THE IMMUNITY FOLLOWING INTRACUTANEOUS AND SUBCUTANEOUS VACCINATION WITH ELEMENTARY BODY SUSPENSIONS OF VACCINIA

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The knowledge of the nature of vaccinia virus gained in recent years and the methods of preparing relatively pure suspensions of the virus which have been developed justify the hope that the technique of vaccination against smallpox may be improved. The potency of vaccine lymph and its relative freedom from bacterial contaminants have been regulated under the Therapeutic Substances Act for several years past; nevertheless, for prophylactic purposes, it is desirable to introduce the use of pure bacteria-free virus in a measured volume of known potency. The usual method of vaccination which involves scratching the skin and applying to the scratch a variable amount of virus-containing lymph cannot be described as an accurate procedure and, unless proper precautions are taken, may invite local secondary bacterial infection. If it could be shown that a satisfactory immunity follows the intracutaneous injection of a measured amount of a bacteria-free suspension of virus, this method would possess obvious advantages; the usual dressing could be dispensed with, the amount of scarring, and the local secondary bacterial infection which is associated with vaccination would be reduced or abolished. In our opinion, the value of vaccinial scars has been exaggerated; they may be useful as visible evidence that the patient has been successfully vaccinated sometime in the past, but they are not an indication of existing immunity to smallpox. It is not usually suggested that any other measure of prophylactic inoculation should be accompanied by a permanent scar as a record of immunization.

Methods for the preparation of bacteria-free suspensions of vaccinia virus have been devised by many workers, but they have not come into general use for the preparation of material for vaccination. Rivers (1931) and Rivers & Ward (1933, 1935) described a method of cultivating vaccinia virus in association with living chick embryo tissue and the use of this material for intracutaneous vaccination in a limited number of patients. While this communication was being prepared for publication, Rivers, Ward & Baird (1939) published an account of the amount and duration of immunity induced by intradermal inoculation of culture vaccine virus. Their work has many interesting features

which bear directly on our own observations, and these will be discussed later. Goodpasture & Buddingh (1935) described a method of cultivating the virus in the chorio-allantoic membrane of the developing chick embryo and the successful use of this material in an extensive series of over one thousand vaccinations; these authors employed the usual method of vaccination by scarification and not the intracutaneous technique. Stevenson & Butler (1933) reported on the cultivation of a dermal strain of virus on the chorio-allantoic membrane and recently (1939) these authors have published an exhaustive study of the characters and immunological properties of this strain. Gallardo & Sanz (1937) reported the successful result of 3483 vaccinations by the subcutaneous route with virus derived either from tissue culture, from the chorio-allantoic membrane or from a bacteria-free suspension prepared from crude vaccine pulp. No mention is made of the occurrence of vesicles at the site of inoculation.

It has been suggested that cultivation of the virus in tissues of different species may modify its virulence and host specificity. There is no definite evidence to support the contention that this would adversely affect its immunizing efficiency. If it were possible, however, to prepare elementary body suspensions directly from ordinary vaccine pulp, the virus would undoubtedly possess the general properties of that ordinarily issued in vaccine lymph. These suspensions would have the advantage of freedom from bacteria and cellular debris. Eagles (1935) described a method of preparing and testing elementary body suspensions from filtrates obtained from rabbit vaccine pulp. Kaiser (1937) described the preparation and use of purified suspensions from vaccine pulp. Behrens & Nielsen (1935) and Behrens & Ferguson (1935) described a method of purifying and preserving the virus of vaccinia derived from vaccine pulp which appeared to be relatively simple and to render the suspension suitable for passage through bacteria-proof filters. We consider that it is essential that any material prepared from ordinary vaccine pulp and intended for human prophylaxis by injection should be filtered; this is the only means of eliminating possible contamination by sporing bacteria without destroying the virus.

Our observations have been undertaken to study the lesions resulting from the intracutaneous injection of bacteria-free elementary body suspensions of vaccinia derived directly from ordinary vaccine pulp, to note any constitutional disturbances or complications which might occur and to investigate the immunity which resulted from this procedure. In the course of these studies a comparison has been made between the immunity resulting from this procedure and that obtained by scarification with the same elementary body suspensions and with ordinary glycerinated lymph.

MATERIALS AND METHODS

Preparation, titration and storage of the elementary body suspensions. The method of Behrens & Nielsen, already referred to, depends upon the iso-electric precipitation of the suspended tissue and the protein in an aqueous suspension of vaccine pulp. The precipitation

is brought about by the addition of a weak organic acid such as $M/400$ citric acid. Immediately after separation of the precipitate the supernatant fluid is brought back to neutrality with sodium carbonate solution.

