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Smallpox Vaccination by Intradermal Jet Injection *

3. Evaluation in a Well-vaccinated Population

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Smallpox vaccinations were performed in a well-vaccinated prison population by multiple-pressure technique and by intradermal jet injection using serial dilutions of vaccine. A total of 140 subjects were divided into groups, and each group was vaccinated by the multiple-pressure technique or by jet injection using 1 of 3 vaccine dilutions with the following calculated titres: ID-Jet 1 = $10^{7.0}$ $TCID_{50}$/ml, ID-Jet 1a = $10^{6.3}$ $TCID_{50}$/ml, ID-Jet 2 = $10^{6.0}$ $TCID_{50}$/ml. Clinical observations were made at intervals during the first week after vaccination. Prevaccination and post-vaccination serum samples were obtained immediately before and 30 days after the vaccination. Results based on clinical and serological evaluations indicated that smallpox vaccination by jet injection with the ID-Jet 1 vaccine was at least as efficacious as vaccination by the standard multiple-pressure technique.

Data already presented (Millar et al., 1969; Roberto, Wulff & Millar, 1969) have shown that jet injection is a feasible method of performing primary vaccination and revaccination of persons with little or no immunity to vaccinia. Serially diluted vaccines administered by intradermal jet injection were as effective as the accepted multiple-pressure techniques. The present study was designed specifically to compare the efficacy of diluted smallpox vaccine administered by jet injection with undiluted vaccine administered by multiple pressure in a group of individuals with relatively high levels of residual immunity.

METHODS AND MATERIALS

Study population

Volunteers were selected from prisoners admitted to the Evaluation and Orientation Section of the Atlanta Federal Penitentiary. Many were in process of transferring from other federal prisons; most had been admitted several times to federal prisons, and had been vaccinated on each occasion. Subjects were assigned alternately to 1 of 4 groups; histories of previous vaccination were obtained; evidence of primary vaccination was confirmed by inspection of primary scars and a prevaccination blood specimen was drawn. Group 1 was vaccinated by multiple-pressure technique using undiluted vaccine. The remaining 3 groups were vaccinated by intradermal jet injection with 0.1 ml of diluted vaccine preparations. The viral concentrations used were 10^7 $TCID_{50}$/ml for group 2, $10^{6.3}$ $TCID_{50}$/ml for group 3 and 10^6 $TCID_{50}$/ml for group 4.

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TABLE 1
STUDY GROUP CHARACTERISTICS

Characteristics	Group 1	Group 2	Group 3	Group 4
Vaccination method and vaccine dilution	Multiple pressure (undiluted)	ID-Jet 1	ID-Jet 1a	ID-Jet 2
Vaccine titre [a]	8.5	7.0 [b]	6.3	6.0
Number of subjects tested	37	35	33	35
Age of subjects:				
Mean	38	38	36	39
Range	26–52	24–51	24–58	25–62
Mean No. of years since last vaccination	7	9	9	11
Mean No. of previous vaccinations	6	4	4	4
No. of subjects with 3 or more previous vaccinations	24	16	20	17
Pre-vaccination neutralizing-antibody titre				
<41	10	7	7	5
41–160	3	11	6	11
161–320	11	9	11	12
321–640	7	6	7	6
>640	6	2	2	1
Geometric mean titre	142	132	139	140

[a] Expressed as log TCID$_{50}$/ml.
[b] The titre of 7.0 is actually for a 1 : 33 volumetric dilution, arbitrarily designated "ID-Jet 1" (expressing dose relationship) thereby accounting for the apparent 0.5 log$_{10}$ discrepancy.

