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WHO EXPERT COMMITTEE ON SMALLPOX

First Report

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WHO EXPERT COMMITEE ON SMALLPOX

Geneva, 14-20 January 1964

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WHO EXPERT COMMITTEE ON SMALLPOX

First Report

The WHO Expert Committee on Smallpox met in Geneva from 14 to 20 January 1964. Dr P. M. Kaul, Assistant Director-General, opened the meeting on behalf of the Director-General. He said this was the first Expert Committee on Smallpox convened by the Organization and its purpose was to review the situation with special reference to control and prevention of the disease. This review should include consideration of the epidemiology of the disease and its recent behaviour in endemic and non-endemic countries; recent findings in laboratory and field research, especially the importance of high potency vaccines for use in revaccination and the observations on the value of chemoprophylaxis; the Organization's programme of eradication in the light of experience since that programme was launched by the Eleventh World Health Assembly in 1958; and the international quarantine requirements for vaccination and revaccination in the light of the increasingly rapid movement of persons between countries and of recent importations of infection into countries normally free from the disease.

Brigadier M. S. Haque was elected Chairman and Professor C. W. Dixon, Rapporteur.

INTRODUCTION

Though means for the prevention of smallpox have been known since the end of the eighteenth century, the disease is still endemic in most countries in Asia and Africa and has not been completely eradicated from Latin America. Europe, North America and Oceania are free from indigenous infections but are becoming increasingly liable to the importation of infection from persons incubating the disease or suffering from mild attacks, as has been shown by recent experience in England, Western Germany, Poland and Sweden.

In the past twelve years the reported number of cases in the world has fallen from about half a million to less than 100 000. The reported cases, however, represent only a fraction of those that occur.

Observations have shown that vaccines of relatively poor potency are adequate for successful primary vaccination but inadequate for successful revaccination. Their use for revaccination gives a false sense of security, since a negative response is often taken as evidence of immunity. Failure in successful revaccination explains in part the continued presence of smallpox in some endemic countries where vaccination is regularly practised.

Though chemoprophylaxis does not lessen the need for routine vaccination, recent observations on its value are important because they have shown that it may be used to supplement the protection afforded by vaccination to contacts. These observations will undoubtedly stimulate study of the value of chemoprophylaxis and chemotherapy in other virus diseases.

As the only source of the virus is man and as vaccination provides good protection for a number of years, eradication of smallpox in endemic areas is well within the compass of modern preventive medicine. Failure to achieve it hitherto has been due to a variety of factors, the most important being (in addition to the use of poor vaccine mentioned above) the incomplete cover of the population because of inadequate health services, shortage of equipment, transport and personnel, and failure to conduct concurrent evaluation of the adequacy of vaccination campaigns. Given energetic action and practical help from countries free from the disease the endemic countries could rapidly bring it under control and ultimately eliminate it.

DEFINITIONS

The definitions given to some of the terms used in this report are shown below:

Variola major: Typical smallpox with a case-fatality rate in the unvaccinated ranging from about 20% to about 50%, depending on the age distribution of the patients and on other environmental and host factors.

Variola minor: Mild form of smallpox with a case-fatality rate in the unvaccinated of less than 5% (synonym: alastrim).

Vaccination: Cutaneous inoculation of smallpox vaccine into a person not previously successfully vaccinated (synonym : primary vaccination).

Revaccination: Cutaneous inoculation of smallpox vaccine into a person who has a vaccination scar or convincing documentary evidence of previous successful vaccination or revaccination.

Repeat vaccination or repeat revaccination: Reinoculation of smallpox vaccine into a person in whom vaccination or revaccination did not produce a major reaction.

Successful vaccination or revaccination: Occurrence of a major reaction following vaccination or revaccination (synonym : take).

Major reaction: 1. Presence of a typical Jennerian vesicle on examination one week *after primary vaccination.*

2. Presence of a vesicular or pustular lesion *or* an area of definite palpable induration or congestion surrounding a central lesion, which may be a scab or ulcer, on examination six to eight days *after revaccination.*

Equivocal reaction: Any response to vaccination or revaccination other than a major reaction.

WORLD MORBIDITY AND MORTALITY

The reporting of smallpox, as of any other communicable disease, is frequently unreliable and the data available to the Committee are not accurate. They convey information on the general trend of the incidence of the disease in different parts of the world. With the improvement in health services and the increasing interest in the control of smallpox, it is probable that reporting will become more complete and this may for a time produce a paradoxical situation with some countries reporting an increasing number of cases and deaths while the real number is in fact decreasing.

The figure overleaf shows the reported incidence of smallpox in Africa, America and Asia for the period 1950-53. During this interval two peaks of high incidence occurred, one in 1950-51 and one in 1957-58, mainly in Asia. The second peak was much lower than the first, with about half the number of cases.

In Table 1 is given the reported world incidence and mortality by continents over the last five years (1959-63). About 88 000 cases and 25 000 deaths were recorded throughout the world in the first 11 months of 1963. Most of these—about 73 000 cases and 24 000 deaths—occurred in Asia, where India, as in previous years, reported the largest numbers —59 000 cases and 18 000 deaths. In second place was Pakistan, which notified over 7000 cases and 5000 deaths. Immediately behind was Indonesia, where 6000 cases but only 132 deaths were reported. Saudi Arabia and Thailand notified no cases in the first 11 months of 1963; both reported relatively large numbers in 1959 and a few cases in 1960, 1961 and 1962.

In Africa fewer cases and deaths were notified in the first 11 months of 1963 than in the years 1961 and 1962. Congo (Léopoldville) reported the highest numbers—over 4000 cases and over 650 deaths. Congo (Brazzaville), Mali, Nigeria, Northern Rhodesia and Tanganyika also reported more than 500 cases in 1963.

