JENNERIAN PROPHYLAXIS BY MEANS OF INTRADERMAL INJECTIONS OF CULTURE VACCINE VIRUS

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PLATE 25

(Received for publication, June 28, 1935)

The successful cultivation of vaccine virus in a medium consisting of minced chick embryo tissue suspended in Tyrode's solution and the use of this virus by means of dermal inoculation for Jennerian prophylaxis in man have been described in previous communications (1, 2). A continuation of investigations of culture vaccine virus has rendered possible at this time a statement regarding the desirability of its intradermal use in man and a presentation of facts pertaining to its preservation and application.

In an earlier report (2) the fact that the titer of our vaccine gradually diminished during the course of successive passages in the medium used was recorded. At that time there was also described a strain of culture virus, designated as the second revived strain, which maintained a constant titer and appeared to have become adapted to *in vitro* cultivation. This second revived strain has now been carried through 130 successive passages in chick embryo tissue and Tyrode's solution during a period of 3 years and still regularly yields when injected intradermally into rabbits typical mild vaccinal lesions. It is assumed, therefore, that serial passage in cultures may be continued much longer, or that by reversion to earlier cultures preserved at low temperatures the necessity of further rejuvenation by passage through animals will seldom arise.

Intradermal Vaccination against Smallpox

Since the cultivation of vaccine virus *in vitro* has made available an active agent free from bacteria and now sufficiently mild in its

action to be injected with safety into the skin of human beings, we believe that intradermal vaccination deserves serious consideration as a means of preventing smallpox. The procedure is quickly and easily executed and the amount of material inoculated can be controlled accurately. The resultant reactions are characterized by erythema and induration without the formation of an open sore and a disfiguring scar. There is, therefore, little opportunity for the occurrence of secondary infections. The absence of a vaccinal scar is not an undesirable feature since the presence of such a scar is no assurance of immunity to smallpox. Furthermore, the reactions produced by intradermal injections of vaccine virus in individuals who have been previously vaccinated are characteristic and more susceptible of interpretation in terms of resistance to infection than are the reactions that follow dermal revaccination.

Method of Intradermal Inoculation

Routine cultures of vaccine virus after being ground in a mortar without an abrasive are tested for the presence of ordinary bacteria. Bacteria-free emulsions are then mixed with equal amounts of sterile glycerol and stored at a temperature several degrees below 0°C. With such material most of our intradermal vaccinations and revaccinations have been conducted in the following manner. 0.1 cc. of glycerolated culture virus diluted 5–10 times with sterile salt solution is injected into the skin of the upper arm or thigh by means of a tuberculin syringe fitted with a 27 gauge needle in a manner similar to that used in making an intradermal tuberculin or Schick test. After the injection has been completed it is essential to cleanse the needle wound thoroughly with alcohol in order to prevent the formation of a vesicle on the surface of the skin. No dressing or special care is indicated; *i.e.*, the arm or thigh can be freely bathed throughout the evolution of the intradermal lesion.

Results of Primary Intradermal Vaccinations

In the clinic at the Hospital of The Rockefeller Institute there is no opportunity of vaccinating a large number of people. Nevertheless, from time to time we are requested to make primary vaccinations, and during the last year and a half 29 infants and children have received the culture virus intradermally.

When an individual who has never had a successful vaccination receives the culture virus intradermally, nothing abnormal is noted at the point of inoculation until 4 to 9 days later. Then a small red papule appears which gradually becomes

larger until the area of induration and erythema is 2 or 3 cm. in diameter (Fig. 1). At the height of the reaction, which is usually 4 to 6 days after its initiation, a secondary zone of erythema, less intense than the primary, makes its appearance around the lesion, remains for a day or two, and then rapidly disappears. The induration gradually becomes less marked and as a rule is no longer discernible 3 weeks after the appearance of the lesion. If the inoculation is made properly there is no vesicle formation to leave a scar.

The 29 infants and children received one intradermal inoculation of the second revived strain of culture vaccine virus (42_2–87_2 culture generations were used) that had been stored with glycerol 6 days to 6 months. All of them reacted with primary takes.

