

## Antibody response following smallpox vaccination and revaccination

BY A. W. DOWNIE, L. ST VINCENT, A. R. RAO AND C. H. KEMPE

*Department of Pediatrics, University of Colorado Medical Center and the  
Infectious Disease Hospital, Tondiarpet, Madras*

(Received 12 April 1969)

The measurement of antibody following vaccination has been recorded in many publications. The inhibition of vaccinia haemagglutinin, because of its technical simplicity, is a method which has been commonly employed in recent years, although the complement-fixation and neutralization techniques have also been used. Some kind of neutralization test is believed to give a better measure of protective antibody than the other tests (Elisberg, McCown & Smadel, 1956; McCarthy, Downie & Bradley, 1958; Espmark & Rabo, 1965). The more recently introduced precipitation test in agar gel, useful as a diagnostic test in smallpox, is usually negative for antibody in postvaccination sera. All four techniques for measuring antibody were used in our studies.

The present study was made as a background to the work on smallpox reported in the following papers (Downie *et al.* 1969*a, b*; Kempe *et al.* 1969). Because of differences in technical methods the titres of antibodies in postvaccination sera reported from different laboratories or even from the same laboratory at different times may not be directly comparable. This difficulty may be partly overcome by the use of an international reference serum of known antibody content. However, in the tests reported below on postvaccination sera the same techniques and reagents were used as in the studies on smallpox sera and the results have been used to assess the significance of our antibody measurements in smallpox. Three different groups of vaccinated individuals were studied: U.S. Army recruits bled 2–4 weeks after vaccination or revaccination, blood donors in Madras with histories of repeated vaccinations and young adults admitted as chickenpox patients to the Infectious Disease Hospital in Madras.

### *Sera*

### MATERIALS AND METHODS

The 210 sera from U.S. Army recruits had been collected for the preparation of vaccinia immune gamma globulin in 1962. The small samples used in the present investigations had been set aside at the time of collection and had been stored frozen at  $-20^{\circ}\text{C}$ . The Madras sera, 45 from blood donors and 42 from chickenpox patients, had been collected in 1960 and stored frozen since that time. All sera were inactivated by heating at  $58^{\circ}\text{C}$ . for 20 min. before use in the tests described below.

Requests for reprints should be addressed to Dr C. H. Kempe, 4200 East Ninth Avenue, Denver, Colorado 80220.

*Tests for precipitins*

All sera were tested undiluted against vaccinia soluble antigen prepared from vaccinia-infected rabbit skin as described by Downie & Kempe (1969). The antigen was used in the optimal dilution of 1/4. Agar in a concentration of 1% was used to prepare a layer 1 mm. thick on microscope slides. Using a plastic template, cups 4 mm. in diameter were cut in three rows so that the distance between neighbouring cups was 1.5 mm. Antigen was placed in the centre row of cups, and sera in the outside rows. Slides were kept in a moist container at room temperature and the tests read after 4, 24, and 48 h.

*Haemagglutinin inhibition*

Blood cells from two fowls were used throughout in a concentration of 0.5% stabilized with 1% normal rabbit serum. Haemagglutinin was prepared from vaccinia-infected chorioallantoic membranes (Downie & Kempe, 1969). To two-fold serial dilutions of serum from 1/10 upwards equal volumes of haemagglutinin diluted to contain four doses were added; after incubation for 1 hr. at 37°C. one volume of fowl red cells was added and the results read after the tests had stood 1–2 hr. at room temperature. These tests were carried out by the micro method in plastic plates.

*Complement fixation*

The same vaccinia rabbit soluble antigen was used as in the precipitation-in-agar-gel tests. The optimum concentration, determined by block titration (Downie & Kempe, 1969) was 1/80. The complement used was pooled preserved guinea-pig serum kept at 4°C. (Richardson, 1941). Two and a half doses of complement were used throughout. Sensitized cell suspension was prepared by mixing 3.0% washed sheep cells with an equal volume of sheep cell haemolysin diluted to contain 4 M.H.D. Serum dilution, complement and antigen each in 0.1 ml. volumes were mixed and kept overnight at 4°C. before adding 0.2 ml. of sensitized cells next day and incubating the mixtures at 37°C. for completion of the tests. A vaccinal immune rabbit serum was titrated with each batch of tests.

*Neutralization tests*

These were made in monkey kidney monolayers in Leighton tubes. Sera were tested in threefold dilutions from 1/10 upwards. Equal volumes of serum dilutions and vaccinia virus suspensions were mixed and held in a water bath at 37°C. for 2 hr. before adding 0.1 ml. volumes to monolayers in Leighton tubes. The tubes were incubated at 37°C for 40 hr., the medium removed and the cell sheets stained with carbol fuchsin before counting plaques. Three or four tubes were used for each serum-virus mixture. The vaccinia virus suspension, prepared from infected monkey kidney tissue cultures, was diluted before use so that 30 to 70 plaques were produced in control tubes. A serum with known antibody content or the International Standard immune serum (pooled convalescent smallpox serum) was titrated with each batch of tests.