Our method has been as follows: 40 grams of vaccine pulp obtained from sheep are ground with pyrex glass powder and a litre of distilled water is added to make a 4% suspension. This is allowed to stand overnight in a refrigerator. Coarse tissue particles are removed by brief centrifugation. Samples are then taken and a preliminary titration made with $N/10$ citric acid to determine the optimal point for precipitation.

Behrens & Nielsen carried out the precipitation at a *p*H which produced a clear supernatant fluid and reported that, whereas about 75% of the protein was removed, the major part of the virus remained in the supernatant fluid. In our experience, when a clear supernatant fluid is obtained there is a substantial loss of virus; it has been our practice to carry out a preliminary titration with acid on a small sample of each suspension and to add sufficient acid to produce definite precipitation but to leave a turbid supernatant fluid. In this way, we have materially improved the yield of virus and have, at the same time, obtained a fluid which will readily pass through bacteria retaining filters.

The predetermined amount of acid is now added to the main bulk of suspension. Immediately after precipitation the suspension is centrifuged at 2000 revolutions per minute for half an hour. The resulting supernatant fluid is decanted and immediately brought back to neutrality with $N/10$ sodium carbonate solution.

Difco Proteose Peptone and sodium chloride are now added to the supernatant to a concentration of 1% and 0·43% respectively. Physiological saline produces agglutination of the elementary bodies of the virus, but the lower concentration of salt does not cause this effect although it is sufficient to prevent further precipitation of any protein which may be left in solution. The presence of 1% peptone has a definite preservative action on the virus. Suspensions containing this substance have been maintained both at 37° C. and at 22° C. for many weeks with only slow deterioration in the infective activity of the virus.

The supernatant fluid is now filtered. In our earlier experiments we relied upon passage through Berkefeld "V" filters, but considerable experience convinced us that complete freedom from bacterial contaminants could not be ensured by means of these filters. It was, therefore, decided to employ the Berkefeld "V" filters as "clarifying" agents and to make a further filtration through "Gradacol" membranes. Membranes with an average pore diameter of 0·7 to 0·8μ are suitable for this purpose and passage through these membranes results in little further loss of virus, provided that a supernatant fluid of suitable turbidity or opalescence has been obtained. Both aerobic and anaerobic sterility tests are made on the final filtrates at 37° C. and at 22° C.

These filtrates form the elementary body suspensions used in the immunization experiments now to be described. They may be stored in the liquid state in the refrigerator for many weeks, but in order to ensure retention of potency over prolonged periods most of our preparations have been desiccated from the frozen state in 1 c.c. amounts in ampoules. After desiccation the evacuated ampoules are filled with dry sterile nitrogen and sealed. This material has been reconstituted as required by the addition of 1 c.c. of sterile distilled water.

During the course of experimental work on the preparations of these elementary body suspensions, it was found that the method of precipitation by acid could be replaced by simple dialysis of the centrifuged suspension of pulp in cellophane bags against running tap water for 6 hr. followed by overnight dialysis against distilled water. This procedure results in the precipitation of a substantial amount of inactive protein and gives a final product comparable with that obtained by iso-electric precipitation. Elementary body suspensions prepared by either method have been used in this investigation.

All the elementary body suspensions used in this work were titrated intracutaneously in the rabbit. The potency of the preparations was such that a definite vaccinial lesion was produced at a dilution of 1:100,000. When we wished to use a weak suspension of this for

human inoculation, dilutions in peptone saline were made from the reconstituted dried material. Many samples were tested by scarification in the rabbit in parallel with the intracutaneous titration; typical vaccinial vesicles resulted, and it was usually found that the limiting titre by scarification was about one-hundredth of that obtained by the intracutaneous route. Samples of several suspensions were shown to be neutralized by anti-vaccinial serum. Stained films prepared from the reconstituted suspensions showed large numbers of elementary bodies together with a fair amount of amorphous "background" material.

Technique of vaccination. Glass syringes of 1 c.c. capacity and fine needles of the type commonly employed for Schick testing were used for giving the intracutaneous inoculations. The skin of the outer aspect of the arm was cleansed with spirit and the needle introduced intracutaneously for a distance of about 8 mm. at such a depth that a typical wheal was produced by the injected material around the point of the needle and away from the site of insertion. This was done in order to prevent, as far as practicable, the fluid leaking back through the needle puncture. The needle used for the test was also employed to withdraw the suspension from the ampoule and was not changed between successive injections. After it had been withdrawn from the skin, however, the needle puncture was carefully swabbed with surgical spirit until all visible leaking had stopped. No dressing was used.

