AMOUNT AND DURATION OF IMMUNITY INDUCED BY INTRADERMAL INOCULATION OF CULTURED VACCINE VIRUS

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Jennerian prophylaxis in man by means of intradermal inoculation of cultured vaccine virus was described (1) in 1935. Since then this type of prophylaxis against smallpox has been used by us, by many private physicians, and by physicians in a number of clinics. For the last 3 years the virus employed has been from generations 50 to 170 of the "second revived" strain of cultured vaccine virus, dried from the frozen state in the presence of gum acacia and sealed *in vacuo* (1, 2). This strain (3) was originally derived from calf lymph vaccine virus supplied by the New York City Board of Health in 1931 and has been propagated since then by serial transfers in a medium consisting of Tyrode's solution and minced chick embryo tissue. Intradermal inoculation of the virus in rabbits reveals that it maintains a uniform potency during continued cultivation. Moreover, the lesions produced in rabbits are less severe than are those caused by other strains of vaccine virus.

When inoculated intradermally in susceptible persons, cultured vaccine virus produces a high percentage of positive reactions. The percentages reported by those to whom the virus has been dispensed have ranged from 80 to 100; it is probable that an average of over 90 per cent has been obtained in a minimum of 6000 to 7000 intradermal vaccinations. In our experience with more than 200 primary vaccinations the incidence of "takes" has been 100 per cent. Typical positive reactions appear as small red papules on the 4th to 9th day after inoculation. Erythema and induration increase until the lesions are 2 to 4 cm. in diameter at their height 4 to 6 days later. Erythema disappears rapidly although induration may persist 4 or 6 weeks. If

the inoculation is made properly, no vesicle forms to leave a scar. Those who have observed or experienced reactions produced in this way have been pleased by the lack of accompanying constitutional symptoms and the absence of open sores.

Knowledge of the amount and duration of immunity to smallpox induced by the intradermal injection of cultured vaccine virus is of cardinal importance. However, no opportunity of observing the incidence of smallpox in a group of individuals vaccinated in this manner has arisen. Nevertheless, from experience it is known that the efficacy of any type of vaccination against smallpox can be tested by revaccination with a potent calf lymph vaccine virus. In spite of this fact, reports of the effect of primary vaccination with cultured vaccine virus on subsequent revaccination with calf lymph virus have been few. In 1935 (1) we described the results obtained in the revaccination of 7 persons who had been successfully vaccinated with cultured virus 13 days to 7 months previously. Of these, 6 were immune to New York City calf lymph virus, while 1, after an interval of 7 months, responded with an accelerated take. In 1937 (4) we conducted revaccinations on a small group of children who had been vaccinated with cultured vaccine virus 1 month to $2\frac{1}{4}$ years previously. In 6 of 14 such children the response to calf lymph virus was that of an accelerated take, *i.e.*, vesicles formed and the reactions were not at their maximum until the 5th or 6th day.

During the last year and a half we have studied a large group of children in order to obtain more complete information concerning the amount and duration of immunity produced by cultured vaccine virus against the New York City calf lymph strain of virus. The results of the study will be reported at this time. In addition, information regarding the immunity produced by cultured virus against other strains of calf lymph vaccine virus, as well as a consideration of the effect that differences in the manner of performing primary inoculations with cultured virus have on subsequent immunity, will be presented.

Methods

At the Rockefeller Hospital there is little opportunity of performing primary vaccinations, in consequence of which it has not been possible in this clinic for us to observe the response of a large number of children to revaccination. However,

at the Children's Prophylactic Clinic of the New York Hospital, cultured vaccine virus supplied by us has been administered intradermally for several years. The facilities and records of this clinic were made available to us through the courtesy of Dr. Samuel Levine and Dr. Parker Dooley.

Children were selected in whom a positive primary vaccination with cultured virus had been observed and recorded and in whom no further prophylaxis against smallpox had been carried out. Each child was revaccinated with New York City Board of Health calf lymph vaccine virus applied to a linear scratch ⅛ inch in length. In addition various groups received on the opposite limb commercial calf lymph virus A or B applied to a linear scratch. All virus used was received fresh each week from the place of preparation and was stored at 0°C. before use. A single observation on the 5th day after revaccination has, as a rule, been all that could be made; the few that could not be seen on the 5th day were seen between the 4th to 7th days.