Vaccine

Lyophilized calf lymph smallpox vaccine from a single commercial production lot[1] was used for all vaccinations. This vaccine was found to have a titre of $10^{8.5}$ TCID$_{50}$/ml, when titrated in primary rhesus monkey kidney cell culture using ½ log dilution steps (Millar et al., 1969; Roberto, Wulff & Millar, 1969). For multiple-pressure use, the vaccine was reconstituted with the standard commercial diluent containing 50% of glycerol (USP) and 0.25% of phenol (USP) in sterile distilled water. Vaccine for jet injection was prepared as follows: Vaccine from a standard 10-dose vial (multiple-pressure use) was reconstituted with 10 ml of physiological saline (0.85% sodium chloride). The preparation was termed "ID-Jet 1 vaccine". A sequential 5-fold dilution in physiological saline yielded vaccine designated as "ID-Jet 1a" and a 10-fold dilution "ID-Jet 2" (Table 1). Calculated titres of these diluted vaccines were: ID-Jet 1 = $10^{7.0}$ TCID$_{50}$/ml, ID-Jet 1a = $10^{6.3}$ TCID$_{50}$/ml, ID-Jet 2 = $10^{6.0}$ TCID$_{50}$/ml.

Vaccination techniques

Each subject was inoculated on the left deltoid area either by the multiple-pressure method or by intradermal jet injection. Multiple-pressure vaccinations were performed by 1 vaccinator with a single pointed needle making 30 tangential pressures through a drop of vaccine.

Intradermal jet injection vaccinations were performed with the automatic hypodermic jet-injection apparatus[2] equipped with the intradermal nozzle

[1] Kindly provided as Dryvax, Lot 177101, by Wyeth Laboratories, Inc., Marietta, Pa., USA.

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

(Millar et al., 1969). Jet-injection inoculations were performed with the control set to deliver 0.1 ml.

Clinical and serological evaluations

The inoculation site was examined immediately after vaccination and then on days 2, 4 and 7. (The day of vaccination was designated " day 0 ".) Maximal vertical and horizontal diameters of erythema or of induration or both were measured and evidence of vesiculation was noted and recorded at each reading. A successful revaccination was defined as a " major reaction " according to the criteria of the WHO Expert Committee on Smallpox (1964), i.e., " . . . 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 ". All other reactions were termed " equivocal ". Samples of blood (10 ml) were drawn just prior to revaccination and again 30 days after vaccination. The sera were separated and frozen at −20°C within 6 hours of collection and remained in this state until serological studies were conducted. Serum-neutralizing antibodies were determined by techniques already described (Millar et al., 1969). A 4-fold rise in titre was considered to be a significant serological response.

RESULTS

Characteristics of the study group

Various characteristics of the study group are summarized in Table 1. All the subjects had been vaccinated several times. At least half of each group reported vaccination within the previous 5 years and had prevaccination neutralizing-antibody titres greater than 160. By chance, the multiple-pressure group was composed of subjects who, according to the background data obtained, had been vaccinated rather more frequently than those in the other groups.

Clinical results

The cutaneous reactions to vaccination were sharply accelerated as expected in a group with significant residual immunity. The proportion of each group (1–4) demonstrating lesions with an area of erythema of at least 1 cm^2 reached a maximum (92%, 86%, 88%, 69%, respectively) on the fourth day after vaccination and declined thereafter; the mean area of erythema in all groups (1–4) also was greatest during the first 4 days.

The proportion of subjects demonstrating major reactions and equivocal reactions is shown in Table 2. The responses of the first 2 groups (recipients of multiple-pressure (undiluted) and ID-Jet 1 vaccines) were similar. Major reactions were demonstrated in about three-fourths of the subjects in both groups. The proportion of major reactions declined in recipients of ID-Jet 1a and ID-Jet 2 vaccines. Only 2 subjects, 1 vaccinated by multiple pressure and 1 with the ID-Jet 2 vaccine, demonstrated no cutaneous response to vaccination.

Although an occasional subject demonstrated a minimal degree of regional adenopathy, no person had any significant systemic reaction following vaccination.

TABLE 2
CUTANEOUS REACTIONS [a]

Group	Vaccination method and vaccine dilution	Vaccine titre [b]	Total No. of reactions	Major reaction		Equivocal reaction	
				No.	%	No. [c]	%
1	Multiple pressure (undiluted)	8.5	37	28	76	9 (1)	24
2	ID-Jet 1	7.0	35	25	71	10	29
3	ID-Jet 1a	6.3	33	18	54	15	46
4	ID-Jet 2	6.0	35	15	43	20 (1)	57

[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.

