REVACCINATION AS A MEASURE OF IMMUNITY TO SMALLPOX

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An interesting annotation in a recent issue of the *Lancet* (1 August 1942), referring to the Glasgow outbreak, has once more emphasized how little is known as to the degree and duration of resistance which vaccination produces to smallpox. The need of an accurate method of measuring such resistance is also stressed. The article specifically draws attention to the old question: in a general vaccination campaign during an epidemic of smallpox, when both likely and unlikely contacts are vaccinated, does the absence of a 'take' indicate that an individual is immune to the disease? *A priori* there are two methods by which the immunity of a person to smallpox might be measured: (1) serological—titration of antibodies in the person's serum, (2) revaccination. The serological method will be first briefly considered. In the above annotation reference is made to a recent method of titrating anti-vaccinal sera by their inhibition of agglutination of chick red blood corpuscles by vaccinia virus (Nagler, 1942). The hope is expressed that such a method will have a variety of uses.

In the writers' opinion it seems doubtful if such a hope will be realized. Although the particular technique is new—as applied to vaccinia virus—methods for the titration of anti-vaccinial sera have been well known for many years. A full discussion is outside the scope of this paper but it must be noted that, if reliance is to be placed on a serological test, it should be generally agreed that a close parallelism exists between the titre of antibodies in a serum and the immunity of the animal to vaccinia virus. Recent literature, including the discussions in textbooks (Levaditi & Lépine, 1938; Van Rooyen & Rhodes, 1940), however indicates that no such agreement exists. The problem is one of some complexity, for not only may the titres of the various antibodies, virus-neutralizing, agglutinins, etc., in the same serum differ widely, but also a complete correlation has not been established between the titre of any particular antibody and the resistance of the animal to vaccinial infection. Of the more recent tests, the mouse protection test, carried out on similar lines to the well-known test for yellow fever, is claimed to give valuable information as to the state of immunity in a population (Haagen, 1936). The method may be useful for titrating antibodies when they are present in a measurable concentration such as following vaccination, and in these cases it is generally agreed that a correlation exists between the titre of virus-neutralizing antibodies and the individual's immunity. No such correlation has been established in those many border-line cases in whose sera the most delicate serological test fails to detect antibodies, but who, if judged by the failure of vaccination, are partly or completely immune.

REVACCINATION

It is generally agreed by modern authorities that the type of reaction following revaccination is a very valuable index of the subject's residual immunity. Although the phenomena of accelerated reactions were known to Jenner and Cory and later studied by Von Pirquet, the modern classification is essentially that of Leake & Thomas (1926) and Leake (1936). The three categories, (*a*) primary takes indicating an absence of immunity, (*b*) vaccinoid reactions indicating some residual immunity, and (*c*) immune reactions ('negatives') indicating complete immunity, are now familiar to all students of vaccination.

In the writers' opinion, it is, however, doubtful if sufficient stress has been laid on those factors essential for the maximum of successful revaccinations. Thus the skill of the operator, the potency of the lymph, and the number of insertions are even more important in revaccinations than in primary vaccinations.

The report of the Committee on Vaccination (1928, p. 83) observes: 'In the course of our investigations it has been shown that, given a potent lymph, it is a simple matter to secure a 100 % insertion success by a technique consisting of the infliction of a single linear incision not more than 6–7 mm. (say ¼ in.) long, merely through the epidermis, and by the single application of the lymph thereto.' The question whether an equally satisfactory percentage is obtained in the vaccinoid reactions in semi-immune persons after a single linear incision does not appear to have been investigated by the Committee. In this connexion it is common knowledge that, even in the hands of experienced vaccinators using potent lymph in revaccination, negatives (immune reactions) are not infrequently followed by partial (vaccinoid) 'takes', even when the revaccination is immediately repeated. The authors, in the course of mass vaccination campaigns in the Sudan during the past few years, have been frequently impressed by this phenomenon.