19 of them were under observation to such an extent that we could determine the time of appearance of the lesions; 2 appeared on the 4th day after inoculation, 2 on the 5th day, 3 on the 6th day, 5 on the 7th day, 2 on the 8th day, 2 on the 9th day, and 1 each on the 10th, 13th, and 17th days. None of the children seemed sick as a result of the vaccinations. While most of them showed an increase in the size of the axillary lymph nodes, only 2 had fever, and in them the elevation of temperature lasted only one day and went no higher than 101°F. One of the subjects that responded with a primary take had been unsuccessfully vaccinated on 3 occasions previously in which calf lymph and the scratch method of inoculation had been employed.

It has not been possible to make tests for immunity to calf lymph in all of the individuals that showed primary takes resulting from intradermal inoculation of culture virus. In 7 instances, however, such tests have been made. 6 infants revaccinated dermally with the New York City Board of Health calf lymph, 13 days, 16 days, 17 days, 19 days, 20 days, and 6 months, respectively, after a primary intradermal vaccinal lesion responded with immune reactions, while 1 infant revaccinated after a lapse of 7 months responded with an accelerated take.

Results of Revaccination by the Intradermal Method

Revaccinations by means of calf lymph applied dermally result in immediate or immune reactions, or in accelerated takes. Most investigators admit, however, that it is difficult to differentiate an immune reaction from no reaction, because trauma alone incident to a dermal vaccination induces a certain amount of reaction that leads to confusion. In view of this fact, we attempted to ascertain whether

intradermal injections of culture virus in individuals previously successfully vaccinated would yield distinct and unmistakable immune reactions or accelerated takes. If such reactions regularly occur, then the absence of vaccinal scars would not be a valid reason for opposing intradermal vaccination, because within a short time one could determine whether an individual is immune to vaccinia or not.

33 individuals, 1 to 65 years of age, who had been successfully vaccinated one or more times with calf lymph, received intradermal injections of culture vaccine virus similar to those used for primary inoculations. All of them, with the exception of 2, responded with reactions comparable either to the immune reactions or to the accelerated takes that follow dermal revaccinations. The earliest reaction to occur appeared within 6 hours after inoculation, while others did not become evident until 2 or 3 days later. Those that appeared rapidly consisted of small red papules accompanied by itching and disappeared in a day or two. The ones that developed more slowly were larger than the others, endured for 3 or 4 days, and gradually disappeared to be followed by a slight desquamation of the involved skin.

The fact that no reactions were observed until from 4 to 17 days after primary intradermal inoculations of the culture virus led us to believe that the immediate and accelerated reactions encountered in revaccinated people were specific for vaccinia. To test the specificity of the reactions further, uninoculated media incubated and handled in a manner similar to that of the cultures were in many instances injected as a control when the revaccinations were made. None of the control injections produced demonstrable reactions. At this time it is of interest to record the fact that cultures of vaccine virus passed through filters which retain all of the infectious agent contain a soluble antigen that is innocuous for the skin of unvaccinated people but capable of producing a reaction in the skin of individuals immune to vaccinia, while filtrates of uninoculated media cause no obvious changes in the skin of either group.

Preservation of Culture Virus

Culture vaccine virus stored in 50 per cent glycerol retains its activity for several years and can be used for vaccination or the initiation of cultures. Such virus, however, does not remain active in the absence of refrigeration, a feature that has interfered with the dis-

tribution of the active agent by mail. In view of the fact that many viruses desiccated in a frozen state retain their activity under conditions ordinarily deleterious, we have attempted to obtain preparations of desiccated culture vaccine virus that are suitable for human use and retain their activity for considerable periods of time in the absence of refrigeration.

Some results obtained with desiccated culture vaccine virus have already been described (2). However, experience has shown that when such virus is dried in small amounts in glass tubes difficulty is encountered in removing the active agent from the containers. The virus apparently adheres to the walls of the tubes to such an extent that when fluid is added and the material from each of several tubes of the same batch is titrated in the skin of the same rabbit uniform results are not realized. Therefore, it became necessary to add to the culture virus some sterile innocuous substance that would act as a protective agent, add bulk to the dried preparations, and go into solution or become suspended with ease upon the addition of fluid. Egg albumen was the material investigated first.

