

SERUM PROTEINS AND ANTIBODY STUDIES IN SMALLPOX.

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SMALLPOX is an acute infectious disease in which the stage of viraemia usually terminates when detectable antibodies appear in the circulation. Response of antibodies, which are protein in nature, has not been found uniform in this disease (Sarkar *et al.*, 1967), and it was thought worthwhile to find out the pattern of protein fractions and their relation, if any, to the development of antibody in the sera of smallpox cases.

MATERIALS AND METHODS.

Samples of blood were collected from smallpox cases admitted in the Infectious Diseases Hospital, Calcutta. The clinical severity of the cases designated as haemorrhagic, confluent and discrete (Sarkar and Mitra, 1967), as well as the period of illness when blood samples were collected, varied. The sera after separation were preserved at -20°C . for varying periods of time before they were tested. Protein fractions in the sera were determined in the Department of Biochemistry of the School by paper electrophoresis using a Beckman Spinco electrophoresis apparatus (Model R), and the calculation of the different proteins was done in a Beckman analytrol machine (model RB). The total proteins were estimated by the Biuret method. Normal values, previously determined in a similar manner and in the same apparatus, were accepted for calculations in the present study.

The technique of haemagglutination-inhibition (HI) tests was the same as was used previously (Sarkar, Chatterjee and Chakravarty, 1964; Sarkar *et al.*, *loc. cit.*).

RESULTS.

Results of paper electrophoresis and those of HI tests along with the period of illness of the patients are presented in Table I. Eleven of the sera (Nos. 9, 10, 18-20, 22-24, 26-28) were freshly collected, 11 (Nos. 2, 6-8, 12, 13-17, 21) were collected about a year ago and 6 sera (Nos. 1, 3-5, 11, 25) were collected three and half years ago. Their results have been compared with those of normal sera in Table II.

It may be noted in Table II that in the sera of smallpox cases albumen is significantly low, and total globulin, α_2 and γ are significantly high. In the three sera (serial Nos. 1, 3 and 5), all the globulin fractions could not be electrophoretically separated and therefore those values were not included in the calculations in Table II.

Of the 28 smallpox cases, 11 were clinically 'haemorrhagic', 3 were 'discrete' and 14 were 'confluent'.

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TABLE I.
Showing protein fractions (in grammes per cent) in the sera of smallpox cases as well as titres of HI antibody.

