SMALLPOX VACCINES

by

Dr Colin Kaplan
The Lister Institute of Preventive Medicine, Elstree, England

General principles of preparation and culture

Smallpox vaccine is a suspension in a suitable medium of infectious vaccinia virus. Most smallpox vaccine is prepared from virus obtained from the skins of animals. Such virus is usually contaminated to some degree by normal skin bacteria of the vaccinifer. The aim in preparing vaccine of this sort is to reduce the bacterial contamination to an acceptable level while at the same time maintaining the potency of the vaccine. The preparation of the vaccinifers is clearly of major importance.

Whatever animals are used they must obviously be healthy. There are geographical differences in the commonly occurring animal diseases. In Britain, for example, tuberculosis and foot and mouth disease are not serious hazards, since government agencies control them very closely. In some countries, however, either or both of these diseases may occur commonly enough to make it essential for vaccine producers to ensure that animals suffering from them are not either used for producing vaccinia virus or introduced into those parts of the production facility where they may contaminate (or potentially contaminate) the environment. Such contamination may result in the spread of the particular disease well beyond the confines of the laboratory, and may also, of course, jeopardize the health and safety of recipients of the vaccine. The first requirement, therefore, is an efficient system of quarantine and veterinary examination of all animals intended for vaccine production. Sheep should be vaccinated against contagious pustular dermatitis (orf) and used only if they are non-reactors, or when soaks on vaccination lesions have fallen off. An additional safety factor is introduced if the
vaccinifers are killed at the end of the incubation period and submitted to a thorough post-mortem examination. Local conditions, customs and beliefs may, however, discourage this step. Animals not killed should be kept under observation for long enough after the harvest to ensure that they are healthy.

Acceptable animals must be prepared for vaccination. This is done by shaving an area on flank or belly, preferably one not liable to soiling by excretions. The shaved area is thoroughly cleansing by washing with hot water and soap. In some circumstances it may be advisable to use an efficient skin disinfectant as well. Quaternary ammonium compounds have been found useful. Finally, before vaccination the prepared area should be well rinsed with sterile distilled water. While it is not essential that animals should be anaesthetized during the skin preparation and vaccination, some authorities think it desirable on humanitarian grounds. Veterinary "nembutal" in appropriate dose is an effective and easily administered anaesthetic.

The prepared skin is vaccinated by rubbing into superficial scarifications a seed virus of adequate potency. Some authorities believe that many, if not most, seed viruses are not of high enough potency. They believe that much effort should be expended to ensure a seed virus of very high infectivity. The extent of the vaccination is governed, as a rule, by local factors, especially whether the animals are to survive or not. After the vaccination the animals are removed to clean, fly-screened quarters during the period of development of the vaccinial eruption. The fodder and fresh water supplies must be adequate, and the pens in which the animals are kept must be cleaned frequently and thoroughly. The so-called incubation period should not be so long that crusts form on the vaccinated area. Four or five days is generally long enough.

Before the vaccinal material is collected the area should once more be cleaned by washing with warm water and soap. The eruption is then scraped from the skin with a curette. If it is possible to kill the animals, exsanguination before the scraping ensures a whiter-looking product. If the animals are not killed, anaesthetization before scraping is desirable.
From the quarantine period onwards, permanent records should be kept of rectal temperatures taken at least twice daily, weight and condition of animals before vaccination, condition during the incubation period, weight of the vaccinal material collected and abnormal post-mortem findings, if any. It should be possible to identify vaccinal material at any stage of its existence and associate it with the permanent record.

After collection, vaccinal material should be stored continuously at temperatures below 0°C except when being processed. Traditionally, virus-containing pulp is reputed to retain its potency indefinitely if properly stored. It may be doubted that temperatures of -10°C to -20°C constitute proper storage indefinitely, but pulps may be expected to retain their potency for many months in this temperature range.