SMALLPOX INCIDENCE IN AFRICA, AMERICA AND ASIA, 1950-63

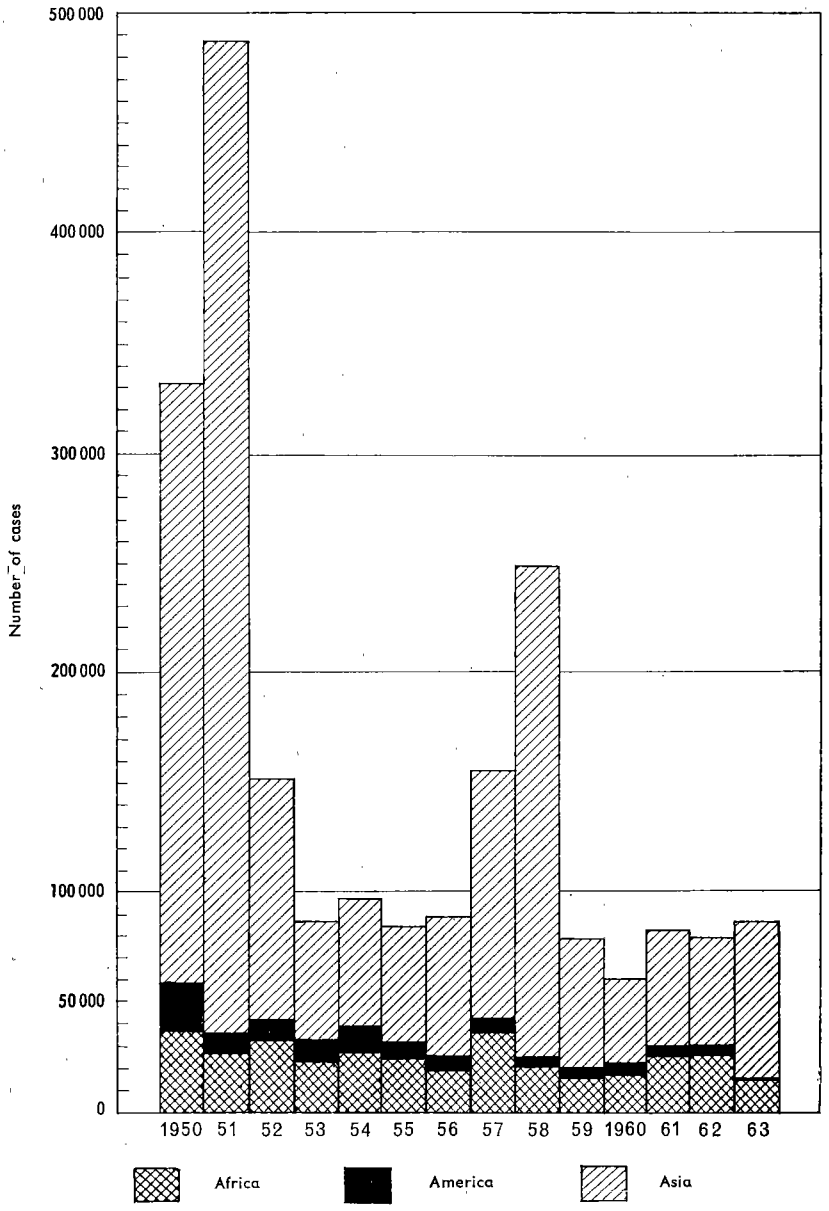

TABLE 1

REPORTED WORLD SMALLPOX MORBIDITY AND MORTALITY, 1959-63

Continent		1959	1960	1961	1962	1963 [a]
Africa	C	13 950	15 851	24 025	24 188	15 078
	D	1 071	1 017	1 798	2 423	1 484
America	C	4 889	3 090	1 939	3 029	241
	D	—	—	—	—	16
Asia	C	58 085	39 221	53 549	46 374	72 973
	D	15 781	9 639	13 081	12 287	24 033
Europe	C	13	47	24	137	145
	D	1	—	4	27	11
Total	C	76 937	58 209	79 537	73 728	88 437
	D	16 853	10 656	14 883	14 737	25 544

[a] Cases and deaths notified to the World Health Organization up to 29 November 1963.

The case fatality appears much higher in Asia than in Africa. Even when allowance is made for unreliable reporting and the inclusion in some countries of cases of chickenpox, this difference seems to be important. This may be explained by differences in the variola strains prevalent in Asia and in Africa (see page 14).

In the Americas, where extensive national control programmes have been carried out, the reported incidence of the disease has decreased in recent years. Lately low mortality rates have been reported and it has been assumed that only variola minor has been present, but in the past outbreaks with relatively high mortality have occurred. Brazil, where reporting is partial, continues to record the largest number of cases. Ecuador reported 45 cases and Colombia only four. Argentina, which had previously reported a few cases every year, did not report any in 1963.

In Europe, five cases were imported during 1963 and a total of 140 secondary cases and 11 deaths occurred as a result of these importations.

In Table 2 are shown the approximate incidence rates in countries which reported more than 500 cases in 1962. Rates were much higher in the relatively sparsely populated countries in Africa than in the densely populated countries in Asia, but Asia was the more important reservoir of the disease because of the large number of cases.

It is in the best interests of all countries to have as accurate reporting as possible. Countries are under obligation to conform to International Sanitary Regulations and should report cases and outbreaks as they occur in accordance with these regulations; all countries should refrain from imposing international control measures in excess of those laid down in these regulations.

Valuable additional information would be obtained by epidemiological investigations in affected areas and this procedure should be utilized by

TABLE 2

SMALLPOX INCIDENCE RATES IN COUNTRIES REPORTING MORE THAN 500 CASES IN 1962

Country	Approximate rate per 100 000 population
Africa:	
Cameroon	20
Chad	35
Congo (Brazzaville)	144
Congo (Léopoldville)	29
Guinea	100
Ivory Coast	58
Mali	38
Niger	34
Nigeria	11
Rhodesia & Nyasaland	12
Tanganyika	10
Togo	38
Uganda	10
Upper Volta	30
Americas:	
Brazil	4
Asia:	
India	10
Indonesia	1
Pakistan	4

health authorities as frequently as possible to supplement their notification services. In view of the need to define the incidence and geographical distribution of the different types of smallpox, information on mortality, clinical type and vaccinial state of cases, especially in outbreaks, is required along with laboratory studies on the viruses isolated.

FACTORS IN TRANSMISSION

Sources of virus

The source of the smallpox virus is man. It is spread from patients with acute infections; long-term carriers do not exist. Though virus is present in large quantities in scabs, virus from the respiratory tract is by far the most important in the spread of infection. The patient must be regarded as infectious from the onset of fever. Virus is liberated over a variable period of time depending on the clinical type of disease. Many patients appear to be most infectious about the third or fourth day, which generally coincides with the appearance of the rash. Virus from the respiratory tract may be present on the skin, clothing and bedding and the patient is infectious long before the maturation of vesicles and discharge of scabs.

The scabs, which are the dried pustules, have long been regarded as an important source of infection and virus can remain alive in them for many months. In practice they are not important sources of infection, but patients should be isolated until the scabs are shed. Subsequent desquamation of the skin is not infectious.

The most likely source of infection is contact with a patient in the early phase of the disease. The mode of infection may be by direct droplet transmission or by resuspension of droplet dust from clothing and bedding. Virus from the respiratory tract appears to die out fairly rapidly.

Personal clothing and bedding contaminated with virus from the respiratory tract frequently give rise to infection in those handling them —persons in the family, chamber-maids in hotels, laundry workers in hospitals and elsewere, and persons handling used clothing from the patient's home. Infection is not transmitted by food. Flies are attracted to the secretions on the skin of the patient, and infection could be conveyed mechanically to the mouth or eyes of small children, particularly in tropical countries.

The corpse may be infectious from body fluids or from contamination of the clothing or shroud. Pathologists, post-mortem technicians and undertakers infected from this source sometimes have a normal attack of smallpox but occasionally develop " inoculation variola " from pricking a finger. Laboratory technicians and laboratory cleaners have also been infected from specimens or glassware.

Aerial spread

Spread of variola major virus from smallpox hospitals by air currents for distances of the order of half a mile has been seriously considered on many occasions over the last 50 years. Variola minor virus does not appear to have been incriminated under these conditions.

It is impossible to deny that virus could be blown a distance of a quarter of a mile and give rise to infection, but it is not the cause of the pattern of the spread of disease sometimes seen around a smallpox hospital. In such an event, infection by missed cases in the community, imperfect disinfection of clothing or irregular contact between the sick and healthy can never be completely excluded.

Though it has been shown that bacteria can be blown along hospital corridors, and rather mysterious patterns of secondary cases of smallpox in hospitals may be due to this method of spread, the possibility of spread by persons or fomites rather than by air currents cannot be eliminated. The early case, not the convalescent with many scabs, is usually the source of virus in these episodes.

The infectious unit in smallpox is probably a relatively large droplet or particle which rapidly sediments near the patient, particularly on his

pillow, and is resuspended in dust particles. Climatic factors such as temperature, humidity, air movement, etc., probably account for part of the variation in the incidence in endemic areas.

Type of case

The infectivity of cases of different degrees of severity varies considerably. The most fulminating types seem less infectious than the severe types, which are very infectious. The moderately severe cases are infectious for at least five to six days. The milder ones, including abortive cases and cases of variola sine eruptione, may be infectious for only a few hours and if the infectious period occurs at night the patient may infect no one, not even those living in the same house, but in the absence of disinfection the environment of the patient may remain infectious for some time.

As far as is known, all strains of variola major are of equal infectivity but variola minor seems to have less epidemic potential.

Extrinsic factors in transmission

Mobility. In the more severe forms of smallpox the patient is confined to bed; the maximum weight of infection is on the other members of the family, particularly those caring for the patient. In the milder forms, particularly in variola minor, the patient may remain ambulant after the initial pyrexial attack. Presumably because of variation in the amount of virus dispersed from the respiratory tract on to clothing etc., it is impossible to predict whether the secondary infections will be few or many.

Family size and composition. Multiple cases in families do not occur as frequently as is sometimes supposed. This is particularly true of variola minor. The larger the household and the greater the number of young susceptible adults in the community, the greater the chance of rapid multiplication of cases.

Occupation. As mentioned above, those who handle bedding and clothing from the patient in the early phases of disease are at greatest risk. Others most likely to be infected are doctors, nurses and other members of hospital staff, undertakers and health inspectors, who have not been satisfactorily vaccinated.

Public health factors in transmission

Early diagnosis. Early diagnosis is very important in preventing spread of smallpox in a community, and is related to the efficiency and the availability of medical care. If the diagnosis is missed and the patient kept at home family members are exposed to considerable risk. If the patient is admitted to a hospital unprepared to handle smallpox cases and is mis-

diagnosed, there is considerable risk to other patients, nursing staff, other hospital workers and visitors.

Control of contacts. The most important single factor in preventing the spread of smallpox is the rapid identification and control of contacts (see page 27).

