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Fractionation of Serum Protein in the Indian Sheep

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Fractionation of serum protein in the Indian sheep was studied. 43.295% albumin, 28.79% ($\alpha + \beta$) globulins and 27.915% γ-globulins were found. The observed values were compared with the earlier studies.

Key Words: Fractionation, Serum, Sheep, Albumin, Globulin.

INTRODUCTION

Protein is one of the most important constituents of the living cells and is abundantly present in all the feeding material available for the nutrition of mammals or other living organisms.

In animals serum protein has an important role in the normal functioning, such as the transport of the organic or the inorganic constituents from one part of the body to another part, maintenance of the normal osmotic pressure differences, antigen or antibody reactions, etc. By contributing to the viscosity of animal plasma the serum proteins provide resistance to the blood flow in the animal system which is essential for an efficient functioning of the heart. The serum proteins are also the source of nutrition for the tissues of the animal body.

In order to study the role of different proteins of serum, methods were developed for the fractionation of the proteins. In the beginning the precipitation of the animal plasma proteins was carried out by the salting out method. The fraction salted out by half saturated ammonium sulphate solution was termed as "globulin" fraction and the fraction obtained by a perfect saturated solution of ammonium sulphate was called the "albumin" fraction. Howe[1] separated the blood serum proteins by using a 21.5% solution of sodium sulphate while the estimation of the separated fractions was done by the Micro-Kjeldahl method. Cohn *et al.*[2] developed another method for the large scale separation of the animal blood plasma by using ethanol at a definite pH, at constant ionic strength using a thermostat maintained at a low temperature. Tiselius[3] developed the electrophoresis technique, while Koing[4] introduced the paper electrophoresis technique by using filter paper as supporting medium for electrophoresis. The paper electrophoresis technique proved to be simple, convenient, reliable and useful in the fractionation of protein mixtures in the biological fluids. This technique was further modified by Flyan and Demayo[5], Kunkel and Tiselius[6], Levin and Oberholzer[7], Boguth[8], Crook *et al.*[9], Jenecks *et al.*[10], Laurell and Laurell[11], Neely and Neill[12], William *et al.*[13], Dunlop and Dickson[14], Campbell[15], Decker *et al.*[16], Ressler and Jacobson[17], Latner and Park[18], Gotz and Bologh[19], Irfan[20], Contreras[21], Mehrotra and Singh[22], etc. The present work is an attempt to study the fractionation of serum protein in the Indian sheep.

EXPERIMENTAL

Animals: Bikanai lambs and adult sheep were maintained at the Department of Physiology, U.P. College of Veterinary Science and Animal Husbandry, Mathura. The animals selected from the flock at random were in the following two age groups:

(i) 1–6 months; (ii) 6 months–1 year.

All the animals maintained under usual farm conditions were healthy.

Blood Samples: Blood samples taken directly from the jugular veins of the animals were collected in sterile 15 mL centrifuge tubes containing 1 mg EDTA for each mL of blood. The samples were centrifuged immediately at 2000 r.p.m. for 15 min. The serum thus separated was kept in refrigerator till further analysis was done.

Preparation of serum

The serum sample was diluted to one-fifth concentration using a buffer of veronal acetate of 8.6 pH and 0.05 ionic strength.

Buffer

2 Litres of veronal acetate buffer of 8.6 pH with 0.05 ionic strength was prepared by using the following constituents:

(i) Sodium diethyl barbiturate 14.7 g
(ii) Sodium acetate 9.7 g
(iii) 0.1 N HCl 0.09 litre
(iv) Distilled water 1.91 litre

Fractionation of the serum proteins

Eight to ten filter paper strips (Whatman No. 3) of 4 cm × 33 cm size were soaked in the buffer solution taken in the glass troughs. The excess fluid was removed by pressing the strips between the folds of laboratory filter paper. The strips were then laid longitudinally over a carrier frame. The strips were set parallel and ceramic chains were laid over to hold the strips in position. The carrier frame was placed in the electrophoresis cabinet in such a way that the free ends of the strips dipped on either side in the buffer solution. The cabinet was closed with its cover. Direct current regulated at 200 V through a voltage stabilizer was passed through the solution for exactly 1 h. After switching off the current 0.02 mL of the diluted serum sample was applied on each of the strips through the window with the help of a 0.1 mL micropipette. Current was allowed to pass through the solution again after closing the window. The voltmeter was adjusted to 90 V and the current strength of 1 milliampere was adjusted through each strip. Electrophoresis at this voltage and current strength was allowed to proceed for a period of 16 h and the switch was turned off. The carrier frame together with strips in position was then transferred into a hot air oven maintained at temperature between 110–120°C and was allowed to accomplish sufficient denaturation of the proteins for 30 min. The carrier frame was removed and was allowed to cool. The ceramic chains were removed and the strips were ready for staining.

Staining

The staining of the strips was done by the procedure developed by Block et al.[23] The dried strips after electrophoresis were dipped in the staining fluid and were kept in it for 16 h. Thereafter the staining fluid was poured off from the bathing trough and the strips were rinsed according to the following procedure described by Jenecks *et al.*[10]

The strips were removed from the electrophoresis cabinet and were dried for 30 min in an oven maintained at (115 ± 1)°C. The strips were cooled and were then transferred to a staining trough in stain bath for 16 h. The strips were then rinsed successively through the three solutions in the following order:

(i) 2% CH_3COOH for 5 min

(ii) 2% CH_3COONa for 10 min

(iii) 10% CH_3COOH + 2% CH_3COONa for 2 min.

The strips were then pressed between the folds of the laboratory filter paper, to remove excess fluid. Thereafter the strips were kept in an oven maintained at (120 ± 1)°C, for 10 min and were taken out for recording of pherograms by electrophoretic scanning.

Recording of pherograms and calculation of relative concentration

The relative concentrations of different protein components obtained as stained bands on paper strips were determined by obtaining pherograms of the coloured strips on suitable paper with the help of an extinction recorder and integrator Model ERL-10 of "Carl Zeiss Jena". The relative concentrations were determined by drawing the distribution curves.

RESULTS AND DISCUSSION

The results of the blood serum protein fractionation by electrophoresis have been shown in Table-1.

TABLE-1
AVERAGE VALUES OF SERUM PROTEIN FRACTIONS IN THE INDIAN SHEEP

Sl.No.	Age	Albumin (%)	(α + β) globulins (%)	γ globulin (%)
1.	Below 6 months	43.07	28.87	28.06
2.	Above 6 months	43.52	28.71	27.77
	Average	43.295	28.79	27.915

The results obtained in the present study can be compared (Table-2) with the studies done earlier by Campbell[15] and N. Ek[24].

TABLE-2
COMPARISON OF THE SERUM PROTEIN FRACTIONS IN SHEEP

Sl.No.	Studies done by	Albumin (%)	(α + β) globulin (%)	γ-globulin (%)
1.	Campbell	52.00	24.00	24.00
2.	N. Ek	48.50	28.70	22.80
3.	Present study	43.295	28.79	27.915

The difference in the values is perhaps due to the particular nutritional or physiological status of the animals and due to the difference in the animals' age groups. The (albumin : globulin) ratio calculated was in agreement with the values reported for buffalo-calves.[25]

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