TABLE 3

NEUTRALIZING-ANTIBODY RESPONSE BY PREVACCINATION TITRE

Prevaccination neutralizing titre range	Group 1 (multiple pressure)			Group 2 (ID-Jet 1)			Group 3 (ID-Jet 1a)			Group 4 (ID-Jet 2)		
	No. of vaccinations	No. of 4-fold rises	%	No. of vaccinations	No. of 4-fold rises	%	No. of vaccinations	No. of 4-fold rises	%	No. of vaccinations	No. of 4-fold rises	%
<160	13	9	69	18	13	72	13	9	69	16	6	38
⩾160	24	3	12	17	6	35	20	0	0	19	3	16
Grand total	37	12	32	35	19	54	33	9	27	35	9	26

Serological results

Neutralizing-antibody responses are presented by group and prevaccination titre in Table 3 and Fig. 1–4. Those subjects with prevaccination titres of less than 40 demonstrated a uniform response to vaccination irrespective of the vaccination method or the dilution of vaccine. Of the 29 subjects with prevaccination titres of less than 40, only 4 (1 in each study group) failed to develop a 4-fold rise in neutralizing-antibody titre. Among those with prevaccination titres of 160 or above, however, the influence of the various initial viral doses can be seen. Of 17 such subjects receiving ID-Jet 1 vaccine, 6 (35%) developed a 4-fold antibody rise. Of the remaining 63 subjects with prevaccination titres of 160 or above, only 6 responded with an antibody rise, 3 after multiple-pressure vaccination and 3 after ID-Jet 2 vaccination. Of the methods used, intradermal jet injection of the vaccine containing 10^7 $TCID_{50}$/ml seems to be the most vigorous immunological stimulus in this, presumably, highly immune population. Among the remaining 3 groups, differences in serological responses were insignificant.

Correlation of dermal reaction with antibody response

Only a gross correlation was observed between the dermal reaction and antibody response (Table 4).

TABLE 4

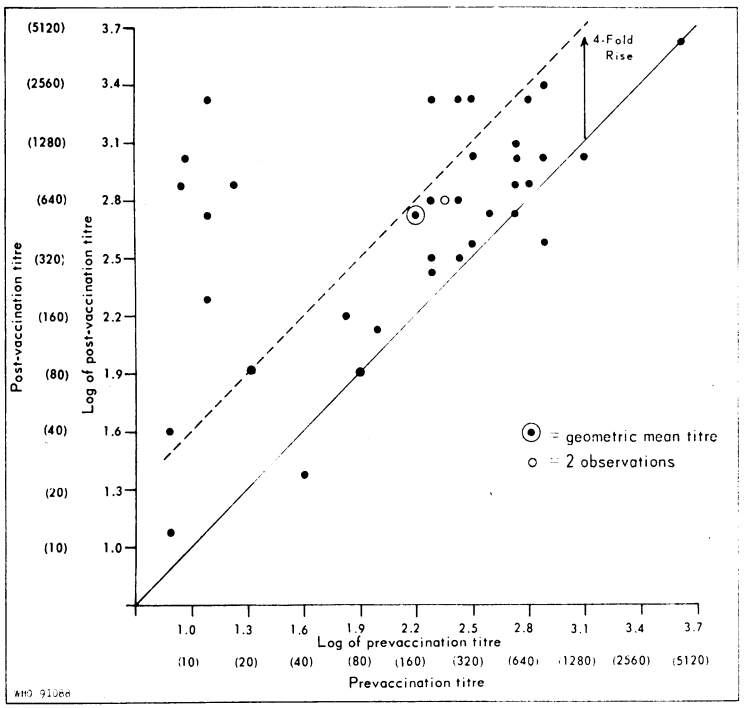
NEUTRALIZING-ANTIBODY RESPONSE BY CUTANEOUS REACTION AND PREVACCINATION NEUTRALIZING-ANTIBODY TITRE

