Advances in the Immunoprophylaxis of Smallpox[1]

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IT is a unique case in the history of medicine when a method and its means for application survive for 160 years almost unchanged, particularly when these 160 years represent a complete revolution in thought and approach to most medical problems. However, this is the case with smallpox vaccination and with the smallpox vaccine, which is even today basically prepared in a very similar way to that devised by Dr. Duquemelle in Rheims, France, in the first decade of the last century (1, 2, 3).

However, this does not mean either that the vaccine is so satisfactory that it needs no improvement or that the tremendous amount of research concentrated on smallpox vaccine was without results. Although in the last twenty years progress in this field has been less dramatic than in other fields of immunoprophylaxis of virus diseases, such as poliomyelitis or measles, laboratory research and field investigations have cleared up many points which were passed from generation to generation of medical people in some kind of mythical form, or at least in dogmatic terms (4).

One of the very much discussed and extremely controversial subjects in this field was the nature and origin of the vaccinia virus itself. At the present time, the generally accepted view is that vaccinia virus represents a separate entity in the group of the animal pox viruses, which includes both mammalian and fowl pox viruses. The interrelationship among the various members of the mammalian pox viruses is a very close one (5). Morphological differences are almost nonexistent; they share several common antigens, although they appear to be serologically distinct. Nevertheless, at one point they can be differentiated: in their host range, in their ability or inability to infect and multiply in various laboratory and non-laboratory animals. Taking all this into account, it appears that vaccinia virus is most closely related to the cowpox virus from which it most probably originates. Phylogenetically the whole group derives probably from one common ancestor and its differentiation must be connected with the domestication of animals by the first agricultural settlers of the human race (6).

The difficulty of tracing the origin of vaccinia virus strains may be exemplified by the history of the Lister Institute's vaccine strain, which is currently used in Great Britain. Allegedly it came from France, where it had been isolated from a soldier who contracted smallpox during the Franco-German war in 1870 (1).

Different vaccine-producing laboratories propagate their virus strains in various animals, mostly ruminants like calves, sheep or water buffaloes, or in rabbits.

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Passage of the strains in humans has been discontinued in most countries, but even today in certain countries alternate passages in different animals are part of the regular procedure in the maintenance of vaccine strains.

Although all vaccinia strains are serologically identical, certain strains may acquire distinct properties, mostly through artificial conditioning. In this way neurotropy can be developed, or affinity towards deeper lying tissues of mesodermal origin. Only those strains which multiply in epithelial tissue without a tendency to generalization are suitable for vaccine production. Nevertheless, even in such strains very distinct differences exist in regard to their pathogenicity in vaccinated humans. However, so far it has not been demonstrated convincingly that the occurrence of postvaccinal neuro-complications, progressive vaccinia or similar generalized conditions can be related to certain more aggressive vaccinia strains (7).

Many problems are encountered in preparation of a vaccine of satisfactory purity. The difficulty of preparing a vaccine of a high potency and free of contaminating bacteria as well can be illustrated by the fact that no regulation requires complete bacterial sterility of the smallpox vaccines. Canadian regulations permit a count not exceeding 500 bacteria per ml. of vaccine, provided complete absence of pathogenic bacteria has been achieved. This is probably the only biological product in which bacterial contamination is tolerated as an almost unavoidable condition. The contamination of the vaccine originates from the skin of the animal in which the vaccine is prepared which becomes invaded by bacteria during the incubation period, after the inoculation of the vaccinia virus. Spraying of the animals with dyes, disinfectants and various chemicals which inhibit bacterial growth to a greater extent than virus multiplication is not a completely satisfactory solution. In this respect progress has been made by the topical use of different antibiotics which have a strong antibacterial activity without inhibiting virus multiplication. During further processing, the antibiotics are removed, so that in the vaccine scarcely any trace of them can be demonstrated. It is not likely that such infinitesimal amounts could induce sensitization in vaccinated persons or allergic reactions in already sensitized individuals.

