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Smallpox Vaccination by Intradermal Jet Injection *

1. Introduction, Background and Results of Pilot Studies

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Jet injection has met with great success in the rapid and effective mass administration of several immunizing agents. The recent development of a jet injector nozzle specifically designed for intradermal inoculation suggested the possible extension of jet injector methodology to mass smallpox vaccination. A total of 156 volunteer subjects, 16 unvaccinated and 140 vaccinated more than 5 years previously, received either undiluted smallpox vaccine by the multiple-pressure technique, or 0.1 ml of various dilutions of smallpox vaccine by jet injector using the new nozzle. Cutaneous and serological responses in revaccinees revealed that jet injection of diluted vaccine with a titre of 10^7 $TCID_{50}$/ml was as effective as multiple-pressure inoculation of undiluted vaccine. Among the small number of primary vaccinees, jet injection of diluted vaccine with a titre of 10^6 $TCID_{50}$/ml appeared as effective as multiple-pressure inoculation of undiluted vaccine. No complications of vaccination occurred.

The findings confirm the utility of the intradermal nozzle for jet injection of smallpox vaccine. In view of the speed of administration and the economy of vaccine, it is suggested that there is a distinct role for jet injection in global smallpox eradication efforts. Further studies on larger numbers of unvaccinated subjects and on persons with significant residual vaccinial immunity are needed to define the optimal concentration of vaccine for mass vaccination by jet injection.

Jet injection is now accepted as an effective, safe, and uniquely rapid means of inoculating many biological agents. Until recently, only subcutaneous or intramuscular injection could be performed with jet-injection equipment; thus the advantages of the technique were not available to the administration of smallpox vaccine, which undergoes multiplication in the epidermis. In 1962, Ismach and his associates [6] developed a special nozzle for the jet injector to permit the dispersion of inoculum in the dermis and epidermis. This nozzle, applicable to smallpox vaccination, offered the possibility of a revolutionary change in the potential speed and effectiveness of mass smallpox vaccination campaigns. With the view that smallpox vaccination by jet injection might favourably affect the progress of global smallpox

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eradication campaigns, the National Communicable Disease Center initiated studies in 1963 to compare the efficacy and safety of smallpox vaccine administered intradermally by jet injection with vaccination performed by the traditional multiple-pressure technique. This paper reviews relevant previous work and describes the results of pilot comparative studies.

BACKGROUND

Intradermal vaccination by jet injection differs in concept from the traditional methods in 2 ways; the antigen is administered intradermally rather than percutaneously, and by jet-injection equipment rather than with the aid of the scarification needle.

Intradermal administration of smallpox vaccine by syringe and needle

The intradermal administration of smallpox vaccine held great interest for workers early in this century. Wright (1918), seeking a method for consistent satisfactory vaccination, reported success in the intradermal inoculation of smallpox vaccine diluted 1:2 in distilled water. To 54 previously unvaccinated men, he administered diluted vaccine by intradermal injection and simultaneously vaccinated them by the scratch technique with undiluted vaccine. A total of 85% of the intradermal inoculations produced " takes " while only 17% of the scratch vaccinations did so. Using this method, Wright felt that there was a reduced chance of bacterial infection, and he considered that the delivery of a known quantity of virus offered distinct advantages as well.

Roberts (1932), after reviewing studies of intradermal vaccination, concluded that successful intradermal vaccination resulted in immunization similar to that following successful vaccination by scarification. However, Roberts and other early workers were concerned with the potential risk of secondary infection following intradermal injection of smallpox vaccines which were heavily contaminated with bacteria. While isolated incidents of abscess formation were reported (Kirstien, F., cited by Roberts, 1932), no major problems developed.

The development by Rivers (1931) and by Goodpasture & Buddingh (1933) of vaccine viruses cultured in chick embryo tissues and free from bacterial contamination increased the interest in the use of intradermal vaccination. In initial studies, intradermal vaccination with the Rivers vaccine appeared to produce erythema and induration, but not vesiculation (Rivers & Ward, 1935; Rivers, Ward & Baird, 1939); Ellis & Boynton (1939) showed in 3000 college students that successful intradermal vaccination with the Rivers strain resulted in typical primary vesicles in no way different from those observed following traditional scratch and multiple-pressure methods. Since 1940, only a few scattered reports have appeared (Pierce & Willoughby, 1943; McCowan, 1940; Victoriano, 1953; Rosenbusch, 1953; Vox, 1953).

The earlier workers, however, showed convincingly that (1) intradermal inoculation of potent smallpox vaccine produces successful vaccination; (2) the systemic and cutaneous responses are clinically similar to those produced by scarification; (3) diluted vaccines can be administered intradermally without a reduction in the " take rate "; and (4) vaccination in this manner is not accompanied by an unusually high incidence of pyogenic complications, despite the use of heavily contaminated vaccines.