Ambulant mild cases. Ambulant mild cases are particularly likely to occur among adolescents and fairly severe but ambulant cases may occur among tramps and other itinerants. Partially immune travellers and persons engaged in the transport industries, notably seamen, may have mild and missed attacks and so carry infection from one place to another.

Incubation period

The date of the onset of fever, and not that of the onset of rash, should be used for the calculation of the incubation period. When this is done, the incubation period of variola major is found to be reasonably constant at 12-13 days. Occasionally it may be as short as nine days or as long as 15 days.

In variola minor the incubation period appears to be the same. Due to the trivial eruption, patients may mis-state the day of onset, thus suggesting a longer incubation period. Where contact with the source of infection lasts for some days precise calculation of the incubation period is impossible.

Exceptionally short incubation periods. In some cases of variola major the incubation period appears to be between eight and 10 days. In many persons, concurrent vaccination has been performed and it is possible that the initial pyrexial attack is due to the vaccination. A shorter incubation period occasionally occurs in very severe cases where any other possible source of infection can be ruled out. In smallpox pulmonary allergy, a virus-pneumonia-like condition occurring in immune contacts, the incubation period is only eight days but these patients are not infectious. It is good public health policy to regard the incubation period to the onset of fever as 12 days and a quarantine period of 16 days is a reasonable safeguard against the spread of infection.

IMMUNOLOGY

Duration of immunity produced by smallpox

In most people an attack of smallpox gives lifelong immunity, but some lose their immunity and again become susceptible. About one patient in a thousand develops a second attack, but the rate depends very

much on the age of the population at risk. Second attacks are usually mild or abortive, but may be severe and fatal. Some mild cases are probably missed because of the patient's previous history of smallpox.

The immunity induced by an attack of variola major is probably greater than that induced by variola minor and immunity is more likely to break down on exposure to variola major than to variola minor because of the greater virulence and infectivity of variola major virus.

Duration of immunity produced by successful vaccination

After successful primary vaccination immunity to variola major is almost complete for three years, but there are individual variations. It has been suggested that, on an average, within one year after primary vaccination the chance of an attack is reduced to 1/1000th of that in the unvaccinated, within three years to 1/200th, within 10 years to 1/8th, within 20 years to 1/2, and after 20 years there is little if any protection from clinical infection. However, the mortality in smallpox patients successfully vaccinated many years before is less than in the unvaccinated, although deaths do occur.

Immunity following successful primary vaccination is more effective against variola minor, where few cases occur within five years and the possibility of a clinical attack within 10 years is still very slight compared with the risk in the unvaccinated. Even 20-30 years after successful vaccination there is considerable immunity to clinical infection.

Because of the problem of interpreting equivocal reactions (see definition on page 5) it is more difficult to obtain accurate information about the immunity following revaccination. The freedom from the disease enjoyed by medical and nursing personnel revaccinated regularly and exposed to occasional risk of infection indicates that regular revaccination induces a high degree of immunity.

Relation between antibody level and vaccination reaction

After successful vaccination or an attack of smallpox there is usually a demonstrable rise in serum antibody. Even with the most sensitive technique, however, there is at best only a rough correlation between the antibody titre and the type of reaction induced by revaccination, so that it is not possible from the antibody level to predict in an individual the skin reaction to revaccination. Persons with a relatively high level of neutralizing antibody tend to give an allergic response following revaccination and this may not result in an increase in antibody titre. On the other hand, persons with an initial low antibody titre are more likely to have a major reaction on revaccination and to show a subsequent rise in level of neutralizing antibody.

Relation between antibody level and immunity to smallpox

The levels of antibody measured by various laboratory techniques have been examined at various stages in the course of the disease, but there are no data providing satisfactory information on the relation between antibody level and immunity to smallpox.

Relation between titre of vaccine and immunity induced

The amount of virus introduced into the skin at the time of vaccination or revaccination determines whether a take will occur (see page 18), but does not determine the subsequent immunizing effect, as this effect is dependent on the extent to which virus multiplies in susceptible cells. A virus inoculum which produces a major reaction (see definition on page 5) after vaccination or revaccination provides a satisfactory stimulus irrespective of the size of the virus dose.

Relation between number of inoculations and immunity induced

Other things being equal, when smallpox occurs many years after primary vaccination there is some evidence (for instance, from early studies in England) that the death-rate decreases as the number of vaccination scars increases. Recent experience in India tends to support this view, but there is not general agreement about it.

The trauma of multiple inoculations and fear of severe reactions induced by them may so increase the avoidance of vaccination that the possible improvement of immunity gained by the individual may be more than offset by the smaller proportion of the population protected.

Passive immunization

Gamma-globulin of human or animal origin has been used in the prevention and treatment of some serious complications of vaccination. When injected at the same time as the vaccination is performed it reduces the incidence of post-vaccinal encephalitis. It can be prepared from the serum of adult donors three to six weeks after a major vaccination reaction, from the serum of convalescent smallpox patients, or from the serum of hyperimmunized calves. It is estimated that in intimate household contacts human gamma-globulin will reduce the attack rate of smallpox by at least 70%. It is no substitute for successful vaccination before contact or successsful vaccination immediately after contact and is of most value in contacts discovered late in their presumptive incubation period. Owing to the limited supply of the human gamma-globulin its use may have to be limited to close contacts who have never

been vaccinated prior to exposure and to those in whom vaccination by itself is strongly contra-indicated.

LABORATORY DIFFERENTIATION OF SMALLPOX VIRUSES

Epidemiological and clinical observations will usually identify an outbreak of smallpox as either variola major or variola minor (alastrim); laboratory methods will differentiate the causal viruses. The virus of variola major produces visible lesions on the chorioallantois or in tissue culture at temperatures as high as 38°C, and an inoculum of 10^5 pock-forming units is lethal for the chick embryo in four days. The virus of variola minor does not produce lesions at these temperatures and is not lethal for the chick embryo with an inoculum less than 10^7 pock-forming units.

Strains have been isolated in Tanganyika which are intermediate in their laboratory characteristics. Though of low chick virulence, they produce some lesions at 38.3 °C but much more at 36 °C. The characteristics of the individual strains have remained constant. The intermediate mortality rates (4%-15%) reported from African countries have been assumed to represent concurrence of variola major and variola minor within the population, but in fact they may be due to the presence of intermediate strains. This possibility can only be resolved by study of the viruses isolated, together with adequate clinical and epidemiological data from further outbreaks in Tanganyika and from outbreaks in other areas in Africa.

LABORATORY DIAGNOSIS OF SMALLPOX

In most cases the diagnosis of smallpox can be made on clinical grounds and does not require laboratory confirmation. However, in patients with atypical, very mild or fulminating infections, laboratory confirmation may be necessary.

The most satisfactory laboratory procedures are those based on the demonstration of the virus or its antigens from lesions in the patient. Diagnosis by methods based on the detection of antibodies in the patient are less satisfactory because they do not differentiate between members of the vaccinia-variola group.

Some laboratory diagnostic procedures are relatively simple but should only be used in a laboratory with a properly trained staff; others, more complicated, should be performed only in special laboratories with standardized reagents. Information on the collection and dispatch of specimens is given in Annex 1 and details of the tests in Annex 2.

Tests recommended at different stages of the disease

The laboratory methods used in the diagnosis of smallpox will depend on the facilities available and the experience of the virologist.[1]

The pre-eruptive illness. In febrile contacts, in the absence of a rash an attempt may be made to isolate virus by culture of blood (buffy coat) or throat garglings on the choriollantoic membrane or in tissue culture. However, these examinations will usually be negative and results will not be available for at least several days, by which time the clinical picture will usually be clear.

Macular and papular stage. The most useful tests, because positive results can be available within two hours, are the demonstration of virus particles in stained smears or by immunofluorescence in scrapings of the skin lesions. Scrapings inoculated on the chorioallantoic membrane or in tissue culture should give positive results within two or three days.

Vesicular stage. Virus is usually demonstrable microscopically in smears made from the bases of vesicles. Virus antigen should be demonstrable in vesicle fluid within four to five hours by the gel-diffusion method or in 18-24 hours by complement-fixation. The virus is readily isolated in culture.

Pustular stage. Gel-diffusion and complement-fixation tests will give positive results with fluid from pustules. Culture will always be positive.

Crusting stage. Extracts of crusts will give a positive test for variola-vaccinia antigen by gel-diffusion or complement-fixation. Virus will be demonstrable by culture.