For the subcutaneous inoculations a hypodermic needle was introduced diagonally into the subcutaneous tissue of the upper arm, at such a depth that no obvious wheal resulted.

All the subjects vaccinated were resident in hospitals or institutions where medical and nursing attention was available at all times. Their arms were inspected on the 3rd, 7th and 10th days after vaccination and measurements taken of the diameter of the inoculation papule and of the surrounding erythema. The presence or absence of pain, general malaise, and involvement of axillary lymph glands was noted and accurate records kept of the evolution of each lesion at the different stages.

Revaccination. In order to test the efficacy of the measure, each individual was revaccinated with Government vaccine lymph by scarification after an interval of not less than 6 weeks. A single scratch insertion about 10 mm. long was made over the deltoid muscle of the left arm and the result recorded on the 3rd, 7th and 10th days.

CLINICAL STUDIES

The present investigation embraces the results of vaccination with suspensions of elementary bodies by intracutaneous and subcutaneous routes. Of the total number vaccinated, 328 in all, 131 were adult members of the nursing staffs of the London County Council Hospital Service, the majority of whom had been previously vaccinated; and 197 were children whose ages ranged from 3 to 14 years and who had not been vaccinated before.

Reactions and clinical picture. Immediately after the intracutaneous injection the inoculation papule became red in colour, due to local trauma. During the first day or two this redness tended to fade but from the 3rd to the 7th day it increased in intensity, became warm to the touch and more "active" in appearance. It was not painful, however, even on palpation. Provided that there was no vesicle formation, the inoculation papule remained until the 7th day at approximately its original size. It then gradually subsided, leaving an area of staining and slight scaly desquamation. There was no sign of local tissue necrosis at any time. In a large proportion of those inoculated, an area of erythema with infiltration of variable extent and degree appeared around the inoculation papule towards the end of the day on which vaccination was

carried out. In primary vaccinations these changes were usually well marked by the 3rd day, but they reached their height about the 7th day. A feature of this reaction was that it was more marked on the distal side of the original injection papule and tended to assume the form of slightly raised well-defined crescentic blotches. When a vesicle developed it usually occurred at the site of the needle puncture, but sometimes it appeared on the summit of the papule on the 3rd or 4th day and had the appearance of a typical umbilicated vaccinia vesicle on the 7th day. In a few instances one or more minute superficial herpetiform vesicles developed in the vicinity of the papule on the 3rd or 4th day. These subsided quickly, leaving only a fine desquamation.

At the beginning, the dosage of elementary body suspension was tentative. The first group consisted of nurses who were required to be vaccinated prior to their undertaking nursing duties. These received 0·1 c.c. of an elementary body suspension which was infective in a dilution of 1:1000 in the rabbit skin when injected intracutaneously. As little or no reaction followed these injections and as, in a considerable proportion, successful revaccination with vaccine lymph showed that little or no immunity had been conferred, double the above dose, i.e. 0·2 c.c. of the same dilution, was given to the second group, again without demonstrable immunity resulting. Subsequent groups were given 0·2 c.c. of suspensions infective at 1:10,000 and finally at 1:100,000. When the higher concentrations of virus were used the local reactions were more intense and there was a definite increase in the incidence of vaccinial vesicles. These vesicles developed most frequently at the site of the needle puncture and, in our opinion, were caused by leakage along the needle track, notwithstanding our attempts to prevent this by repeated swabbing with spirit.

Intracutaneous vaccination of adults. In all, 131 nurses or members of the medical staff were vaccinated by the intracutaneous route (Table I). Of these, fourteen had not previously been vaccinated, eighty-seven had been successfully vaccinated more than 10 years previously and thirty had been successfully vaccinated less than 10 years previously. None showed any untoward local reaction or constitutional disturbance. Among the twelve previously

Table I. *The immunity resulting from the intracutaneous vaccination of adults with an elementary body suspension of vaccinia virus*

	Not previously vaccinated	Successful vaccination more than 10 years previously	Successful vaccination less than 10 years previously	Totals
	(*a*)	(*b*)	(*c*)	
Vaccinated with elementary body suspension	14	87	30	131
Subsequent vaccination with lymph by scarification:				
Positive	7	9	—	16
Negative	5	58	24	87
No subsequent vaccination	2	20	6	28

unvaccinated individuals, five were subsequently immune to revaccination by scarification; of the sixty-seven who had been successfully vaccinated more than 10 years previously, fifty-eight were immune, while each of the twenty-four vaccinated less than 10 years previously was immune. It was not possible to determine whether the intracutaneous vaccinations rendered any of those who had been previously vaccinated immune to subsequent revaccination by scarification, or whether they were already immune.