The mean counts for each serum dilution-virus mixture were plotted on graph paper as a percentage of the negative serum-virus control; the titre of the serum was determined by the point where the graph crossed the 50% line.

The titres recorded for all techniques indicate dilutions of sera before admixture with virus or antigen.

### Precipitins

### RESULTS

None of the postvaccination sera produced a precipitin line in the tests in agar gel. Among the 45 Madras blood donors there were seven persons who had suffered from smallpox years previously; these sera also were negative for antibody by the precipitation test.

Table 1. *Incidence of haemagglutinin-inhibiting antibodies in three groups of vaccinated or revaccinated individuals*

Titre	Percentage of sera		
	U.S. Army recruits (210)	Madras	
		Chickenpox (42)	Blood donors (45)
< 10	59	55	13
10	34	36	56
20	6	7	22
40	1	2	9

### Haemagglutinin-inhibition antibody

The results of these tests for the three groups of sera are shown in Table 1. The numbers of sera in the three groups differed and the results have been expressed as percentages for comparative purposes.

There is no significant difference in the titres shown by the Army recruits and the chickenpox patients in Madras. The titres for the blood donors in Madras are higher than those for the first two groups. The results were not significantly affected by the inclusion of the seven blood donors with a history of smallpox. Five of these had an HI titre of 1/10, one had a titre of 1/20, and one had a titre of 1/40.

### Complement-fixing antibody

The results of tests on the sera expressed in percentages are shown in Table 2.

Again there is little difference in the results between the sera of the U.S. Army recruits and the sera from the Madras chickenpox patients. The sera from the blood donors in Madras show rather more positive results but the difference is not significant. Of the seven donors with a history of smallpox, five had a titre of less than 1/5, one had a titre of 1/5, and one had a titre of 1/10.

*Neutralization tests*

The titres for individual sera are shown in Fig. 1. The results are shown for the second batch of 100 sera from U.S. Army recruits, as there was insufficient serum remaining from the first batch of 110 sera to permit testing at a dilution of 1/10.

Table 2. *Complement-fixing antibody in three groups of vaccinated or revaccinated individuals*

Titre	Percentage of sera		
	U.S. Army recruits (210)	Madras	
		Chickenpox (42)	Blood donors (45)
< 5	93	95	89
5	4	0	2
10	3	5	9
20	0	0	0

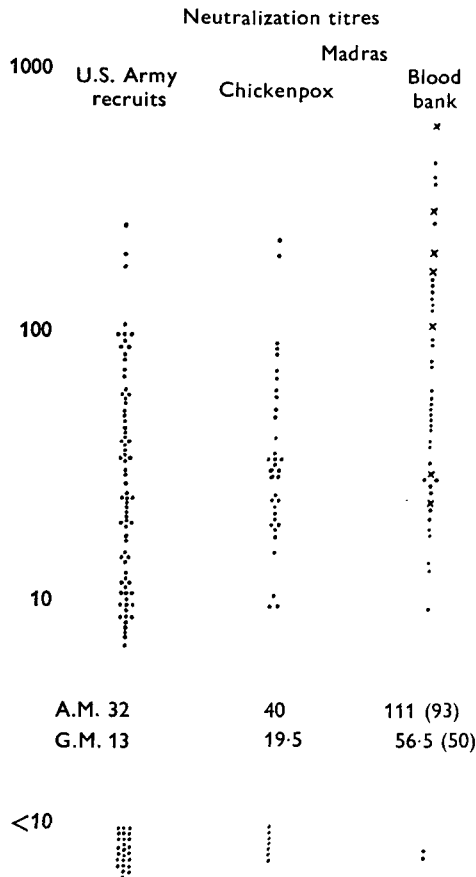


Fig. 1. Neutralizing antibody in three groups of vaccinated or revaccinated individuals. A.M. = arithmetic mean; G.M. = geometric mean. Figures in parentheses give the A.M. and G.M. after exclusion of seven persons with history of smallpox. ×, History of smallpox.