Tests for antibody. Tests for antibody in the patient's serum are never as satisfactory as the methods outlined above, but may be the only practicable laboratory procedure in mild cases in patients who never develop an eruption, or in the retrospective diagnosis of patients from whom no crusts or other material are available for the detection of virus or virus antigen. After the vesicular stage of illness, the serum of most patients will contain a high level of antibody demonstrable by the haemagglutination-inhibition or complement-fixation tests, using either a vaccinia or a variola antigen. Convalescent smallpox sera will usually precipitate with a vaccinia or variola antigen in a gel-diffusion test, while post-vaccination sera usually do not. Recent vaccination may make these serological tests uninterpretable, but antibody levels are usually considerably higher after smallpox infection than after vaccination.

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[1] In the hands of an electron microscopist experienced in virology, typical virus particles may be demonstrable in material from skin lesions at any stage of the disease. This method cannot be regarded as a routine diagnostic procedure.

SMALLPOX VACCINES

General principles of preparation and control

Smallpox vaccine is a suspension, in a suitable medium, of infectious vaccinia virus, at a concentration which has been shown to be adequate to infect non-immune persons by the cutaneous route. The effectiveness of a vaccine is determined by its level of infectivity at the moment it is applied to the skin and not solely by the virus content at the time of production. It is imperative that handling in storage, shipment and application be such that adequate potency is preserved.

Different strains

Many different strains of vaccinia virus are used for the preparation of smallpox vaccine; the origins of few, if any, are known. It is very unlikely that any of them was derived from variola virus. Some vaccine strains are more pathogenic for man than others. There is no evidence that strains producing severe local lesions and marked systematic disturbances confer better protection than strains producing milder clinical reactions. The less pathogenic strains, provided they produce adequate immunity, should therefore be preferred for vaccine production.

There may be differences in the duration of immunity following successful primary vaccination with different strains of vaccinia virus. For example, prolonged, repeated passage of a strain in tissue culture or on the chorioallantoic membrane may lessen the immunizing potency. The seed used in tissue cultures or in eggs for vaccine production should not be more than five passages removed from the animal host (calf or sheep) used for propagating the stock.

Many producers of smallpox vaccine have maintained their seed virus by alternate propagation in the skin of the vaccinifer of choice and a small animal such as the rabbit. This is a traditional procedure, but with modern facilities for either freeze-drying or deep sub-zero storage of a primary seed lot it has no advantages over a seed lot system recommended by the WHO Study Group on Requirements for Smallpox Vaccine.[1]

Studies in man are essential to ensure that the strains which are used will result in consistently successful revaccinations and will evoke immunity of good quality as shown by protection against smallpox and by immunity to challenge with potent vaccine one or more years after primary vaccination. Preference is to be given to strains which meet these requirements in man and also prove less neurotropic and skin-necrotizing in laboratory

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1959, **180**, 13.

animals. Studies of vaccine strains in laboratory animals may ultimately permit correlation of laboratory findings with protection in the field.

Animals used for production

Most smallpox vaccines are prepared from virus grown on the skin of animals. Calves, water-buffalo calves, sheep or other animals may be used according to availability and preference.

Adequate precautions must be taken to ensure that no disease transmissible to man exists in the animal. This requires proper inspection, quarantine and post-mortem examination (or observation after the vaccine is harvested).

Bacterial contamination is minimized by scrupulous cleanliness and scrubbing of the area to be vaccinated. The vaccinial eruption is harvested at the vesicular stage; four to five days after inoculation is generally long enough. Later harvests yield a greater mass of material but this has a lower titre and the total yield of effective vaccine is less. The harvest is processed to reduce bacterial contamination to the minimum while retaining maximum virus potency. Bacteriological testing should always include examination for the presence of anaerobic organisms, especially *Clostridium tetani*, and of certain specified aerobic organisms.[1]

Vaccines grown in chick-embryo membranes

Vaccines prepared from chick-embryo chorioallantois should be bacteriologically sterile. Egg vaccines when freeze-dried are not as yet as thermostable as freeze-dried vaccines of animal origin. Production of vaccine in eggs eliminates the need for animal husbandry and the necessity to clean up a contaminated product.

Vaccines from tissue culture

The production of smallpox vaccine on tissue cultures is still experimental. These vaccines must be shown to be at least as safe and effective as the best traditional vaccines before they are accepted for general use.

Liquid and dried vaccines

Liquid vaccines deteriorate rapidly even at moderately high atmospheric temperatures and after exposure to sunlight. They should be discarded after seven days if stored at 0°C-10°C and after 24 hours if stored at higher temperatures. Freeze-drying a liquid vaccine provides a more stable product which is resuspended in liquid for use. *After reconstitution this vaccine is as labile as liquid vaccine.* Both types of vaccine have their

[1] See: Study Group on Requirements for Smallpox Vaccines (1959) *Wld Hlth Org. techn. Rep. Ser.*, **180**, 17.

place in the control of smallpox. Potent liquid vaccines are satisfactory in temperate countries with good communications and adequate facilities for cold storage. In hot countries and where communications are bad, freeze-dried smallpox vaccine should be used because its stability obviates many of the difficulties associated with transport and storage. In these countries it is especially valuable to use dried vaccine for intending travellers, airmen and seamen. With dried vaccine it should be possible to reduce the number of vaccine producers in a region and ensure a high standard of both practice and product in vaccine establishments.

Killed and attenuated vaccines

Attenuated smallpox vaccines are those which by extensive passage have become markedly less pathogenic for man; at present they have a reduced immunizing potency. These and also killed vaccines have been subjected to much study in recent years. There is no evidence that such preparations are effective in preventing infection with the virus of variola major, even though they may evoke demonstrable antibodies. They may have a place in providing a minimal active immunity under cover of which one can safely infect the complication-prone individual with standard strains known to be effective in affording protection. If used, they may make the interpretation of reaction to the standard vaccine difficult.

Importance of potency of vaccines in primary vaccination and revaccination

Before a vaccine is issued for use material from final containers should satisfy at least one of the potency tests described in the report of the WHO Study Group on Requirements for Smallpox Vaccine.[1] It cannot be too strongly stressed that testing a vaccine at only one dilution may give very misleading results. A proper assay of virus should be undertaken. The potency should be not less than that recommended by the above-mentioned Study Group.[1] Permanent records should be kept of all bacteriological and potency tests.

In primary vaccination, essentially 100% successful vaccinations result if the vaccine has potency of 5×10^7 pock-forming units on the chorioallantoic membrane. The success rate in revaccination is lower than in primary vaccination. Presumably this is related to the immunity of the subject. Vaccines of considerably reduced potency can give a high take-rate in primary vaccinations, but even in the hands of a skilful vaccinator the revaccination take-rate with such a vaccine is less than that obtained with vaccines of higher titre. For revaccination, therefore, vaccine of high potency (over 10^8 pock-forming units per ml) is necessary if protection is to be ensured. Potency tests of batches of vaccine in which even

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1959, **180**.

large numbers of persons are primarily vaccinated, though useful, may give misleading results. Some batches of vaccine should also be tested by a large number of revaccinations, preferably in comparison with a standard reference vaccine applied simultaneously to the other arm by the same vaccinator using the same technique. Field observations, supported by laboratory studies, should be made on freeze-dried as well as liquid vaccine to ensure that deterioration has not occurred after release from the manufacturer during shipment and storage in laboratories and clinics. The Committee noted that WHO had made arrangements for potency tests to be carried out in an international laboratory on early batches of vaccine from new laboratories producing freeze-dried vaccines and periodically on vaccines used in eradication campaigns.

VACCINATION AGAINST SMALLPOX

Vaccination is the attempt to create a limited infection of the individual with vaccinia virus, using the cutaneous route. The object is to produce, with minimal systemic involvement, a localized infection which leaves a small scar. Potent infectious material must be applied to the target cells, which lie in the basal layers of the epithelium. Virus deposited in the subcutaneous tissue has little if any capacity to infect.

Site for vaccination

Various areas of the body have been used for primary vaccination in the attempt to conceal the scar; the best area for primary vaccination and for revaccination is the outer aspect of the upper arm, over the insertion of the deltoid muscle or behind the midline. Social customs and dress habits may, however, influence the choice of the site. With good techniques, small unobtrusive scars are the rule.

Preparation of vaccination site

Vigorous cleansing of the vaccination site, as for a surgical operation, is of no advantage and may produce slight abrasions which may become the site of secondary lesions. Chemical agents should not be used. If the skin is dirty it should be wiped clean.