| Serial number. | Day of illness. | Total protein. | Albumen. | Total globulin. | GLOBULIN-FRACTIONS : | | | | H.I. titre. | Type of cases, |
|----------------|-----------------|----------------|----------|-----------------|----------------------|------------|---------|----------|-------------|----------------|
| | | | | | α_1 | α_2 | β | γ | | |
| 1 | 3 | 7.00 | 2.69 | 4.31 | 2.21 | 0.37 | 2.14 | 2.10 | < 10* | H |
| 2 | 3 | 8.15 | 3.32 | 4.93 | 0.84 | 0.37 | 2.14 | 1.58 | < 10 | H |
| 3 | 3 | 10.9 | 0.53 | 10.37 | 0.24 | 8.95 | | 1.18 | < 10 | H |
| 4 | 3 | 7.25 | 3.02 | 4.13 | 0.41 | 1.40 | 0.88 | 1.54 | 10 | D |
| 5 | 4 | 10.21 | 3.42 | 6.78 | 0.78 | 4.29 | | 1.71 | .. | H |
| 6 | 4 | 6.10 | 2.59 | 4.51 | 0.52 | 1.16 | 0.52 | 1.31 | 10 | H |
| 7 | 4 | 9.50 | 3.53 | 5.97 | 0.41 | 1.11 | 0.57 | 3.88 | 320 | C |
| 8 | 5 | 7.90 | 3.32 | 4.58 | 0.54 | 1.45 | 0.63 | 1.96 | < 10 | H |
| 9 | 5 | 5.90 | 2.65 | 3.25 | 0.30 | 0.75 | 0.54 | 1.66 | 120 | C |
| 10 | 5 | 8.00 | 3.36 | 6.64 | 0.56 | 0.78 | 0.72 | 2.58 | < 10 | C |
| 11 | 5 | 8.40 | 3.06 | 5.34 | 1.84 | 1.27 | 0.34 | 1.89 | 40 | C |
| 12 | 6 | 7.70 | 3.19 | 4.51 | 0.37 | 0.37 | 1.65 | 2.12 | < 10 | H |
| 13 | 6 | 6.55 | 2.62 | 3.93 | 0.25 | 0.42 | 0.72 | 2.54 | < 10 | H |
| 14 | 6 | 6.80 | 1.83 | 4.97 | 0.97 | 0.98 | 1.36 | 1.66 | < 10 | H |
| 15 | 6 | 7.25 | 3.36 | 3.89 | 0.60 | 0.93 | 0.74 | 1.62 | < 10 | H |
| 16 | 6 | 7.90 | 3.26 | 4.64 | 0.30 | 1.94 | 0.51 | 1.86 | > 1280 | D |
| 17 | 6 | 7.70 | 3.16 | 4.54 | 0.66 | 1.27 | 0.74 | 1.87 | > 1280 | D |
| 18 | 6 | 8.90 | 4.74 | 4.16 | 0.34 | 0.40 | 1.21 | 2.21 | 320 | C |
| 19 | 6 | 7.50 | 3.33 | 4.17 | 0.29 | 0.75 | 0.83 | 2.30 | 320 | C |
| 20 | 7 | 7.95 | 3.84 | 4.11 | 0.36 | 0.30 | 1.29 | 2.16 | 320 | C |
| 21 | 7 | 7.35 | 3.11 | 4.21 | 0.36 | 1.11 | 0.63 | 2.14 | > 1280 | C |
| 22 | 9 | 6.60 | 3.02 | 3.58 | 0.35 | 1.02 | 0.48 | 1.73 | 640 | C |
| 23 | 9 | 7.50 | 3.58 | 3.92 | 0.40 | 0.99 | 0.58 | 1.95 | 40 | C |
| 24 | 9 | 8.10 | 4.28 | 3.82 | 0.15 | 0.87 | 0.64 | 2.16 | > 1280 | C |
| 25 | 9 | 7.90 | 2.94 | 4.96 | 0.41 | 1.44 | 0.55 | 2.56 | 640 | C |
| 26 | 17 | 8.10 | 3.78 | 4.32 | 0.10 | 0.75 | 0.64 | 2.83 | 1280 | C |
| 27 | 17 | 8.50 | 2.35 | 6.15 | 0.10 | 1.01 | 0.61 | 4.43 | 160 | C |
| 28 | 17 | 7.40 | 3.81 | 3.59 | 0.34 | 0.38 | 0.96 | 1.91 | 640 | C |

* Indicates reciprocal of serum dilution inhibiting haemagglutination. — Indicates area where the fractions were not separated.
H — haemorrhagic ; D — discrete ; C — Confluent.

TABLE II.
Showing comparison of results of protein estimation of smallpox sera with those of normal sera.

| | | Total protein. | Albumin. | Total globulin. | α_1 . | α_2 . | β . | γ . |
|--------------------|-----------|----------------|----------|-----------------|--------------|--------------|-----------|------------|
| Normal sera (25) | { Maximum | 8.45* | 5.55 | 4.0 | 0.47 | 0.97 | 1.25 | 1.90 |
| | { Minimum | 6.7 | 2.79 | 2.05 | 1.13 | 0.28 | 0.53 | 0.91 |
| | { Mean | 7.55 | 4.58 | 3.09 | 0.25 | 0.51 | 0.87 | 1.46 |
| Standard-deviation | | 0.45 | 0.71 | 0.54 | 0.08 | 0.18 | 0.22 | 0.30 |
| Smallpox sera (28) | { Maximum | 10.9 | 4.74 | 10.37 | 1.84 | 1.45 | 1.65 | 4.43 |
| | { Minimum | 6.60 | 0.53 | 3.25 | 0.10 | 0.30 | 0.34 | 1.18 |
| | { Mean | 7.77 | 3.07 | 4.87 | 0.41 | 0.96 | 0.86 | 2.23 |
| Standard-deviation | | 1.26 | 0.79 | 1.02 | 0.124 | 0.133 | 0.165 | 0.176 |
| Significance | | N.S. | H.S. | H.S. | N.S. | H.S. | N.S. | H.S. |