Various Forms of Vaccine

The *liquid glycerinated vaccine* is still the most popular type especially suitable for individual use. The last major advance in its preparation dates back about twenty years, when phenolization up to 0.5% proved to be very useful in cutting down contamination without impairing too much the potency of the vaccine.

The major disadvantage of the liquid vaccine is its instability, its rapid loss of potency at temperatures above its freezing point. While it can be kept at $-30°$ C. in practically unchanged condition for at least 15 months, and probably much longer, at $+4°$ C. a demonstrable loss in potency can be observed after 5 to 6 months. At room temperature the loss of potency is such that after 1 week the number of takes would be reduced significantly. This heat lability of the liquid vaccine renders it unsuitable for mass vaccination in tropical areas where smallpox is still endemo-epidemic. This was one of the major reasons why eradication programs could not be started successfully until recently.

The most important progress in the field of vaccine production has been the

development of a highly potent, and to a great extent purified, sterile and extremely stable *freeze dried smallpox vaccine* (8, 9). The idea of having the vaccine preserved in the dried state is about as old as the liquid vaccine itself. The first dried vaccine produced in a practical way and administered to several thousands of people in the tropics was made available by L. Otten who worked in the Dutch East Indies before World War II. His vaccine was remarkably stable at high temperatures, gave a very satisfactory percentage of takes, but unfortunately it was highly contaminated with bacteria and very crude. He did not use any purification method and his drying method was that of using H_2SO_4 in a desiccator. He was the first to demonstrate the importance of having the dried vaccine sealed in vacuum (10, 11).

The present dried vaccines are purified either by physical means, using various numbers of alternate low and high speed centrifugation cycles, or by chemical means, using derivatives of the fluorocarbon group for the removal of non-specific substances. These vaccines are sterile, or contain only a very small number of bacteria. They are highly potent, with a titre adjusted prior to freeze drying in a manner to ensure a theoretical 100% take in primary vaccination. And finally, the dried vaccine is stable at $+4°$ C. for a number of years, at $+37°$ C. for at least 1 year, and for several months at $+42°$ C. Even after exposure to 100° C. for 1 hour it still retains sufficient potency for vaccination. Once reconstituted, its stability is comparable to that of the liquid glycerinated vaccine (12, 13).

The dried vaccine has been used successfully in eradication programs in South America and in many parts of South East Asia. It comes in vials of 10, 25 or 50 doses and it is very suitable for mass vaccination.

Attempts to overcome the difficulties in using animals for vaccine production date back at least thirty years. They resulted in two kinds of vaccine preparations: the egg vaccine and the tissue culture vaccine.

The *egg vaccine* is based on the great sensitivity of the chorioallantoic membrane of the developing chick embryo towards the vaccinia virus. Its attractiveness lies mainly in the ease of producing bacteriologically sterile vaccine, in the rapidity by which vaccine lots can be prepared and in the economy of the method (14). However, when propagated continually in eggs, the virus tends to lose in its immunogenic properties and to acquire certain undesirable properties, such as an affinity towards mesodermal cells and inducement of hemorrhagic pocks on the allantoic membrane. This can be overcome by preparation of great amounts of seed virus of known qualities which can then be used for production of a great number of vaccine lots. The economy of this method lies in the fact that approximately 50 doses can be prepared per egg. The egg vaccine may be prepared both in liquid or in freeze dried form, although its stability seems to be somewhat less than that of calf vaccine.

The egg vaccine never gained wide acceptance. The potential danger of using eggs infected with viruses of fowl leucoses or other avian viruses may be one of the reasons for this. It has been used, however, on a fairly large scale in Texas since 1939, and also on a limited scale in India, Sweden and Holland (15). According to all reports, it compares favourably with the classical vaccine. It should be mentioned also that cases of post-vaccinal neuro-complications are known after administration of the egg vaccine.