The time at which a reaction to vaccine virus is at its maximum and not the size of the lesion is considered to be the correct index of susceptibility (5). Reactions are usually classified as no reaction, immune reaction, accelerated take, primary take. Due to the impossibility of making frequent observations on the revaccinated children only two types of reaction are recorded, namely, immune reactions and accelerated takes. Immune reactions are those which showed on the 5th day only a small papule or some evidence that a mild response to inoculation had been present. The children who showed at this time no evidence that the virus had been effectively introduced into the skin were excluded from consideration. Thus, a few rapid immune reactions may have been missed, but the number was not great enough to affect significantly the results of the study. Accelerated reactions comprise those which on the 5th day showed the presence of a vesicle surrounded by a zone of erythema. The use of vesicle formation as one of the criteria for classification has insured the inclusion in the group of accelerated takes of even the mildest of this kind of reaction, *viz.*, one which heals without the formation of an enduring scar. It is possible that some of the reactions may have been in an early stage when the results were recorded and that the time at which the maximum sizes were reached approached closely the time at which primary takes would have been at their height. However, from observation of some children later than the 5th day after inoculation and from the size of resultant scars which we have seen, we believe that most of the accelerated reactions were correctly classified.

Results of Revaccination with New York City Calf Lymph Vaccine Virus of Children Who Had Received One Successful Intradermal Inoculation of Cultured Vaccine Virus

331 children who had received one inoculation of cultured virus resulting in a primary take 1 month to 3 years and 9 months previously were revaccinated with New York City calf lymph virus (Table I).

Of these, 82 or 25 per cent responded with immune reactions, while 249 or 75 per cent showed accelerated takes. Most of the accelerated reactions were mild in character, presenting a small vesicle surrounded by a zone of erythema 0.5 to 1 cm. in width. Frequently on the 5th day the contents of the vesicles were drying or inquiry revealed that the lesions had been larger or as large on the preceding day. Children responding in this manner did not present the usual symptoms and signs that as a rule accompany primary vaccination with calf lymph. There were others, however, in whom the lesions presented no signs

TABLE I

Results of Revaccination with New York City Vaccine Virus of Children Who Had Received One Successful Intradermal Inoculation of Cultured Virus

Number of children revaccinated	Time between primary and secondary vaccinations	Immune reactions		Accelerated takes	
		Number	Per cent	Number	Per cent
39	1–6 mos.	13	33	26	67
76	6 mos.–1 yr.	25	33	51	67
185	1–2 yrs.	37	20	148	80
31	2+ yrs.	7	23	24	77
Total 331		82	25	249	75

Distribution of age at time of primary vaccination similar for all groups.

of regression on the 5th day and who experienced later fever and lymph gland enlargement accompanying the presence of a central pustule in a zone of erythema and induration of considerable extent. Nevertheless, healing of these lesions was rapid and the scars which resulted were small and superficial.

Analysis of the data obtained in this group of 331 children revealed that the proportion of immune individuals was fairly constant and bore no relation to the interval which had elapsed between the primary vaccination with cultured virus and revaccination with calf lymph (Table I). It is true that the percentage of accelerated reactions was slightly higher in the children revaccinated after 1 year and that the more severe reactions were observed in this group, but the figures obtained give little indication that susceptibility to calf lymph virus increased with the lapse of time within the limits of the observations.

It is known that infants shortly after birth (6) are somewhat resistant to infection with vaccine virus. Furthermore, it has been demonstrated (6) that such infants after a successful vaccination rapidly lose their immunity, many being fully susceptible a year later. The results obtained in our group of 331 children, the majority of whom were first vaccinated between the ages of 6 months and 1 year, indicate that the differences in the age at which the primary vaccinations were performed had no influence on the proportion of children

TABLE II

Results of Dermal Revaccination Made with New York City Vaccine Virus to Ascertain the Duration of Immunity Produced by One Successful Intradermal Inoculation of Cultured Virus in Relation to the Age of Children at Time of Primary Vaccination

Number of children revaccinated	Age when first vaccinated	Immune reactions		Accelerated takes	
		Number	Per cent	Number	Per cent
69	6–9 mos.	15	22	54	78
98	9 mos.–1 yr.	29	30	69	70
55	1–2 yrs.	14	25	41	75
35	2–3 yrs.	7	20	28	80
53	3–5 yrs.	11	21	42	79
21	5+ yrs.	6	29	15	71
Total 331		82	25	249	75

Distribution of interval of time between primary and secondary vaccinations similar for all groups.

who retained complete immunity during the period of observation (Table II).