Group	Vaccination method and vaccine dilution	Vaccine titre [b]	Number of subjects vaccinated	Fraction showing 4-fold or greater rise in antibody titre [a]					
				Major reaction			Equivocal reaction		
				Prevaccination titre ⩽160 [c]	Prevaccination titre ⩾160 [c]	Total [c]	Prevaccination titre ⩽160 [c]	Prevaccination titre ⩾160 [c]	Total [c]
1	Multiple pressure (undiluted)	8.5	37	8/9 (89)	3/19 (16)	11/28 (39)	1/4 (25)	0/5 (0)	1/9 (11)
2	ID-Jet 1	7.0	35	10/13 (77)	4/12 (33)	14/25 (56)	3/5 (60)	2/5 (40)	5/10 (50)
3	ID-Jet 1a	6.3	33	5/6 (83)	0/12 (0)	5/18 (28)	4/7 (57)	0/8 (0)	4/15 (27)
4	ID-Jet 2	6.0	35	3/9 (33)	2/6 (33)	5/15 (33)	3/7 (43)	1/13 (8)	4/20 (20)
Total				26/37 (70)	9/49 (18)	35/86 (41)	11/23 (48)	3/31 (10)	14/54 (26)

[a] i.e., number with 4-fold antibody rise/number with dermal reaction.
[b] Expressed as log $TCID_{50}$/ml.
[c] Percentages in parentheses.

FIG. 1

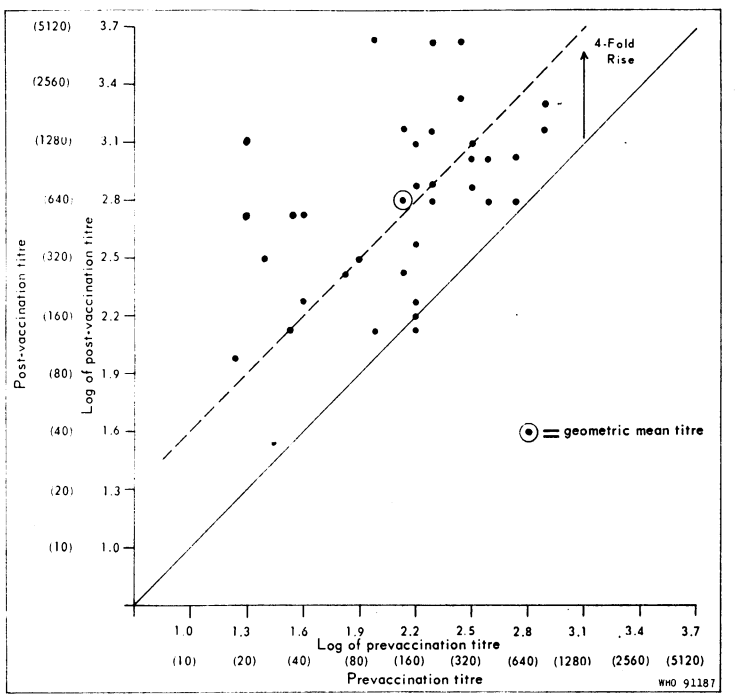
PREVACCINATION AND POST-VACCINATION NEUTRALIZING-ANTIBODY TITRES[a] COMPARED FOR MULTIPLE-PRESSURE VACCINATION METHOD[b]