Methods of vaccination

The Committee reviewed the different methods of applying the vaccine. Because vaccines of high potency are now available the use of instruments such as the rotary lancet, which causes considerable trauma and severe reactions, is contra-indicated. The multiple pressure method or the single

short (6-mm) scratch should be used routinely (see Annex 3). With the multiple pressure method it is possible for the vaccinator to vary the amount of virus inserted in vaccination and revaccination by applying fewer or more strokes without increasing the area of skin involved.

The Committee urged the introduction of the less traumatic methods, realizing that this must be done without disorganizing existing programmes.

Dose of vaccine

The amount of vaccine applied to the skin was, in the past, based on the quantity necessary for large multiple inoculations. With the small inoculations of modern vaccination techniques only a small droplet of vaccine need be applied to the skin, so that one " dose " can effectively vaccinate at least five and perhaps more persons. Vaccine should never be diluted to increase the number of " doses ", since this may make an effective vaccine useless.

Number of inoculations

With potent vaccines and good technique, one inoculation is sufficient for routine primary vaccination. Inoculation at more than one site is indicated for contacts of smallpox patients, and may be used for revaccination, especially if the first revaccination fails. The inoculations should be not less than 2.5 cm apart.

Vaccination reaction

A successful primary vaccination is one which, on examination after one week, shows a typical Jennerian vesicle.

A successful revaccination is one which, on examination one week (six to eight days) later, shows a vesicular or pustular lesion *or* an area of definite palpable induration or congestion surrounding a central lesion, which may be a scab or ulcer. These reactions should be termed " major reactions "; all others should be termed " equivocal reactions ". A major reaction indicates virus multiplication with consequent development of immunity. An equivocal reaction may be the consequence of immunity adequate to prevent virus multiplication or may be an allergic skin response elicited by inactive vaccine or poor technique. Since these cannot be differentiated, vaccine potency should be checked and revaccination repeated. A second reading should be made after six to eight days, and, if the result is still equivocal, revaccination should again be repeated.

Age for primary vaccination

The age of choice for primary vaccination is based on a number of factors, including the risk of smallpox infection of the unvaccinated child, the presence and duration of maternal immunity, the risk of the complica-

tions of vaccination and the influence of the normal primary vaccination reaction on the general health of the subject.

Vaccination can be accomplished in the first few days of life, but at this age the presence of passive maternal immunity may interfere with the take and the degree of immunity may be reduced. In non-endemic areas there are advantages in waiting for decline in maternal immunity and greater immunological maturity. Vaccination may be conveniently and successfully carried out in the third or fourth month of life but the age may be varied to fit a general scheme of infant immunization. At this age, persisting maternal antibody may produce an earlier maximal vaccinal reaction with less systemic symptoms. However, in endemic areas, primary vaccination should be carried out as early as possible, preferably in the neonatal period, and repeated about 12 months later.

Frequency of revaccination

Revaccination is necessary to maintain or reinforce immunity conferred by previous vaccination. In a non-endemic area, it is desirable to maintain in the general population a level of immunity high enough to minimize the risk of serious complications when revaccination is required. Revaccination at five- to 10-year intervals will generally serve to maintain adequate immunity. The intervals can be adjusted to suit convenience, such as school entry, etc.

For those at special risk, for example, hospital and public heath personnel, a level of immunity is required which will ensure that there is no risk of smallpox on exposure. This can be achieved by revaccination at least every three years. If exposure is probable, revaccination should be more frequent. In endemic areas revaccination of the general population should be carried out every three to five years.

Complications of vaccination

Vaccinia virus has a low but definite pathogenicity for man, producing a localized necrotic lesion, regional lymphadenopathy and systemic symptoms of malaise and fever. Virus dissemination to the eczematous skin of a vaccinated person may lead to eczema vaccinatum. A vaccinated person may also cause eczema vaccinatum in family or other contacts with eczema. Occasionally, virus is transplanted from the vaccination site to the face, eye, or elsewhere to establish secondary lesions. The mode of development of post-vaccinal encephalitis is not known; this complication is rare, and one of its most puzzling features is its geographical variation. In the rare immunologically deficient individual, the usual immune mechanisms may fail and result in progressive vaccinia. In this condition, and in eczema vaccinatum, human vaccinal gamma-globulin

is frequently effective. In some of the progressive lesions, however, the defect appears to be cellular rather than humoral. In these complications chemotherapeutic agents (see page 23) seem to be of some value.

Prevention of complications

The intramuscular injection of 2 ml of 16% human gamma-globulin at the time of primary vaccination significantly reduces the frequency of post-vaccinal encephalitis. Most epidemiological studies have shown that this complication is less frequent when vaccination is performed in the first year of life.

A second approach to the prevention of complications is to create a preliminary active immunity. Conclusive results are not yet available from the studies at present in progress.

Complications can be minimized by avoiding the routine vaccination of persons whose medical history and physical status suggest the possibility that complications may occur. In endemic areas or in contacts of smallpox cases there are no absolute contra-indications. The presence of eczema in the subject (or his close contacts) is a contra-indication to *routine* vaccination. Vaccination should not be routinely performed on any person who is sick, is suffering from leukaemia, has evidence of defective immune mechanisms, is on corticosteroid treatment, or has a septic skin condition. When such persons have to be protected vaccination should be done under the cover of gamma-globulin or chemoprophylactics.

Simultaneous administration of vaccines

In immunization programmes the use of mixed vaccines or the simultaneous administration of more than one vaccine saves staff, time and money.

Recent small-scale controlled studies of simultaneous smallpox and BCG vaccination on opposite arms showed no interference between them (as indicated by the characteristics of the skin lesions). No increase in complications occurred.

Mixed smallpox and yellow fever (Dakar strain) vaccines have been extensively used in Africa with good immunological responses and no increase in complications. During the Second World War yellow fever vaccine, prepared with the non-neurotropic 17D strain, and smallpox vaccine were used simultaneously on a large scale without ill effect. In a recent study on 600 children in West Africa, smallpox, yellow fever (17D strain) and live measles vaccines were mixed and administered by jet injection and effective levels of immunity as indicated by antibody studies were obtained.

CHEMOPROPHYLAXIS AND CHEMOTHERAPY

Of the chemical compounds recently studied for their antiviral effect, isatin-β-thiosemicarbazone was shown to have protective effect against vaccinial infection in mice. Derivatives have shown marked activity against vaccinial and variola infection in tissue cultures and laboratory animals. N-methyl-isatin-β-thiosemicarbazone, because of its low toxicity for animals and man, and its high level of activity against vaccinia and variola viruses in the laboratory, has been tested in prophylactic trials against smallpox.

In over 1100 household contacts given the drug by mouth, three mild cases of smallpox occurred. In a comparable group of contacts who did not receive the drug there were 78 cases of smallpox and 12 deaths. The drug was effective even when given more than six days after contact. Further trials with different dosage schedules are in progress.

A related compound, 4-bromo-3-methyl-isothiozole-5-carboxaldehyde-thiosemicarbazone, has been tested in therapeutic trials in hospital patients. There is some slight indication that the drug may have an effect in reducing the mortality in the severe types of smallpox in vaccinated patients, but little effect in severe cases in unvaccinated patients. These trials are continuing with an increased dose of the drug.

Other preparations such as 5-iodo-2-deoxyuridine, 6-aza-uracyl-riboside, and sulfone derivatives are being studied but none has yet been tested against smallpox.

Some or all of these drugs may have a prophylactic, and possibly therapeutic, value against smallpox. Their prophylactic use may prove of considerable value in the control of the disease by decreasing the sources of infection. It should, however, be emphasized that their antiviral effect lasts only a short time. In endemic areas their use in no measure lessens the urgent need to produce lasting protection of the population by vaccination and periodic revaccination.

Since a number of chemical compounds show sufficient antiviral activity to merit field trial, they should be submitted to carefully planned comparative field studies in endemic areas under the aegis of a responsible research organization.

SMALLPOX ERADICATION

The global eradication of smallpox is well within the bounds of possibility. The only reservoir is man; infection is manifest; carriers do not exist; and successful Jennerian vaccination provides effective immunity.

Its eradication is a matter of concern to all countries as those now free constantly run the risk of the introduction of the infection from endemic areas.

