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SERUM PROTEIN ELECTROPHORESIS AS A VALUABLE FORENSIC TOOL FOR ANIMAL SPECIES IDENTIFICATION

(With 4 Tables and 4 Figures)

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التحليل الكهربي لبروتين مصل الدم كوسيلة تشخيصية للتفريق بين الفصائل الحيوانية المختلفة في مجال الطب الشرعي

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في هذه الدراسة تم فحص ١٦ فصيلة حيوانية لمعرفة المكونات المختلفة لبروتين مصل الدم (بري_ألبيومين وألبيومين وجلوبيولين ومكونات الجلوبيولين). تم تجميع مصل الدم من الفصائل الحيوانية المختلفة السليمة إكلينيكيا (الأبقار والجاموس والجمال والأغنام والماعز والأرانب والخيول والحمير والبغال والكتاكيت والرومي والحمام والكلاب والقطط والجرذان والفئران البيضاء). تم حساب القيمة النسبية والحقيقية لمكونات بروتين مصل الدم وكذلك حساب نسبة الألبيومين/الجلوبيولين. وقد أسفرت النتائج أن بروتين مصل الدم قد انفصل بواسطة التحليل الكهربي باستخدام أسيتات السليلوز إلي مكونات أساسية علي النحو الآتي:

١ــ أربع مكونات (ألبيومين وألفا وبيتا وجاما جلوبيولين) في الأبقار والجاموس .
٢ــ ست مكونات (ألبيومين وألفا١ والفا٢وبيتا١وبيتا٢ وجاما جلوبيولين) في الجمال والأرانب والخيول والحمير والبغال.
٣ــ سبع مكونات (ألبيومين وألفا١ والفا٢وبيتا١وبيتا٢ وجاما١و جاما٢ جلوبيولين) في الأغنام والقطط والكلاب.
٤ــ خمس مكونات (ألبيومين وألفا وبيتا١وبيتا٢ وجاما جلوبيولين) في الماعز.
٥ــ سبع مكونات (بري-ألبيومين وألبيومين وألفا١ والفا٢وبيتا١وبيتا٢ وجاما جلوبيولين) في الكتاكيت والرومي والحمام.
٦ــ خمس مكونات (ألبيومين وألفا١ وألفا٢ وبيتا وجاما جلوبيولين) في الجرذان والفئران البيضاء.

وقد أوضحت نسبة الألبيومين إلي الجلوبيولين وجود فرق بين هذه الفصائل الحيوانية المختلفة.

ومما سبق ذكره من مكونات بروتين مصل الدم المختلفة في هذه الفصائل التي تم دراستها أنه يمكن استخدام اختلاف هذه المكونات وكذلك اختلاف نسبة الألبيومين إلى الجلوبيولين كوسيلة تشخيصية للتفريق بين هذه الفصائل الحيوانية وخاصة في مجال الطب الشرعي.

SUMMARY

Sixteen animal species were investigated to define the reference values of different serum protein constituents (pre-albumin, albumin, globulin and globulin fractions). Serum samples were obtained from clinically healthy animal species (cows, buffaloes, camels, sheep, goats, rabbits, horses, donkeys, mules, chicken, turkeys, pigeons, dogs, cats, albino mice and albino rats), 5 samples from each species. The relative (%) and absolute values of the electrophoretic fractions of serum proteins were determined using cellulose acetate electrophoresis technique. Albumin/globulin ratio was also calculated. The obtained results revealed that protein fractions were clearly resolved into: (1) four fractions (albumin, α-, β- and γ-globulin) in cows and buffaloes. (2) Six fractions (albumin, α_1-, α_2-, β_1-, β_2- and γ-globulin) in camels, rabbits, horses, donkeys and mules. (3) Seven fractions (albumin, α_1-, α_2-, β_1-, β_2-, γ_1- and γ_2-globulin) in sheep, dogs and cats. (4) Five fractions (albumin, α-, β_1-, β_2- and γ-globulin) in goats. (5) Seven fractions (pre-albumin, albumin, α_1-, α_2-, β_1-, β_2- and γ- globulin) in chicken, turkeys and pigeons. (6) Five fractions (albumin, α_1-, α_2-, β- and γ-globulin) in albino mice and rats. The albumin/globulin ratio of the investigated animal species indicated difference between each others. The reference values of the protein fractions which defined herein as well as albumin/globulin ratio could be used as a diagnostic tool for animal species identification in the field of forensic medicine.

Key words: Forensic medicine - animal species identification - cellulose acetate electrophoresis.

INTRODUCTION

The electrophoretic technique is the current standard of reference for the fractionation of the serum proteins in clinical biochemistry. The marked advances in technology of the past decade have made this previously elaborate technique into a widely used, routine clinical biochemical test procedure. Its current widespread use is commensurate with its reflection of a variety of changes in serum protein patterns in

disease. Although only a few changes in pattern can be considered as diagnostic of specific disease, the results of electrophoresis, properly interpreted, can be one of the most useful diagnostic aids (Kaneko, 1997).