Certain adults who had previously been vaccinated developed an early and comparatively extensive area of redness around the site of inoculation. This area was slightly raised with a well-defined margin, but was not tender. On a number of these individuals a series of intracutaneous tests were carried out by injecting small quantities of the individual constituents of the elementary body suspension, viz. (*a*) 1% peptone solution, (*b*) a solution of sheep protein, and (*c*) a suspension of vaccine virus killed by heat. These tests showed that the early hyperaemia and induration which developed around the injection was due to the protein of the virus. The reactions which followed the injection of the suspension of killed virus were identical with those early reactions experienced by the same patients when they were vaccinated. No reactions resulted from the injection of the other materials.

In view of the disappointing immune response to intracutaneous vaccination in these adults an attempt was made to detect changes in circulating antibody before and after vaccination of a small group of nurses. Virus neutralization tests were carried out with samples of serum taken just before vaccination and again after intervals of 14 days and 6 weeks. Some of the nurses showed evidence of a slight increase in neutralizing antibody 14 days after vaccination, others none at all, but no correlation could be obtained between the neutralization tests, the size of the initial intracutaneous lesion or the immunity to subsequent revaccination. The attempt was, therefore, abandoned.

Intracutaneous vaccination of children. After it had been demonstrated in adults that intracutaneous vaccination with the elementary body suspension produced trivial local reactions with absence of constitutional disturbance, it was decided to carry out primary vaccinations by this method in a group of children, whose ages ranged from 3 to 14 years. As a preliminary, six children were given 0·2 c.c. of a suspension infective at a dilution of 1:1000; one developed a vesicle and was subsequently found to be immune to revaccination, while the other five had mild local reactions but were subsequently found to be non-immune. To the remainder (101 children) 0·2 c.c. of a suspension infective at a dilution of 1:10,000 was given. All were revaccinated by scarification after an interval of not less than 6 weeks. The results are summarized in Table II and Table III (group A).

A study of both of these tables reveals that of the 107 children vaccinated intracutaneously, sixty or 56% developed a vesicle following the primary intracutaneous inoculation. These vesicles were typical vaccinial "takes" with

no appreciable difference in their appearance and evolution to that following vaccination by scarification; a small but unmistakable scar was visible 6 weeks later. With three exceptions the sixty "vesicle-producers" were found to be completely immune on revaccination. On the other hand, of the forty-seven who did not produce vesicles only nineteen were immune.

Table II. *The immunity resulting from intracutaneous vaccination of a group of children subdivided into those that produced vesicles and those that did not*

	Primary intracutaneous vaccination		
	Vesicle	No vesicle	Totals
Revaccination:			
Immune	39	18	57
Not immune	2*	25	27
			84

* These two cases developed definitely modified reactions on revaccination which, though not completely negative, did not proceed to true vesicle formation.

Mention must be made of the three "vesicle-producers" who differed from the rest of the series. Following intracutaneous vaccination all three developed typical umbilicated vaccinial vesicles, pustules and scabs. Two of them had anomalous positive reactions on revaccination 6 weeks later, but these, however, were not typical vaccinial "takes". They were mild reactions which persisted to the 7th day and appeared then as localized, raised, inflammatory areas about 5 mm. wide along the length of the scratch. They did not form true umbilicated vaccinial vesicles or pustules and there was no glandular involvement. A thin linear scar resulted. The third case was not available until 19 weeks afterwards, when revaccination resulted in the development of a typical unmodified vaccinial lesion.

Measurements of the papules, vesicles, pustules and surrounding erythema are not given in this paper because it is considered that they are without significance. It was observed that the degree of local reaction produced by the intracutaneous and subcutaneous vaccinations had apparently no relation to the production of immunity as indicated by the results of revaccination by scarification. In some instances, these local reactions were quite intense with demonstrable involvement of the regional lymph glands; yet subsequent vaccination by scarification 6 weeks later produced an unmodified "take". Conversely, the production of a small vesicle, with a trivial local reaction, was followed by a negative response to revaccination. This negative response consisted of a mild redness around the scratch and disappeared during the first three days.

The relative efficiency of vaccination by different methods. It was decided to compare the immunity resulting from scarification with an elementary body suspension with that following either intracutaneous injection of the suspension or scarification with vaccine lymph. Sixty-eight children were divided into three groups: twenty-three (group A) received the suspension intracutaneously;

twenty-four (group B) were vaccinated by scarification with the same suspension in 50% glycerine and twenty-two (group C) were vaccinated by scarification with vaccine lymph. The results are recorded in Table III.