Preservation of Culture Vaccine Virus in the Presence of Egg Albumen

Mixtures of culture virus and egg albumen were desiccated and handled in the following manner.

3 parts of culture virus, ground in a mortar without an abrasive until the particles of tissue are finely dispersed, are thoroughly mixed with 1 part of sterile egg albumen. The mixture is then distributed in 0.25 cc. amounts into small sterile test tubes, frozen rapidly by means of solid CO_2 in alcohol, and placed in a refrigerating box at $-4°C.$ in the presence of $CaCl_2$. Air is evacuated from the box by means of a Cenco-Hyvac pump, and throughout the period of desiccation the pressure in the box is maintained at approximately 1–2 mm. of mercury. Drying of the virus and egg albumen occurs rapidly. The tubes are usually allowed to remain in the box for 24 hours after which they are removed and placed in a desiccator at room temperature. When the tubes have returned to the temperature of the room they are removed from the desiccator and quickly sealed in a hot flame. The dried material appears as white foam-like pellets which can be freed with ease from the sides of the tubes and pulverized. It is readily miscible in water or saline solution.

A number of samples of culture virus have been dried with egg albumen and have been shown to retain their activity for considerable periods of time when held at room temperature. One experiment, summarized in Table I, showed by intradermal tests in rabbits that the virus kept under such conditions for $3\frac{1}{2}$ months was still active.

Desiccated mixtures of culture virus and egg albumen may be used for dermal vaccination of human beings.

A tube is opened aseptically, the dried material is pulverized in the container, a small amount of the powder is taken up on the end of a sterile wooden applicator and is rubbed on a scarified area of skin of the upper arm or thigh. The moist serous exudate resulting from the scarification dissolves the powder sufficiently

TABLE I

Record of Intradermal Titrations in Rabbits of Vaccine Virus, Culture 66_2, Desiccated with Egg Albumen and Stored at Room Temperature

Time of titration	Titer of virus
Before desiccation	10^{-5}
Immediately after desiccation	10^{-5}; 10^{-6}
5 days " "	10^{-5}
16 days " "	10^{-6}
1 mo. " "	10^{-5}
2 mos. " "	10^{-4}
$3\frac{1}{2}$ mos. " "	10^{-3}

to cause it to adhere to the skin. The evolution of the vaccinal lesions induced in this way is normal and similar to that caused by moderately potent commercial vaccine virus.

Eleven of 12 children who received dermal applications of the virus dried with egg albumen responded with typical primary takes. One of them was sensitive to the albumen and a large urticarial lesion developed almost immediately at the site of inoculation. This disappeared, however, and the evolution of the vaccinal lesion was entirely normal.

Egg white is a highly antigenic substance. In view of this fact, although the use of mixtures of virus and albumen for dermal vaccination is not attended by harmful results even in individuals already sensitive to egg white, one hesitates to advocate the use of such

preparations for intradermal vaccination. Inasmuch as we were particularly interested in developing a method of preserving the culture virus for intradermal use in human beings, we sought for harmless non-antigenic substances that would protect the active agent during the processes of drying and resuspension. Purified gum acacia has been found to act satisfactorily.

Preservation of Culture Vaccine Virus in the Presence of Purified Gum Acacia

A 30 per cent solution of gum acacia, purified for intravenous administration, was obtained from Eli Lilly and Co. Intradermal injections of this material in concentrations as high as 7.5 per cent caused no perceptible reactions in human beings. Mixtures of culture virus and acacia were desiccated with ease, and when dry they went back into solution readily upon the addition of water or saline solution. It appeared that we had found an ideal means of preserving the virus with the exception that the apparatus used for freezing and drying necessitated the exposure of the desiccated material to atmospheric pressure and moisture before the containers were sealed. We were aware, therefore, that a method of desiccation which would permit the containers of the dried product to be sealed while under a vacuum would be an improvement. Recently, Mudd and his associates (3, 4) have described an apparatus designed for this purpose, and we have adapted it to the preservation of our culture virus.

