of vaccine under different conditions of storage, climate, and transport. It is considered undesirable at the present time to attempt to lay down any specifications which might restrict further developments. The following remarks should therefore be considered only as a guide which will certainly have to be modified as experience is accumulated.

Vaccine has been administered intramuscularly, subcutaneously, or intradermally. The optimum dosage schedule has not yet been determined. At the present time, the recommended dose is 1 ml intramuscularly or subcutaneously, or about 0.2 to 0.5 ml intradermally in two sites. The first two doses have usually been given at an interval of about one month and followed by a reinforcing dose several months later. Recent experience in the USA suggests that a significant degree of protection may be conferred by a single dose.

The provocation of paralytic poliomyelitis by inoculations, particularly intramuscular inoculations which cause local reactions, seems now to be established. There is no reason to assume that inoculation of poliomyelitis vaccine is completely free from this risk, although its very slight local irritant action may minimize the risk.

The assessment of the risk of provocation, if any, is of importance in deciding whether to carry on mass vaccination campaigns during epidemics of poliomyelitis. Opinions of the probable frequency with which provocation may occur as a result of poliomyelitis vaccination vary from zero to relatively high figures, and it is clear that no opinion is fully supported by adequate evidence.

It is recognized that health authorities may be faced with a virtual necessity to carry out mass vaccination of populations threatened with a large epidemic. It is impossible, for the reasons stated, to give clear guidance on the possible dangers of provocation, which would in any case be set against the benefits of the immunity produced. It is nevertheless essential that health authorities should be aware that the absence of danger has not yet been proved.

It would therefore seem wise to conduct mass vaccination programmes at times when the incidence of poliomyelitis is usually low. This is recommended if only because of the danger that the chance occurrence of poliomyelitis in a vaccinated child might be ascribed to the vaccine even though the latter may be perfectly safe. Mainly for the reasons mentioned above, it seems inadvisable to vaccinate family contacts of established poliomyelitis cases.

Duration of immunity following vaccination is at present unknown. Neutralizing antibodies may be detected for a few years at least. More information is needed on the relationship between the antibody level and the resistance to infection, but there are grounds for believing that the presence of detectable antibody implies a significant degree of resistance to the paralytic disease.

Discussion of some of the other questions mentioned above will be found in other sections of this review.

## 8. LIVE VIRUS VACCINES<sup>1</sup>

The group considered reports on the present position regarding vaccination against poliomyelitis with attenuated virus vaccine.

---

<sup>1</sup> A summary of the information presented to the group in a report by Professor A. B. Sabin and in working documents by Dr Hilary Koprowski will be found in Annexes 3 and 4 respectively.

It was agreed that living, attenuated poliomyelitis vaccine was in the early experimental stages of development. Studies with the best strains currently available should be encouraged ultimately to provide a sufficiently large field experience of the possibility and safety of using such a product under different conditions—e.g., among infants during the first few months of life possessing various levels of placentally transmitted antibody; among children possessing different levels of antibody produced by inactivated virus vaccine; and among other individuals as a primary method of immunization.

## 9. DESIGN AND TECHNIQUES OF SEROLOGICAL SURVEYS

The basic idea of serological surveys is that of determining the local frequency of a given infection by ascertaining how many people in a given area have developed antibodies to a given causative agent or sub-type of that agent. In essence, these surveys are often a means of determining the historical and also the current prevalence of a given infection. They differ from estimates based on attack-rates of clinical cases recorded within a given area during a given period of time in that the serological approach detects with some degree of accuracy the subclinical and inapparent infections as well as the clinical. Thus the approach is comparable to Schick-test surveys for diphtheria or tuberculin-test surveys in various age-groups within different segments of a population. In the case of poliomyelitis one can also determine the relative frequency with which representatives of each of the three types of poliovirus have caused infection. Furthermore, the survey offers a more accurate means of determining the incidence of this disease in various socio-economic levels of the population than do case-finding and case-reporting.

Assuming that vaccination against poliomyelitis may be accepted as a recognized public health procedure in the near future, a problem which will confront health authorities will be to decide which age-groups in the various sections of the population seem to be in most need of immunization and to estimate the benefits likely to be obtained in relation to the cost.