Table III. *The relative efficiency of vaccination intracutaneously or by scarification with elementary body suspension and that of vaccination by scarification with vaccine lymph*

	(A) Intracutaneous injection of E.B.S.			(B) Inoculated by scarification with E.B.S.	(C) Inoculated by scarification with vaccine lymph.
	Vesicle	No vesicle	Total	All produced vesicles	All produced vesicles
Revaccination:					
Immune	18	1	19	23	22
Not immune	1*	3	4	1*	—
Totals	—	—	23	24	22

* Reference to these cases is made in the text.

Nineteen of the children vaccinated intracutaneously developed vesicles, and of these eighteen were completely immune. Of the four children who did not produce vesicles, one was immune and the other three developed typical vesicles on revaccination. All the children vaccinated by scarification with the suspension developed typical vaccinial vesicles; twenty-three out of twenty-four were completely immune on revaccination. The two children in groups A and B who developed vesicles but were not immune deserve mention; they were not available for revaccination until 19 weeks after the primary vaccination and, on revaccination, developed typical unmodified pustules. The twenty-two children vaccinated with vaccine lymph all developed vesicles and were all immune on revaccination.

This observation showed that when inoculated in the ordinary way by scarification, the elementary body suspension produces typical vesicles and that a satisfactory immunity resulted; it also confirmed our previous experience of the correlation between vesicle production and immunity.

The fact that three children (Tables II and III, groups A and B) who had developed vesicles at primary vaccination were found to be not immune on revaccination 19 weeks later is of interest, but does not invalidate the comparison between the immunity of the different groups after 6 weeks. The number of children tested after the longer interval is too small to be significant, but it does suggest that the duration of insusceptibility to vaccination should be investigated in any future study. Kitasato (1911) and Donally & Nicholson (1934) have already reported that a surprising number of successfully vaccinated individuals are susceptible to revaccination after a short interval.

Subcutaneous vaccination of children. In order to prevent fluid from leaking back through the needle puncture and the subsequent formation of a vesicle, it was decided to vaccinate a further group of forty-five children by injecting the elementary body suspension subcutaneously. Twenty-four children re-

ceived 0·2 c.c. of a suspension infective at a dilution of 1:10,000 and the remainder the undiluted suspension infective at 1:100,000. The local reactions following the subcutaneous vaccinations, even with the undiluted virus, were found to be distinctly milder, and the appearance of vesicles less frequent than in the intracutaneous series.

Many of the children showed no local lesion although some of these had palpable axillary adenitis. There was no general constitutional disturbance in any member of the group. Of the forty-five cases, eleven developed vesicles and were all immune on revaccination; of the thirty-four who did not develop vesicles, eleven were immune and twenty-three not immune on revaccination.

Table IV. *The immunity resulting from the subcutaneous vaccination of a group of children subdivided into those that produced vesicles and those that did not*

	Primary subcutaneous vaccination		
	Vesicle	No vesicle	Totals
Revaccination:			
Immune	11	11	22
Not immune	—	23	23
			45

Thus there is no evidence that subcutaneous injection produces a stronger immunity than the intracutaneous route; in this series we again obtained the same correlation between vesicle production and immunity. It is noteworthy that only three out of the twenty-four children, or 12%, who received the diluted suspension developed vesicles, compared with eight out of twenty-one, or 33%, who received the undiluted suspension. These figures may be compared with the 56% of those inoculated in the intracutaneous groups who developed vesicles.

Since the studies in these groups of children indicated that there was a correlation between the production of a vaccinial vesicle and the subsequent development of immunity, a more detailed analysis was made of the first group of adults (Table I (group *a*)) in order to ascertain whether the same correlation existed. It was found that, of the twelve nurses who had not been previously vaccinated, all those who developed vesicles on intracutaneous vaccination were immune to subsequent vaccination, whereas, of the eight who did not produce vesicles on intracutaneous vaccination, only one was subsequently immune.

Table V. *The immunity resulting from the intracutaneous vaccination of a group of adults not previously vaccinated* (see Table I (*a*)) *subdivided into those that produced vesicles and those that did not*

	Primary intracutaneous vaccination		
	Vesicle	No vesicle	Totals
Revaccination:			
Immune	4	1	5
Not immune	—	7	7
			12

Table VI summarizes the results obtained in all the groups vaccinated intracutaneously and subcutaneously with the elementary body suspension.