Because of failure to demonstrate any notable advantages of intradermal vaccination, investigators lost interest in the technique. Nevertheless, these early findings became relevant with the advent of a jet-injection technique for intradermal inoculation.

The development of jet injection

The history of the development of jet injection has been reviewed by Hingson, Hamilton & Rosen (1963). While the principle of jet inoculation was introduced over 100 years ago, widespread application of the technique came only after the Second World War. The most promising large-scale application has been in mass administration of immunizing agents, e.g., typhoid, poliomyelitis, diphtheria–pertussis–tetanus (DPT), and other antigens (Hingston, Hamilton & Rosen, 1963; Batson, Wall & Landy, 1949; Lipson et al., 1958; Anderson, Lindberg & Hunter, 1958; Warren et al., 1955).

As the design of jet-injector nozzles permitted only subcutaneous or intramuscular (rather than dermal) delivery of antigen, little work was attempted with smallpox vaccine. Elisberg, McCowan & Smadel (1956), using a hand-operated, multiple-dose jet injector equipped with the standard subcutaneous nozzle, inoculated 21 previously unvaccinated individuals with a chorio-allantoic membrane (CAM) vaccinia virus preparation containing 10^5 CAM infectious units. Altogether, 20 subjects developed typical primary cutaneous reactions; sera

obtained from 14 of them showed haemagglutination-inhibition (HI) antibodies. Of 10 revaccinated adults, 2 showed " accelerated " reactions while the remaining 8 had " immediate " reactions. Serological evidence of successful revaccination was found in 2 out of 9 individuals whose sera were studied for HI antibodies. Subcutaneous inoculation of the same vaccine by syringe and needle revealed no cutaneous or serological response. These workers vaccinated 1056 military recruits by jet injection; only 1 failed to develop either an " immediate ", an " accelerated " or a " primary " reaction. The results indicated that smallpox vaccine administered in this way yielded satisfactory results without untoward reactions. Success was attributed to the small amount of virus trapped in the superficial skin layers as the stream of vaccine passed through to the deeper tissues.

Meyer et al. (1964) attached a short plastic sleeve to the nozzle of the injector, thereby raising it from the skin, with the expectation that an increased amount of vaccine would be delivered superficially. Among 300 unvaccinated children who received 1.5×10^6 tissue culture infective doses ($TCID_{50}$) of smallpox vaccine in this manner, 97% developed typical primary cutaneous vaccinial reactions. Of 237 children, 99.6% showed evidence of seroconversion by HI antibody determination.

A nozzle specifically designed to deliver injected materials intradermally was introduced by Ismach in 1962. Benenson & Ismach (personal communication) observed that jet injection with this nozzle produced a small superficial bleb similar to that which followed intradermal inoculation with syringe and needle. In July 1962, Millar & Henderson (unpublished data), using the new device, inoculated 41 previously vaccinated young adults with a diluted smallpox vaccine. A total of 32 subjects had cutaneous evidence of satisfactory revaccination 8 days later. These findings prompted a further clinical and serological trial of intradermal administration of smallpox vaccine by jet injection.

Objectives of the present study

The study was designed to compare the efficacy of vaccination performed by the traditional multiple-pressure technique with vaccination performed by jet injection, employing the newly developed " intradermal nozzle ". Moreover, an attempt was made to define the vaccinia virus concentrations that would be adequate to ensure successful vaccination by this means.

MATERIALS AND METHODS

Study population

A total of 156 adult males were recruited on the basis of vaccination history from volunteers in the Atlanta Federal Penitentiary and the Georgia State Prison; none claimed vaccination within the previous 5 years. In all, 140 bore scars of previous successful vaccinations; the remaining 16 had neither old vaccination scars nor a history of vaccination and were considered to be unvaccinated.

The 140 subjects with vaccination scars were divided into 6 approximately equal groups (Table 1). Group 1 was vaccinated with undiluted, reconstituted lyophilized smallpox vaccine by multiple-pressure technique; the remaining 5 groups were vaccinated with the jet injector, receiving 0.1 ml each of different dilutions of the vaccine, designated respectively, ID-Jet 1, ID-Jet 2, ID-Jet 3, ID-Jet 4 and ID-Jet 5. The 16 primary vaccinees were so allocated that at least 1 subject received vaccination by each of the 6 alternative methods.

Vaccine

Lyophilized calf lymph smallpox vaccine from a single commercial production lot [1] meeting standards set by the Division of Biologics Standards, National Institutes of Health, was used for all vaccinations. The vaccine, when titrated in primary rhesus monkey kidney tissue culture tubes using half log dilution steps, had a titre of $10^{8.5}$ $TCID_{50}$/ml.