In countries with accurate records of the age incidence of paralytic poliomyelitis over a number of years (one or two decades), a reasonable decision and estimate could be made on that basis alone. However, in many countries and in special areas of certain countries such records are not available or are incomplete. In these countries, serological surveys can often serve to give information on the general immunological status

of these population groups. From them one can often determine which are the most vulnerable local age-groups, and, granted that a certain percentage of paralytic cases can be prevented in these age-groups through vaccination, one might be able to make better estimates of benefits which could be gained than could be done in the absence of such information.

Surveys are time-consuming and expensive. Consequently, they should not be undertaken without a well-defined purpose and without careful planning and assurance that facilities for doing the work properly are available. It may be mentioned here that once a survey of poliomyelitis antibodies has been decided upon, its various objectives should be carefully considered.<sup>1</sup>

The indications for carrying out a pre-vaccination poliomyelitis antibody survey are, therefore :

(1) that the information desired within a given population cannot be obtained from existing data based on the local age incidence of paralytic poliomyelitis over an adequate period of recent years, i.e., at least one decade ; and

(2) that facilities, time, personnel, and local interest exist for carrying out such work.

If a serological survey is decided upon, its statistical adequacy should be given serious consideration. Primarily the sample or samples of the population chosen should be truly representative and should be of adequate size. This has not been sufficiently emphasized in the past. Indeed, many of the surveys hitherto published cannot be accepted as valid on statistical grounds. The solution of this problem would appear to be a compromise between requirements for statistical significance at a given level and the practicability of collecting and examining large numbers of sera.

The group noted that there was urgent need for guidance on the conduct of these surveys in many countries.

## 10. CONCLUSIONS

### 10.1 General

1. The group considered that, subject to the application of the safeguards contained in paragraphs 2, 3, and 4 below, the results obtained with poliomyelitis vaccine in mass immunization campaigns already carried

---

<sup>1</sup> These objectives need not necessarily be limited to measurements of poliovirus antibodies ; for it is quite possible that if enough serum is obtained and stored (either in a frozen or lyophilized state) that serum may also be used for other antibody surveys, if it is so desired.

out in various countries justified the conclusion that countries with a high incidence of paralytic poliomyelitis should plan to bring vaccination into routine use at an early date. In countries with a low incidence of paralytic poliomyelitis a decision to vaccinate should only be made after a careful review of the many other factors discussed in this review.

2. The group recommended that, especially in countries starting vaccine production on a large scale for the first time, every effort should be made to incorporate in the vaccine strains of virus attenuated as far as is consistent with the maintenance of adequate antigenicity after inactivation. If this course were followed, the danger arising from failure to detect traces of residual active virus would be minimized.

3. The production and testing of poliomyelitis vaccine require considerable experience and the highest technical skill if accidents are to be avoided. It is recommended that they should not be attempted unless technical personnel and equipment of a very high standard are available, and until the technical staff have been thoroughly trained in the essential techniques. In view of the difficulty and expense of meeting this recommendation, countries with limited resources might consider some form of co-operation.

4. The continued successful use of poliomyelitis vaccination, as well as some future developments in this field, may depend on knowledge of the characteristics of the prevalent poliomyelitis viruses in various parts of the world and of other viruses causing similar clinical syndromes. It is recommended that WHO should enlist the co-operation of national laboratories with the existing network of WHO regional poliomyelitis laboratories in order to facilitate the collection, interchange, and study of poliomyelitis viruses, and that laboratories co-operating in the network should also be encouraged to exchange and study viruses which may be responsible for clinical disease resembling poliomyelitis. Cases of such infections may be mistaken for poliomyelitis and therefore regarded as vaccine failures unless thoroughly investigated.

## 10.2 Further research

Many problems for the solution of which further research is needed have been indicated in this review. Only the most important of these will be mentioned here. Several of these studies are already under way.

1. It is recommended that investigations be continued with a view to the precise definition of the underlying mechanisms of the inactivation process of poliovirus, especially during the final stages. The safety of the vaccine is fundamentally dependent on knowledge of this process. The occurrence of occasional and unexpected failures of inactivation and the fact that there are divergent views on the process are a cause for concern.

2. There is now good evidence that the resistance of man to paralytic poliomyelitis is related to the presence of neutralizing antibody in the serum. However, further studies are needed to define more precisely (a) the level of antibody necessary to prevent paralysis, and (b) the level of antibody which will prevent infection.

3. Estimates of the antigenicity of all vaccines in routine production depend upon laboratory tests on animals. These tests are of real value only when the results have been related to the effect in man. It is therefore recommended that further investigations should be carried out to determine the correlation between the results of laboratory tests of antigenicity, antibody production in man, and the actual protection afforded to man.