Criterion for eradication

The Committee considers that the term "smallpox eradication" implies the elimination of the disease the world over. It may also be applied to the elimination of the disease from continents or large regional areas. It is not applicable to individual countries, especially if they are contiguous with countries where the disease is endemic. For these countries the term "national smallpox control" should be used.

Successful national smallpox control may be said to have been achieved if no indigenous disease has occurred for three successive years and if such local outbreaks as may have occurred from imported cases have been rapidly controlled.

Countries which have succeeded in controlling smallpox have to maintain an effective control programme until the disease has been eliminated from that region.

"Regional eradication" will be reached when all the countries in the region have achieved successful national control.

"Global eradication" will be reached only when the disease is shown to be absent from all countries of the world. This will take years to accomplish and one prerequisite, the importance of which will increase as the global incidence decreases, is adequate case-finding and reporting.

Until then each country must maintain in its health services a permanent immunization programme or apply combined isolation and immunization measures when the disease is reintroduced.

It is essential that countries exposed to a high risk of the introduction of smallpox should maintain an adequate degree of immunity in the population by vaccination of new members of the population (newborn infants and immigrants), and by periodic revaccination of persons of all ages —schoolchildren, adolescents and adults—especially those individuals (and their families) whose occupation brings them into frequent contact with international travellers. In view of the increasing volume of international traffic, it is desirable that the International Sanitary Regulations should be strengthened to increase the safeguard against the reintroduction of smallpox to countries where control has been achieved.

The WHO smallpox eradication programme

In 1958 the Eleventh World Health Assembly resolved that an effort should be made to eradicate smallpox and to this end the Twelfth World Heath Assembly requested the Director-General to prepare a programme of advice and help to countries on the basis that campaigns would be primarily

the responsibility of national governments. Since 1959 WHO has provided assistance in the form of vaccines (donated by Member States), refrigeration and laboratory equipment, and other supplies. It has also provided consultant advice in the laboratory and in the field; has arranged training courses on the production of freeze-dried vaccine; and has held two conferences on smallpox eradication programmes, one in Africa and one in Asia. In the planning of campaigns stress has been laid on the necessity for prior assessment of the public health problems represented by smallpox and on the need for sufficient numbers of trained staff and for adequate amounts of potent vaccine.

Information on the programme was given to the Committee by the Secretariat; by the members from India and Pakistan, where intensive national control programmes are in progress; and by the member from Ghana, where the problem is being tackled by improving the routine control measures.

The report of the careful work by independent appraisal teams who studied the control programmes in three different parts of India was of great value to the Committee, and their findings are probably applicable to most other national control programmes in endemic areas. The emphasis placed by the Organization on the use of freeze-dried vaccine has been abundantly justified. Where liquid vaccines have been used the percentage of major reactions has usually been low in the revaccinated. In all programmes, therefore, very high priority must be given to providing adequate quantities of potent freeze-dried vaccine and to ensuring that they are stored and handled with the precautions necessary to maintain the optimum potency up to the moment when the vaccine is applied to the skin. For the application of the vaccine the methods which cause the least trauma (see Annex 3) should be used. The target set by the Organization—namely, that 80% of each segment of the population should be vaccinated—was found in practice to be unsatisfactory in some cases. Follow-ups showed that, though the numbers of vaccinations made represented 80% or more of the estimated population, there were often sections (e.g., infants under one year of age, men working in the fields during the day) where the proportion vaccinated was only about 30%. Since in some cases the vaccine was not of uniformly high potency when applied, the proportions successfully vaccinated—i.e., in whom immunity developed—may have been even lower. The continuing occurrence of smallpox in areas where the vaccination programme had been completed was proof that many remained unprotected.

The Committee felt that campaigns would proceed with greater success if separated in the three definite phases shown below.

Preparatory phase. This should include epidemiological assessment of smallpox; recruitment of personnel and their training; provision for ade-

quate supplies of potent vaccine and of equipment for its storage and distribution; provision of transport; arrangements for the education of the population in the importance of smallpox and the success of vaccination; preparation where necessary of special legislation; preparation of a detailed plan (which should be submitted to WHO for consideration; definition of the chain of responsibility for the programme from the centre to the periphery of the health services.

Attack phase. In the attack phase it is imperative to concentrate on areas with high densities of population, whether they be urban, rural or mixed, where the disease persists and from which spread to other areas is likely to occur. After these densely populated areas are solidly protected, the maximum effort should be transferred to the contiguous areas. This is particularly pertinent if vaccine supplies are limited and have to be used to the best possible advantage. In this maximum effort vaccination must be performed with potent vaccine by properly trained vaccinators. Each vaccination and revaccination must be examined after 6-8 days and vaccination must be repeated in those who do not show a major reaction. The target must be to cover 100% of the population. Special attention should be paid to the age-groups in which the disease most frequently occurs, as shown by analysis of age-specific attack rates, and to the newborn children and pregnant women, in whom mortality is very high. In the major centres the attack phase should be completed within six months if possible.

Control phase. As soon as one of these high density areas has been successfully vaccinated as planned the control phase may be put into operation. Depending on circumstances it may be the responsibility of the normal public health services or combined with other special control programmes such as malaria eradication, or the control of yaws or tuberculosis. In either case an increase in the staff will be necessary if this phase is to be successful. Those responsible will undertake the vaccination of newborn children, immigrants and floating populations and conscientiously follow the normal revaccination programme. They will be required to make an epidemiological investigation of each outbreak or sporadic case which occurs and to control the spread by " ring " vaccination (see page 28) around these foci of infection and by other control measures. In this phase attention should, where relevant, be paid to raising the general level of care and amenity in smallpox and infectious disease hospitals so as to diminish the fears of some populations and encourage them to seek admission instead of hiding patients.

In many countries the family registers compiled for the smallpox programme will be found useful for other social and health programmes.

The Committee was impressed by the work which has been accomplished and was convinced that smallpox eradication would be achieved

by sustained application of the Organization's programme over a period of years in the major endemic areas, where 1000 million people live and where the risk of infection poses a threat to themselves and to all other nations. The speed at which eradication will be accomplished will depend on how much practical help is to be given by the countries already free of the disease.

Control of smallpox in countries normally free of the disease

Smallpox has often been prevented in the past by detecting cases in persons arriving at seaports because the incubation period of the disease exceeded the duration of the journey from the country of infection to the country of destination. With air travel it is exceptional for a patient to be diagnosed on arrival at an airport. He usually develops symptoms days afterwards, by which time he may have moved freely in more than one country.

Early, accurate diagnosis is essential, and the clinical diagnosis of smallpox should form part of the teaching programmes of all medical schools. In the absence of cases useful knowledge can be acquired from good photographs. Doctors in general and hospital practice and especially those employed as port and airport medical officers need periodic reminders through medical journals, scientific meetings, etc., of the possibility of smallpox in any acute febrile condition if the person, or a close relative, has recently arrived from, or passed through, an infected country. Severe smallpox may resemble purpura, acute leukaemia, meningitis, pneumonia or even the " acute abdomen ". It is easy to confuse the milder benign types, particularly in the vaccinated, with chickenpox. In an adult who has just arrived from abroad a diagnosis of chickenpox should not be made without seriously considering the possibility of smallpox.

Immediate discussion (notification) with the health officer is essential whether the suspected person is in hospital or not. The health officer should have on immediate call a clinician with special experience of smallpox and if possible specialized virus laboratory facilities. Immediate appropriate action must be taken by public health authorities when smallpox is suspected clinically, and, since the laboratory provides later confirmation, it is more important to have access to an experienced laboratory, even if this occasions some delay, than to use a local but inexperienced laboratory.

Persons with suspected smallpox should be isolated immediately and investigations to trace close contacts instituted. Close contacts should be vaccinated, as soon as they are identified, with a highly potent vaccine, using two or three inoculations to accelerate the development of immunity. At this stage, every hour counts.

It is of the utmost importance that a scheme of priority is followed in the organization of vaccination and revaccination. The classification of contacts is most helpful.

Class 1: " Inner ring " contacts. These are members of the same household, persons working in close proximity during the early stages of illness, neighbours and visitors having actual contact with the patient, the patient's room or patient's clothing or bedding. These contacts should be kept under daily surveillance (including the taking of temperature) for 16 days. Though this can be left to medical auxiliary staff for the first nine days of the presumptive incubation period, thereafter contacts should be seen by a doctor. It may be convenient for the contact to spend these last few days in hospital. If he does develop even a mild attack, this " source " is then already under full control.