For multiple-pressure vaccination, this vaccine was reconstituted with the standard commercial diluent containing 50% glycerol (USP) and 0.25% phenol (USP) in sterile water. Vaccine for jet-injection use was prepared as follows. To a vial containing 0.3 ml of smallpox vaccine reconstituted for multiple-pressure vaccination, 10 ml of Hanks' solution was added. The preparation was termed the " ID-Jet 1 " vaccine. Sequential 10-fold dilutions were made in Hanks' solution yielding vaccines designated " ID-Jet 2 ", " ID-Jet 3 ", " ID-Jet 4 " and " ID-Jet 5 ". Calculated virus concentrations of these diluted vaccines are shown in the second row of Table 1.

Vaccination techniques

Each subject was inoculated on the left deltoid area, either by multiple pressure or by intradermal

[1] Kindly provided as Dryvax, Lot No. 177101A, by Wyeth Laboratories, Inc., Marietta, Pa., USA.

TABLE 1
PREVACCINATION CHARACTERISTICS OF SUBJECTS BY GROUP

Characteristic	Groups					
	1	2	3	4	5	6
Vaccination method	Multiple pressure (undiluted)	ID-Jet 1 [a]	ID-Jet 2 [a]	ID-Jet 3 [a]	ID-Jet 4 [a]	ID-Jet 5 [a]
Vaccine titre [b]	8.5	7.0 [c]	6.0	5.0	4.0	3.0
Number	23	22	22	24	24	25
Age:						
Mean	41	34	30	32	34	32
Range	30–61	22–67	22–46	21–40	24–56	21–40
Mean No. of years since last vaccination	16	20	18	20	20	20
Prevaccination neutralizing antibody titre:						
<10	8	5	3	9	11	9
10–40	5	10	6	7	5	6
41–160	7	4	9	4	4	6
161–640	3	2	4	3	3	4
>640	0	1	0	1	1	0
Geometric mean titre	23	27	38	25	22	27

[a] Intradermal jet injection of 0.1 ml of vaccine.
[b] Expressed as log $TCID_{50}$/ml.
[c] The titre of 7.0 reflects the 1 : 33 volumetric dilution described in the text.

et injection. Multiple-pressure vaccinations were performed by 1 vaccinator with a single-pointed needle making 30 tangential pressures through a drop of undiluted vaccine. Intradermal jet-injection vaccinations were performed with an automatic hypodermic jet-injection apparatus,[1] equipped with the new intradermal nozzle. The mechanical principles of injector operation and design have been described by Benenson (1959) and a diagram of the intradermal nozzle is given in Hendrix, Nichols & Hirsch (1966).

The nozzle consists essentially of a central injection tip with an eccentric aperture and a surrounding cup. The nozzle cups the skin into a small mound and angles the injection stream tangentially into the superficial skin layers. Roberto (unpublished data, 1966), after injecting Indian ink into the skin of pigs, showed that while small amounts of injected material reached the subcutaneous tissue, the bulk of vaccine was contained within the dermal layer.

In the present studies, jet-injection inoculations were performed with the dose adjustor set at the 0.1-ml calibration. The nozzle was placed against the skin of the left deltoid area with the injector at approximately right angles to the long axis of the arm. The arm and injector were held firmly in place during the injection. In most subjects, a drop of vaccine remained on the surface of the skin after inoculation.

Evaluation of results

The cutaneous responses following revaccination were interpreted according to the recommendations of the WHO Expert Committee on Smallpox (1964, p. 20). A " major reaction " is termed " 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 ".

[1] Manufactured by the Scientific Equipment Manufacturing Corporation, Lodi, N.Y., USA.

Anything less than this is judged an " equivocal reaction ". The " major reaction " is held to be indicative of virus multiplication (and the consequent development of immunity) while the " equivocal reaction " may be due to a degree of pre-existing immunity sufficient to prevent virus multiplication, to application of inactive vaccine, or to faulty technique.

In this study, the site of vaccination was examined immediately following inoculation and then daily for the next 14 days. The extent of erythema and induration of the developing lesions, as well as the presence or absence of vesiculation, were observed and recorded daily. Photographs were made of the lesions at each examination.

The neutralizing antibody response was measured as an additional index of response to vaccination. Previous work by McCarthy, Downie & Bradley (1958) suggests that neutralization antibody is superior to either complement-fixing or haemagglutination-inhibition antibody for quantitative assay of serological responses.

A 10-ml sample of blood was obtained from each subject immediately prior to vaccination and again 30 days after the vaccination. Sera were separated, frozen, and transported in solid carbon dioxide to the laboratories of the National Communicable Disease Center, Atlanta, Ga. They were kept frozen at –20°C until serological tests were performed.

The neutralizing antibody titre was determined by a method described by Cutchins, Warren & Jones (1960), modified as follows:

Dried smallpox vaccine,[1] passed in monkey kidney tissue cultures, was used as antigen. The maintenance fluid for monkey kidney tissue culture tubes was high-cystine, altered Eagle's medium (the pH being adjusted to 7.4 with sodium bicarbonate).