Table VI. *Summary of results: the immunity resulting from all the primary intracutaneous and subcutaneous vaccinations subdivided into groups of those that produced vesicles and those that did not*

	Primary vaccination		
	Vesicle	No vesicle	Totals
Revaccination:			
Immune	72	31	103
Not immune	3*	58	61
			164

* Two out of these three cases developed definitely modified reactions on revaccination which, though not completely negative, did not proceed to true vesicle formation.

The figures contained in this table were submitted to Dr L. S. Penrose of the Eastern Counties Institution for his opinion on their statistical significance. His reply may be summarized as follows:

The association between vesicle production and immunity for the figures given in this table is $+0{\cdot}63, \pm 0{\cdot}06$.

The measurement of association is, therefore, ten times the standard error, and the chances that this association arose by random sampling are exceedingly small, e.g. about 1 in 1000. Dr Penrose expressed the opinion that there is a highly significant association between vesicle formation and the development of immunity.

The strain of virus used by us in all these observations was the same as that used in the preparation of the Lister Institute vaccine lymph, and has been propagated for many years alternately in sheep and rabbits. The Government vaccine lymph used for subsequent revaccination by scarification is prepared from a strain propagated in calves. In view of the failure of a large proportion of those vaccinated intracutaneously to develop a satisfactory immunity, it was thought desirable to determine whether vaccination with one strain conferred complete protection against revaccination with the other. Cross-protection tests were done on susceptible individuals who were vaccinated by scarification with lymph prepared from one strain, and revaccinated 6 weeks later with lymph prepared from the other. Thirty-three individuals successfully vaccinated with lymph prepared from sheep were immune on revaccination with calf lymph, and thirty-nine successfully vaccinated with calf lymph were immune on revaccination with lymph prepared from sheep. Therefore, one may conclude that the protection was complete and reciprocal.

The immunity of rabbits resulting from vaccination. In order to investigate the cause of the disappointing immune response of children to intracutaneous vaccination it was decided to carry out parallel observations on rabbits.

Eighteen rabbits were divided into three groups of six; one group were given a single intracutaneous injection of 0·2 c.c. of undiluted elementary body suspension. None of these injections produces vesicles though there was some central necrosis of the skin over the lesions. The second group was inoculated with the same suspension by scarification of one

small area of skin. The third group received a similar inoculation by scarification with ordinary glycerinated lymph. Two rabbits from each group were tested for immunity to vaccinia after intervals of 2, 4, and 6 weeks. The test for immunity was the titration by scarification on these rabbits of ascending dilutions of a standard glycerinated lymph that was known to produce confluent or semi-confluent vesicular lesions in normal rabbits, in a dilution of 1:10,000. The immune response of all these rabbits was satisfactory. A few abortive scattered papules were produced in the areas inoculated with the lower dilutions of glycerinated lymph but these did not proceed to true vesication. There was no significant difference in the response of the rabbits of the different groups or between those tested after the shorter or longer intervals. It appears therefore that intracutaneous vaccination in rabbits produces an immunity comparable with that obtained by scarification and that vesicle production is not of importance.

Five groups of six rabbits were then given single intracutaneous injections of ascending dilutions of the suspension. The first group received an injection of 0·2 c.c. of a 1:10 dilution of the suspension, the second received 0·2 c.c. of a 1:100 dilution, and so on to the last group, which received an injection of a 1:100,000 dilution, the limit at which the suspension produced a visible lesion. Those rabbits that received an injection of a dilution of 1:1000 or higher did not show any central necrosis of the skin over the vaccinial papule. The immunity of these rabbits was similarly tested after intervals of 2, 4 and 6 weeks.

All the rabbits that had received a single injection of suspension up to and including a dilution of 1:1000 showed an immunity in every way comparable with the group of rabbits in the previous experiment. Of the rabbits that had received an injection of the 1:10,000 dilution, five out of six showed an immune reaction, whereas the sixth rabbit, after an interval of only a fortnight, produced a typical confluent eruption. There was no evidence of a decrease of immunity after 6 weeks. Of the six rabbits that had received the 1:100,000 injection, two produced immune reactions and the remaining four showed typical confluent eruptions. The last two to be tested, after an interval of 6 weeks, failed to show any immunity; therefore there may have been some decrease in resistance with the passage of time.

It is of some interest that such good protection against revaccination is afforded by a dilution of the virus that is only ten times the minimal infecting dose. This result accords with our observation that in children the immune response at 6 weeks is the same irrespective of whether the children receive the suspension undiluted or diluted a hundred times. The duration of immunity after the smaller dose has not been investigated.