Tissue culture vaccine. Attempts to produce smallpox vaccine by propagation of the vaccinia virus *in vitro* were made far back in the heroic days of the tissue culture technique. With the development of the newer methods of cell propagation these attempts became more and more numerous. Since vaccinia virus is one of the most easily propagated viruses in tissue cultures, it seemed quite reasonable to believe that all the animals involved in vaccine production would soon become part of the past and give way to the more elegant method of vaccine production performed in tubes and flasks. Unfortunately, so far this is not the case, the main reason being that the virus yield in the harvested cells and cell fluid is below the desirable level. The virus has to be concentrated at least ten times to be comparable to the calf vaccine. The cells and tissues used mostly for preparation of this type of vaccine are chick fibroblasts, bovine embryonic skin, and bovine embryonic tongue epithelium. Numerous reports have been published on successful vaccination of several thousand persons, both for primary and revaccination purposes, with a fair success rate in general (16, 17).

The advantages of a tissue culture vaccine are so obvious, having in mind primarily the purity of the product, simplicity of its preparation and economy, that it is to be hoped that satisfactory methods of production will be developed soon.

Evaluation of Potency

The greatest progress in vaccine production has been made probably in the field of vaccine evaluation, potency testing and correlation of vaccine potency to takes in primary vaccinees and revaccinated persons of different age groups. Much of the credit in this respect should go to the World Health Organization whose teams in the last few years have cleared up a great number of epidemiological and immunological problems concerning the prevention of smallpox.

Until recently, the rabbit scarification test was the only one used routinely for vaccine potency determination. It is still the only one prescribed by government regulations. Most likely it will never be omitted, since it gives a good indication of the effect of the vaccine when applied to the human skin. However, it cannot be considered as a quantitative test in the true sense. There are two methods, used more and more widely, which by determination of the number of infective units in a certain amount of the vaccine make it possible not only to predict the percentage of takes in susceptible persons, but also to adjust the concentration of the vaccine in such a way as to ensure its optimal potency. These methods are the determination of the number of pock-forming units on the dropped chorioallantoic membrane of the chick embryo and the determination of the number of plaques in monolayer cell cultures caused by virus particles present in a given amount of vaccine. It has been generally observed that vaccines containing between $5 \times 10^7 - 10^8$ infectious particles per milliliter will give 100% takes, and those containing approximately 2×10^6 infectious units will vaccinate successfully only 50% of susceptible persons. However, it seems that a positive take will render to the vaccinee a degree of protection which is independent of the potency of the vaccine. Based on combined laboratory and field experience, standards were set up by the WHO which are a great help both to the vaccine producer and to those who are applying the vaccine. The standards were set up

to ensure 100% takes in primary vaccination and a high percentage of takes in revaccination. Persons with partial immunity resulting from a previous vaccination need to be vaccinated with a highly potent vaccine in order to induce a maximal percentage of positive reactions (18–21).

Live Vaccine Versus Inactivated Vaccine

The controversy of live vaccine versus inactivated vaccine has never affected smallpox vaccination, since no doubts exist so far that only live vaccine can give adequate protection against the disease. However, it has been generally recognized as desirable to have an inactivated vaccine, capable of inducing a low degree of basic immunity which would be increased at a later stage by application of a live vaccine of full strength. It has been assumed that even a low level of active immunity would be sufficient to prevent viremia, which seems invariably to follow primary vaccination with live smallpox vaccine. In this way it has been hoped to prevent the postvaccinal spread of the vaccinia virus and consequently to eliminate the danger of postvaccinal neuro-complications, generalized and progressive vaccinia.

So far three types of inactivated smallpox vaccine have been described: the *formaldehyde inactivated,* the *ultraviolet light* and the *gamma ray irradiated* vaccines. Reports on these vaccines are controversial. They seem to induce some serological response which, however, is inferior to the response induced by the live vaccine both qualitatively and quantitatively.