Anti-vaccinial gamma-globulin has some protective value and, if supplies permit, should be given, in addition to immediate vaccination by multiple insertion, to close contacts in whom the vaccinal immunity state was inadequate before contact. The prophylactic value of chemotherapeutic agents (see page 23) promises to be even better for this purpose and may be a most valuable tool in the hands of the health officer.

Class 2: " Outer ring " contacts. Such contacts comprise visitors and neighbours who have entered the house of the sick person but have no known contact with him or his immediate environment, and persons at the same workplace but not in close proximity with the patient.

Class 2 contacts are also important, and in countries where housing and environmental conditions are bad can best be dealt with by being included in Class 1.

Class 3: Remote or doubtful contacts. This class includes persons who live or work in the same locality but definitely have no contact with the infected home or inmates. This group is administratively difficult to deal with—the hundreds of potential contacts in the market-place, at the crowded railway station or at the football match which the patient attended. For administrative reasons it is obvious that the health officer will have to draw the line between Classes 1 and 2, the important contacts, and Class 3, of little importance. It is often argued that all, even the most remote, contacts should be vaccinated. This action may theoretically ensure complete safety, but the argument overlooks the practical difficulties and the fact that in the attempt to achieve complete coverage, the Class 1 and 2 contacts are frequently neglected by not ensuring that their vaccinations are successful. The smaller the number of health personnel available, the stronger the argument for concentration on Class 1 and 2 contacts.

Limitation of the number of epidemiologically unnecessary vaccinations should be related to the risk of serious complications, loss of working

time and the cost to the community, including public health or general practitioner service costs.

If diagnosis is made sufficiently early a smallpox outbreak does not spread quickly and control is effected not by indiscriminate vaccination but by vaccinating well those who are at greatest risk.

The hazard of infected bed linen and clothing, particularly from hotels and public institutions, reaching laundries and infecting sorters should be stressed. Disinfection measures are required, particularly of clothing and bedding.

The high proportion of cases among doctors, nurses and medical auxiliaries in recent outbreaks in countries where the disease is normally absent emphasizes the need to maintain a high level of immunity in public health and hospital staff.

International certification of vaccination or revaccination

The Committee was informed of the dicussions that had taken place in recent meetings of the World Health Assembly and the WHO Regional Committee for Europe on the adequacy of the present international requirements, particularly for revaccination.

The Committee stressed that the risk of international transfer of smallpox infections could best be reduced by ensuring the use of highly potent vaccine satisfactorily administered to intending travellers and could only be eliminated by the eradication of smallpox. An important practical contribution to the solution of the present problem would be to make available freeze-dried vaccine especially for revaccinations carried out in hot climates.

They did not support a proposal that revaccination should be read at the fourth day, since the result at this stage was difficult to interpret. A satisfactory reading could be made between the sixth and eighth days. If the result of the reading was equivocal the revaccination should immediately be repeated and preferably more than one inoculation should be made. If two inoculations were used, a second reading, though desirable, was not essential.

In the case of those known to be vaccinated or revaccinated within the previous five years, it would be technically acceptable to give three inoculations of a potent vaccine at the same time. This procedure must, however, be reserved for those who present evidence that they have had :

(*a*) a successful primary vaccination, or

(*b*) a successful revaccination, or

(*c*) a revaccination the result of which is not recorded as successful. Persons in this last category, however, may have a severe reaction from

the three inoculations if the previous revaccination failed to raise the immunity.

The Committee also considered the interval after which a person with a primary vaccination should be permitted to undertake international travel, and was of the opinion that an interval of eight days after inoculation of vaccine which resulted in a successful primary vaccination, read on the sixth to eighth day, was acceptable.

RECOMMENDATIONS

The success of the smallpox eradication programme within a reasonable period of time is directly linked on the one hand with the amount of practical assistance in the form of technical advice and the supply of vaccine and other essentials which the smallpox-free countries are prepared to give to the endemic countries, and on the other with the efforts which the endemic countries are prepared to put into the setting-up of effective programmes on a national or regional basis.

The Committee's principal recommendation is that WHO should take all steps in its power to increase the international co-operation so that the success of the programme will be ensured in the shortest possible time.

Other recommendations, some of which are contained in the appropriate sections of the text, are given below:

Close regional co-operation is necessary in the planning and execution of national vaccination programmes.

Countries in which notification of cases and deaths is defective should make an effort to effect improvements as an essential part of the smallpox control campaign. Determination of the vaccinial status of cases and of the population at risk could be of considerable value in increasing knowledge of smallpox in relation to vaccination. Inquiries of this nature could well be made in some endemic countries.

In all national vaccination campaigns, independent concurrent evaluation of the results is essential for the timely identification of deficiencies in the programme.

Studies on the variations in strains of variola virus from different parts of Africa should be continued and extended, and the laboratory studies should be combined with clinical and epidemiological observations in order to obtain sound information on the importance of the differences.

Further studies of vaccine strains used by different producers should be made and the laboratory findings compared with the results of vaccina-

tion and revaccination in the field in order to be certain that the present potency requirements for strains are satisfactory.

In order materially to improve acceptance rates in all countries, smallpox vaccines as potent as those at present available but causing less reaction and fewer complications should be developed.

The multiple pressure or the single-scratch method of vaccination should be used universally and the more traumatic methods should be given up.

Many chemical compounds for therapeutic and prophylactic use will become available for test in the near future. They should be tested in controlled studies under the aegis of a responsible research organization.

The terms " major reaction " and " equivocal reaction " (see pages 5 and 20) should replace other terms at present in use.

ACKNOWLEDGEMENTS

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Dr W. Ferreira, Medical Officer, Virus Diseases, WHO, and

Dr A. A. Sidky, Medical Officer, Virus Diseases, WHO.

Annex 1

COLLECTION AND DISPATCH OF SPECIMENS

Collection

Material from skin lesions. For microscopic demonstration and isolation of virus, material should be obtained by scraping macules, papules or the base of vesicles with a Hagedorn needle or small scalpel. The scraping should be smeared on clean slides. Five or six lesions should be sampled; gross admixture of blood is undesirable. The slides should be allowed to dry in air and should not be placed in a fixative or in a disinfectant. They should be separated from each other by means of rubber bands or small pieces of cardboard, wrapped in greaseproof paper, and placed in a container for dispatch to the laboratory.

Vesicle fluid and pustular fluid. They are best collected in small glass capillary tubes which are put in a screw-capped bottle or other suitable container for dispatch. If capillary tubes are not available, the material may be spread thickly on glass slides and allowed to dry in air. In the laboratory, the material on the slides may be washed off in a small volume of saline and used for the detection of antigen and/or for culture.

Blood. If blood is to be used for examination for virus in the first few days of illness, a few millilitres should be collected into citrate. If obtained for antibody studies, the blood should be allowed to clot and the serum then removed.

Mouth washings. The patient should be made to gargle or wash his mouth with 5-10 ml of physiological saline or broth; the fluid should be collected in sterile screw-capped bottles.

Scabs. At least six scabs per patient should be collected and placed in a screw-capped bottle for dispatch.

Dispatch

All the specimens mentioned above and the instruments used for their collection must be regarded as highly infective. It is therefore essential that all specimens and containers should be securely packed in metal, wooden or strong cardboard containers before being sent to the laboratory.

Specimens should reach the laboratory with the least possible delay. If they cannot be delivered by hand they should be sent by the most rapid

means of transport available and the receiving laboratory should be informed by telephone of the estimated time of their arrival. Packages sent through the post should conform to national and international postal requirements.

With all specimens sent to the laboratory, details should be given of the patient's age, name, address, history of contact, history of vaccination, date of onset of illness, and date of appearance of rash (if any).

Annex 2

LABORATORY TESTS FOR DIAGNOSIS OF SMALLPOX

Microscopic demonstration of virus particles

Smears from skin lesions (see Annex 1) should be fixed and stained for virus particles by Gutstein's, Hertzberg's or Gispen's modification of Morosow's technique. If such smears are properly prepared from early cases of smallpox, innumerable virus particles will often be found. When the lesions have become pustular, the results are not satisfactory. The only other lesions which may present a similar picture are those of vaccinia or cowpox. In smears from varicella or herpes simplex, elementary bodies are scanty, stain poorly and appear smaller.

The simplest staining method, that of Gutstein, is described below.