Again traditionally, vaccinal pulp has, in the past, been ground into so-called lymph by comminuting it with 40-60 per cent. glycerol in such devices as the Chalybeus Grinder or the Doerring Roller Mill. The vaccinia virus may, today, be extracted more rapidly and at least as efficiently by homogenizing the pulp in any of several machines (e.g. the Silverson Laboratory Mixer, the Servall Omnimix or the Waring Blender). Storage of the homogenate at low temperature in the presence of 40-60 per cent. glycerol is accompanied by a steady decrease in the contamination by viable bacteria over a period of months. The same end result may be more rapidly achieved by treating an aqueous extract or homogenate with phenol or some other suitable bactericidal substance. Glycerol may subsequently be added both to prevent the multiplication of such viable bacteria as may remain and to impart a suitable viscosity to the vaccine.

If the material is treated only by the addition of glycerol bacteriological tests must be repeated at intervals to determine whether it is fit for issue. With phenol or other suitable substance, however, bacteriological testing is seldom necessary more than twice. When the minimum standard of bacterial purity is attained it may be thought expedient to make pools of approximately equal volumes
for dispensing. The minimal standard of bacteriological purity to be aimed at is that suggested in the Technical Report Series, No. 180. Bacteriological testing should always include tests for the isolation and identification of anaerobic organisms as well as certain specified aerobic organisms.

It is believed by some that a certain amount of bacterial contamination of smallpox vaccine is not only innocuous, but actually useful in promoting a satisfactory level or longer duration of immunity. In the absence of favourable evidence such opinions should be regarded with reserve and not taken as the occasion for relaxing efforts to improve the elegance and bacterial purity of the product.

Before a vaccine is issued for use it should have been shown to have a satisfactory potency when material from final containers is tested. At least one of the potency tests in Technical Report Series No. 180 should be used. It cannot be too strongly stressed that testing a vaccine at only one dilution may give very misleading results. Ideally a proper assay of virus should be undertaken. The potency should be not less than that recommended in No. 180 of the Technical Report Series. Permanent records should be kept of all bacteriological and potency tests.

Strains of virus

Many different strains of vaccinia virus are used for the preparation of smallpox vaccine. The origins of few, if any, of them are known. It is highly improbable, however, that any of them was derived from variola virus (see Herrlich et al. (1963) Arch. Virusforsch. 12, 579). Some vaccine strains are more pathogenic than others (see Polak et al. (1963) Bull. Wld Hlth Org. 29, 311). There is no evidence that strains producing severe local lesions and marked systemic disturbance confer better protection than strains producing milder clinical reactions. The less pathogenic strains should, therefore, be preferred for vaccine production. The practice of occasionally passaging the vaccine strain in the skin of, for example, the donkey, to restore or increase the "virulence" of the seed is illogical and undesirable. Other things being equal the strain of choice should produce compact, well-defined lesions (pocks) on chick embryo chorioallantois, with a minimum of necrosis: it will thus be suitable for assay by the pock-counting technique.
Many producers of smallpox vaccine maintain 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 where there are facilities for either freeze-drying or deep sub-zero storage of a primary seed lot it has no advantages over a seed lot system as recommended in the Technical Report Series No. 180.

Animals used for vaccine production

The animals most commonly used as vaccinifers are young bovines in which tuberculosis is less common than in older animals. In many Asian countries water-buffalo calves (*Bubalus bubalis*) are more readily available than cow calves (*Bos taurus*) and are accordingly much used as a source of vaccinia virus. Vaccinia virus is potentially infectious for a wide range of hosts any of which may, theoretically, be used as a vaccinifer. In practice, however, those producers not using bovines usually use the smaller two-toed ungulates such as sheep or goats. Despite the smaller yield of virus-containing material from such animals they may have certain advantages over larger animals for some producers.