Two obvious factors are concerned in such failures, viz. lack of skill of the vaccinator—a not uncommon factor in Africa—and the potency of the lymph. It has been observed that lymphs which have given quite a high percentage of 'takes' in suceptible individuals (primary reactions) have often given indifferent results in the group of vaccinoids.

Such results are in accordance with those obtained by the Japanese investigators (Kii, 1926; Kasai, 1926) in their work on the titration of lymph potency in the semi-immune animal. Their results, which are very interesting and suggestive, have been more recently fully confirmed by the present authors (unpublished

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experiments). By this method the differences between lymphs of high and low potency are perhaps contrasted even more satisfactorily than with the standard method of titration on rabbits. We found further that some lymphs of border-line potency, i.e. those which would pass the standard as recommended by the Smallpox and Vaccination Commission of the League of Nations (1927), completely failed to give reactions. In other semi-immune animals (rabbits and sheep) a given dilution of lymph rubbed on to several equal scarified areas of the animal has produced varying results, some areas being completely negative (immune reactions) and others showing scattered abortive papules or vesicles (vaccinoids). The similarity between these findings and the failures observed in human revaccination in certain individuals is striking.

In the application of revaccination for estimating residual immunity, it therefore seems that both potency of lymph and number of applications (insertions) have to be considered. The present work is an attempt to evaluate these two factors, as well as to determine the effect of a virus suspension of specially high potency.

Methods. Two vaccines were used, one being a standard vaccine lymph (sheep) prepared in the Stack Laboratories and issued for routine vaccination in the Sudan; the other being a specially prepared and concentrated suspension of elementary bodies of vaccinia (hereafter referred to as E.B.'s) prepared from sheep pulp. The method of preparation by differential centrifugation is essentially that of Macfarlane & Salaman (1938).

Testing of potency. Both vaccines were titrated on the same rabbit. For potency testing, a specially bred albino strain, of very uniform and high susceptibility, is now used in these Laboratories for all routine titration of vaccine lymphs.

The technique of scarification is that employed at the Government Lymph Establishment, Colindale, and kindly communicated by the Director, Lieutenant-Colonel W. D. H. Stevenson. One shaven flank was used for the vaccine lymph, the other for the E.B.'s. The results are shown in Table 1.

Table 1. *Titration of two preparations of vaccinia virus*

Vaccine	Dilutions						
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Lymph no. 14	++	++	+	0	0	–	–
E.B.'s	–	–	++	(4)	(1)	(1)	0

++ = confluent reactions; + = semi-confluent. Figures in brackets show number of vesicles. 0 = negative; – = not carried out.

In accordance with our standard procedure for lymphs which give a confluent reaction in 10^{-4} dilution, no. 14 was diluted 1 in 2, so as to approximate as closely as possible to routine conditions. The fallacies of end-point titration by scarification of vaccinia virus and, in particular, of vaccine lymph are recognized, but in the present work such inaccuracies do not materially affect the issue. The aim was to employ a preparation of much higher potency than a standard lymph and, as judged by titration, it is considered that this aim has been realized.

Vaccination. The vaccinations were carried out by one of us (M. A. H.) on a batch of 107 recruits for the Sudan Defence Force, by permission of Kaimakam Buchanan Bey, A./P.M.O. All recruits had previously been vaccinated at least once, generally in infancy, while some had been revaccinated in 1940 or the beginning of 1942 and many showed well-marked scars. No record of such vaccinations was available, but the intervals elapsing since the last vaccination are not essential to this investigation, which is only concerned with an assessment of the existing immunity at the time of revaccination.

Routine vaccination in the Sudan is by two linear incisions each 1 cm. long on the skin over the deltoid, made by a surgical needle. A drop of lymph is placed on the skin and incision is made through it. No dressings are used.

In the present series two pairs of incisions were used on the same arm, the upper pair for batch no. 14 and the lower for the E.B.'s.

It may be noted here that no differences whatever could be observed in the degree of reaction of the primary takes of the two vaccines in spite of the definitely higher potency of the E.B.'s.