There are well over 200 plasma proteins described and quantitated mainly in diseased man and many of which change only subtly or not at all. The proteins of an individual or of a species are synthesized under genetic control, it would be expected that variations in proteins would occur between individuals and between species. These variations are reflected in the species differences of the normal serum protein electrophoresis (SPE) patterns. Thus, in ruminants such as the cow, the normal SPE pattern exhibits an albumin, one α, one β and one γ fraction of globulin (Kaneko, 1997).

Routine serum protein electrophoresis is recognized as the most reliable assessment of animal protein profiles in health and disease and has replaced biochemical determination of albumin and A/G ratio in the ability to predict abnormalities of clinical significance. The importance of considerable species differences to overall interpretation of animal electrophoresis is well established and constitutes a continued challenge to the veterinarians and to the providing laboratories to continue the pursuit of species-specific, even age- and gender-specific, reference ranges. Patterns of various diseases continue to emerge as more scrutiny is applied to use this tool in animal diagnostics for overall health assessment as an adjunct to specific disease diagnosis and for both prognostic and therapeutic monitoring. Electrophoresis has applications in biology, biochemical research, protein chemistry, pharmacology, forensic medicine, clinical investigations, veterinary science and food quality control.

Protein electrophoresis can be performed on either serum or plasma. Serum is generally preferred because the fibrinogen in plasma will often obscure the electrophoretogram in the β-γ region (Tietz, 1987). With electrophoresis, proteins are separated based on their migration rate in an electrical field. The rate of migration depends on the charge and the size of protein, the strength of the electrical field, and the medium through which the proteins are migrating (Kaneko, 1997). The rate of proteins migration will be affected by the pH and the ionic strength as well as composition of the buffer system used (Tietz, 1987; Kaneko, 1997). The standard buffer is barbital pH 8.6 at which the majority of plasma proteins will carry a negative charge (Kaneko, 1997). The

medium of choice for routine diagnostic purpose is either agarose (Baker and Valli, 1988; Matthews, 1982) or cellulose acetate (Kaneko *et al.*, 1997) because each is relatively simple to use and provides accurate results. Other media that can be used include starch or polyacrylamide gel.

Serum albumin, SA is the major plasma protein, fulfilling several physiological functions: as transport molecule (for fatty acids, bilirubin, hormones, drugs, ions), in colloid osmotic regulation, and as easily accessible protein reserve (Andersson, 1979). In humans, nutrition, stress, altered hormonal balance and liver diseases are known to affect SA levels, causing major disorders (Birke *et al.*, 1979). General investigations on animal serum albumin are rare, and protein or sequence data are available only for a few species (Doolittle, 1984; Brown *et al.*, 1989; Sargent et al ., 1981).

Species identification by electrophoresis is routinely used in the food industry in order to prove adulterations, especially of meat (Bauer and Hofmann. 1987), fish (Rehbein, 1992) or dairy products (Mayer and Hoertner , 1992). In forensic sciences, unknown blood samples are often (Divall, 1985; Righetti *et al.*, 1991), but not necessarily identified by electrophoretic methods. Protocols based on protein reactivity with specific antibodies (Benjamin *et al.*, 1987) or DNA analysis (Gill, 1989) are preferred for special human applications (e.g. distinguish between individuals).

The principal aim of this study was to establish general protein patterns of serum samples of different healthy animal species and to compare between them as a useful tool for species differentiation using cellulose acetate electrophoretic technique.

MATERIALS and METHODS

Sixteen animal species were investigated in this study. Blood samples were obtained by jugular puncture and were allowed to clot at room temperature. Serum samples were obtained from clinically healthy individuals of different animal species (5 samples from each species) as following: Cows (balady), buffaloes, camels, sheep, goats, rabbits, horses, donkeys, mules, chicken (meat producing chicken), turkeys, pigeons, dogs, cats, albino rats, and albino mice. Serum samples harvested and stored at -4°C until analyzed by cellulose acetate

electrophoresis. All hemolyzed or lipemic samples were discarded and another samples were collected.

(A) Determination of total serum protein:

Total protein was determined by the Biuret method as described by Kachmar (1970).

(B) Electrophoretic patterns of serum protein:

Serum proteins were electrophorsed according to the procedure of Helena Laboratories Publications (Kohn, 1958). Titan III Cellulose Acetate Plates, Electra HR buffer, and Ponceau S stain were used. The electrophoretic patterns of serum proteins were scanned and graphed by Auto-Scanner Flur-Vis to reveal the densitometer tracings.

RESULTS

The obtained results were summarized in Figures 1 - 4 and Tables 1 - 4.