Discussion

The outstanding fact that emerges from a study of the immunity that is developed as a result of intracutaneous or subcutaneous vaccination with our strain of virus is a rather surprising one; there appears to be a definite correlation between the production of a vaccinial vesicle and the subsequent development of immunity. With three exceptions, the seventy-five children who showed a vesicle at the primary vaccination were completely immune on revaccination by scarification. Two of the three children classified as not completely immune developed definitely modified reactions on revaccination without true vesicle formation; the one remaining child produced a typical unmodified vesicle. Of those children who did not develop a vesicle, fifty-eight out of eighty-nine were not immune on revaccination. The immunity of these children bore no relation to the intensity or size of the intracutaneous lesion produced by vaccination; some of the children who responded with a trivial

area of induration were completely refractory to revaccination with vaccine lymph, whereas other children who had shown a considerable area of induration surrounded by extensive hyperaemia produced a typical vaccinial vesicle on revaccination. It is particularly surprising to note that many children, in whom there had been sufficient proliferation of virus to cause palpable axillary adenitis, were not immune as a result of this infection.

Rivers *et al.*, in the paper already referred to, describe the immunity resulting from the primary intracutaneous vaccination of 331 children. The immunity produced was tested by scarification with New York City Board of Health calf lymph after an interval of from 1 month to 3 years and 9 months. These authors state that if the intracutaneous inoculation is done properly no vesicle forms; therefore one can assume that in their series of children only a small proportion, if any, developed a vesicle on primary vaccination. They classified the results of revaccination as either "Immune reactions" or "Accelerated takes"; there is some discussion as to the nature and significance of the accelerated takes, but it appears that the children showing this type of reaction developed vesicles surrounded by a zone of erythema and the reactions were classified as accelerated if the vesicle was present on the 5th day after revaccination. Of the whole group of 331 children, 82, or 25 %, were immune and 249, or 75 %, responded with accelerated takes. It appears that these authors consider that this result is disappointing, since they recommend that individuals who are vaccinated intracutaneously with culture virus should be revaccinated 6 months to a year later with a potent vaccine lymph, in order to obtain a satisfactory immunity to smallpox. Apparently they attribute this failure to develop a solid immunity following intracutaneous vaccination to the attenuation of their strain of culture virus. It is, however, possible that if they had vaccinated a group of children by scarification with this material, and thus produced vesicles, they might have experienced the same results as ourselves, namely, that a very high proportion of these children would have been immune. There can be no question of attenuation of the strain of virus used by us; it is precisely the same as that issued in Lister Institute glycerinated lymph, and, moreover, when applied by scarification has been shown to produce a firm immunity to revaccination with Government vaccine lymph.

It is possible that if we had been able to make daily observations of our revaccinated children we might have been able to classify them into groups of more or less accelerated reactions. However, we decided that the mere fact that a typical vaccinial vesicle appeared on revaccination 6 weeks after primary intracutaneous vaccination was an indication that a sufficient degree of immunity had failed to develop.

The satisfactory results recorded by Goodpasture & Buddingh (1935) in their series inoculated with virus cultivated in the chorio-allantoic membrane of the chick may well be due to the fact that their cases were vaccinated by scarification, and a vesicle was thus produced at the primary inoculation.

It may be that a complete immunity to subsequent scarification with vaccine lymph can only be obtained by the production of a vaccinial vesicle; if so, this would be an unexpected and disappointing result, since it had been hoped that intracutaneous vaccination with a bacteria-free suspension of the virus would eliminate vesicle formation, with consequent reduction in the incidence of secondary local bacterial infection, constitutional disturbances and scarring, and would make it possible to dispense with dressings over the site of inoculation. There are many points connected with the nature of vaccinial immunity and with the tissue affinities of various strains of virus which await elucidation, but experiment had not so far suggested that the development of a vesicle is a necessary precursor of the immune state. Ordinary Jennerian vaccination, however, which results in a firm immunity to revaccination, does involve the formation of a vesicle. It is possible that further investigation with other strains of virus which may not be so exclusively epidermotropic may show that, by the use of a suitable strain, a satisfactory immunity can be developed without vesicle formation. We have had some indications that different strains of vaccinia virus produce different types of lesion in the chorio-allantoic membrane of the developing chick embryo. The nature of these differences is being investigated in the hope that the results may throw some light on the type of strain which is most suitable for use by the intracutaneous route as an immunizing agent in man. In any further investigation, it will be necessary to devise more stringent precautions to prevent the accidental development of vesicles after intracutaneous and subcutaneous inoculation.

