

ELECTROPHORETIC STUDY OF PROTEIN PATTERNS OF SERUM FROM NORMAL NON-PREGNANT AND PREGNANT WOMEN

By

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The serum protein fractionation has always been a difficulty in routine clinical work. Most of the laboratories employ tedious methods of fractional precipitation with various concentrations of salts to separate albumin from globulin. A simple method of fractionating proteins on filter paper suitable for clinical laboratories has been developed in Germany, the United States and Sweden; Wieland and Fischer (1948), Gremer and Tiseleus (1950); Durrum (1950), and Turba and Enenkcl (1950). Electrophoresis as a method of separation of protein fractions has proved valuable in analytical studies. Up to the present there have been quite a few reports regarding the utility of this method in investigating the comparative patterns of serum protein. The following paper reports the results obtained of variations in serum albumin and α_1 α_2 β and γ - globulins in normal non-pregnant and pregnant women.

MATERIAL AND METHOD

Blood samples were obtained from the subjects attending the ante-natal clinic of the local hospital. Blood was drawn without haemolysis and collected in dry test-tubes and was allowed to clot. After clotting the blood was centrifuged at low speed and the serum thus obtained was used for the study.

A 'Perspex' apparatus supplied by ADCO, India Ltd., was used for electrophoresis which allows 6 paper strips measuring 36 x 4 cm. to be run at the same time with temperature control. All the analyses were made in 'Veronal' buffer at pH 8.5 and 0.05 μ (1.84 gm. of diethyl barbituric acid and 10.3 gm. of sodium diethyl barbiturate in one litre of distilled water). Filter paper strips (Whatman 3 mm) were stretched taut on the bakelite frame kept in the apparatus for equilibration whereafter 25 microlitres of serum were applied by a calibrated micropipette. A potential of 180 volts (direct current) was applied for 20 hours. At the completion of the run the papers were dried for 10 minutes at 105° C. and the protein denatured. The strips were stained with 1 per cent bromophenol blue (B. D. H.) in ethanol (95 per cent) saturated with mercuric chloride. Surplus dye was washed with methanol several times till the last wash was free from the dye and again dried.

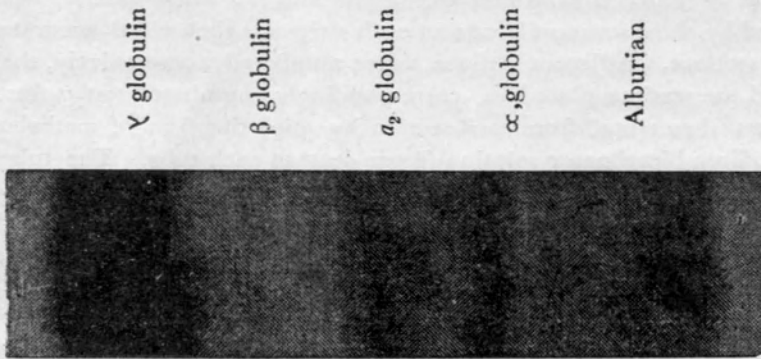
The standardisation and estimation of the protein fractions was that adopted by Paton *et al.* (1954) and Flynn and De Mayo (1951). The area enclosed by the outermost bands on each strip was then ruled accurately into 5 mm. sections. Adjacent sections were numbered consecutively, the strips cut and the sections placed in correspondingly numbered test tubes. The dye was then eluted from each section by pipetting 5 ml of methyl alcohol with sodium bicarbonate solution (4 per cent) to each tube. The tubes were allowed to stand at room temperature for an hour by which time the extraction was complete. The optical density of each was next determined in a photoelectric colorimeter at 595 $m\mu$. A blank was also prepared by eluting a section of the strip which was protein free but subjected to the same staining procedure. The more dense solution corresponding normally to the albumin fraction usually required dilution of 1 part to 5. The values obtained for the optical densities of the globulins were multiplied by an empirical factor of 1.6 which corrects for the lower binding power of the globulins by the bromophenol blue; Cremer and Tiseleus, (*loc cit.*). The corrected optical densities were next plotted against distance and the resultant area under the curve was divided as in the classical Tiseleus experiment into symmetrical peaks whose combined areas equal that of the total. The area of the component sections were next determined. These areas proportional to the protein concentration and therefore the per cent composition were calculated. With the protein concentration known the absolute values of albumin and globulin fractions were determined. Example of curve is shown in Figure 2. The total protein and albumin/globulin ratio were done by the usual micro-Kjeldahl's method.