DISCUSSION

Hematochemistry constitutes an increasingly useful aid in zootechnical and veterinary research. It permits the study of specific pathological alterations of certain blood constituents and recognition, under strictly controlled experimental conditions, of the existence of metabolic alterations of different origin. Many factors can influence the level of a particular blood constituents: genetic type, feeding, macro- and micro-environment, rearing technique, age, physiological state and sex as well as pathological factors. Moreover, methods of sampling and obtaining the biological material and the method of analysis can also influence the results.

Proteins in the blood of mammals contain fractions which have been identified as albumin, alpha, beta and gamma globulin. Each main fraction consists of subfractions which exhibit individual and genetically determined variation (Andersen, 1984). It has been reported that different species of animals have characteristic protein profiles (Phillis, 1976). Albumin/globulin ratio was reported to be varied according to the species of animal (Kaneko *et al.*, 1997).

Serum contains a variety of small molecules, as well as hundreds of different serum proteins. Serum proteins are frequently separated and characterized by electrophoresis in the clinical laboratory to determine

the concentration of various proteins and to detect abnormal protein species.

Serum albumin is the major plasma protein in quite a number of species and thought to be a protein undergoing rather rapid evolutionary changes (Doolittle, 1984). All animals investigated in this study were mammals, except the chicken, pigeon and turkeys. Most mammalian serum albumin had rather similar electrophoretic behavior.

Figures 1 and 3 show that cow's and buffalo's serum resolved into albumin and single alpha (α), beta (β) and gamma (γ) globulin zones. The obtained results in this study for electrophoretogram of serum protein of cattle and buffaloes are in agreement with that recorded by Schalm *et al.* (1975); Yoshida (1986,1991) and Kaneko et al .(1997).

In the present study (Table 1), the absolute concentration (g/l) of total serum protein, albumin and globulin were 70.93, 32.58 and 38.35 for cows, and 73.64, 36.63 and 37.01 for buffaloes. The relative concentrations (%) of globulin fractions (Table 2) (α, β and γ) were 14.93, 17.72 and 21.42 for cows, and 12.76, 15.95 and 21.54 for buffaloes. Albumin/globulin ratio (A/G ratio) was 0.85 and 0.99 in cows and buffaloes respectively. On the absolute basis (g/l) these globulin fractions were α (10.59), β (12.57) and γ (15.19) for cows, and α (9.4), β (11.75) and γ (15.85) for buffaloes.

Today, the fractionation of serum proteins by means of zone electrophoresis with cellulose acetate membrane is performed as a routine work for clinical diagnosis. This electrophoresis is highly efficient in separating the serum proteins in a short time and dealing with many samples at ones. Electrophoresis with cellulose acetate membrane make it possible to separate the serum proteins in animals. Excellent electrophorograms produced by it have been presented for dogs (Fukuda and Shibanai, 1971) and horses (Berrier, 1969). The serum proteins of cattle, however, have not clearly fractionated. Especially, α-globulin has not been separated into two fractions, α_1 and α_2 as yet. Furthermore, the diagnostic significance of changes in α_1 and α_2 has been clarified in human medicine (Hirayama *et al.*, 1964). Therefore, it considerably significant for a clinical diagnosis in cattle to divide α-globulin into α_1- and α_2-gl obulin (Kawamura *et al.*, 1974).

Serum proteins of cattle has hardly been observed that α-globulin was divided into two fractions, α_1- and α_2-globulin, and that β- and γ-globulin were separated clearly from each other. In dogs (Fukuda and Shibanai, 1971) and horses (Mattheeuws *et al.*, 1966), the serum proteins

have been divided distinctly into 5 or 6 fractions, such as albumin, α_1-, α_2-, β_1-, β_2- and γ-globulin. It is generally assumed that the low separation of the serum proteins in cattle and buffaloes may be attributed to the high viscosity and the small difference in isoelectric point among the protein fractions of serum. By vertical electrophoresis with polyacrylamide gel, however, the α- globulin fractions of cattle are divided into two parts (Schmidt, 1968). Ek (1969) reported that use of a modified buffer solution containing 0.38 g of calcium acetate in 1.0 L of barbital buffer solution and by use calcium lactate, 3 gm (Kawamura *et al.*, 1974), it was clearly possible to separate β-globulin from γ-globulin, but not to divide α- globulin into two fractions. From the results obtained by Kawamura *et al.* (1974), it is presumed that even α-globulin of bovine serum may be separated into α_1- and α_2-globulin by using modified buffer barbital solution containing 1 gm and 2 gm calcium lactate.

