SERUM PROTEINS. ELECTROPHORESIS

Plasma is a slightly yellow liquid due to bile pigment and opalescent due to fat content. It consists of: 90% water and 10% solid residue of which:

- **9% are organic substances:**
  - -Nitrogen - proteins: fibrinogen, albumin, globulin
  - -proteinaceous: urea, uric acid, ammonia, creatine, creatinine
  - -without nitrogen: glucose, lipids, oxalic acid, and citric acid.

- **1% is mineral substances**: Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, $HCO3^-$

Plasma protein is a mixture of fractions. The total amount of protein in plasma is called proteinemia whose normal values are between 6.5 -8 g%. Proteinemia that is more than 8 g% is called hyperproteineamia and when if is less than 6 g% is called hypoproteinaemia.

Proteins are amphoteric substances whose dissociation and electric charge depend on: the pH environment in which they are suspended and their isoelectric-point. Suspension in an 8.6 or more alkaline pH causes protein to charge molecules negatively. Placed in a continuously electric field, protein molecules will migrate to the anode (+). How electric load is different for different molecules migration speed will not be identical. After a period of time it will separate into groups with equally or near the molecular speed. Speed of migration depends on: size and shape of the molecule; characteristics of the environment in which the migration occurs; load electricity.

Electrophoresis is the phenomenon of migration of ions from a solution under the action of a continuous electric field: A^+ cations move towards the cathode (-) and anions B^- to anode (+).

MATERIAL REQUIRED

- Electrophoresis apparatus - charger (current source)
  - room electrophoresis: plastic box containing two side tanks that communicate through a bridge, ferries are equipped with one electrode;
- Support for migration: filter paper, agar, acryl amide, cellulose acetate;
- pH = 8.6 buffer
- Dyes
- Fixing and wash bath
- Eluant (0.4% NaOH)

PROCEDURE

Filter paper is cut in strips and marked at one end (to know the place for submission of plasma); these paper strips are soaked with buffer and fixed in the machine. Serum (0.01 ml) is applied to the starting line with micropipettes. After 10-16 migrating hours bands are removed, dried and then stained 20 minutes in wash solution until the background paper discoloration is colorless, leaving only the colored spots representing protein fractions.

After staining bands on filter paper remain five colored spots corresponding to the order of migration rate: albumin, globulin $alpha_1$; globulin $alpha_2$; beta globulins; gamma globulins.

The amount of dye attached to each fraction is directly proportional with the amount of protein existing in the fraction. Evaluation can be done:

-by direct photometry
-dye-elution of each fraction and measuring the optical density; fractions are cut into small fragments, centrifuge with NaOH solution; after 2 hours at 70 ° C is added to alcohol, cool and add glacial acetic acid. Mix and centrifuge. Samples are read at photo colorimeter.

Graphical representation of the read values allows drawing a curved named electroforeogram.

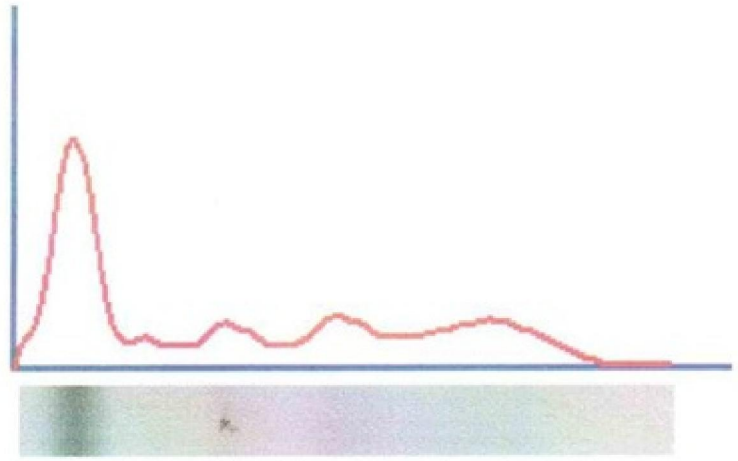

Figure no. Normal electroforeogram

Extinction values are recorded for each fraction as above curve that traces and device. Calculation of percentage values of serum protein fractions is based on the existence of a direct proportionality between the total area and total protein and between the amount of protein contained in each fraction and the area circumscribed by the curve that it represents.

INTERPRETATION

Normal values:

58 ± 4% albumin (4.5 g)
alpha$_1$ globulin 4 ± 1% (0.3 g)
alpha$_2$ globulin 8 ± 1% (0.47 g)
beta globulin 10 ± 2% (0.9 g)
gamma globulin 18 ± 2% (1.45 g)

The albumin / globulin = 1.5
Change of this report or an electrophoresis fraction is named disproteinemia.

Table no. 3 Pathological changes in serum protein fractions

No	**Patological condition**	**Total proteins**	**albumin**	**α_1 glob.**	**α_2 glob.**	**β glob.**	**γ glob.**
1	An acute inflammation, after surgery, myocardial infarction	Normal	Slight decrease	Normal	Marked increase	-	-
2	Chronic inflammation	Normal	Slight decrease	-	-	-	Marked increase
3	Nephropathy	Decrease	Marked decrease	-	Increase	Increase	-
4	Exudative enteropathy	Decrease	Decrease	-	Low Increase	Low Increase	Low Increase
5	Liver destructive processes	Decrease	Marked decrease	-	-	-	Marked increase
6	Multiple Myeloma	Increase	Decrease	-	-	-	Marked increase

Electrophoreogram correct interpretation requires knowledge of a total protein levels. For example, both a liver and a chronic infection phase synthesis of gamma-globulins antibody increase and decrease in proportion to albumin, the difference being the amount of low protein levels and increased liver disease in the second case.