4. Further information of the effectiveness of the vaccine in very young children is required.

5. Studies of the effect of mass vaccination of the susceptible age-groups on the incidence of infection with poliovirus in the vaccinated population are needed.

6. The duration of the immunity conferred by the vaccine should be further studied by every available means.

7. Further investigations are needed on the development in the laboratory, the isolation in the field, and the selection of attenuated strains of poliomyelitis viruses which might be used either in inactivated vaccines or in live virus vaccines.

8. The design and techniques of serological surveys as they apply to poliomyelitis should be further investigated, particularly with regard to the statistical adequacy and problems of uniformity.

---

## Annex 1

POLIOMYELITIS VACCINE ANTIGENICITY  
AND POTENCY TESTS

## Antigenicity Tests\*

A vaccine antigenicity test should fulfil two requirements :

1. It should be quantitative.
2. The quality measured should be correlated to the capacity of the vaccine to afford protection against the paralytic disease.

In vitro methods of assay of antigenicity have been devised (measurement of antibody-combining capacity according to Krech or of complement-fixing capacity according to Le Bouvier). As, however, antigenic activity in vitro is not necessarily associated with immunizing capacity, it seems hardly justifiable to rely upon such methods for the present purpose.

Protective effect is, of course, most adequately estimated by means of a challenge technique. But the challenge should be administered so as to resemble the "natural" infection as much as possible. It is doubtful whether the mouse challenge test fulfils this requirement. Besides, an animal which, like the mouse, possesses a high natural resistance to infection with poliovirus seems *a priori* hardly suited for such tests. The use of chimpanzees, which seem to be the best choice in this respect, is obviously out of the question.

It would therefore seem that at present we will have to resort to methods by which the capacity of the vaccines to stimulate production of neutralizing antibodies is measured. Such tests may be designed according to either one of two principles.

1. In the monkey test described by Salk, the antibody titre obtained after a full course of three inoculations is determined, and the mean titre in a group of animals serves as a measure of the immunizing capacity of a vaccine.
2. The alternative to a test of this type is the antigenic extinction limit titration by which the minimum amount of vaccine needed to elicit a demonstrable immune response is determined. The use of guinea-pigs for this purpose is described below.

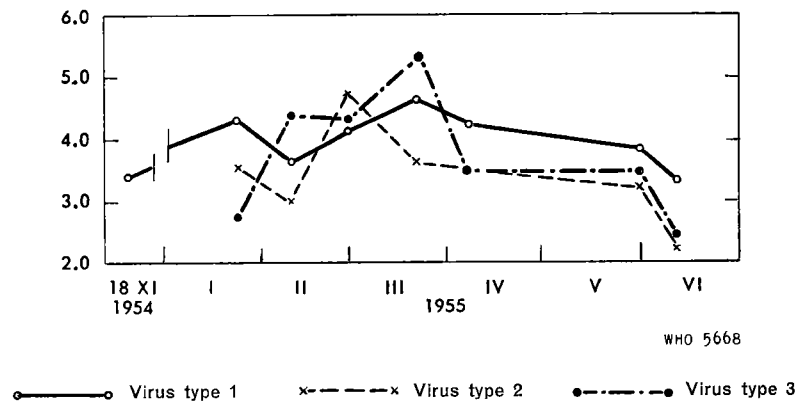
---

\* Presented by Professor S. Gard

Whether or not the protective effect in man is correlated to the neutralizing titre established has not yet been studied. However, the gamma-globulin experiments in chimpanzees as well as in man seem to indicate that even traces of circulating antibody might suffice to prevent invasion of the virus. If so, the guinea-pig test would appear to be well suited as an antigenicity test of vaccines, provided that a correlation exists between the responses in man and the test animals. Experience in this respect is still limited, but the results so far obtained appear promising.

Although duplicate experiments in guinea-pigs or titrations repeated within a short period of time usually give consistent results, certain irregularities have been observed when experiments more widely spaced in time were compared. Fig. 1 shows results obtained over a seven-month period from November 1954 to June 1955. The values recorded are immunogenic indices (ratio of TC infectivity titre to extinction limit titre) obtained on titration of consecutive lots of live virus of all three types.

