WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTÉ

EXPERT COMMITTEE ON SMALLPOX

Geneva, 14-20 January 1964

Smallpox/WP/9
12 December 1963

ENGLISH ONLY

THE RELATION BETWEEN POTENCY OF VACCINE USED IN SMALLPOX VACCINATION AND IMMUNITY PRODUCED

by

Dr M. F. Polak
Rijks Instituut voor de Volksgezondheid,
Utrecht, Netherlands

It is, for various reasons, appropriate to discuss this subject separately for primary vaccination and for revaccination.

Potency and immunity after primary vaccination

It can be stated, for all practical purposes, that the development of a manifest infection, assuming the shape of "typical primary vaccinia", is a prerequisite for immunity after cutaneous application of smallpox vaccine. Consequently, to achieve immunization, the vaccine potency must have at least such a level as to assure a take rate near to 100 per cent. This level is known from vaccination studies in young adults (Cockburn et al., 1957; Polak et al., 1962) and amounts to 5-10 x 10^7 PFU/ml as assessed on the chorio-allantoic membrane. For young children in the first year of life a somewhat higher level is probably required (Espmark, as presented by Dostal, 1962). However, variation of infectivity between the strains of vaccinia virus as used now all over the world cannot be precluded.

It may be asked whether application of vaccines of potencies exceeding this level will result in an immunity of better quality. To the writer's knowledge there are no published reports of studies on human beings that give a definite answer to this question, but it is often observed that a higher potency will cause acceleration of pock formation. It is indeed comprehensible that the application of a higher potency vaccine may result in an initial infection of

a larger number of susceptible cells. The number of cells available for primary infection sets, however, a limit to the quantitative relation between vaccine potency and speed of pock formation. It cannot be stated at what level of potency this limit is attained, but it may well be above 10^8 PFU/ml.

The study of Cross, Kaplan & McClean (1958) relate to the comparative use of three full-potency vaccines giving a success rate of 100 per cent. in young adults, and their heat-degraded derivatives (potencies 1-4 x 10^6 PFU/ml) with an average take rate of 42 per cent. After one year the successfully vaccinated subjects of both groups were challenged by revaccination with a potent vaccine. The results are given in Table 1. No difference in the immunity status as indicated by the reaction to revaccination was observed.

If the interval between primary vaccination and challenge procedure is, however, shortened from one year to one week, an effect of vaccine potency is clearly observed. Cross (1959) found that on the eighth day after primary vaccination with a heat-deteriorated vaccine giving a take rate of 50 per cent., a considerable proportion (50 per cent.) of the vaccinees showed small vesicles of 1/4 in. or less instead of the normal size of 1/2 in. that is observed with a scratch of 1/4 in. Revaccination of these "poor takes" on the eighth day with standard potent vaccine by a 1/4 in. linear scratch resulted in a 1/2 in. vesicle eight days later, while the original vesicle had now extended to the full 1/2 in. size. These results were obtained in two groups of 25 and 50 persons with poor takes. "On the other hand, revaccination, on the eighth day, of 50 typical primary cases with vesicles of 1/2 in. or more in length produced no takes at all. Of 50 subjects with poor takes who were revaccinated with a potent vaccine three weeks after primary vaccination, 45 failed to take, and the remainder showed an accelerated reaction. A poor take due to the use of deteriorated vaccine seemed therefore to be associated with delay in the production of immunity, although a satisfactory level of protection was eventually reached."

Disregarding the time factor, we may assume that infection as well as ensuing immunity is an "all or none" phenomenon after primary vaccination of human beings. This opinion can be given only in the case of vaccination by one cutaneous lesion.

If two or more scratches are made in each individual of a group, we can expect a graded response after the use of low potency vaccine. Some persons will not show one vesicle for each lesion made and the ensuing immunity may be influenced by the number of vesicles observed, being the highest if vesicles appear in all lesions made.

The last-mentioned point has a bearing on the interpretation of seemingly contradictory results obtained in animal experiments.

Attention is drawn, in the first place, to the observations of Groth & Münsterer (1935). These authors tested the effect of vaccination of rabbits by the intracutaneous (five injections of 0.1 ml per animal) and by the cutaneous (0.25 ml on a scarified area of 60 sq. cm) method. Nine tenfold vaccine dilutions from 10^{-1} to 10^{-9} were made and for both methods of vaccination nine rabbits were used per vaccine dilution, making a total of 2 x 9 x 9 = 162 animals. In accordance with their experience in vaccination of children, the authors noticed a relation between the vaccine dilution applied and the time interval between vaccination and maximal skin reaction. This interval was increased for higher vaccine dilutions.