Table 2. *Results of vaccinations with two preparations of vaccinia virus*

Type of reaction	Batch no. 14 insertions		E.B.'s insertions	
	One only	Both	One only	Both
P.	—	12	—	12
V.	38*	23	19*	48
I.R.	*	34	*	28

P. = primary take; V. = vaccinoid reactions; I.R. = immune reaction.

* Where one insertion only was positive, the other insertion giving an immune reaction. The figures in the I.R. column for one insertion would therefore be the same as in the vaccinoid column.

DISCUSSION

It is convenient to discuss each category of reaction in turn.

(*a*) *Primary takes.* The results are identical with both vaccines, each giving a 100 % insertion success rate, and fully confirm the assertion of the Committee on Vaccination noted above.

(*b*) *Vaccinoid reactions.* Here the results show an interesting contrast. The total number of reactors is approximately the same with each vaccine, viz. 61 with lymph no. 14 and sixty-seven with the E.B. suspension, but the difference in the insertion success rate is striking.

With no. 14 only 23 out of 61 (37·7 %) took in both insertions as compared to 48 our of 67 (71·6 %) with the E.B. suspension. The difference, 33·9 %, is equal to approximately 3·8 times its standard error (8·74). Since the odds against observing so great a difference as the result of chance are nearly 15,000 to 1, the difference is clearly significant and indicates the advantage of the higher potency of the E.B. suspension. Nevertheless, even with this preparation, 19 out of 67 failed to take in more than one insertion.

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In the case of lymph 14, Table 2 shows that 38 persons gave vaccinoid reactions in one insertion, yet failed to take in the other, that is, out of 76 insertions 38, or 50 %, were failures. If only one insertion had been made in each person it is a reasonable assumption that with a failure rate of 50 % only 19 out of 38 would have reacted, and that the total figure in the vaccinoid group would therefore be 42 instead of 61. It is assumed, of course, that none of the 23 persons who reacted with both insertions would have been missed with one insertion.

In the case of the E.B.'s the corresponding figures would be 57 instead of 67.

(c) *Immune reactions.* There is no significant difference between the two vaccines, 31·7 % (no. 14) compared with 26·1 % (E.B.'s).

A more detailed analysis of the protocols showed that 13 persons giving immune reactions with no. 14 gave vaccinoid reactions with the E.B.'s—seven in both insertions and six in one insertion, while six of those who were negative with E.B.'s gave vaccinoid reactions with no. 14, one in both insertions and five in one insertion. It is difficult to say if these discrepancies are of any significance. It is sometimes far from easy to read certain border-line reactions, while the factor of the total number of insertions has also to be borne in mind, since irrespective of the dose of vaccine used, it is reasonable to assume that the larger the number of insertions the greater the chances of a take in one of them. The importance of the number of susceptible cells exposed to the action of the virus has been emphasized by the recent work of Sprunt (1941). This worker concludes on the basis of experimental evidence 'that in addition to the amount of virus injected the chance of a lesion also depends on the tissue mass (number of cells) exposed to the virus shortly after injection and that the larger the number of host cells per virus particle the greater the probability of a lesion'. In partially immune animals, the optimal conditions for the multiplication of virus in the skin must be still more exacting, including the exact depth of the insertion in the epithelium and the actual dose of virus introduced into the incision.