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

FIG. 2

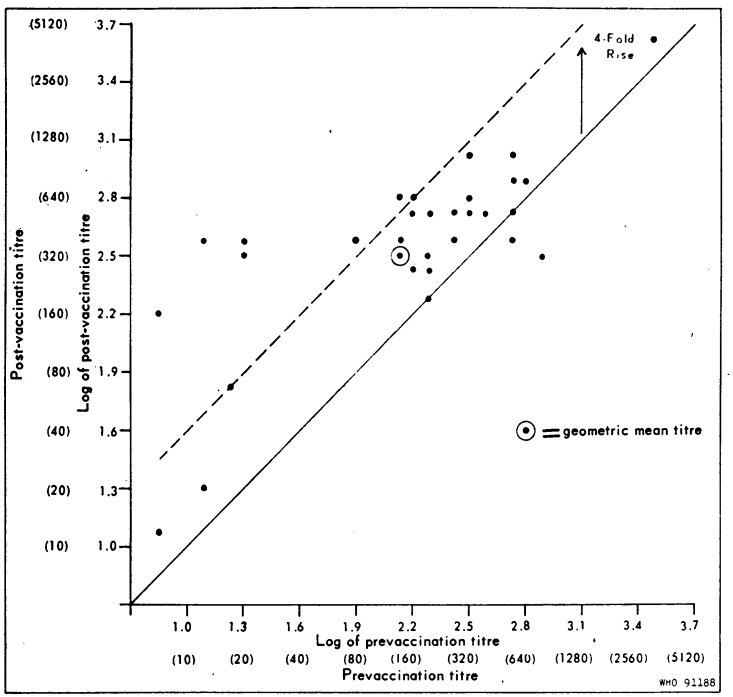
PREVACCINATION AND POST-VACCINATION NEUTRALIZING-ANTIBODY TITRES[a] COMPARED FOR ID-JET 1 VACCINE[b]



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

FIG. 3

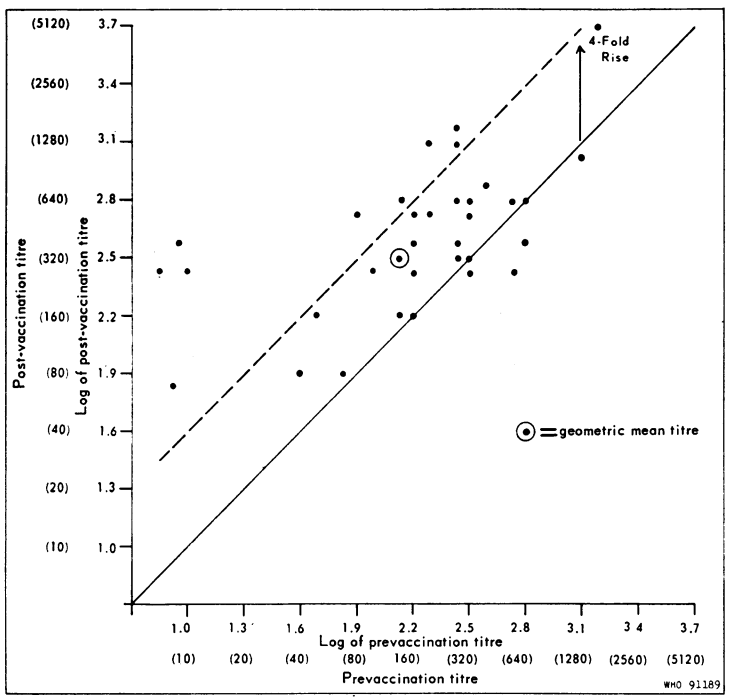
PREVACCINATION AND POST-VACCINATION NEUTRALIZING-ANTIBODY TITRES [a] COMPARED FOR ID-JET 1a VACCINE [b]



[a] Logarithms of titres are plotted.
[b] Vaccine titre, $10^{6.3}$ $TCID_{50}$/ml.

FIG. 4

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.

The presence of vesiculation, the area involved and the time of development of the vesicle, the extent of erythema and induration, and combinations of these measurements did not show close correlation with the neutralizing-antibody response. In general, however, there was a greater proportion (41%) of 4-fold responses among those with major reactions than that observed (26%) among those who had equivocal reactions. This correlation was no more specific for those with prevaccination titres less than 160 than for those with prevaccination titres greater than 160. In the ID-Jet 1 group alone, there was no correlation between dermal response and antibody rise. In this group, approximately half of the subjects showing either a major reaction or an equivocal reaction demonstrated 4-fold antibody responses.

