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## RELATION BETWEEN ANTIBODY LEVELS AND VACCINATION REACTION:

by

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Many studies have been performed in the attempt to determine a numerical factor which is a measure of immunity. In the case of vaccinia, such studies have the added academic advantage that the significance of a circulating antibody can be tested directly by challenge with the living infectious agent.

Neutralizing antibodies were the first antibodies discovered to appear after vaccination or clinical smallpox (Béclère et al 1899). They have been measured by the ability of the test serum, when mixed with virus, to prevent infection when injected into the skin of susceptible animals or to prevent pock formation when placed on the chorioallantoic membrane, or plaques on tissue cultures, using vaccinia or variola as virus. The presence of a factor which can prevent the characteristic activity of the virus was generally taken to connote immunity. The development of complement fixing antibodies has been taken as proof of infection with the vaccinia-variola group of viruses; the introduction of the haemagglutination-inhibition test (Nagler 1944) provided a technically simpler and apparently more sensitive in vitro test. Lately, the inhibition of I. cell agglutination by vaccinia virus was introduced with the hope that this measured actual immunity. (Mayyasi et al 1959).

All these tests show rises in serum titre after vaccination and after smallpox and persist for variable lengths of time. Their relationships to immunity can be inferred only if there is a correlation between titre level and reaction after vaccination.

Neutralizing antibodies appear after disease or vaccination and persist for many years. However, Sato and Kuroki (1929) presented the neutralizing

potency of the sera of a group of persons before and after revaccination. The prevaccination sera of 11 of 36 persons who developed only an allergic reaction contained no neutralizing antibody as measured in the rabbit skin; only one of those failed to show a rise in neutralizing potency after vaccination. On the other hand, of 32 who developed lesions considered by the authors to be significant, all but three had evidence of persisting neutralizing antibody at the time of vaccination.

Elisberg et al (1956) cite a 6 month old infant whose serum did not neutralize vaccinia virus on the chorioallantois of the embryonated egg but responded to a potent calf lymph vaccinia challenge with only an immediate reaction. McCarthy, Downie and Bradley (1958), showed that variola neutralizing antibodies, measured by reduction in the pock count on the chorioallantois, persisted for over 20 years; revaccination after such an interval is expected to result in accelerated or primary reactions. Cutchins, Warren and Jones (1960) showed by a plaque reduction test with vaccinia virus in a monkey kidney tissue culture that neutralizing antibody persisted for at least 10 years. Driessen (1936) compared serum reduction in the number of hemabsorption centres per monkey kidney tissue culture tube, using vaccinia virus, with the reaction to vaccination and concluded that "there is even no indication of any relationship". Among 22 individuals studied by Pincus and Flick (1963), 5 had neutralizing titres (serum dilution producing 50% pock reduction on the chorioallantois with vaccinia virus) of 16 or higher; 3 developed accelerated reactions to vaccination. The complement fixing antibody was reported by McCarthy, Downie and Bradley to appear in only 14 of 43 army recruits with successful primary vaccination and persisted for only a few months. After revaccination, about half had complement fixing antibodies at a 1:5 level or higher; none of 12 sera tested more than one year after revaccination were positive at this level. More direct information derives from personal work done with C. H. Kempe; a group of medical students were vaccinated and the reactions classified by the day of the greatest diameter of erythema as determined by daily measurements (Benenson et al 1952). The results were compared with the complement fixation titres, using a CAM vaccinia antigen (Table 1).

Vaccination Reaction	Complement fixation titre					Total students
	<2	2	4	8	16	
Primary	2	3	-	-	-	5
Accelerated	-	14	15	7	3	39
Allergic	-	12	22	18	-	52

TABLE 1 Vaccinia Complement fixing antibody titre and reaction to vaccination in 98 medical students

Although those who developed primary takes were essentially devoid of antibody 10 of 39 developed an accelerated reaction despite a serum titre of 1:8 or higher; complement fixation titres evidently can not be used to predict the vaccination reactions with any degree of reliability.