Slides smeared with scrapings are fixed by flooding with methanol for 10-30 minutes; alcohol is added as needed to prevent drying. The methanol is washed off with distilled water. A freshly prepared mixture of equal parts of 1% aqueous solution of methyl violet and 2% aqueous solution of sodium bicarbonate is filtered on to the slide. Gentle heat is applied until steam rises and this heating is repeated three or four times during a five-minute period. The stain is then flushed off the slide with distilled or tap water and the slide is blotted dry on filter-paper.

The smear is examined under an oil-immersion lens. A tentative positive report is made only if innumerable elementary bodies are seen. They are of uniform size, about one-quarter that of *Staphylococcus*, uniformly deeply stained and confined to the area of the smear. A smear from a vaccinial lesion has an identical appearance and constitutes an excellent comparison slide.

Virus particles are less numerous in vesicle fluid, and material from pustular lesions is unsatisfactory.

Immunofluorescence

This method for detecting virus particles in smears from early lesions is relatively simple if the necessary reagents and equipment are available. The indirect technique, using an immune rabbit serum to treat smears, followed by an antirabbit serum coupled with a fluorescent dye, gives more reliable results than the direct technique. Results to date do not suggest that this method has any great advantage over microscopic examination of stained smears.

Identification of antigen in the early stages of the disease

Gel-diffusion technique. The precipitation technique is a reliable diagnostic procedure if sufficient material is obtained from the skin lesions of a suspected patient. Vesicle or pustular fluid or extracts in saline of one or two crusts should suffice. A hyperimmune animal serum is used, though smallpox convalescent serum will sometimes give satisfactory results.

The older technique of carrying out precipitation tests in tubes, using a clarified crust extract against hyperimmune serum, has now given place to the gel-diffusion method. The test is best carried out on microscope slides. A 1-mm layer of agar is prepared on a slide, using a 1%-1.5% concentration of agar in isotonic phosphate-saline buffer at pH 7.3 containing 0.01% thromersal. This agar layer is made by permitting molten agar to harden between two slides (one preferably of Plexiglass or Perspex for easy removal) separated at the ends by 1-mm glass slides. Reservoirs or cups 4 mm in diameter, with centres 5-6 mm apart, are prepared.

Antigenic extracts are made from scabs by extraction with the phosphate buffered saline used for preparing the agar. Crusts are crushed with a glass rod and allowed to stand one hour at 37°C with a few drops of buffer to make approximately a 10% suspension w/v.

The immune serum should be placed in one cup and vesicle or pustular fluid or crust extracts placed in surrounding cups. It is always advisable to include in this test a known positive extract of smallpox or vaccinial material, and extracts should also be tested against a normal rabbit serum as an additional control. The slide is held in a humid atmosphere at room temperature.

If a suitable hyperimmune serum is used precipitate lines appear in the agar between the antigen and antiserum cups in two hours, linking with those in the positive control within four to five hours. If a weaker serum is used, results may be easier to read after 24 hours. A good convalescent herpes zoster serum may be included, as this will give a positive result in 24 hours with vesicle fluid if the case should be one of varicella.

Complement-fixation. The complement-fixation technique is a very sensitive method of detecting smallpox antigen in vesicle or pustular fluid

or extracts of crusts or in the blood serum of patients with fulminating infections. Such material should be tested with a hyperimmune rabbit serum and known positive and negative control preparations should be included with all diagnostic tests. Overnight fixation in the cold gives more satisfactory results than a shorter period of fixation at room temperature or at 37°C. The results should be available within 18-24 hours. This technique for detecting antigen is more sensitive than the agar gel-diffusion method, but the results are not so quickly available and the technique is rather more complicated.

Virus isolation. This is the most reliable and sensitive of all laboratory techniques for the diagnosis of smallpox. *It should always be used as a confirmatory test to supplement any other diagnostic method employed.* Isolation of virus may be carried out on the chorioallantoic membrane of 12-day chick embryos or in tissue cultures. Antibiotics are added (penicillin 500 μg/ml and streptomycin 500 mμ/ml) to the material from the patient's lesions before inoculation. This technique should give positive results at all stages of the disease, from the appearance of the first macule to the disappearance of all crusts from the patient's skin. In patients with severe, and particularly with fulminating, infections, virus may be isolated from the blood in the first day or two of illness. The buffy coat from the blood is more likely to give a positive result than the whole blood. Virus may sometimes be recovered from mouth washings during the first 10 days of disease.

The typical appearances produced on the chorioallantoic membrane within three days will enable a tentative specific diagnosis to be reached by the experienced worker without serological confirmation.

Tissue culture (preferably human or monkey cells) may be used instead of chorioallantoic membrane for isolation of virus. The presence of virus in the tissue culture may be detected by immunofluorescence or the development of Guarneri bodies in 24 hours, by haemabsorption of fowl cells in 48 hours, or by cytopathogenic effect in two to four days. These time intervals may be shortened when the virus content of the inoculum is high. Experienced workers may be able to identify the specific virus by the character of the inclusion bodies or the pattern of cytopathogenicity.

When confirmation of the identity of a virus isolated on the chorioallantoic membranes or in tissue culture is required, this may be done by demonstration of specific variola-vaccinial antigel by haemagglutination, complement-fixation or gel-diffusion techniques, using a specific antivaccinal serum prepared in the rabbit; or specific neutralization of the virus by immune serum may be determined in tissue culture or on the chorioallantoic membranes. These tests, however, will not distinguish variola from vaccinia virus. The differential identification can be confirmed

by the nature of the lesions on the chorioallantoic membranes and in tissue culture and by the fact that vaccinia virus will produce demonstrable lesions at an incubator temperature of 39°C-40°C while neither kind of variola virus will grow at this temperature.

Serological methods in the later stages of the disease

These methods are concerned with the detection of antibody in the patient's serum after the first few days of illness. In these tests either vaccinia or variola can be used as antigen. The examination of a specimen taken during the first few days of illness and one after the first week is desirable to demonstrate a rise in antibody titre. The early specimen is, however, frequently not available and the significance of a result has to be assessed on one specimen, with appropriate consideration of the patient's vaccination history.

Haemagglutination-inhibition. This technique for measuring antibody will often give high titres (over 1 : 1000) and is technically simpler than the complement-fixation test. Vaccinial haemagglutinin prepared in eggs is the antigen used. Since haemagglutination-inhibiting antibody may persist at low levels for some years after vaccination against smallpox, paired sera should be used, if available. Antibody rise can be demonstrated after the fifth to sixth day of illness.

Complement-fixation. This may be the best technique for detecting an increase in antibody in smallpox patients but requires a high level of technical competence. Either crust extract (treated with 0.2% formol) from known smallpox patients or vaccinial antigen prepared in the rabbit is used. The test usually becomes positive about the seventh or eighth day of illness, at serum dilutions up to 1/640. A positive result in a serum dilution of 1/20 or over would usually be significant in a patient not recently vaccinated.

Precipitating antibodies. Most convalescent smallpox sera used undiluted will form lines of precipitate in agar gel-diffusion against a vaccinia or variola antigen; this is not usually observed in post-vaccination sera.

Neutralization. The serum of patients with suspected smallpox may be tested for its power to neutralize vaccinia or variola virus on the chorioallantoic membrane or in tissue culture. This test is rather more time-consuming than either complement-fixation or haemagglutination-inhibition. Neutralizing antibodies may persist in the serum for many years after smallpox vaccination so that only a very high titre (greater than 1/1000) can be considered significant in a single test on a vaccinated individual when the serum-virus mixtures are allowed to react for two hours at 37°C before inoculation.

Annex 3

TECHNIQUES OF VACCINATION

In the *multiple pressure technique* a small drop of vaccine is placed on the skin and a series of pressures is made within the smallest possible skin area (not more than 1/8 inch or 3 mm in diameter) with the side of a sharp needle held tangentially to the skin. The pressures are made with the side of the needle, not the point. For revaccination, 30 strokes are completed in a few seconds, using an up-and-down motion perpendicular to the skin. For primary vaccinations, not more than 10 strokes are necessary. Immediately after the pressures are made, remaining vaccine is wiped off the skin. No signs of bleeding should occur. No dressing should be used.

The *scratch technique* consists of a single linear scratch not more than 1/4 inch or 6 mm in length performed through the vaccine with a needle or another suitable instrument. The scratch should not be too superficial. The needle should not draw blood but the scratch should be deep enough so that slight oozing occurs after a few seconds. The vaccine is rubbed into the scratch with the side of the needle, and no dressing is necessary.

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