An Electrophoretic Study of Leprosy Serum and its possible relationship with Haemagglutination Titre

MRS GOURI BANERJEE* and A. N. ROY Indian Institute for Biochemistry and Experimental Medicine, Calcutta

Investigation on haemagglutination reaction of serum of leprosy patients have been made by various authors; and Gernez-Rienz *et al* (¹), Floch and Sohier (² and ³). Pissier and Sacret (⁴), Viet (⁵), Roy and Banerjee (⁶ and ⁷) got positive haemagglutination titre in sera of leprosy patients. Relationship between gammaglobulin content of serum protein and antibodies is now considered an interesting problem immunologically. Haemagglutination reaction is an antigen-antibody reaction, so a study of electrophoretic pattern of serum proteins and its possible relation with Haemagglutination titre of sera of leprosy cases was undertaken. The result of this study is presented in this paper.

MATERIALS AND METHODS

Separation of sera from clotted blood

Thirty-two cases of leprosy patients were examined. They were clinically diagnosed leprosy patients without any evidence of tuberculosis. Microscopic examination of smear made from skin lesions was also done. Ten normal adults selected from the workers of the Indian Institute for Biochemistry and Experimental Medicine were examined as controls. Ten ml. of blood was taken from the vein of each person in fasting state in the morning. Blood samples were kept in refrigerator at 4°C for serum to separate by coagulation and clear serum obtained by centrifugalization.

Paper-electro phoresis

The method of separation of different protein fractions of serum was that of Ganguli (⁸). Usually 20 cm Whatman filter paper No. I, was used, bromophenol blue and acetic acid solution were used for staining and washing respectively. The quantitative determination of the different fractions of serum was carried out according to Ganguli(⁹).

Preparation of sera for electrophoresis

One ml. serum of each case was taken in small test tubes and trace of bromophenol blue was added. The dye combined with the albumin and acted as an indicator for the length of the run given.

Haemagglutination reaction

The same method was followed as was done in our preliminary study $(^{6})$.

RESULTS

The distribution of different fractions of albumin and globulin from sera of normal and the leprosy cases is represented in Tables I and II.

Total serum protein and distribution of its various fractions and haemagglutination titre in normal human subjects (Results of protein fraction in the average of 10 individual cases)									
Total Protein %	Albumin	α1	G a2	lobulin β	γ	– Total Globulin	A.G. Ratio	No. of Cases	Haemagglu- tination Titre
7.6	58.5	2.8	4.6	10.4	23.7	41.5	1.4	6 3	Negative 1 :2 negative

TABLE I

*Present address: Institute of Post-graduate Medical Education and Research, Calcutta 20.

DISCUSSION

In an earlier investigation Roy and Banerjee (7)demonstrated that there was no correlation between haemagglutination and tuberculin skin sensitivity reaction in leprosy. In the present study which was undertaken to see the possibility of a relationship between the serum protein fractions especially globulin fractions and the haemagglutination titre of sera of leprosy cases, it may be observed from Tables I and II that the albumin-globulin ratio between the normal and leprosy sera alters appreciably. Compared with normal sera (Table I) the globulin fractions of the leprosy sera got elevated (more than double) and the A:G ratio became less than normal as shown clearly in another table retained in our records. Similar observation was reported by Mayama (10) while studying the electrophoretic distribution of serum proteins in leprosy. His report indicates that α and β globulins were found to be elevated but the γ globulin was markedly raised while there was a drop in the albumin fraction. But no attempt was made by him to correlate between haemagglutination titre and globulin fractions.

The investigation reported herein shows that with the increase in the globulin fraction there is a simultaneous increase in the haemagglutination titre in 81 per cent of the leprosy cases studied. The low titre value in the rest (19 per cent) of the leprosy cases may be due to some reasons which need further investigation for such low value, although there is an increase in globulin fractions. So it will not be unreasonable to say that there is a correlation between the increase in haemagglutination titre and globulin fractions in this disease. The result appears to be consistent with the view that haemagglutination titre is an index of antibody formation and that antibodies are modified globulins (¹¹).

SUMMARY AND CONCLUSION

Haemagglutination reaction of sera of 32 bacteriologically positive and negative leprosy cases was studied and the protein fractions of their sera were investigated by paper electro-phoresis.

A decrease in albumin and an increase in globulin were demonstrated. Albumin-globulin ratio was less than one. A correlation between the increase in haemagglutination titre and globulin components of the serum proteins in leprosy cases was observed, these data have been statistically verified and found to be significant.

ACKNOWLEDGEMENTS

Our thanks are due to Dr J. C. Ray, M.D., F.N.I., former Director of this Institute, for his interest and facilities given for this study. To Dr D. K. Roy, D.PHIL, D.SC., of the Department of Biochemistry of this Institute and Dr N. C. Ganguli, D.SC., of the Department of Applied Chemistry, University College of Science, Calcutta, we are grateful for their helpful assistance in this work. Director of Vagrancy Home (leprosy), Government of West Bengal and the medical officer-in-charge of the Home were very kind to offer us facilities to study the leprosy patients in the home and out thanks are due to them.

Laboratory assistance rendered by Shri Sudhir Das and Miss Snigdha Datta is highly appreciated.

References

- I. GERNEZ-RIENX, G., MONTESTRUCE, E. and TACQUET, A. (1952). Bull. Acad. Nat. Med., 136, 375.
- 2. FLOCH, H. and SOHIER, R. (1952). Arch. Inst. Pasteur de la Guane et du territoire del Inini. Publication, 272.
- 3. FLOCH, H. and SOHIER, R. (1953). Bull. Soc. Path. Exact., **46**, 916.
- 4. PISSIER, M. and SACRET, E. (1953). Marco Med., 32, 779.
- 5. VIET, M. (1954). Ann. Inst. Pasteur, 86, 76.
- 6. ROY, A. N. and BANERJEE, G. (1955). Ann. Biochem. Explt. Med., 15, 55.
- 7. ROY, A. N. and BANERJEE, G. (1957). Ann. Biochem. Exptl. Med., 17, 111.
- 8. GANGULI, N. C. (1956). Annal. Chem., 28, 1499.
- 9. GANGULI, N. C. (1956). Clinicia Chimica. Acta., 1, 413.
- MAYAMA, AKIRA (1954). Science Repts Research Inst. Tohoku University Sec. C., 5, 273.
- 11. HEIDELBERGER, M. (1956). Lectures in Immunochemistry, Academic Press, New York.