FIG. 1. VARIATIONS IN IMMUNOGENIC INDEX AT DIFFERENT TIMES OF THE YEAR



Of each type one and the same strain was used throughout. The immunogenic index seems to be a fairly fixed strain characteristic, and in spite of the fact that each titration represented a separate lot of virus the recorded indices may serve as a reasonably accurate indicator of the ability of the animals to respond to immunization. The largely parallel trend of the three types further supports this assumption. One may conclude, therefore, that the ability to respond undergoes a seasonal variation, reaching a minimum in February-March (a high index implies a poor response).

A similar seasonal variation in resistance to infection in guinea-pigs has been attributed to vitamin and other dietary deficiencies. It is not yet known whether the poor performance in antigenicity tests in late

winter and early spring can be explained in the same way. If so, it should be possible to eliminate or reduce the irregularities observed.

In any event, these observations lead to the conclusion that antigenicity tests should always include the simultaneous titration of a reference vaccine and that the results should be referred to that obtained with such a preparation.

**Potency Test currently used at the State Bacteriological Laboratory,  
Stockholm \***

Serial tenfold dilutions of the vaccine are inoculated intradermally in 0.2 ml. amounts in groups of five guinea-pigs, each weighing approximately 250 g. Two weeks later a booster inoculation of the same amount of the same dilution is administered by the same route. The animals are bled seven days after the booster. Neutralization tests against 100 tissue culture  $ID_{50}$  of virus are set up on each serum undiluted (after inactivation at 56°C). Two tissue-culture tubes are seeded from each serum-virus mixture. Virus and uninoculated controls are included as usual. Readings are taken after six days. A less than 2- degeneration in at least one tube is recorded as a positive result (see Table I). On this basis the immunogenic extinction limit is calculated according to the method of Reed & Muench.

**TABLE I. SAMPLE NEUTRALIZATION TEST TITRATION**

| Vaccine dilution | Neutralization * |   |   |   |   | Result |
|------------------|------------------|---|---|---|---|--------|
| 10 <sup>0</sup>  | +                | + | + | + | + | 5/5    |
| 10 <sup>-1</sup> | +                | + | + | + | + | 5/5    |
| 10 <sup>-2</sup> | +                | + | + | - | + | 4/5    |
| 10 <sup>-3</sup> | +                | + | - | - | - | 2/5    |
| Extinction limit |                  |   |   |   |   | 2.7    |

\* Five sera per vaccine dilution, two tissue-culture tubes per serum

*Comments.* Extinction limits in guinea-pigs are only slightly influenced by (a) the route of inoculation, and (b) the interval between sensitizing dose and booster, at least in the range of from 10 days to 10 weeks. Preliminary results indicate that this may not be true in human beings. Thus,

\* Presented by S. Gard, T. Wesslén, A. Fagraeus, A. Svedmyr & G. Olin (to be published in 1956 in *Archiv für die gesamte Virusforschung*)

in one experiment the response to subcutaneous administration of two doses three weeks apart paralleled closely the results obtained with the same vaccine in guinea-pigs, whereas intracutaneous inoculations with the same interval yielded inferior results, particularly with type 1 (see Table II). In additional groups participating in the same experiment inoculations were spaced also at six weeks; the results in these groups are not yet available.

**TABLE II. A COMPARISON BETWEEN THE IMMUNITY RESPONSE TO POLIOMYELITIS VACCINE IN MAN AND IN GUINEA-PIGS**

| Route of inoculation   | Concentration of vaccine | Responses *  |              |              |
|--|--------------------------|--------------|--------------|--------------|
|  |                          | virus type 1 | virus type 2 | virus type 3 |
| Man:<br>Subcutaneous inoculations<br>in man,<br>2x1.0 ml,<br>3 weeks apart | 10 <sup>0</sup>          | 14/14        | 16/16        | 16/16        |
|  | 10 <sup>-1</sup>         | 4/9          | 8/10         | 10/11        |
|  | 10 <sup>-2</sup>         | 4/7          | 10/13        | 7/15         |
|  | 10 <sup>-3</sup>         | 1/12         | 5/17         | 1/12         |
|  |                          | 1.6**        | 2.5**        | 1.9**        |
| Man:<br>Intradermal inoculations<br>in man,<br>2x0.45 ml,<br>3 weeks apart | 10 <sup>0</sup>          | 9/18         | 11/12        | 12/13        |
|  | 10 <sup>-1</sup>         | 1/13         | 8/12         | 8/16         |
|  | 10 <sup>-2</sup>         | 0/12         | 11/18        | 7/18         |
|  | 10 <sup>-3</sup>         | 1/8          | 10/12        | 2/13         |
|  |                          | 0.0**        | 3.0**        | 1.4**        |
| Guinea-pig:<br>Antigenicity test   | 10 <sup>0</sup>          | 5/5          | 5/5          | 4/4          |
|  | 10 <sup>-1</sup>         | 3/5          | 3/5          | 3/5          |
|  | 10 <sup>-2</sup>         | 1/5          | 3/4          | 2/5          |
|  | 10 <sup>-3</sup>         | 0/5          | 0/5          | 0/5          |
|  |                          | 1.3**        | 2.0**        | 1.5**        |

