Ind. Jour. Med. Res, 57, 1, January, 1969.

RELATION BETWEEN THE NEUTRALIZING AND HAEMAGGLUTINATION-INHIBITING ANTIBODIES IN SMALLPOX.

J. K SARKAR[1], S N CHATTERJEE[2], A C MITRA[3]

(From the Department of Virology, Calcutta School of Tropical Medicine)

AND

A MONDAL[4].

(From the Infectious Diseases Hospital, Calcutta)

[Received for publication, March 27, 1968]

INTRODUCTION

NEUTRALIZING (N) and haemagglutination-inhibiting (HI) antibodies are the two antibodies most commonly searched for in smallpox and alastrim patients, as well as in individuals after vaccination, although complement fixation (CF) tests (Downie and McCarthy, 1958) and recently agar gel flocculation tests (Ray *et al*, 1966) have also been carried out occassionally in such cases In the sera of alastrim patients, McCarthy and Downie (1953) did not find complete correlation between N, HI and CF antibodies, whereas, in healthy individuals after vaccination, Mastikova *et al* (1961) found complete correlation between HI and N antibodies The present work was undertaken to see if there is correlation between HI and N antibodies in the sera of smallpox patients

MATERIALS AND METHODS.

Samples of blood were collected from smallpox cases soon after admission in the Infectious Disease Hospital Calcutta The selection of patients was random, and all the cases were virologically positive, virus having been isolated either from blood or from skin lesions or from both Blood was allowed to clot, brought back to the laboratory in ice and the separated sera were preserved at −20°C

Patients were classified into three clinical types – haemorrhagic' (very severe), 'confluent' (moderately severe) and 'discrete' (mild) as per criteria stated previously (Sarkar *et al*, 1967) Amongst the haemorrhagic cases, there were a few 'variola pustulosa haemorrhagica' (Maegraith, 1956) patients who were comparatively less severe clinically Day of illness was calculated from the day of initial fever

The HI and N tests were carried out in the techniques followed previously (Sarkar *et al*, *loc cit*)

RESULTS

One hundred and thirty-five cases of the present series included 45 'haemorrhagic' (including 6 of 'Variola pustulosa haemorrhagica'), 45 'confluent', and 45 'discrete'. The results of these cases along with their period of illness are presented in Table I Titre of virus has been shown only in those cases whose blood was virologically positive

1. Professor 2 Assistant Professor. 3. Assistant Research Officer (*Present address* — Superintendent, Vaccine Institute, Calcutta Corporation) 4 Professor.

TABLE I.

Showing HI and neutralizing titre in the sera of smallpox cases on different days of illness.

Case number.	Clinical type.	Days of illness.	Virus titre (per ml. of serum).	HI titre.	Neutralizing titre.
1	Haemorrhagic	2	2×10^3	<10*	<10†
2		2	3×10^4	<10	<10
3		2	2×10^4	<10	<10
4		2	1×10^3	<10	<10
5		3	7×10^3	20	<10
6		3	7×10^3	<10	<10
7		5	7×10^2	40	10
8		2	3×10^4	<10	<10
9		4	5×10^2	40	10
10‡		3	*Nil*	80	10
11‡		6	*Nil*	80	10
12		3	$2{\cdot}5 \times 10^4$	<10	<10
13		2	1×10^3	<10	<10
14		3	$3{\cdot}5 \times 10^4$	<10	<10
15‡		4	*Nil*	80	10
16		3	6×10^2	<10	<10
17		3	3×10^4	20	<10
18		3	3×10^4	20	<10
19		4	2×10^2	40	10
20		3	1×10^5	<10	<10
21		3	1×10^6	<10	<10
22		3	2×10^4	<10	<10
23		3	$2{\cdot}5 \times 10^4$	<10	<10
24		2	2×10^4	<10	<10
25		3	$5 \quad 10^4$	<10	<10
26		4	3×10^4	<10	<10
27‡		6	*Nil*	320	40
28		3	2×10^3	20	<10
29		3	3×10^3	40	<10
30		4	3×10^3	<10	<10
31‡		6	*Nil*	320	40
32‡		6	*Nil*	80	10
33		2	$2 \cdot 10^5$	<10	<10
34		2	2×10^5	<10	<10
35		4	2×10^4	<10	<10
36		2	3×10^4	<10	<10
37		4	1×10^4	20	<10
38		3	1×10^2	<10	<10
39		2	2×10^4	<10	<10
40		4	1×10^2	<10	<10
41		3	2×10^2	<10	<10
42		3	3×10^3	<10	<10
43		3	$2{\cdot}5 \times 10^4$	<10	<10
44		4	4×10^4	20	<10
45		2	2×10^5	<10	<10
46	Confluent	4	*Nil*	160	20
47		5	*Nil*	160	40
48		5	1×10^3	80	10
49		5	*Nil*	80	10
50		5	*Nil*	160	20
51		4	*Nil*	80	10
52		6	*Nil*	320	40

TABLE I (*Contd.*).