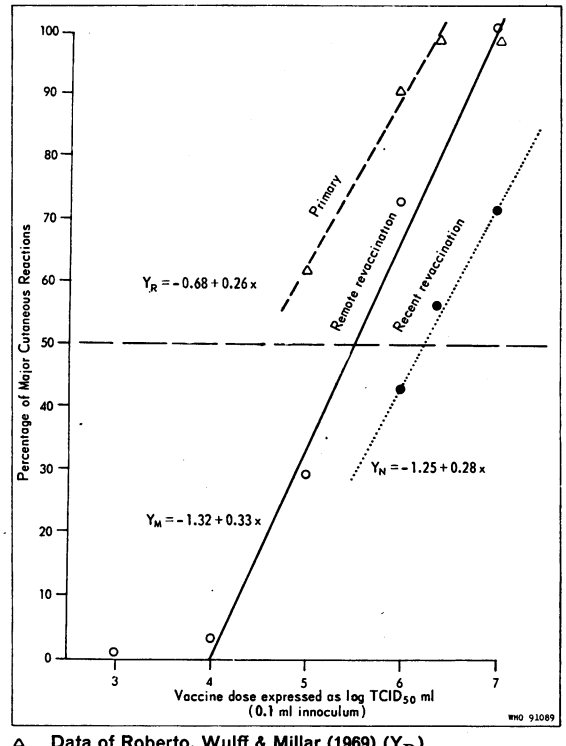
DISCUSSION

In the present study, it was found that successful smallpox revaccination could be accomplished by jet injection in well-vaccinated subjects. It is apparent that jet injection using a smallpox vaccine with a titre of $10^{7.0}$ $TCID_{50}$/ml is at least as reliable in inducing cutaneous and antibody responses as the multiple-pressure method using undiluted vaccine. The serological response in subjects with high prevaccination neutralizing-antibody titres was better following the jet injection of ID-Jet 1 vaccine than by any other method. This suggests that the amount of vaccinia virus delivered to target cells by this method was greater than by either multiple-pressure vaccination with undiluted vaccine or jet injection of the more dilute vaccines. Smallpox vaccination with both ID-Jet 1a and ID-Jet 2 vaccines did not produce cutaneous results comparable to vaccination by the multiple-pressure method or the ID-Jet 1 vaccine. The over-all serological neutralizing-antibody responses, however, were similar with these diluted vaccines to those induced by the multiple-pressure method.

The analysis of these data permits delineation of a jet-injection vaccination dose–response relationship for groups with varying immunity to vaccinia virus. In a previous article, Millar et al. (1969) defined this response for a group of remote revaccinees with limited residual immunity; Roberto et al. (1969) defined this relationship for primary vaccinees and in the present study it was determined for well-vaccinated subjects. This composite analysis is presented in Fig. 5. As might be expected, the infecting dose by jet injection necessary to produce

FIG. 5

JET INJECTION VACCINATION RELATIONSHIP BETWEEN CONCENTRATION OF VACCINE AND PROPORTION OF MAJOR REACTIONS INDUCED [a]



△ Data of Roberto, Wulff & Millar (1969) (Y_R)
○ Data of Millar et al. (1969) (Y_M)
● Data from this paper (Y_N)

[a] In groups with differing immunities.

a major reaction in 50% of a group of human volunteers is directly proportional to the immune status of the population studied. For primary vaccinees, this theoretical vaccine dose expressed as log $TCID_{50}$ is approximately 4.5; for remote revaccinees, 5.5; and for well-vaccinated subjects, approximately 6.2.

