ELECTROPHORETIC SEPARATION OF PLASMA PROTEINS IN NORMAL INDIANS AND IN PATIENTS SUFFERING FROM VARIOUS DISEASES*

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Different fractions of plasma proteins have different physiological role in the body. In diseased states these fractions may be increased or some new fractions may appear. Electrophoretic studies of the plasma proteins in different diseases would indicate some of the pathological changes produced in the body under these conditions. The present investigation deals with such studies in normal Indians and in patients suffering from diabetes mellitus, gastroenteritis, syphilis, cirrhosis of liver and pneumonia.

MATERIALS AND METHODS

Collection of blood. Blood was withdrawn from the anticubital vein of patients suffering from diabetes mellitus, syphillis, cirrhosis of liver and pneumonia when admitted into the hospital and before any tratement was given.

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In patients suffering from acute gastroenterities, blood was withdrawn when dehydration of the patients was corrected by saline transfusion. Normal subjects were students of this department and blood was collected in the early morning before breakfast. Blood samples were collected in phials containing crystals of potassium oxalate.

Determination of total plasma proteins. Nitrogen was determined in a digest of 0.1 cc plasma by the micro-Kjeldahl method. Nitrogen was also estimated in an aliquot of protein-free blood filtrate by micro-Kjeldahl method. The difference in the two nitrogen values multiplied by 6.25 gave the total plasma protein value.

Determination of fibrinogen. Fibrinogen in 1 cc plasma was separated as fibrin by the method of Cullen and Van Slyke (1920), nitrogen in fibrinogen was determined by the micro-Kjeldahl method, the value was multiplied by 6.25 and fibrinogen content of plasma determined.

Determination of albumin and globulins. 1 cc plasma was diluted 3 times with a buffer solution prepared by dissolving 10.3 gm. sodium diethyl barbiturate and 1.84 gm. diethyl barbituric acid in water and bringing the volume to 1 liter. This buffer has ionic concentration 0.05 and pH 8.6. Whatman No. 1 filter paper was cut into strips measuring 4x41 cm., a line was drawn at the point at which protein specimens were to be applied, four such strips were soaked in the buffer solution, 0.02 cc of diluted plasma was applied with a micro-pipette on the paper strips and electrophoretic separation of plasma carried out in a L. K. B. Paper Electrophoresis-apparatus for 12 hours with a current of 6 mA and 300 volts according to the method of Hardwick (1954). The filter paper strips were dried, immersed in 0.1 per cent bromophenol blue in absolute ethyl alcohol saturated with mercuric chloride for 30 minutes, washed in 0.5 percent acetic acid until the background was clear and dried at first in room temperature and then in an electric even at 100°C. The filter paper strips were then read in a Photovolt Photœlectric Densitometer Model 425 and the optical density curves of the coloured zones plotted on a graph paper. The total area of the plotted curve was equivalent to the total plasma protein content of the sample used for the electrophoresis which was determined by the micro Kjeldahl method. The resultant area of the curve was divided into symmetrical peaks whose combined areas equalled that of the total. Areas of component sections of the graph were measured by cutting out the curves and weighing in a micro balance. The weight of the component sections of the curves were proportional to the area and the protein value of each section was calculated from the total protein value. γ-globulin was not determined from the graph because γ-globulin and fibrinogen zones in the electrophorogram were almost inseparable. The value was obtained by deducting the sum of the albumin, α-globulin, β-globulin and fibrinogen values from the total protein value. The results are given in Table 1.

TABLE I.

Different fractions of plasma proteins (%)