The Mudd apparatus (Fig. 2) used by us consists of a cylindrical glass bulb to which on one side near the top is fused in a horizontal position a long hollow glass side arm or manifold with 24 apertures. On the opposite side, the bulb is attached by a short side arm and rubber stopper to a trap which in turn is connected by pressure tubing to a Cenco-Hyvac pump. The bulb and trap are placed in a mixture of solid CO_2 and alcohol in a thermos jug. The material to be dried is frozen in pyrex glass containers which are then suspended from the apertures of the side arm or manifold by means of units consisting of rubber tubing, glass tubing, and a hollow rubber cork. Then the pressure in the apparatus is reduced rapidly to a low level, the material to be dried remains frozen, and the moisture drawn from the containers condenses in the large bulb immersed in the freezing mixture. The containers of the dried virus are sealed, while the vacuum pump is still running, by means of a torch equipped with 2 tips opposing each other and supplied with a mixture of oxygen and gas.

The method which we now use for the preservation of culture vaccine virus to be employed for vaccination of human beings is as follows.

1 part of a sterile 30 per cent solution of gum acacia is mixed with 11 parts of culture virus which has been ground in a mortar without an abrasive until the particles of tissue are finely dispersed. 0.4 or 0.8 cc. of the mixture are placed in each of a number of ampoules. The ampoules, blown from pyrex glass tubing with an internal diameter of approximately 5 mm., have necks about 8.5 cm. in length, flat bottoms, and a capacity of at least 2 cc. The virus is placed in the ampoules by means of a syringe with a long needle, after which the neck of each ampoule is inserted into one end of a short piece of pressure gum rubber tubing the other end of which receives an arm of a short piece of glass tubing bent to form a right angle. The other arm of the glass tube passes through a gum rubber stopper that fits an opening in the manifold of the drying apparatus. Each time before they are used, the bent glass tubes, with one arm pushed through its hollow rubber stopper and closed by a gauze plug and the other arm inserted into its short piece of rubber tubing, are wrapped separately in paper and sterilized in an autoclave. After 24 units consisting of ampoules, rubber tubing, glass tubing, and stoppers have been assembled in the manner described the contents of the ampoules are frozen by immersion in a mixture of solid CO_2 and alcohol. Then the units are rapidly connected with the drying apparatus by insertion of the rubber stopper of each unit into an aperture of the manifold after removal of the gauze plug from the end of the glass tube. The vacuum pump is started immediately, and within a few minutes the outer surface of the ampoules becomes covered with frost which gradually evaporates as desiccation proceeds. Desiccation is completed within 5 or 6 hours, and the necks of the ampoules are then sealed by means of an oxygen torch while the vacuum pump is still running.

When the dried material is desired for use an ampoule is opened and 2 cc. of sterile water or saline solution is added by means of a syringe and needle. In this manner a 1 to 2.5 or a 1 to 5 dilution of the virus is effected depending on whether 0.8 cc. or 0.4 cc. of virus have been placed in the ampoules before desiccation. The 1 to 2.5 or 1 to 5 dilutions of the virus may be used for intradermal vaccination of human beings, or by means of further dilutions they may be used for intradermal titrations in rabbits.

In view of previous experiences we have assumed that the virus dried in the presence of acacia will remain active for long periods of time when stored at low temperatures. However, we wanted to know definitely whether it would retain its activity under conditions encountered during transportation requiring several days or weeks. Consequently, we have tested numerous batches of the dried culture

TABLE II

Summary of Results of Experiments Performed to Determine Whether Mixtures of Culture Vaccine Virus and Gum Acacia (2.5 Per Cent) Frozen, Desiccated, and Sealed in Vacuo Would Retain Their Activity for a Reasonable Period of Time When Stored at 37°C.

No. of experiment	M20	M21	M22	M23	M25	M28	M29A	M29B	M30	M31	M32	M33	M34	M35
No. of culture generation	81_2	52_2	52_2	83_2	84_2	86_2	88_2	119_2	89_2	89_2	78_2	79_2	52_2	54_2
Titer before desiccation	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-6}	10^{-5}	10^{-5}	10^{-4}	10^{-5}	10^{-6}	10^{-6}	10^{-4}	10^{-5}	10^{-4}
Titer immediately after desiccation	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-6}	10^{-5}	10^{-5}	10^{-6}	10^{-4}	10^{-4}
Titer after 2 wks. at 37°C.	10^{-5}	10^{-4}	10^{-3}	10^{-4}	10^{-3}	10^{-4}	10^{-4}	10^{-3}	10^{-4}	10^{-5}	10^{-4}	10^{-3}	10^{-4}	
Titer after 1 mo. at 37°C.					10^{-2}									10^{-4}

virus that had been stored at 37°C. for 2 and 4 weeks. From the results of the experiments, summarized in Table II, it is obvious that the dried virus retains its activity remarkably well under such conditions.