After inactivation (30 minutes at 56°C) the sera were diluted in 4-fold steps in phosphate-buffered saline (PBS) starting with the dilution 1:4. Virus dilutions (30–60 pock-forming units per 0.1 ml) were made in PBS with 20% skimmed milk. Equal amounts of virus and serum dilutions were incubated for 24 hours at 36°C in a water-bath. Four rhesus monkey kidney tissue culture tubes were inoculated with each virus–serum mixture. Cultures were incubated at 36.5°C for 40 hours. Antigen controls, a rabbit immune serum control and a negative serum control, were incorporated in each test.

[1] Dryvax; Wyeth Laboratories.

After the 40-hour incubation period, 0.2 ml of a 0.04% solution of neutral red in distilled water was added to each culture. The tubes were then incubated in a stationary rack for an additional 30 minutes at 37°C and 1 hour at room temperature (20°C–22°C). Plaques with a diameter at least 1 mm, which developed during the first infectious cycle, were counted.

The average number of plaques in the virus controls (6 cultures) and in the tubes which were inoculated with the virus–serum mixture was calculated. The percentage of plaque inhibition in the different serum dilution steps was plotted on probability paper against the logarithm of the serum dilution and the 50% plaque reduction titre was determined.

RESULTS

Characteristics of study group

Prevaccination characteristics of the groups of volunteers studied are summarized in Table 1. Each grouping was considered essentially equivalent in all relevant aspects at the outset of the study.

Performance of the jet injector

There were no mechanical failures of the injector in inoculating any of the subjects. Immediately after a jet injection, a clearly visible superficial bleb was present at the inoculation site in each inoculated individual. A small amount of liquid remained on the skin surface at the inoculation site indicating that somewhat less than 0.1 ml had actually penetrated the epidermis.

Evaluation of clinical results

Revaccinees. Lesions following jet injection were not grossly different in character from those following the multiple-pressure technique. In most instances erythema and induration developed during the first 24 hours, with maximal skin responses noted between the second and eighth days.

The frequencies of cutaneous responses on day 7 are summarized in Table 2, using the WHO Expert Committee on Smallpox (1964) criteria for interpreting revaccination reactions. All subjects vaccinated by the multiple-pressure technique with undiluted vaccine, and all those vaccinated with the ID-Jet 1 vaccine by jet injection, developed major reactions. A progressively diminishing proportion of subjects in groups 3, 4 and 5 showed major reactions; no subject developed a major reaction from the ID-Jet 5 vaccine. Conversely, equivocal

TABLE 2
CUTANEOUS REACTIONS[a] IN REVACCINATED SUBJECTS

Group	Vaccination method	Vaccine titre[b]	Number of subjects vaccinated	Major reaction		Equivocal reaction	
				No.	%	No.	%
1	Multiple pressure (undiluted)	8.5	23	23	100	0	—
2	ID-Jet 1	7.0	22	22	100	0	—
3	ID-Jet 2	6.0	22	16	73	6	27
4	ID-Jet 3	5.0	24	7	29	17 (2)[c]	71
5	ID-Jet 4	4.0	24	1	4	23 (8)[c]	95
6	ID-Jet 5	3.0	25	0	—	25 (13)[c]	100

[a] Criteria of the WHO Expert Committee on Smallpox (1964).
[b] Expressed as log $TCID_{50}$/ml.
[c] Values in parentheses indicate number of subjects with no dermal response.

reactions occurred with increasing frequency in groups 3, 4, 5 and 6. Among subjects receiving the ID-Jet 5 vaccine, over half developed no reaction at all.

No complications of vaccination were observed. During the first 7 days following vaccination, a total of 33 individuals complained of sore arms and 5 developed axillary lymphadenopathy. These occurred most frequently among those vaccinated by multiple-pressure or by jet injection with ID-Jet 1 and ID-Jet 2 vaccines (Table 3).

Primary vaccination

Among the 16 adults apparently vaccinated for the first time, 12 developed typical Jennerian vesicles. These included all of those who received either the undiluted vaccine by multiple pressure, the ID-Jet 1 vaccine or the ID-Jet 2 vaccine. Only 1 of the 3 individuals vaccinated with the ID-Jet 3 vaccine developed a primary take; no takes were recorded in 2 subjects receiving the more dilute vaccines (Table 4).