Subjects	Total protein	Albumin	α — globulin	β — globulin	γ — globulin	Fibrinogen
DIABETES (14)	7.90* ±.27	3.31 ±.14	1.29 ±.10	1.21 ±.10	1.69 ±.08	0.40 ±.03
t	1.1	7.2	1.5	4.0	3.2	2.9
Gastroenteritis (6)	6.64 ±.23	2.10 ±.10	0.75 ±.08	1.37 ±.10	1.89 ±.20	0.53 ±.04
t	6.3	16.9	3.6	5.4	2.4	5.1
Syphillis (8)	7.33 ±.38	3.07 ±.17	1.70 ±.24	0.84 ±.11	1.42 ±.16	0.30 ±.03
t	2.4	7.6	2.3	0.56	0.17	0.27
Cirrhosis of liver (6)	6.40 ±.02	1.75 ±.12	1.17 ±.11	0.86 ±.12	2.10 ±.22	0.51 ±.05
t	16	21	0.46	0.71	3.0	3.7
Lobar pneumonia (6)	6.98 ±.26	2.55 ±.22	1.04 ±.12	1.09 ±.19	1.39 ±.35	0.71 +.11
t	4.4	8.0	0.5	1.6	0	3.4
Normal subjects (22)	8.22 ±.11	4.64 ±.11	1.11 ±.06	0.77 ±.05	1.39 ±.05	0.31 ±0.2

*Mean with Standard Error. Figures in parenthesis indicate the number of subjects.

Discussion

In normal persons plasma albumin was 4.64 *per cent* and the value compared well with the value reported by Koiw *et al* (1952), Ricketts and Sterling (1949) and Satoskar and Lewis (1954). α—globulin was 1.11 *per cent* and although the value was slightly higher than that reported by Western workers (Koiw *et al, loc. cit.;* Ricketts *et al, loc. cit*) it was more than double of the value obtained by Satoskar and Lewis (loc. cit.) who worked with Indian subjects in Bombay. β—globulin was 0.77 *per cent* and did not differ from the value reported by other workers. γ—globulin was 1.39 *per cent* and the value was considerably lower than the value reported by Satoskar and Lewis, and only slightly higher than the value obtained by Western workers. Fibrinogen value was 0.31 *per cent.* Total plasma protein value was 8.22 *per cent.* The value was slightly higher than the value observed by other workers. The difference in our results with those of Satoskar and Lewis might be due to difference in the diet consumed or due to the determination of total protein by the copper sulphate method which is likely to give less accurate result than the micro-Kjeldahl method.

Patients suffering from diabetes mellitus had low plasma albumin, high β—globulin, γ—globulin and fibrinogen, and no change in a— globulin and total protein values. The results are in close agreement with those of Schneider *et al* (1946), Young and Webber (1953) and Klinger (1953). Low value of plasma albumin might be due either to decreased synthesis or increased utilization for the formation of tissue protoplasm. Increased β— globulin might be due to increased cholesterol content of blood of diabetics. The higher concentration of γ — globulin usually indicates the presence of increased amounts of antibodies (Tiselius and Kabat, 1939).

In patients suffering from acute gastro-enteritis total plasma protein and albumin diminished possibly due to dilution of the plasma by saline transfusion. The packed cell volume, on the average, was 36 per cent in these cases. While a — globulin fraction of plasma diminished, β— globulin, γ—globulin and fibrinogen contents increased considerably.

In syphilitic patients total plasma proteins and albumin diminished. There was no change in β— globulin, γ — globulin and fibrinogen. Unlike our observations Cooper *et al* (1946) and Merklen *et al* (1951) reported increased γ — globulin contents of serum in patients suffering from syphillis. We observed an increase in the a — globulin content of plasma.

In patients suffering from lobar pneumonia total protein and albumin diminished. There was no change in the different globulin fractions. Fibrinogen content was very high.

In patients suffering from cirrhosis of liver we observed diminution in total protein and albumin possibly due to diminished synthesis in the liver. There was a slight increase in β— globulin. γ — globulin and fibrinogen

increased markedly. Brante (1952) also observed an increase in β— globulin in liver cirrhosis.

The changes in the different components of plasma proteins observed in the different diseases, however, were not specific.

Summary.

Different fractions of plasma proteins were determined in 22 healthy adults, in 14 patients suffering from diabetes mellitus, in 8 patients suffering from syphillis and in groups of 6 patients suffering from acute gastro-enteritis, eirrhosis of liver and lobar pneumonia.

Total protein and fibrinogen were determined by micro-Kjeldahl method. Albumin and different fractions of globulins were determined by paper electrophoresis.

While albumin diminished in all the diseased conditions studied, the other constituents varied differently in the different diseases. The different fractions of plasma proteins did not change specifically in the diseased conditions studied.

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