Vaccines grown in chick embryonic membranes

Several producers, notably the State of Texas, United States of America, successfully prepare vaccine from virus propagated in chick embryonic chorioallantois. Such vaccine should be bacteriologically sterile. It may be doubted, however, that production methods based on growth of virus in chick embryos are suited to the conditions in many tropical countries, where eggs would in any case be more usefully employed as articles of diet. Apart from this, the necessity for ensuring a regular supply of eggs to producing laboratories might lead to relatively great expenditure of money and effort on poultry farms and poultry husbandry generally. And in some parts of the world there might have to be too great a diversion of trained laboratory staff from other objectives, such as pathological services, to the production of smallpox vaccine.
Vaccine derived from tissue culture

The production of smallpox vaccine by tissue culture methods is clearly a worthwhile aim. At present it is an experimental exercise. Successful large-scale production of a vaccine known to contain no adventitious viruses has not yet been attained. Even when it is, traditional methods ought not to be abandoned unless new methods can assure vaccines at least as safe and effective as the best traditional vaccines.

Liquid and dried vaccines

Both types of vaccine have their place in the control of smallpox. Liquid vaccines are more useful in temperate climates and in countries with short internal distances and good communications. In hot climates and under conditions of difficult communications, dried smallpox vaccine is the material of choice since its stability obviates many of the difficulties associated with transport and storage. In addition, when all, or a very large part, of a region's requirements are met by dried vaccine, it should be possible to reduce the number of vaccine producers in that region and ensure that a high standard of both practice and product is maintained in vaccine establishments.

Killed and attenuated vaccines

The term "attenuated" is best not used in relation to smallpox vaccine. It is used, generally without definition, to mean either reduced infectivity or reduced virulence. It is usually difficult, if not impossible, to determine, even from the context in which it occurs, the sense assumed by the user. If smallpox vaccine strains are not of attenuated (i.e. reduced) virulence or pathogenicity they should not be used in the production of vaccine.

Killed or non-infectious smallpox vaccines have been the subject of research for many years. Many methods of killing have been investigated, but a preparation consistently stimulating vaccinia-neutralizing antibody in individuals previously unvaccinated has only recently been attained. Despite the accumulation of traditional lore about local reactions at the site of revaccination there is no
real information about the relative importance of neutralizing antibody and allergic hypersensitivity in immunity to vaccinia virus, let alone immunity to smallpox. The subject is still in the experimental stage. The occurrence of modified local reactions to challenge with live virus after immunization with non-infectious antigen gives information about the allergic state of the individual, but the non-occurrence of modified reactions cannot be assumed to imply lack of immunity.

If killed smallpox vaccine is shown to be immunogenic (i.e. to possess protective antigenicity), its main usefulness will differ according to the endemicity of smallpox. In areas where the disease is endemic and there is a measurable risk of unprotected individuals being infected, a prime function of the killed vaccine would be to immunize eczematous and other infants in whom vaccination with live virus might be hazardous. It could also be used to confer a basic immunity on unprotected adults entering an area of high endemicity who might otherwise be at risk of vaccination complications. In areas where smallpox is very well controlled or eradicated there may be some resistance to conventional vaccination on the ground that risks from vaccination are greater than the potential risks from smallpox. Immunization with an effective killed antigen should help to overcome any reluctance to vaccinate infants and assure a sufficiently high rate of immunity to prevent the rapid spread of smallpox introduced into such a community.

Importance of potency of vaccines in primary and revaccination

The insertion success rate in revaccination is lower than in primary vaccination. This is presumably related to the immunity of the subject. Vaccines of considerably reduced potency can be made to give a high take-rate in the unvaccinated, but even in the hands of a very skilful vaccinator the revaccination take-rate with such a vaccine is less than normal. For revaccination, therefore, especially in epidemic conditions, vaccine of high potency is essential if protection of the population is to be assured. Potency tests involving the vaccination of rabbits with only one dilution of vaccine, or the primary vaccination of even a large group
of infants may give very misleading results. If tests in man are to be used, each batch of vaccine should be tested in a sufficiently large group of revaccinations, preferably by more than one vaccinator. Proper potency assays, however, are preferable. They may be performed as recommended in Technical Report Series No. 180.

It may be thought worthwhile to make available two strengths of smallpox vaccine; one of not more than the recommended minimum potency for primary vaccination, and a second with a minimum potency of about five times the present minimum, for revaccination. This is technically feasible and is the practice in at least one Scandinavian country. There may, of course, be some administrative difficulties, but they should yield to a little ingenuity.