Results of serological evaluation

Revaccinees. There was a wide range of prevaccination neutralizing antibody titres in each group. No single group, however, had more than 4 subjects with prevaccination titres higher than 160; the prevaccination geometric mean antibody titres for the separate groups were similar, ranging from

TABLE 3
ARM PAIN AND AXILLARY LYMPHADENOPATHY AMONG REVACCINATED SUBJECTS

Group	Vaccination method	Vaccine titre[a]	No. of subjects vaccinated	No. of subjects experiencing:	
				Sore arm	Regional lymph nodes
1	Multiple pressure (undiluted)	8.5	23	16	1
2	ID-Jet 1	7	22	9	2
3	ID-Jet 2	6	22	5	2
4	ID-Jet 3	5	24	1	0
5	ID-Jet 4	4	24	1	0
6	ID-Jet 5	3	25	1	0

[a] Expressed as log $TCID_{50}$/ml.

TABLE 4

NEUTRALIZING-ANTIBODY RESPONSE IN REVACCINATED SUBJECTS BY CUTANEOUS REACTION AND PREVACCINATION NEUTRALIZING-ANTIBODY TITRE

Group	Vaccination method	Vaccine titre	Number of subjects vaccinated	Prevaccination antibody titres: [a] major reactions			Prevaccination antibody titres: [a] equivocal reactions			Total [b]	%
				≤160 [b]	>160 [b]	Total [b]	≤160 [b]	>160 [b]	Total [b]		
1	Multiple pressure (undiluted)	8.5	23	16/20	1/3	17/23	—	—	—	17/23	73.9
2	ID-Jet 1	7.0	22	17/19	1/3	18/22	—	—	—	18/22	81.8
3	ID-Jet 2	6.0	22	12/15	0/1	12/16	0/3	2/3	2/6	14/22	63.6
4	ID-Jet 3	5.0	24	1/6	0/1	1/7	1/14	0/3	1/17	2/24	8.3
5	ID-Jet 4	4.0	24	0/1	—	0/1	0/19	0/4	0/23	0/24	0
6	ID-Jet 5	3.0	25	—	—	—	0/21	0/4	0/25	0/25	0

[a] Expressed as log $TCID_{50}$/ml.
[b] Number of subjects with 4-fold antibody rise/number of subjects with dermal reaction.

22 to 38. The geometric mean titres following vaccination were strikingly higher in groups 1, 2, and 3 than in the remaining 3 groups (240, 462, 272, compared with 28, 20, 22, respectively).

An analysis of neutralizing antibody responses is presented in Table 4 and Fig. 1–4. Correlations between 4-fold or greater increases in antibody and cutaneous responses are presented. In the summation of results in all groups, a correlation between increase in antibody and an observed major cutaneous reaction is apparent. Of 69 subjects developing major reactions, 48 (69.2%) showed evidence of a 4-fold or greater increase in antibody titre. Of the 71 individuals who were judged to have equivocal reactions, 3 (4.2%) developed 4-fold increases in antibody.

Differences between groups 1, 2, and 3 are not significant. A sharp decline, however, is noted both in serological and cutaneous responses between the first 3 groups and those receiving ID-Jet 3 vaccine. No response was observed among those receiving ID-Jet 4 and ID-Jet 5 vaccines.

Primary vaccinees. Among the 12 individuals who developed primary reactions, 7 developed detectable neutralizing antibody (Table 5).