The situation is not better with the haemagglutination inhibiting antibody. Collier and van Thiel (1949) found that 58% of those who responded to revaccination with an accelerated or primary reaction had had HAI antibodies at the time of vaccination; the maximal titre observed was 1:1280; this was found in a subject who developed a primary reaction as well as in two who resisted infection. Similar results were obtained in our medical student study (Table 2). Elisberg et al (1956) could not relate prevaccination HAI titres with the reaction to vaccination by jet injections.

Driessen also concluded that "there is no distinct correlation between serum titre (both neutralizing and HAI) and skin reaction after revaccination".

Vaccination Reactions	Haemagglutination Inhibition Titre									Total students
	<5	5	10	20	40	80	160	320	640	
Primary	-	-	1	2	2	-	-	-	-	5
Accelerated	10	1	5	8	4	7	2	1	-	38
Allergic	7	2	1	11	16	8	5	-	2	52

TABLE 2 Vaccinia haemagglutination inhibiting titre and reactions to vaccination in 95 medical students

The hope that the correlation would be better with the serum factor which inhibits vaccinia agglutination of I cells has not been substantiated.

In a study carried out with F. C. Cadigan, we found that among 40 vaccinees

who developed major vaccination reactions, 29 had pre-existing L cell agglutination inhibiting antibody; this ratio is no different from the 21 of 29 minimum whose sera contained pre-existing antibodies.

Thus, the presently available serological tests all are poor indications of the immunity state. The correlations between the neutralizing and HAI antibodies (Driessen 1953), or the CF and HAI titres (McCarthy et al 1958, Benenson and Kempe 1951) suggest that these are all separate antibodies, and indicate prior contact with the antigens of this virus group. Thus, this level in a group is a measure of prior experience and the greater this has been, the more immune the group. So far as the individual is concerned, the measure of immunity to vaccinia is best determined by the reaction to vaccination.

The serological tests are of use in demonstrating whether antigenic stimulation has occurred. Thus, Sato and Kuroda (1929) showed that all but 2 of 36 revaccinated subjects with early reactions with only erythema or papule developed a rise in neutralizing antibody. Benenson and Kempe (1951) demonstrated that the allergic reaction following revaccination with inactivated vaccine evoked no serological responses in the HAI and/or CF tests. McCarthy, Downie and Bradley (1958) found a rise in variola neutralizing antibody in 8 of 15 persons with only an early reaction. Pincus and Flick (1963) reported that significant rises in both neutralizing and HAI antibodies could even occur after vaccinations which elicited no local skin reaction.

Conclusion: It is evident that the level of no serum antibody now known can be taken to indicate whether immunity to vaccinia does or does not exist. Their presence does indicate prior contact with the antigens of the vaccinia-variola group and, therefore, a high level in a population may correlate with the level of herd immunity.

#### Relation between Antibody Levels and Immunity to Smallpox

Data directly relating antibody levels and immunity to smallpox are not available, but inferences are made based on known relations to vaccinia and on observations made early in the disease before antibodies should have appeared in response to the clinical infection.

Although it is widely believed that the presence of neutralizing antibody should confer immunity to variola, Downie and McCarthy (1958) (J. Hyg. 56:479) cite a fatal case in which a high variola neutralizing titre was present on the second day of disease. Downie (1951) (Lancet 1, 419) reported a case of mild smallpox in a nurse whose blood contained a considerable titre of neutralizing antibody just before exposure; she had been vaccinated two years earlier.

Herrlich (1958) has noted the presence of high levels of HAI antibodies, complement fixing (V antigen) antibodies, and/or precipitating antibodies (Ouchterlony technique) as early as the third day of primary hemorrhagic smallpox in previously vaccinated pregnant women; and raises the question whether this form of disease involves allergic factors.

Epidemiological data indicate that immunity to smallpox persists longer after vaccination than does immunity to the percutaneous introduction of vaccinia. Since correlation between serum titres and immunity to vaccinia cannot be established, it would then be even more difficult to establish a relation between titres and immunity to smallpox.

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