We have been able to vaccinate and revaccinate only a few people with mixtures of virus and acacia. In every instance, however, the intradermal inoculation of the material resulted in primary reactions, accelerated takes, or immune responses.

Inasmuch as we are in no position to vaccinate a large number of people, the dried mixture of culture virus and acacia is being supplied to a group of physicians who will determine the rapidity of appearance and duration of immunity resulting from its intradermal use. Already more than a thousand individuals have been vaccinated and in the course of time reports concerning the value of intradermal vaccination with the culture virus will appear in the literature.

DISCUSSION

Intradermal vaccination of human beings against smallpox has been tried by a number of investigators and Roberts (5) has recently reviewed the literature on the subject. It should be clearly understood that we are not advocating the intradermal use of ordinary calf lymph or cultures of vaccine virus recently initiated from calf lymph or other sources, because such materials (1, 5) used intradermally may result in more severe constitutional disturbances and damages to the tissues at the site of inoculation than those caused by their dermal application. Although our culture virus came originally from the New York City Board of Health calf lymph it is free from ordinary bacteria and has now been propagated for such a length of time *in vitro* that mild reactions are regularly produced when it is injected intradermally in rabbits and human beings.

Our second revived strain of culture virus can be used with safety for intradermal vaccination of human beings, and when handled properly it induces a high percentage of takes which are followed within a reasonable length of time by an immunity to a standard dermal strain of vaccine virus. Consequently, we are convinced that the intradermal use of culture virus should be seriously considered as a method to be employed more extensively for protection against smallpox.

Knowledge concerning the duration of immunity produced by the culture virus is not available yet. Nevertheless, this will be obtained gradually. When the facts regarding the matter are presented, investigators and physicians should not be surprised to find that a certain number of successfully vaccinated individuals will have lost their immunity within a period of a year. At least that is the case with people who have been successfully vaccinated for the first time with calf lymph. For instance, Kitasato (6) found that 13.3 per cent of people successfully vaccinated with calf lymph were again susceptible a year later. Donnally and Nicholson (7), using a standard calf lymph, vaccinated 500 infants at birth. Fifty-two of the successfully vaccinated babies were revaccinated at from 13 to 16 months of age, and 30.8 per cent of them again exhibited primary takes. The facts just mentioned are not generally appreciated and indicate that in many countries revaccinations are not practiced with sufficient frequency and regularity.

It may be found that the immunity induced by one intradermal inoculation of culture virus will not be as enduring as that produced by potent calf lymph. If such proves to be the case, the advantages of the intradermal method of administration are so obvious and the amount of reaction and inconvenience caused by the culture virus is so inconsequential that two or more intradermal inoculations, made at the same time in different areas of skin, or more frequent revaccinations may meet with general favor as means of prolonging or renewing an immunity to smallpox.

This discussion should not be concluded without calling attention to the fact that the direct evidence in our possession only shows that the culture virus will prevent an infection with calf lymph. Such evidence leads us to believe that individuals vaccinated with culture virus are protected against smallpox. We cannot be absolutely certain that this is true, however, until people vaccinated with the culture virus have been exposed to smallpox.

CONCLUSIONS

The second revived strain of culture vaccine virus has been propagated through 130 culture passages during a period of 3 years. It seems to be adapted to *in vitro* cultivation and still has an intradermal titer (rabbits) of 1 to 100,000 or 1 to 1,000,000.

Intradermal inoculations in human beings of 0.1 cc. amounts of culture virus diluted from 2.5 to 10 times result in primary takes in unvaccinated people and immune reactions or accelerated takes in individuals previously successfully vaccinated. Primary takes produce an immunity to standard strains of calf lymph.