TABLE 5

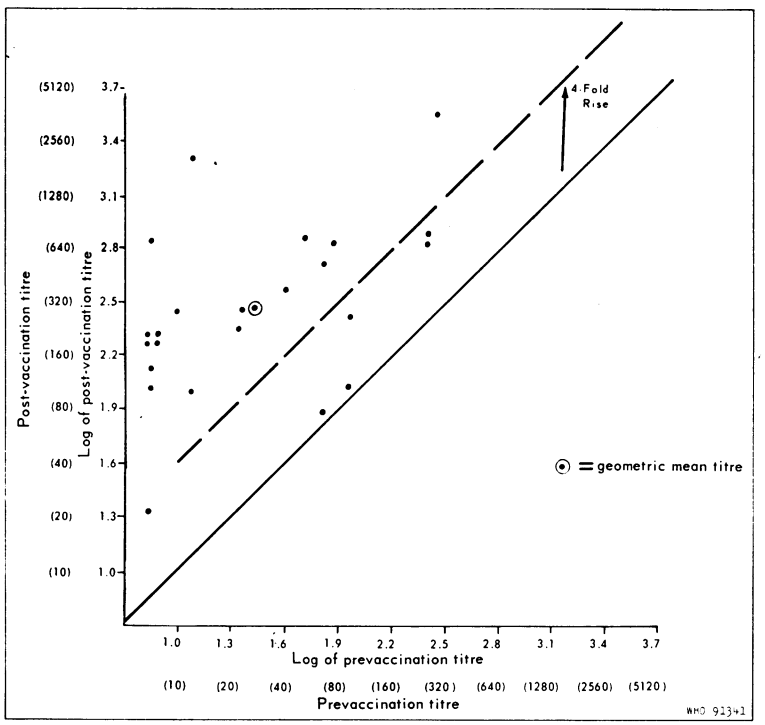
ADULT PRIMARY VACCINATIONS; CUTANEOUS AND NEUTRALIZING-ANTIBODY RESPONSES

Group	Vaccination method	Vaccine titre [a]	No. of subjects vaccinated	Jennerian vesicle	Neutralizing antibody	
					No. of converters	Titre range
1	Multiple pressure (undiluted)	$10^{8.5}$	2	2	1	20
2	ID-Jet 1	10^{7}	5	5	4	16–24
3	ID-Jet 2	10^{6}	4	4	2	8–16
4	ID-Jet 3	10^{5}	3	1	0	—
5	ID-Jet 4	10^{4}	1	0	0	—
6	ID-Jet 5	10^{3}	2	0	0	—

[a] Expressed as $TCID_{50}$/ml.

FIG. 1

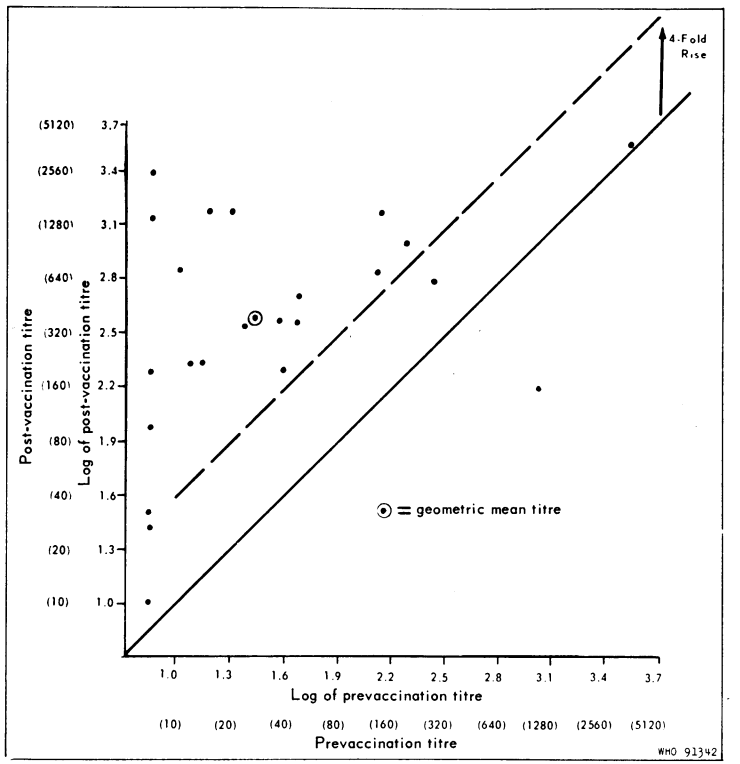
PREVACCINATION AND POST-VACCINATION NEUTRALIZING-ANTIBODY TITRES [a] COMPARED FOR MULTIPLE-PRESSURE VACCINATION [b]



[a] Logarithms of titres are plotted.
[b] Vaccine titre, $10^{8.5}$ $TCID_{50}$/ml.

FIG. 2

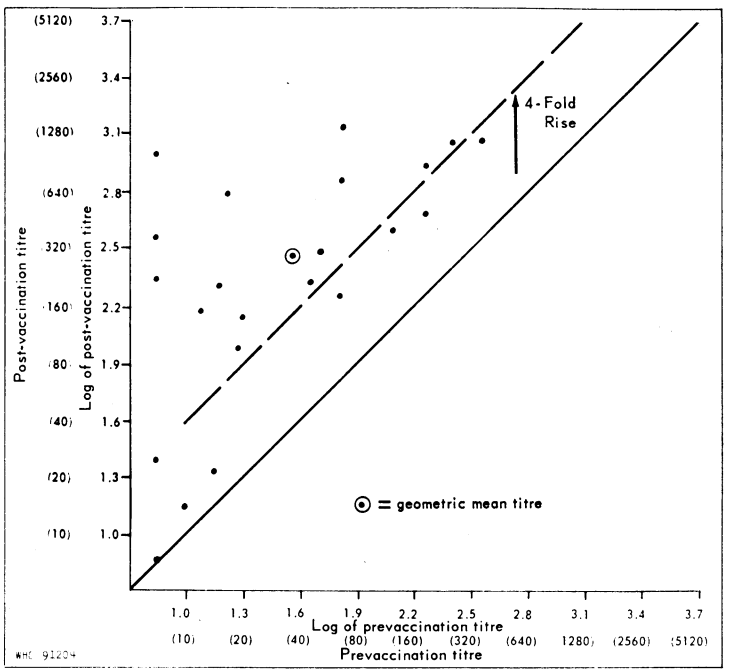
PREVACCINATION AND POST-VACCINATION NEUTRALIZING-ANTIBODY TITRES [a] COMPARED FOR ID-JET 1 VACCINE [b]



[a] Logarithms of titres are plotted.
[b] Vaccine titre, 10^{7} $TCID_{50}$/ml.