Revaccination by the cutaneous method was performed in three groups of 27 rabbits (three animals per dilution) after one, three and six months respectively. This challenge procedure was followed for the group of 81 rabbits primarily vaccinated by the intracutaneous method, as well as for the group primarily vaccinated by the cutaneous (scarification) method. The state of immunity existing at the time of challenge was assessed by counting the number of pustules after five days. The average numbers, per vaccine dilution as applied in primary vaccination, are given as percentages of the non-vaccinated controls in Table 2, after one, three and six months, for the intracutaneous group as well as for the cutaneous group.

In the first group, the averages observed after three months are higher than after six months. This is explained by the authors by a more intensive scarification procedure. This point is, however, for the present question, of minor importance.

The - not quite regular - increase in the number of pustules per column is of considerable interest and seems to confirm the thesis of a relation between the potency of vaccine used for primary vaccination and the immunity produced.

It is, however, questionable whether this conclusion really contradicts the observations in human beings mentioned above. There is a major point of difference in the procedures of primary vaccination as described by Groth & Münsterer for rabbits, and the scratch method (or the multiple pressure method) as applied in humans.

Such difference is quite clear when we consider that cutaneous vaccination of rabbits was performed with 0.25 ml vaccine on an area of 60 sq. cm. The response observed after various described modifications of vaccine titration in rabbits by the scarified skin procedure is graded from confluent through semi-confluent to decreasing number of discrete pustules. In contrast to the effect in humans, this response to primary vaccination is not an "all or none" phenomenon and it is reasonable to expect an immunizing effect at least partly proportional to the pustulated area.

The ratio between vaccine volume and scarified skin area in rabbits is not the same for the various modifications of the cutaneous method. In WHO Technical Report Series No. 180, for instance, this ratio is 0.2 ml/10 sq. cm for the recommended potency test in the scarified skin of rabbits. Even if we compare this ratio with estimated figures from human vaccination (0.015 ml per scarified area of 1 x 6 mm or 2 x 3 mm) it appears that for actual vaccination of human beings a relative excess of vaccine fluid is applied.

There is another difference with the revaccination study in human beings, cited above. Cross (1959) tested the effect of revaccination only in persons with positive primary vaccination. Groth & Münsterer (1935), however, report, for instance, an average number of 0.6 pustules in nine rabbits after primary cutaneous vaccination with vaccine dilution 10^{-7}. It would appear that for some animals the ensuing revaccination challenge was in fact a primary vaccination after a preceding failure.

As may be seen in Table 2, Groth & Münsterer found also some quantitative effect of the immunizing dose when primary vaccination was performed by five intracutaneous injections of 0.1 ml per animal. Such intracutaneous application of vaccine is not - apart from the species difference - equivalent to a one scratch cutaneous vaccination of humans. First, the multiplicity of lesions enables, for marginal dosage, a graded response of 0, 1, 2, 3, 4 or 5 foci of virus development. Second, the injected fluid will diffuse into the dermal connective tissue - if it is not already partially injected there - and there will be a shift in the ratio between susceptible cells and virus particles.

In the study of Soloviev & Mastyukova (1959) smallpox vaccines from 17 producing centres were compared. Potency tests (two rabbits per vaccine lot) were performed according to Groth's method. Serial tenfold dilution up to 10^{-10} were made of each vaccine lot. A rabbit was intracutaneously injected with 0.1 ml of each dilution and the results were read on the fourth day by recording the final dilution which caused infiltration and by measuring the diameter of the areas of infiltration. Immunity was tested by titration of haemagglutination inhibiting antibody and by the survival rate after intracerebral injection of neurovaccine. Vaccines of high potency were found to be better immunizing agents than vaccines of low potency.

It should be pointed out, however, that:

(1) apart from differences in potency, the various vaccines were prepared from a number of vaccinia strains. The significance of potency should preferably be studied for portions from one batch or closely related batches of one vaccinia strain to avoid eventual influence of interstrain differences;

(2) the assessment of immunity status was done on rabbits who had been subjected to a vaccine potency test. Therefore, a high potency vaccine with an end-point of, for instance, 10^{-6} had caused a larger number of vaccinia foci in a test animal than a vaccine of low potency (10^{-2}). The ensuing immunity may have depended on the number of vaccinia foci and gives not a true comparative picture of the immunizing effect after a number of vaccinia foci caused by a high potency vaccine and an identical number of foci caused by a low potency vaccine.

The results of these animal experiments on the relation between potency and immunizing effect, although they confirm such a relation, do not enforce us to assume a similar relation in the actual circumstances of vaccination of human beings, if we restrict the problem to a comparison between potencies of closely related vaccine batches producing an equal number of vaccinia foci per individual.