Espmark (1965) has performed a similar theoretical analysis for vaccination with the multiple-pressure technique. In his study, the theoretical vaccine dose expressed as log $TCID_{50}$ necessary to produce a cutaneous reaction in 50% of individuals tested was approximately 5.6 for primary vaccinees (by extrapolation), 6.1 for remote revaccinees, and between 6.6 and 7.1 for well-vaccinated subjects. The consistency of the dose–response relationship among immunity groups, as seen in these studies and in the work of Espmark, supports the comparability of

smallpox vaccination by jet injection with the traditional multiple-pressure method. It suggests also that the jet-injection method is considerably more efficient in delivering vaccinia virus to target cells than is the multiple-pressure method. Comparing Espmark's data, as characteristic of typical multiple-pressure vaccination, with those presented in this paper, as characteristic of typical intradermal jet injection vaccination, this difference in efficiency may be estimated. It appears that intradermal jet injection vaccination requires only a vaccine with a concentration of about one-tenth that required for effective multiple-pressure use. With some consistency, this appears to apply generally to primary vaccination and to revaccination, both early and late.

In conclusion, smallpox vaccination by intradermal jet injection using vaccine with a titre of 10^7 $TCID_{50}$/ml is as effective a means of immunization as smallpox vaccination by the presently accepted multiple-pressure technique using undiluted vaccine. The first two studies in this series demonstrated the efficacy of intradermal jet injection in primary vaccinees and remote revaccinees. This paper shows that it is effective also in populations of well-vaccinated individuals.

RÉSUMÉ

LA VACCINATION ANTIVARIOLIQUE PAR INJECTION INTRADERMIQUE SOUS PRESSION: 3. ÉVALUATION DE LA MÉTHODE DANS UNE COLLECTIVITÉ PRÉSENTANT UN NIVEAU D'IMMUNITÉ RELATIVEMENT ÉLEVÉ

On a montré précédemment l'intérêt que présente la méthode de l'injection sous pression pour la primo-vaccination antivariolique et pour la revaccination de sujets chez lesquels l'immunité résiduelle est nulle ou faible. Dans ce troisième et dernier article, la méthode est évaluée sous l'angle de son efficacité lorsqu'il s'agit de vacciner des sujets bénéficiant d'une protection relative.

Cent quarante volontaires déjà vaccinés à une ou plusieurs reprises ont été revaccinés soit par pressions multiples à l'aide d'un vaccin non dilué contenant $10^{8,5}$ doses infectantes de culture de tissu ($DICT_{50}$) par millilitre, soit par injection sous pression de vaccins dilués titrant 10^7, $10^{6,3}$ ou 10^6 $DICT_{50}$/ml. Les réactions cutanées ont été examinées et mesurées les 2e, 4e et 7e jours suivant la vaccination, leur interprétation étant basée sur les critères recommandés par le Comité OMS d'experts de la Variole (1964). Les réponses sérologiques ont été appréciées par titrage des anticorps neutralisants dans les sérums prélevés avant et 30 jours après la revaccination.

Le taux d'apparition de réactions majeures a été de 76% chez les sujets vaccinés par pressions multiples à l'aide de vaccin non dilué; chez les sujets vaccinés par l'injecteur sans aiguille, les taux ont été de 71% (vaccin titrant 10^7 $DICT_{50}$/ml), de 54% (vaccin titrant $10^{6,3}$ $DICT_{50}$/ml) et de 43% (vaccin titrant 10^6 $DICT_{50}$/ml). Après injection sous pression de vaccin titrant 10^7 $DICT_{50}$/ml, les titres d'anticorps sériques se sont accrus dans la proportion de 4 fois ou plus chez 54% des sujets. Chez les sujets vaccinés par les autres méthodes, une élévation correspondante des titres n'a été relevée que dans 26 à 32% des cas. La corrélation entre l'intensité des réactions cutanées et l'amplitude des réponses sérologiques a été très approximative.

Ces résultats, ainsi que ceux des deux études précédentes, montrent que l'efficacité de la vaccination par injection sous pression d'un vaccin titrant 10^7 $DICT_{50}$/ml n'est en rien inférieure à celle de l'inoculation d'un vaccin non dilué par pressions multiples, la dose optimale pour les vaccinations de masse étant de 0,1 ml.

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