RESULTS

During 1956-57 more than 121 specimens were examined by electrophoresis. Average values and the range for the various protein fractions obtained for normal non-pregnant and pregnant is shown in Table I and typical patterns in Figure 1. They agree reasonably well with those values given by Lagercrantz (1945), by Coryell *et al.* (ii 1950) and Levin and Oberholzer (1953) except that the proportion of albumin is a little low i. e. 62 percent of total protein as compared to the value reported by Paton *et al.* (*loc cit.*). However this is a relatively small difference and may be due to other factors as diet (the average Indian women consumes relatively lesser amount of protein) and not necessarily to the method used.

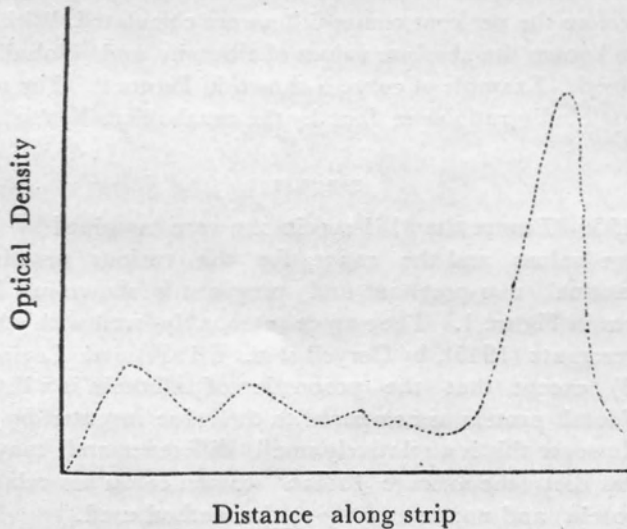
This method has been successfully used to follow the alterations in the serum protein fractions which normally accompany pregnancy. The mean values obtained are recorded in Table 1. There is a great degree of agreement with values reported by Coryell *et al.* (*loc cit.*) and by Macy and Mack (1952). The most conspicuous difference from normal non-pregnant serum is the fall in albumin level which occurs in the trimester and continues until delivery.

Fig. 1.



Typical pattern of serum obtained by paper electrophoresis and stained with bromophenol blue normal serum from non-pregnant woman.

Fig. 2.



Typical curve obtained with serum by quantitative technique - normal non-pregnant

It is not solely due to haemodilution since a fall occurs when the values are expressed per 100 gm. of serum protein. Accompanying this fall there is absolute rise in all the globulin fractions except γ globulin, the level of which

TABLE-I

Serum Protein Contents GM. Per 100 ml. of serum in Normal Non-pregnant and Pregnant Women :

Observation.	No. of subjects.	Albumin.	α_1 Globulin.	α_2 Globulin.	β Globulin.	α Globulin.	Total Protein.	Albumin Globulin ratio.
1. Non-pregnant Range.	17	3.6 to 4.8	0.09 to 0.27	0.24 to 0.59	0.41 to 0.87	0.61 to 1.32	6.12 to 7.8	1.7 to 2.4
2. Non-pregnant Mean.	..	4.25	0.19	0.39	0.57	1.06	6.46	1.92
3. Pregnancy : Upto 25 weeks :	13	3.5	0.24	0.43	0.69	0.94	5.80	1.52
4. Pregnancy : 25-30 weeks :	15	3.1	0.23	0.50	0.80	1.02	5.85	1.22
5. Pregnancy : 31-34 weeks :	11	3.0	0.27	0.56	0.86	0.96	5.65	1.14
6. Pregnancy 35-36 weeks :	19	2.9	0.21	0.59	0.87	1.05	5.62	1.06
7. Pregnancy : 37 weeks.	22	2.80	0.20	0.60	0.91	1.03	5.54	1.03

falls. In the women who developed toxæmia there was a general accentuation of the changes. A more complete analysis of the results from toxæmia, pre-eclampsia and eclampsia will be reported later.

SUMMARY

The technique of protein electrophoresis has been used in clinical work and has been found to be of potential value in the clinical studies of serum proteins owing to simplicity, cheapness and suitability for multiple analyses. Only minute quantities of serum are required. Details are given of a quantitative method of paper electrophoresis which provides for a clear separation of albumin and α_1 , α_2 , β and γ globulins in 25 microlitres of serum. The sera of normal non-pregnant and pregnant women have been analysed for the protein components. This method is also useful to follow the alterations in the serum protein fraction that normally accompany pregnancy.

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