Potency and immunity after revaccination

The skin reaction after revaccination is more varied than after primary vaccination. A broad distinction can be made between takes (with vesiculation) and immediate (early) reactions. The percentage of takes is, as in primary vaccination, dependent on the vaccine potency. According to the investigations of Espmark (Dostal, 1962), in revaccination over 10 years after the last vaccination an eightfold more potent vaccine is needed to get the same number of takes than in primary vaccination of children aged five to 12 months. Although such exact data are meagre in the literature, there is a general consensus of opinion that a highly potent vaccine is needed to avoid a large percentage of immediate (and merely traumatic) reactions. This was recently confirmed in the WHO International Assay on Smallpox Vaccines (Krag & Weis Bentzon, 1963) so far that four vaccines of different origin were classified in the same sequence according to the effect in revaccination and the potencies assessed by scarificatkon (rabbit skin) and by pock counts (Cam).

The immunizing effect of an immediate reaction is dubious. McCarthy et al. (1958) found in only eight of 15 persons with "early reaction without vesiculation" after revaccination a subsequent rise in neutralizing antibody. Moreover, such early reactions can also be elicited by application of heat-inactivated vaccine. Thirteen persons with primary type or vaccinoid reactions after revaccination all had a marked rise in neutralizing antibody.

Conclusions

On account of foregoing evidence and considerations, we may say that vaccine potency can influence the immunity produced, as far as it has an effect on the take rate; this take rate is to be understood as the frequency of individuals with takes in the vaccinated group, as well as the number of takes per individual. This last point is, however, reserved for discussion in section 3.3 of the preliminary agenda.

In addition, potency has some effect on the speed of the immunizing process.

The early reactions after revaccination, however, cannot be divided into immunological "takes" or "failures" by inspection of the local symptoms.

REFERENCES

Cross, R. M. (1959) Lancet, I, 1092

Cross, R. M., Kaplan, C. & McClean, D. (1958) Bull. Wld Hlth Org. 19, 123

Dostal, V. (1962) Progr. med. Virol. 4, 259

Groth, A. & Münsterer, H. O. (1935) Z. Imm. forsch. 85, 139

Krag, P. & Weis Bentzon, M. (1963) Bull. Wld Hlth Org. 29, 299

McCarthy, K., Downie, A. W. & Bradley, W. H. (1958) J. Hyg. (Lond.), 56, 466

Polak, M. F. et al. (1962) Bull. Wld Hlth Org. 27, 311

Soloviev, V. D. & Mastyukova, Y. N. (1959) Bull. Wld Hlth Org. 20, 1099

TABLE 1. RESULTS WITH REVACCINATION OF HUMAN BEINGS AFTER PRIMARY VACCINATION WITH VACCINES OF HIGH AND LOW POTENCY (Cross et al., 1958)

Vaccines used for successful primary vaccination in one linear scratch		Challenge after one year with potent vaccine		
		Negative	AR	V
Unheated[a]	"P" dried	2	12	2
	"Q" dried	3	15	2
	glycerolated	0	10	1
	total	5	37	5
	percentages	10	79	10
Heated[b]	"P" dried	2	39	4
	"Q" dried	4	34	1
	glycerolated	1	12	2
	total	7	85	7
	percentages	7	85	7

[a] Causing 100 per cent. takes in primary vaccination

[b] Causing 46 per cent., 39 per cent. and 37 per cent. respectively takes in primary vaccination

Negative = no local reaction
AR = accelerated reaction. Vesicle appears on the fourth day; does not extend beyond the original 1/4 in. scratch; causes considerable itching; crusts over by the eighth day.
V = vesicle appears on the fourth to fifth day; extends to double the 1/4 in. scratch; does not crust over until about the twelfth day.

TABLE 2. RESULTS WITH REVACCINATION ON SCARIFIED SKIN OF RABBITS AFTER INTRACUTANEOUS OR CUTANEOUS PRIMARY VACCINATION WITH SERIALLY DILUTED VACCINES (GROTH & MÜNSTERER, 1935)

log vaccine dilution for primary vaccination	Primary vaccination					
	intracutaneously, 5 x 0.1 ml			cutaneously, 0.25 ml per 60 sq. cm		
	cutaneous revaccination after 1, 3 and 6 months with 0.25 ml, diluted 1:100 (titre $>10^{-5}$) average number of pustules, expressed in percentages of controls					
	1	3	6	1	3	6
-1	17	43	27	3	41	61
-2	34	65	48	12	52	56
-3	44	71	52	20	62	77
-4	52	72	81	23	69	85
-5	58	73	81	28	58	75
-6	65	82	51	55	85	94
-7	56	93	75	50	71	98
-8	74	98	81	64	67	104
-9	94	82	88	61	78	103
Controls	100	100	100	100	100	100

Averages calculated from three animals, in five groups from two animals through intercurrent death