Case number.	Clinical type.	Days of illness.	Virus titre (per ml. of serum).	HI titre.	Neutralizing titre.
53	Confluent	5	*Nil*	80	10
54		5		320	20
55		12		320	40
56		9		640	40
57		11		2560	320
58		11		2560	320
59		12		640	80
60		12		1280	160
61		12		1280	80
62		4		80	10
63		6		160	40
64		6		320	40
65		6		160	20
66		6		160	20
67		11		1280	160
68		7		640	80
69		8		320	40
70		6		160	20
71		7		160	40
72		9		320	40
73		7		160	20
74		6		160	40
75		6		160	20
76		8		160	20
77		6		160	10
78		12		1280	160
79		12		1280	160
80		9		640	40
81		12		1280	160
82		8		320	40
83		6		160	20
84		6		160	20
85		12		640	80
86		8		640	80
87		12		1280	80
88		6		160	20
89		5		160	20
90		5		80	10
91	Discrete	4	*Nil*	40	10
92		3		20	<10
93		4		80	10
94		5		80	10
95		4		40	10
96		4		20	<10
97		4		40	10
98		4		160	20
99		4		40	10
100		8		160	20
101		8		320	40
102		3		20	<10
103		6		80	10
104		4		80	10
105		3		10	<10
106		5		80	10
107		4		10	<10

TABLE I (*Concld.*).

Case number.	Clinical type	Days of illness.	Virus titre (per ml of serum).	HI titre	Neutralizing titre
108	Discrete	10	*Nil*	320	20
109		10		160	10
110		10		320	40
111		6		320	40
112		3		<10	<10
113		7		160	20
114		6		320	20
115		7		160	20
116		12		320	40
117		8		80	10
118		7		160	20
119		10		320	40
120		8		160	10
121		10		80	10
122		4		80	10
123		7		160	20
124		9		160	40
125		8		160	20
126		6		80	10
127		7		160	40
128		11		160	20
129		6		80	10
130		7		80	10
131		8		160	20
132		12		640	40
133		6		80	10
134		12		320	80
135		12		640	80

*Indicates highest dilution of sera inhibiting haemagglutination

†Indicates highest dilution of sera giving 50 per cent or more reduction of pocks in comparison to those of the control

‡Indicates cases of 'variola pustulosa haemorrhagica'

Statistical analysis of the results of HI and N tests show a significant correlation (correlation coefficient = 0·88) between the HI titre (x) and the N titre (y). This has been obtained in a transformed scale, as given by the following regression equation of X & Y :

$$Y = 0{\cdot}5256\ X - 0\,8342,$$

$$\text{where } X = \frac{\log (x/10)}{\log 2} \text{ and } Y = \frac{\log (y/10)}{\log 2}$$

DISCUSSION.

In smallpox as well as in many other viral infections, neutralizing antibody is usually considered more important than HI or other antibodies, from the point of recovery from the illness. At the same time, the process of N test carried out in animals is more elaborate and time-consuming than that of HI test But in the present work, the correlation between the results of HI and N tests, as has been found in the sera collected

ed on different days of illness from the smallpox cases of varying clinical severity, will certainly raise the importance of HI test, which is already in great use for the purpose of diagnosis (Collier and Schonfeld, 1950 ; Downie and McCarthy, *loc. cit.*), survey (Downie *et al.*, 1961), as well as for assessment of immunity status after vaccination (Mastikova *et al.*, *loc. cit.*).

SUMMARY AND CONCLUSIONS.

Sera of 135 smallpox cases (45 haemorrhagic, 45 confluent and 45 discrete), collected on different days for illness, were tested for the level of neutralizing and haemagglutination-inhibiting antibodies. Significant correlation was obtained between the results of two tests.

The authors are indebted to Dr. K.K. Mathen, Professor of Statistics, All-India Institute of Hygiene & Public Health, Calcutta, for the statistical analysis shown in the paper. Grateful thanks are also due to Dr. J.B. Chatterjea, Director of the School for encouragement and to Dr. P. Manji, Superintendent, Infectious Disease Hospital, Calcutta, for his help.

REFERENCES.

COLLIER, W.A., and SCHONFELD, T.K. (1950) — Studies on the serological diagnosis of smallpox. *Med. Jour. Aust.*, **ii**, 36.

DOWNIE, A.W., HOBDAY, T.L., VINCENT, L.S., and KEMPE, C.H. (1961) — Studies on smallpox antibody level of sera from samples of the vaccinated adult population of Madras. *Bull. Wld. Hlth. Org.*, **25**, 55-61.

DOWNIE, A.W., and MCCARTHY, K. (1958) — The antibody response in man following infection with viruses of the pox group-antibody response in smallpox. *Jour. Hyg. Camb.*, **56**, 479-487.

MAEGRAITH, B. (1956) ... ... — Price's 'Text book of Medicine', 9th ed., 158-168. Oxford University Press, London.

MASTIKOVA, Y.N., SARAYEVA, N.T., KOZACHENKO, N.F., YARO SLAVSKAYA, N.V, RAIKHSTADI, G.N., and SHVARTSMAN, N.N. (1961) — A study of results of smallpox vaccination, *Problems of Virology*, 6, 207-211.

MCCARTHY, K., and DOWNIE, A.W. (1953) — The serum antibody response in alastrim. *Lancet*, **i**, 257-260.

RAY, S., SARKAR, J.K., CHATTERJEE, S.N., and MITRA, A.C. (1966) — Flocculating antibody in smallpox. *Bull. Sch. Trop. Med.*, Cal., **15**, 85.

SARKAR, J.K., and MITRA, A.C. (1967) ... — Virulence of variola virus isolated from smallpox cases of varying severity. *Ind. Jour. Med. Res.*, **55**, 13-20.