FIG. 3

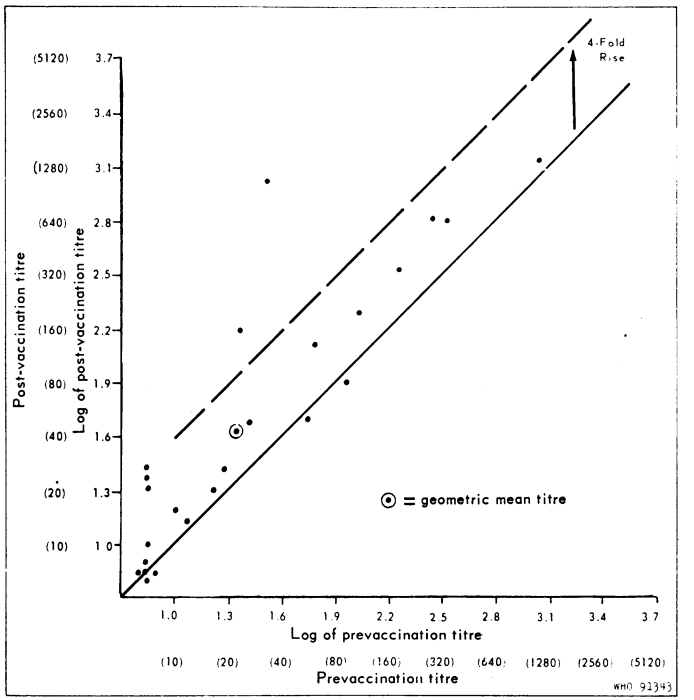
PREVACCINATION AND POST-VACCINATION NEUTRALIZING-ANTIBODY TITRES [a] COMPARED FOR ID-JET 2 VACCINE [b]



[a] Logarithms of titres are plotted.
[b] Vaccine titre, 10^6 $TCID_{50}$/ml.

FIG. 4

PREVACCINATION AND POST-VACCINATION NEUTRALIZING-ANTIBODY TITRES [a] COMPARED FOR ID-JET 3 VACCINE [b]



[a] Logarithms of titres are plotted.
[b] Vaccine titre, 10^5 $TCID_{50}$/ml.

DISCUSSION

Vaccination confers immunity to smallpox. At least 4 factors seem to be involved in recovery from vaccinial infections and probably play a role in protection against smallpox: (1) humoral antibody production (McCarthy, Downie & Bradley, 1958); (2) a delayed hypersensitivity phenomenon (Friedman & Baron, 1961; Pincus & Flick, 1963); (3) interferon production (Wheelock, 1964; Scientific Committee on Interferon, 1962; and (4) cellular resistance measurable *in vitro* (Stienberger & Rights, 1963). Insufficient evidence is at hand to assess the importance of any single factor, or to determine the synergistic effect of the various factors.

Except for natural challenge with variola virus, the definitive test of immunity, cutaneous and neutralizing antibody responses are the most useful indices in man for comparing smallpox vaccines, dilutions of vaccine, and techniques of administration. The visible skin response presumably provides information reflecting the phenomena of delayed hypersensitivity and interferon production, which often exert a primary effect in local control of vaccinial infections; the serological evaluation provides information regarding protection which may be afforded by humoral antibody.

The present study demonstrates that successful intradermal vaccination by jet injection produces a response which is indistinguishable, clinically and serologically, from that following successful vaccination by the multiple-pressure technique. Within the statistical limits set by the small numbers involved, there is no evidence to suggest a difference with respect to the safety of the 2 techniques.

In this study, the ID-Jet 1 vaccine administered intradermally by jet injection produced results in revaccinated persons as good as, or better than, those with undiluted vaccine administered by multiple pressure. From the very limited number of observations in primary vaccination, it would appear that the ID-Jet 2 vaccine might be as effective for primary vaccination as undiluted vaccine by the multiple-pressure technique.

This study showed a reasonably consistent relationship between neutralizing antibody and dermal response patterns. The WHO criteria for interpreting dermal responses are useful in the main for classification of cutaneous responses in " late " revaccination, i.e., in persons with limited residual immunity. However, the antibody and dermal response patterns were not always in agreement. As noted, only 70% of the subjects developing major reactions also developed 4-fold or greater neutralizing antibody rises, and nearly 5% of those with equivocal cutaneous responses *did* develop 4-fold increases in antibody titre. Thus, in assessing the full effect of the vaccine, it is important to measure as many of the parameters of response to vaccination as possible.