\* The numerator represents the number positive and the denominator the number inoculated.

\*\* Extinction limit titres

## Annex 2

### SELECTION OF STRAINS FOR INACTIVATED POLIOMYELITIS VACCINE \*

It has been demonstrated that neurotropic activity and the capacity for extensive multiplication in various extraneural tissues is determined by different genetic complexes in the virus. It has furthermore been

\* Extract from a report by Professor A. B. Sabin

demonstrated that neurotropic activity is best measured by quantitative titrations by the intracerebral and spinal routes in monkeys, and that different strains of poliomyelitis virus exhibit a very wide spectrum of activity ranging from the highest, in which minimal amounts are paralytogenic by the intracerebral route, to the lowest, in which only amounts in excess of  $10^5$  or  $10^6$  TCD<sub>50</sub> of virus produce only occasional mild and non-progressive paralysis by the spinal route. Among the primates, the lower motor neurones of the monkey have been shown to be the most susceptible and those of the chimpanzee to be highly resistant, so that strains which are fully active for monkeys by the spinal route and even moderately active by the intracerebral route are not paralytogenic for chimpanzees in amounts of  $10^6$  to  $10^7$  TCD<sub>50</sub> by the spinal route.

The epidemiological behaviour of the most virulent poliomyelitis viruses in nature suggests that human adult neurones are at least as resistant as those of the chimpanzee, and that the neurones of infants and young children may be the most resistant of all. Although the human alimentary tract has been found to be more susceptible than that of the chimpanzee, and the alimentary tract of monkeys to be the most resistant, a comparative study in human beings, chimpanzees, and cynomolgus monkeys of at least one attenuated strain of poliomyelitis virus has shown that the same does not obtain for the intramuscular route—man having been found to be the most resistant.

### Annex 3

#### PRESENT STATUS OF WORK ON IMMUNIZATION OF HUMAN BEINGS WITH LIVING ATTENUATED POLIOMYELITIS VIRUS \*

The two basic facts which form the foundation of experiments on the immunization of human beings with living, attenuated poliomyelitis virus are :

- (1) that neurotropic activity and capacity for multiplication in the alimentary tract are determined by distinct genetic complexes in the virus, and
- (2) that primates occupy an inverse position with regard to susceptibility of the nervous system and the alimentary tract—the nervous system being more resistant and the alimentary tract more susceptible in the higher primates (see Table I).

---

\* Presented by Professor A. B. Sabin

**TABLE I. COMPARATIVE SUSCEPTIBILITY OF NERVOUS SYSTEM AND ALIMENTARY TRACT OF PRIMATES TO POLIOMYELITIS VIRUSES**

| Type of cells    | Most susceptible $\longrightarrow$ Most resistant |                    |                          |        |
|------------------|---|--------------------|--------------------------|--------|
| Neurones         | Monkey (lower motor)                              | Monkey (brainstem) | Chimpanzee (lower motor) | [Man]  |
| Alimentary tract | Man   | Chimpanzee         | Cynomolgus               | Rhesus |

The results of tests on many strains indicate that neurotropic activity is not an "all or none" characteristic of poliomyelitis virus, but rather that a great multiplicity of so-called "virulence genes" provide strains with a wide spectrum of activity that has been measured quantitatively in monkeys and chimpanzees. Strains of the three immunological types exhibiting varying degrees of low neurotropic activity have been segregated experimentally either by passage in rodents or by selective propagation in tissue culture, and have also been found in nature among healthy children who had no contact with clinically recognized cases during non-epidemic periods. Despite many experimental manoeuvres and the screening of a total of 69 strains from healthy children from different parts of the world in two different laboratories (49 in Cincinnati, Ohio, and 20 in New Haven, Conn.) no strain that is completely devoid of neurotropic activity on the most sensitive lower motor neurones of the monkey has been found. However, strains of each of the three types which are not paralytogenic for chimpanzees and possess only minimal activity after the inoculation of very large amounts of virus intraspinally in monkeys have been found.