The formol-inactivated vaccine is being used in Germany and Holland on quite a large scale in primary vaccination of adults and children in specific cases. Reports state that after vaccination with the live vaccine more than 50% of the persons pretreated with the inactivated vaccine show a modified response of a mild type (22, 23, 24, 25). However, there are reports which deny any beneficial effect of the pre-treatment with killed vaccine (26, 27). There is at least one known case of postvaccinal encephalitis in a child vaccinated as described above (28).

The U.V. and the gamma ray irradiated vaccines were used in selected groups in England with encouraging results. However, it does not seem likely for the near future that killed vaccine will enter into routine use (29–31).

Hyperimmune Antivaccinia Gamma Globulin

Sera of actively immunized animals or humans have been used successfully for many decades both for prophylactic and therapeutic purposes in many infectious diseases. Results achieved in this field with *hyperimmune gamma globulins* are even better due to their purity and higher concentration of immune substances. For reasons unknown these substances were, until recently, completely omitted in any aspect of smallpox prophylaxis or therapy. Now it seems to be proved that hyperimmune antivaccinia gamma globulin deserves to have its place among the other preparations with an effect that covers three aspects of the field: prevention of smallpox in contacts during the incubation period (32, 33); prevention of postvaccinal neuro-complications (34); and therapy of postvaccinal skin lesions due to hematogenic dissemination of the vaccinia virus (35).

Hyperimmune gamma globulin in conjunction with vigorous smallpox vaccination reduces smallpox cases in contacts by 70 to 100% compared to control groups to which only vaccine had been administered.

In Holland, simultaneous administration of smallpox vaccine and hyperimmune gamma globulin became the routine procedure for the Dutch Army. The number of neuro-complications was reduced by 77%.

According to German reports, when given to children simultaneously with the vaccine, hyperimmune serum or gamma globulin alleviates significantly side effects of vaccination, such as high temperature and general malaise. When given in too great an amount, even the local reaction may be completely suppressed (36).

The therapeutic effect of the hyperimmune gamma globulin is not so much to be relied upon. In several cases of postvaccinal encephalitis and progressive vaccinia its application remained without effect, but in many cases of eczema vaccinatum and generalized vaccinia it has been used with very good results.

There are two more substances which should be briefly mentioned in this context. The first is Interferon, a proteinlike substance produced by infected cells both *in vivo* and *in vitro*. It probably plays an important role in cellular immunity. It exerts a strong antiviral effect on a broad spectrum. It is not likely that Interferon will ever play any significant role in smallpox prophylaxis, but it seems to be effective in the treatment of vaccinial skin complications and vaccinial keratitis (37).

The other substance introduced recently is Marboran, a thio-semicarbazone compound. Its antiviral effect has been known for many years. According to recent reports, it has been most successfully used in smallpox contacts in India. Among 1,100 contacts treated with this chemical only 3 mild cases of smallpox occurred, while in a carefully selected control group of the same number there were 78 cases, 18 of which were fatal (38). Marboran has been used with most dramatic results in almost hopeless cases of necrotic vaccinia (39). It should be pointed out that this compound is the first chemical agent successfully used in a virus disease.

Towards Eradication

We now have all the necessary knowledge and technical skill needed for the eradication of smallpox. According to the WHO approximately 1 billion people are still living in endemo-epidemic areas. Even those parts of the world where smallpox has ceased to be indigenous must maintain all the traditional measures in order to prevent a rapid spread of imported infections. Due to rapid and more intensive traffic between all parts of the world the sanitary vigilance has to be even more intense than two or three decades ago (40).

On the other hand, eradication of smallpox is feasible. The cost would not amount to more than 10 cents per person in the areas concerned. And after smallpox is eradicated, or at least reduced to a non-dangerous level, antismallpox vaccination as we have known it for one and a half centuries could be abolished, at least in the Western World. When that time comes, and it may not be too long, the abolition of general smallpox vaccination will represent the greatest recognition of Edward Jenner's genius.

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