Judged by the above results it is apparent that, provided at least two insertions are made, the results with a standard vaccine lymph are almost as satisfactory as with a virus preparation of much higher potency. On analogy the results after four insertions, as in the old method, would be presumably still more satisfactory. If, however, only one insertion is employed, a considerable number of border-line cases will be missed. Unless, therefore, definite experimental evidence to the contrary is brought forward, the question asked in *The Lancet* can be answered as follows: Provided that persons are revaccinated with a potent lymph and in at least two insertions, a negative reaction (i.e. an immune reaction) is practically certain proof that the individual is fully immune at that particular time. It is of course realized that, strictly speaking, such a conclusion is directly applicable only to anti-vaccinial immunity, its applicability to anti-variolar immunity being an inference. The same limitation necessarily applies to any other method, apart from the old one of variolization, of determining immunity to smallpox in terms of vaccinia virus. All evidence, however, suggests that persons possessing complete immunity to vaccinia are also fully immune to smallpox. There remains the question: What are the chances of the vaccinoid reactors—in particular those border-line cases as above who may fail to react with one insertion—being infected with smallpox? As it is no longer feasible to carry out test variolizations, the older observations of Jenner (1798) and Brown (quoted by Morosov, 1938) on variolization in vaccinated persons are of particular interest in this connexion, since their results appeared to be very similar to those of modern revaccination. In more recent times, Müller (1932) has studied the problem by observing the results of revaccinations carried out on patients during the incubation period of smallpox. He concludes that persons showing 'modified pustules' (vaccinoids) must be considered as susceptible to smallpox, although the disease assumes the mild or varioloid form. It seems, therefore, wiser to be on the safe side and assume that although the chances are small, the vaccinoid reactor may contract modified smallpox under certain conditions, e.g. intensity of exposure to variola virus such as is liable to occur during epidemics. The obvious advantage of revaccination over any serological method for the determination of anti-vaccinial or anti-variolar immunity, is that it is at the same time an index of existing immunity and in positive reactors (primary and vaccinoid takes) a method for reinforcing the immunity. As Blaxall (1930) remarks: 'This operation (revaccination) has the great advantage that if the immunity is low the test inoculation will strengthen it, if high there is no discomfort to the patient.' Since the general abandonment of the old four insertions method with cross-hatching, official opinions have differed as to the number of insertions required. Thus in Great Britain, the first schedule to the Vaccination Order 1930 (para. 7) specifies one insertion for all ordinary cases of vaccination and revaccination—the scratch being not more than $\frac{1}{4}$ in. long. In cases 'where maximum protection against smallpox is desired or where the circumstances make it specially desirable to avoid risk of failure, the public vaccinator may, if he considers it necessary, increase the number of such insertions'. 'The number should not exceed four.' The latter clause presumably envisages vaccination during a smallpox epidemic, and it would therefore be interesting to learn of the average number of insertions practised during the recent (1942) Glasgow outbreak.

In the British army two insertions are preferred.* 'There should be two linear incisions of $\frac{3}{4}$ in. long and the total area of vesicle formation should not be less that half a sq. inch' (quoted in *Memoranda on Med. Diseases in Tropical and Sub-Tropical Areas,* War Office, London (1941)).

In the U.S.A., although the multiple-puncture method seems to be the favourite, scarification by one insertion

* Since the above was written, *Bulletin* No. 14 Army Medical Department (issued by the War Office, London, S.W. 1, September 1942) gives the following instructions for revaccinations: 'As in primary vaccination the lymph is applied through a single linear incision about a quarter of an inch long.'

(⅛ in. long) is also advocated by leading authorities (Rosenau, 1935).

On the other hand, Russian and German practice favours three insertions.

The results in the present paper indicate the possible fallacies in the one insertion technique in revaccination and provide experimental verification of the advantages of at least two insertions such as laid down for the British Army.

SUMMARY

1. Serological methods for the determination of smallpox immunity are briefly discussed, and it is concluded that they are unlikely to provide a diagnostic method of sufficient accuracy.

2. Experimental work is recorded which shows certain differences in the response of previously vaccinated individuals, revaccinated simultaneously with a standard vaccine lymph and an elementary body suspension (vaccinia) of high potency. The significance of these differences is discussed.

3. The results indicate that although the advantage of the E.B. suspension over vaccine lymph is relatively insignificant, that of two insertions over one is very marked. In revaccination the routine use of one insertion only may result in a certain number of semi-immunes (vaccinoids) being erroneously reported as immunes.

4. It is concluded that if the two insertion technique be practised, persons showing immune reactions (negatives) may be considered, in all probability, as possessing full immunity to vaccinia-variola virus.

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