The results of approximately 100 tests on 80 adult volunteers with strains of each of the three types have been summarized.

The properties of the available strains are such that only the oral route has provided susceptible tissue for multiplication of virus. The activity of four type 1, two type 2, and two type 3 naturally occurring or experimentally segregated strains has been compared; and certain variations have been encountered in the amount of virus excretion, detection of traces of virus in the blood, time and level of antibody formation, and appearance of a certain proportion of virus particles with increased intracerebral activity in monkeys but still innocuous intraspinally in chimpanzees.

It is believed that there is no immediate prospect of developing or finding strains completely devoid of neurotropic activity in the monkey, but that from among the strains that have been tested in human volunteers it is now possible to select one of each type that possesses only minimal

activity in the spinal cord of monkeys and at the same time the greatest stability on multiplication in the alimentary tract.

The importance of the potential appearance of virulent variants during multiplication in the alimentary tract and the spread of the virus must be considered. It has been pointed out that because of the very broad spectrum of neurotropic activity suggestive of the operation of a large number of "genes" or genetic groups or configurations, a large number of mutations, step by step, would be required to convert the very low neurotropic activity of the selected strains to a point where they might become paralytogenic for chimpanzees. It has also been pointed out that the fact that extensive dissemination of endemic strains of poliomyelitis virus of low virulence can continue in certain populations for many years without evidence of the appearance of highly virulent variants also indicates that reversion to high virulence is an uncommon phenomenon under natural conditions.

The spread of virus from vaccinated to unvaccinated persons by faecal contamination was possible, but under ordinary hygienic conditions, particularly during the winter, this might be expected to be limited. Where poorer hygienic conditions prevail, this would only substitute the spread of highly attenuated strains for the more neurotropic viruses that are already being disseminated.

#### Annex 4

### STUDIES OF IMMUNIZATION OF MAN AGAINST POLIOMYELITIS WITH LIVING ATTENUATED VIRUS\*

Three strains have been used in this work: the MEF 1 and TN strains representing type 2 and the SM strain representing type 1 virus. The MEF 1 strain was used after 70 passages in developing chicken-embryos. The TN strain is a rodent-adapted virus. The type 1 SM strain is also rodent-adapted and can be maintained in chick-embryo tissue culture for several generations. All these strains are non-pathogenic for monkeys when inoculated intracerebrally, but occasionally a monkey injected intraspinally with a high concentration of virus will be paralysed. The type 2 TN and MEF 1 strains are non-cytopathogenic for tissue-grown fibroblasts or epithelium of either simian or human origin. The type 1 SM strain is cytopathogenic.

In clinical studies conducted during the past five and a half years, 150 subjects have received the TN (type 2), 75 the SM (type 1), and 18

---

\* Summary of working documents submitted by Dr H. Koprowski

the MEF 1 (type 2) strains. None of the 243 subjects, the majority of whom were children 9 months to 15 years old, had antibodies against the type of virus administered. All received the virus by the oral route only, either in liquid or in capsule form. No subject showed signs of sickness which could be attributed to the feeding of virus. The non-cytopathogenic type 2 viruses were encountered in the stools of virus-fed subjects only occasionally, and then at very low concentration. These strains were found to be non-contagious for non-immune contacts. The cytopathogenic SM (type 1) strain was always excreted in stools after its oral administration; the duration of alimentary infection varied individually. When eight children, non-immune to all three types of virus, were kept in intimate body contact for three hours daily for 20 consecutive days with six other children who were excreting the SM (type 2) strain, three of the eight became infected. This ratio of contact infection after an unusual degree of exposure seems to indicate a low degree of contagion of the SM strain. Preliminary studies on virus isolated from infected contacts (second human alimentary passage) indicated that it was of no greater pathogenicity for monkeys than the original inoculum.

On several occasions both virus types were administered simultaneously with immune serum globulin; they were also fed, with no untoward reaction, to infants, one to three months old, possessing maternal antibodies. Passive immunity failed to have any effect upon either the duration of alimentary infection or the formation of active antibodies.

All subjects who actually ingested TN and SM virus developed the corresponding antibodies, and it has been shown that, in those tested, antibodies have persisted for five years after a single oral administration of the particular virus.