The differences in response to ID-Jet 1 and ID-Jet 2 vaccines are worthy of attention. The ID-Jet 1 vaccine appears to produce optimal results in revaccinees, as well as primary vaccinees. Responses among revaccinees to ID-Jet 2 vaccine appear to be slightly reduced.

The quantity of virus particles needed to ensure a successful vaccination by jet injection may be calculated. Since the minimum required titre of vaccine appears to be 10^6–10^7 $TCID_{50}$/ml, the minimum quantity of delivered virus (0.1 ml) to ensure successful vaccination of subjects with limited residual immunity is approximately 10^5–10^6 $TCID_{50}$.

Espmark (1965), in studying the multiple-pressure technique in man, showed that the vaccine titres needed to ensure successful takes in 50% of " late " revaccinees varied from $10^{5.9}$ $TCID_{50}$/ml to $10^{6.4}$ $TCID_{50}$/ml. The theoretical vaccine titre required for a 50% take rate in our studies was approximately $10^{5.6}$ $TCID_{50}$/ml,[1] or about ½ log lower than that determined by Espmark. This difference probably relates to the fact that virtually all the vaccine virus dose is administered by jet injection compared with the unknown (but obviously lower) quantity of virus delivered through the skin by the multiple-pressure technique.

The limited number of primary vaccinations reported demands an extension of these studies with the jet injector to deal with this important group. Studies of recent revaccinees will assist in defining more precisely minimum doses of vaccinia virus required for field use. It now appears certain, however, that the advantages of mass smallpox vaccination by jet injection, that made jet injection so popular for mass inoculations of other antigens, can no longer be denied.

[1] See Fig. 5 (composite graph) in Neff et al. (1969).

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RÉSUMÉ

LA VACCINATION ANTIVARIOLIQUE PAR INJECTION INTRADERMIQUE SOUS PRESSION: 1. INTRODUCTION, HISTORIQUE ET RÉSULTATS D'ÉTUDES PILOTES

La technique de l'injection sous pression a été appliquée avec un remarquable succès à l'administration rapide et efficace de toute une série d'agents biologiques. La mise au point récente d'un dispositif d'injection spécialement conçu pour les inoculations intradermiques a laissé entrevoir la possibilité de recourir à ce procédé lors des campagnes de vaccination antivariolique de masse. En 1963, le Centre national des Maladies transmissibles des Etats-Unis d'Amérique a entamé un ensemble d'études visant à comparer, sous l'angle de l'efficacité et de l'innocuité, les résultats obtenus par la technique de l'injection sous pression et par la technique classique des pressions multiples. On s'est en outre efforcé de préciser la concentration du vaccin permettant d'assurer un taux de prise satisfaisant en cas d'emploi d'injecteurs sans aiguille.

On a choisi un échantillon de 156 volontaires adultes, de sexe masculin: 140 d'entre eux avaient déjà subi une vaccination antivariolique; les 16 autres, sans cicatrices vaccinales et sans antécédents de vaccination, ont été considérés comme n'ayant jamais été vaccinés. Les premiers ont été répartis en 6 groupes d'importance sensiblement égale. Le groupe 1 a été vacciné par la technique des pressions multiples à l'aide de vaccin lyophilisé reconstitué et non dilué. Les 5 autres groupes ont été vaccinés par la technique de l'injection sous pression à l'aide de vaccins contenant respectivement 10^7, 10^6, 10^5, 10^4 et 10^3 doses infectantes de culture de tissu ($DICT_{50}$) par millilitre. Quant aux 16 volontaires non encore immunisés, ils ont subi la primo-vaccination, l'un d'entre eux au moins étant vacciné suivant l'une des six méthodes sus-mentionnées. Un examen de la réaction cutanée a été effectué quotidiennement pendant 14 jours chez chaque sujet, les résultats étant interprétés conformément aux critères recommandés par le Comité OMS d'experts de la Variole (1964).

L'injecteur sans aiguille a fonctionné sans aucun ennui mécanique. Une vésicule superficielle nettement visible est apparue à l'endroit de l'inoculation chez tous les sujets. Chez les volontaires soumis à la revaccination, l'étude des réactions cutanées et des titres d'anticorps sériques a permis de constater que l'injection sous pression de vaccin dilué contenant 10^7 $DICT_{50}$/ml était aussi efficace que l'administration de vaccin non dilué par la technique des pressions multiples. Des résultats similaires ont été enregistrés chez les primo-vaccinés avec le vaccin contenant 10^6 $DICT_{50}$/ml. On n'a pas observé de complications postvaccinales.

Il ressort de cette étude que les réactions, tant cutanées que sérologiques, suscitées par l'injection intradermique sous pression sont en tous points semblables aux réactions observées après vaccination par la technique des pressions multiples. En raison de ses avantages (rapidité, économie de vaccin), la vaccination antivariolique par injection sous pression est appelée à jouer un grand rôle dans les programmes visant à l'éradication de la maladie.

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