It can be seen that the majority of the sera neutralized vaccinia virus in a dilution of 1/10 or higher although many were negative in the H.I. and complement fixation tests—an observation reported by others (Herrlich, Mayr & Munz, 1956; McCarthy *et al.* 1958). The neutralizing titres were slightly higher for the chickenpox sera than for the Army recruits—arithmetic means 40 and 32, geometric means 19.5 and 13 respectively. The sera from Madras blood donors showed significantly higher levels of antibody—arithmetic mean 111, geometric mean 56.5. If the results of the seven sera from patients with a past history of smallpox are omitted from this group, the arithmetic mean for the remaining 38 was 93 and the geometric mean 50, titres significantly higher than those from the first two groups.

#### DISCUSSION

The results in general suggest that the neutralization test permits the detection of antibody which may not be detected by the HI and CF tests with the techniques used. It has been shown in previous studies that neutralizing antibody may persist for years after vaccination, when HI and CF tests for antibody have become negative, and that after revaccination a marked rise in neutralizing antibody may not be accompanied by a corresponding rise in HI and CF antibody (McCarthy *et al.* 1958). The differences in mean titre of neutralizing antibody between the three groups of sera may be explained in part by the previous history of smallpox vaccination. We have no information about previous vaccination in the Army recruits, but vaccination 2–4 weeks before the collection of blood samples had been successful. Of the chickenpox patients, a history of revaccination was available for only 20 of the 42 individuals in the series, whereas 41 of 45 blood donors had a history of revaccination. Many of the chickenpox patients were boys who had come from outside Madras to work with Madras families, whereas the blood donors were mostly native to Madras where vaccination and revaccination were more commonly carried out.

It seems a little surprising that the titres in the Army recruits were relatively low as these recruits were bled 2–4 weeks after vaccination or revaccination, the time when maximum antibody response might be expected; the time since vaccination or revaccination of the other two groups was variable although usually longer. The HI and CF antibody response following primary vaccination is not usually great and revaccination may fail to induce increase in titre in these antibodies. After successful revaccination the neutralizing antibody titres are generally considerably higher than after primary vaccination, (McCarthy *et al.* 1958), but the titres in the Army recruits are not dissimilar to those previously recorded in revaccinated individuals.

#### SUMMARY

Three groups of post-vaccination sera were studied for vaccinia antibody by precipitation, haemagglutinin-inhibition, complement-fixation and neutralization tests. All sera were negative by precipitation and many by haemagglutinin-inhibition and complement-fixation tests, but most showed neutralizing activity

at serum dilutions of 1/10 or higher. The differences in antibody titres between the three groups of sera were most probably related to the past history of revaccination.

This investigation was supported in part by Public Health Service Grant AI-1632-16 VR from the National Institute of Allergy and Infectious Diseases, by the World Health Organization and by the Marcus T. Reynolds III Fund.

#### REFERENCES

- DOWNIE, A. W. & KEMPE, C. H. (1969). Variola and other pox virus infections. In *Diagnostic Procedures for Viral and Rickettsial Diseases* (ed. Lennette & Schmidt), 4th ed. New York, American Public Health Association.
- DOWNIE, A. W., FEDSON, D. S., ST VINCENT, L., RAO, A. R. & KEMPE, C. H. (1969*a*). Haemorrhagic smallpox. *J. Hyg., Camb.* **67**, 619-29.
- DOWNIE, A. W., ST VINCENT, L., GOLDSTEIN, L., RAO, A. R. & KEMPE, C. H. (1969*b*). Antibody response in non-haemorrhagic smallpox patients. *J. Hyg., Camb.* **67**, 609-18.
- ELISBERG, B. L., MCCOWN, J. M. & SMADEL, J. E. (1956). Vaccination against smallpox. II. Jet injection of chorioallantoic membrane vaccine. *J. Immun.* **77**, 340-51.
- ESEMARK, M. A. & RABO, E. (1965). The formation of neutralizing antibody following smallpox vaccination in young infants with maternal immunity. *Acta paediat. scand.* **54**, 341-7.
- HERRLICH, A., MAYR, A. & MUNZ, E. (1956). Das Antikörperbild der Variola-Vaccineinfektion. 1. Unterschiedliche Antikörperentwicklung bei der Vaccineinfektion des Kaninchens, Affen und Menschen. *Zentbl. Bakt. ParasitKde* (I. Abt. Orig.), **166**, 72-83.
- KEMPE, C. H., DEKKING, F., ST VINCENT, L., RAO, A. R. & DOWNIE, A. W. (1969). Conjunctivitis and subclinical infection in smallpox. *J. Hyg., Camb.* **67**, 631-6.
- MCCARTHY, K., DOWNIE, A. W. & BRADLEY, W. H. (1958). The antibody response in man following infection with viruses of the pox group. II. Antibody response following vaccination. *J. Hyg., Camb.* **56**, 466-78.
- RICHARDSON, G. M. (1941). Preservation of liquid complement serum. *Lancet* *ii*, 696-7.