Culture virus mixed with purified gum acacia (2.5 per cent), frozen, desiccated, and sealed *in vacuo* retains its activity for a month at 37°C., and when the dried virus is resuspended in saline solution it is suitable for intradermal vaccination of human beings.

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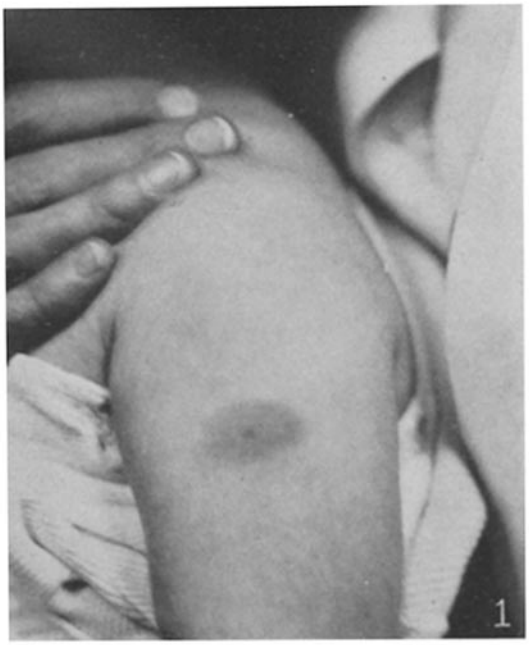
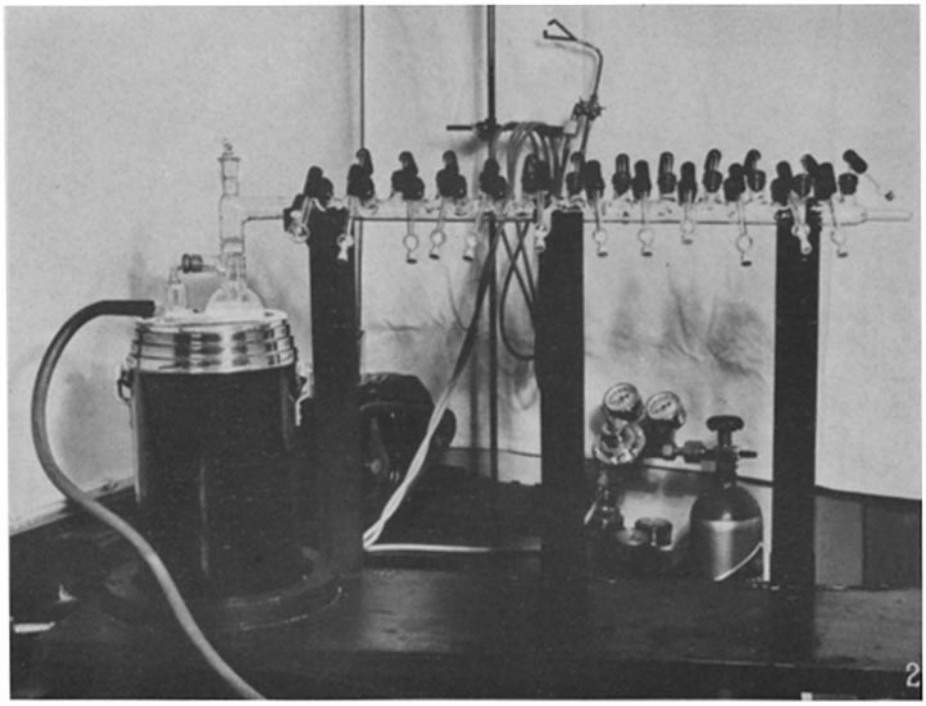
EXPLANATION OF PLATE 25

FIG. 1. Reaction produced in the skin of a young child by culture vaccine virus introduced intradermally. The lesion made its appearance 10 days after inoculation and was photographed 2 days later.

FIG. 2. The Mudd-Flosdorf drying apparatus used in the preservation of culture vaccine virus to be employed for intradermal vaccination of human beings. The tubes containing the virus are closed at the distal end with rubber stoppers. Later we decided that glass containers constructed so that rubber stoppers would not be needed are preferable.





Photographed by Joseph B. Haulenbeek

(Rivers and Ward: Intradermal use of culture vaccine virus)