*Indicates gramme per cent.

H.S. = highly significant.

N.B.—N.S. = not significant.

In the statistical analysis (Table II) the difference with the normal was considered highly significant (H.S.) if the probability value was less than 0.01, significant (S), if it was more than 0.01 but less than 0.05 and not significant (N.S.) if it was above 0.05.

DISCUSSION.

It is a known fact that any morbid condition may affect the protein fractions of human serum either quantitatively or qualitatively, and in pathological states there may be hyperglobulinemia (Riberio *et al.*, 1961). The increase in the globulin fractions usually parallels the antibody titres as demonstrated by various serologic and immunologic tests. The hypo-albuminemia is, probably, set up as a compensating mechanism in order to maintain the colloido-osmotic condition of the plasma (Riberio *et al.*, *loc. cit.*). The hypoalbuminemia in the smallpox cases in the present series may be explained in this light.

Reports on the analysis of the protein composition of antiviral sera by physical and chemical fractionation procedures, in most instances, have not shown significant alterations in either the relative or absolute quantities of the individual serum proteins (Dimopoulos, 1961). There have also been reports in the literature on a number of antiviral sera that have shown alteration similar to that found in antibacterial, antitoxic and antiprotein sera (Dimopoulos, *loc. cit.*). Electrophoretic analysis of sera from trachoma patients in the acute and cicatricial stages have shown a decrease in the albumin/globulin ratio (Yamanaka and Nakanishi, 1952). In poliomyelitis, α_1 and α_2 globulins were increased, but there was no change in the β fraction, whereas in measles, hypergamma globulinemia without any change in the total protein was found (Riberio *et al.*, *loc. cit.*). Krishnamurthy (1956) studied serum proteins of 40 chicken-pox cases and he found relative decrease in the albumen, normal α_1 , increased α_2 and high increase of β and fractions of globulin.

Hyperglobulinemia with hypo-albuminemia have been recorded in the smallpox cases of the present series. Similar was the experience of Connly *et al.* (1953), but they also found hypoproteinemia. No immunological studies were conducted by the latter workers, who postulated that the increase in globulins was the result of liver damage or increase in antibody titre. In the present series HI antibody titre was not found proportional to total globulin or any of its different fractions. In the 10 haemorrhagic cases, who had practically no HI antibody response, total globulin as well as the fractions of globulin (including γ are high in some of the sera. Thus, the results of the present series go in favour of the postulation made by Connly *et al.* (*loc. cit.*). It may be mentioned here that neutralizing antibody was estimated in a proportion of these sera as well as in sera of other smallpox cases and the titres of HI and neutralizing antibodies were found to correlate.

No definite relation could be established between the duration of illness and any of the protein fractions in the sera.

SUMMARY.

1. Protein fractions have been estimated by paper electrophoresis in 28 sera of smallpox cases of different clinical severity collected on 3rd to 17th days of illness. The results were compared with those of 25 normal sera determined previously in similar manner.
2. Haemagglutination-inhibiting titres of all the sera were determined.
3. There was no change in the total proteins of the smallpox sera, but albumin was diminished and total globulin increased. Of the globulin fractions α_2 and γ were significantly increased.
4. HI titres did not correspond to total protein or any of the protein fractions including γ globulin.

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