Results of Revaccination with Commercial Strains of Vaccine Virus of Children Who Had Received One Successful Intradermal Inoculation of Cultured Vaccine Virus

Reports in the literature concerning the duration of immunity in children to vaccine virus are conflicting. Moreover, in attempting to evaluate the results of different workers, one is confused by a lack of uniformity in classification or description of the type of reaction produced by revaccination and by the fact that the relative potency

of the viruses used for the primary vaccinations and revaccinations was either not known or not stated. A mildly acting virus does not always fully protect for a great length of time against a virulent strain, and results obtained by revaccination with a mild strain may not parallel those secured by revaccination with a potent virus. The New York City vaccine virus is a strain of high uniform potency. Consequently, it seemed of interest to compare the results obtained by

TABLE III

Results of Dermal Revaccination with Commercial Strains of Vaccine Virus of Children Who Had Received One Successful Intradermal Inoculation of Cultured Virus

Number of children revaccinated	Revaccinated with New York City virus		Revaccinated with commercial virus A		Revaccinated with commercial virus B	
	Per cent immune reactions	Per cent accelerated takes	Per cent immune reactions	Per cent accelerated takes	Per cent immune reactions	Per cent accelerated takes
78	22	78	72	28		
82	35	65			55	45

Distribution of age at time of primary vaccination and interval of time between primary and secondary vaccinations similar in both groups.

means of its use in the revaccination of children with those secured by revaccination with other strains of calf lymph vaccine virus.

Two commercial preparations of calf lymph virus, A and B, were chosen because they are products widely used in the United States. 78 of the 331 children who were revaccinated with New York City calf lymph received at the same time an inoculation with commercial lymph A; a second group of 82 children received in addition to New York City virus an inoculation of commercial calf lymph B. Of the 78 children, 17 or 22 per cent responded with immune reactions to New York City virus, while 56 or 72 per cent responded with immune reactions to calf lymph A; of the 82 children, 29 or 35 per cent were immune to New York City virus, while 45 or 55 per cent responded in that manner to calf lymph B. These figures, recorded in Table III, show discrepancies that may result from the use of different strains of virus.

Effect of Differences in Primary Inoculation of Cultured Vaccine Virus on Subsequent Revaccination with New York City Board of Health Calf Lymph

At this point it seemed of value to learn whether the administration of large doses of the mildly acting cultured virus or the production of 2 intradermal lesions at the same time would influence the resultant immunity. Accordingly, a group of children at the New York Hospital Clinic were given 2 intradermal inoculations, one in each arm or thigh, of cultured vaccine virus. There were no untoward results. The simultaneous evolution of 2 intradermal vaccinal lesions produced by cultured vaccine virus apparently caused the children no more inconvenience than that evoked by a single reaction. From this group of children, 66, whose records showed that they had had 2 successful simultaneous primary vaccinations, were revaccinated dermally with New York City calf lymph 2 to 6 months later. Of the 66 children, 18 or 27 per cent responded with immune reactions, while 48 or 73 per cent showed accelerated takes. Comparison of these figures (Table IV) with those obtained in the group of 331 (Table I) who received only a single injection of cultured virus for primary vaccination shows that the introduction of a double amount of this virus and the production of 2 primary lesions instead of one did not alter the percentage of children who retained for 6 months complete immunity to the New York City calf lymph.

As stated previously, the virus which has been used during the last 3 years was obtained from the 50th to the 170th culture generations of the "second revived" strain. This virus was selected for human inoculation because it produced mild reactions and maintained a constant potency for man and rabbit. However, from previous experience (2) with the original strain we had noted that the infectivity of the virus diminished on repeated passage in culture and that a change in the character of the lesions produced by it in rabbits also occurred during serial transfer of the virus in the medium used. It occurred to us that a gradual change might have taken place in the "second revived" strain, less marked than that noted in the original one but still great enough to influence the amount of protection produced against a highly potent strain of vaccine virus or against

smallpox. Therefore, it seemed important to determine whether continued cultivation of the "second revived" strain had resulted in a loss of some of its antigenicity essential for the development of a lasting immunity. In order to make this determination, cultured virus from the 20th to the 30th generations of the "second revived" strain was prepared for human inoculation and tested in rabbits and in man.