Recent experimental work in animals has modified the view originally advocated by Besredka (1922), and Levaditi & Nicolau (1923), that immunity from smallpox or vaccinia is due to the infected ectodermal cells, and is independent of any circulating antibody. Although there is now little doubt that the immunity is general and humoral, it may be that, with the strains of vaccinia in common use for vaccination, proliferation of the virus in the epidermis is in some way necessary to ensure the development of a firm immunity in man. The observations of Dible & Gleave (1934) are of interest in this connexion. These workers studied the lesions in a fatal human case of generalized vaccinia, and concluded that the lesions were characterized by a "focal degeneration of stratified epithelium" rather than an acute infective granuloma formed from cells of the reticulo-endothelial system. The involvement of the corium was trivial compared with the epithelial changes, and they attributed the discrepancy between their observations and those of other workers to the method by which the lesions were produced and to differences between the structure of human skin and that of experimental animals. The epithelial lesions in their case bore a striking resemblance to those of smallpox, and they suggested that conclusions based upon the study of the disease in the rabbit might not be strictly applicable to the condition in man.

It may be suggested that it is not justifiable to test the immunity resulting

from intracutaneous vaccination by the method of scarification which primarily involves the epidermis only. Such criticism would imply the acceptance of the hypothesis of the "autonomy of the tissues". Apart from the difficulty of detecting and evaluating small increases in circulating antibody or of interpreting the significance of intracutaneous lesions in immune or partially immune subjects, we consider that any satisfactory method of vaccination should produce a firm immunity to revaccination by any route. Smallpox is not primarily a disease of the skin; it is spread by droplet infection and the primary lesion is in the respiratory tract; and yet insusceptibility to cutaneous vaccination with vaccine lymph has always been accepted as the criterion of immunity to this disease.

The group of children vaccinated by the subcutaneous route present certain interesting features. The local lesions were trivial or absent, and there was no noticeable constitutional disturbance although several children developed palpable axillary glands. The employment of this route, however, did not enhance the immune response. Eleven of these children produced vesicles and they were all immune; of the remaining thirty-four who did not produce vesicles, eleven were immune and twenty-three were not immune when tested by revaccination.

The experimental observations on the immunization of rabbits with this material are of some interest. In this animal the immunity developed does not depend upon vesicle production; there was no significant difference between those groups that had been inoculated intracutaneously, and those vaccinated by scarification. None of the former group developed vesicles. Therefore there would appear to be a difference between the nature of the immunity response of rabbits and of children. The other interesting fact that emerged from the animal experiments was the degree of immunity that was developed as a result of a single injection of quite a small dose of virus. In fact, until the limiting infective dose of virus was reached, there was no demonstrable diminution in the degree of immunity up to 6 weeks after vaccination. It is possible, of course, that the immunity following the larger doses may have been of longer duration, but this point was not investigated. So far as it goes, this result agrees with our experience in children that there is no demonstrable difference in the degree of immunity that results when the virus suspension is used undiluted or diluted ten or a hundred times.

The lesions produced in rabbits following the intracutaneous injection of vaccinia virus are much more severe than those produced in children or adults by the injection of the same dose. The lesion in the rabbit is followed by satisfactory immunity, whereas in man it may not be, unless a vesicle is formed. In view of the very mild lesions in man and the lack of constitutional disturbance, it is possible that with the strains of virus in common use, there is relatively little intracutaneous proliferation of the virus, and this may explain the disappointing immune response. The local reaction in man may be

largely a response to the injection of virus protein, rather than a response to the active proliferation of living virus.

Summary

1. This study includes the results of intracutaneous and subcutaneous vaccination with a bacteria-free elementary body suspension prepared directly from vaccine pulp: 131 adults and 197 children were inoculated.

2. The preparation of the elementary body suspensions of vaccinia virus is described.

3. The character of the lesion and the clinical course of the vaccinations are described.

4. The immunity resulting from intracutaneous vaccination was tested by revaccination by scarification with vaccine lymph after an interval of not less than 6 weeks. A control group was vaccinated by scarification with the same elementary body suspension and similarly tested for immunity.

5. It appears that there is a definite correlation between the appearance of a typical epidermal vesicle following the primary vaccination and the subsequent development of immunity. Seventy-two out of seventy-five individuals who developed vesicles were completely refractory to revaccination; two of the remaining three developed modified lesions on revaccination without vesicle formation. Fifty-eight out of eighty-nine subjects who did not develop vesicles produced typical vaccinial reactions on revaccination.

6. The result of comparable immunization experiments on groups of rabbits are briefly described and discussed.

7. The significance of these results in the vaccination of children and adults against smallpox is discussed in the light of other recent observations on the same subject. The influence of the tissue affinities of the strains of vaccinia virus in common use on their intracutaneous and subcutaneous proliferation is considered.

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