Intradermal inoculation of the virus in rabbits revealed that the infectivity or titer was essentially the same as that of generation 50 to 170, but the lesions produced by the early generations were more edematous and more hemorrhagic and necrotic than were those

TABLE IV

Results Obtained by Dermal Revaccination with New York City Vaccine Virus of Children Who Had Been Primarily Vaccinated Intradermally in Several Different Ways with Cultured Virus

Type of primary inoculation	Number of children revaccinated	Number of immune reactions	Per cent of immune reactions	Number of accelerated takes	Per cent of accelerated takes
Single inoculation with virus from 50–170th culture generation	331	82	25	249	75
Double inoculation with virus from 50–170th culture generation	66	18	27	48	73
Single inoculation with virus from 20–30th culture generation	54	33	61	21	39

produced by later generations of the active agent. Each of 7 volunteers was inoculated intradermally with 0.1 cc. of a 1:10 dilution of the virus. The lesions produced by this material were larger and more severe than those caused by virus from later generations; however, the reactions were not severe enough to cause anxiety regarding the use of the material. Consequently, a study of immunity produced by it was carried out in a group of children at the New York Hospital Prophylactic Clinic. 54 children were inoculated intradermally with cultured virus from generations 20 to 30; 2 to 6 months later they were reinoculated dermally with the New York City calf lymph virus. Of the 54 children, 21 or 39 per cent responded with accelerated takes, while 33 or 61 per cent showed immune reactions (Table IV). The accelerated takes were mild and healed quickly leaving only small superficial scars. These results are significantly different from those

obtained in children primarily vaccinated with virus from culture generations 50 to 170.

DISCUSSION

Vaccination against smallpox by means of dermal application of potent calf lymph vaccine virus is efficacious. Nevertheless, considerable inconvenience and, at times, danger are associated with this type of vaccination which always leaves an ugly scar. In view of these facts, many people in the United States have never been vaccinated. To overcome opposition to vaccination certain health officials minimize the inconvenience and speak of the scar as a "badge of health." Thus, they leave the impression that a person with a scar is protected against smallpox and is not in need of revaccinations at regular intervals. As a matter of fact, all that a scar indicates is that an individual has been vaccinated; it does not show that the person is immune to smallpox. That can be determined only by the results of revaccination with a potent calf lymph virus. Moreover, the longer a person has gone since a primary vaccination, the more likely is he to have lost protection and the greater is his need of revaccination. Some individuals lose immunity more rapidly than do others; this appears to be particularly true of young children and infants. Therefore, revaccinations should be made at regular intervals; in the presence of smallpox epidemics revaccinations should be made regardless of when primary vaccinations or revaccinations were performed.

With the idea that vaccination against smallpox can be made a safer procedure, that mutilation is not an essential feature of the procedure, and that a scar gives the individual as well as the health officer a false sense of security, we undertook a number of years ago to prepare a vaccine virus that could be used in a manner less objectionable than that now employed with calf lymph virus. From the results obtained by us in the use of cultured vaccine virus for Jennerian prophylaxis in man we have become convinced that the ideas which prompted the work are entirely sound.

Continued cultivation of vaccine virus in the medium used by us has brought about a qualitative change in the active agent which makes it possible to introduce considerable amounts of the material intradermally without danger and inconvenience to patients. It has been found, however, that the amount of immunity produced by the

cultured virus, as tested by means of a highly potent calf lymph vaccine virus, would probably not be considered sufficient for complete protection against smallpox. On the other hand, when commercial vaccines widely used in the United States are employed for testing the immunity induced by the cultured virus, the results might be considered satisfactory. At present we are suggesting that primary vaccinations be made intradermally with our cultured virus and that revaccinations be made dermally six months to a year later by means of a potent calf lymph virus. In this way vaccinated individuals will not become sick and will not be subjected to the dangers associated with primary vaccinations with calf lymph virus, but will obtain a solid and lasting immunity to smallpox. It is possible and highly probable that a cultured virus can be developed which will be suitable for intradermal use and will not require prompt dermal revaccinations with a potent calf lymph virus to produce an enduring immunity. These are matters for future investigation.

CONCLUSIONS

Continued cultivation of vaccine virus in a medium consisting of minced chick embryo tissue and Tyrode's solution has resulted in a virus qualitatively changed to such an extent that considerable amounts of it can be injected intradermally into human beings without danger or inconvenience.

Individuals who are vaccinated intradermally with the cultured virus should be revaccinated dermally six months to a year later with a potent calf lymph virus in order to obtain a satisfactory immunity to smallpox without being subjected to the dangers and inconvenience associated with primary vaccinations with calf lymph virus.

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