Kawamura (1974) reported that the total serum protein was 75 g/l and the relative values (%) of its fractions in the cattle by using cellulose acetate electrophoresis technique were: albumin (44.9), α_1-globulin (6.8), α_2-globulin (11.3), β-globulin (10.5), γ-globulin (24.9), and A/G ratio was 0.86. Yoshida (1986) reported that the total protein was 72.7 g/l and the absolute concentration (g/l) of serum protein fractions of the cattle by using cellulose acetate electrophoresis technique were: albumin (35.8), α-globulin (9.9), β-globulin (6.3), γ-globulin (20.5), and A/G ratio was 0.97. Yoshida (1991) reported the total protein was 72.3 g/l and the absolute concentration (g/l) of serum protein fractions in cattle by using cellulose acetate electrophoresis technique were: albumin (35.6), α- globulin (9.9), β- globulin (6.4), γ- globulin (20.3), and A/G ratio was 0.97. Kaneko *et al.* (1997) reported that the total protein was 71 g/l and the absolute concentration (g/l) of serum protein fractions in cattle by using cellulose acetate electrophoresis technique were: albumin (32.9), total globulin (38.1), and A/G ratio was 0.86. Shrikhand and Sarode (1999) found that total serum protein of cows was 72.5 g/l, albumin was 33.7 g/l and globulin was 38.3 g/l and A/G ratio was 0.87.

Inspite of the several studies performed on the blood constituents of the dromedary (one-humped), little information is available concerning the electrophoretic pattern of the serum proteins. The results obtained in this study (Table 1) showed that the absolute concentration (g/l) of total serum protein, albumin and globulin were 69.18, 33.64 and 35.54 in camels respectively. The relative values (%) (Table 2) of

globulin fractions (α_1, α_2, β_1, β_2 and γ) were 5.31, 7.53, 6.69, 7.82 and 24.03 respectively. A/G ratio was 0.95. Previous works on camel serum showed that it resolved into single alpha, beta and gamma globulin zones (Boid *et al.*, 1980 and Abdo *et al.*, 1987) which are differ from the results obtained in the present study (where it resolved into α_1, α_2, β_1, β_2 and γ zones). Abdo *et al.* (1987) reported that the electrophoretic pattern of serum protein and its fractions in camel by using cellulose acetate strips electrophoresis technique showed that: total protein (58.2 g/l), albumin (62%), α-globulin. (2%), β-globulin (15.4%), γ-globulin (20%), and A/G ratio was 1.38. The obtained results in this study for dromedary (one-humped) are in- agreement with those reported for other species. In this study, the electrophoretic patterns of all investigated serum proteins for ovine, bovine, feline and poultry showed that globulin predominates over albumin. In horses, cows, dogs, cats, rabbit, the relative proportion of albumin to globulin are nearly equal (Wesibroth *et al.*, 1974; Swenson, 1984 and Kaneko *et al.*, 1997). The results therefore indicate species differences concerning the proportion of albumin and globulin. It had been also reported that not only the difference in the species of animals can affect the concentration and distribution of blood proteins but also the difference in the breed of one species have a relevant effect as reported in cattle (Balmacida and Bottari (1974), turkeys (Al-Heeti *et al.*, 1985), and chickens (Ross *et al.*, 1978).

In the present study (Table 1), the absolute concentrations (g/l) of total serum protein, albumin and globulin were 71.25, 30.51 and 40.74 for sheep, and 64.24, 30.41 and 33.83 for goats. The relative concentrations (%) (Table 2) of globulin fractions (α_1, α_2, β_1, β_2, γ_1 and γ_2) were 7.11, 8.89, 10.25, 11.13, 14.72 and 5.08 respectively in sheep. In goats, globulin fractions (α, β_1, β_2 and γ) were 9.48, 11.96, 8.56 and 22.67 respectively. A/G ratio was 0.75 and 0.90 in sheep and goat respectively. On the absolute basis (g/l) these globulin fractions in sheep were α_1 (5.06), α_2 (6.34), β_1 (7.30), β_2 (7.94) , γ_1 (10.48) and γ_2 (3.62). Our obtained results are in-agreement with that observed by Dobson (1966), Fox *et al.* (1970) and Kaneko *et al.* (1997). Fox *et al.* (1970) found that sheep total protein was 72 g/l and absolute concentration of its fractions were (g/l): albumin (38.1), α_1-globulin (3.8), α_2-globulin (7.3), β-globulin (9.1), γ-globulin (13.7), and A/G ratio was 1.12, while Kaneko *et al.* (1997) found that total serum protein was 72 g/l and the absolute concentration of its fractions in sheep by using cellulose acetate electrophoresis were (g/l): albumin (27), globulin total (44), α-globulin

(5), β_1-globulin (10), β_2-globulin (7), γ_1-globulin (16), γ_2-globulin (8), and A/G ratio was 0.61. Turner and Wilson (1962) discussed the difficulties of comparing results of electrophoretic examination by different researchers. In most studies, paper electrophoresis was used; it defines fewer protein bands than does electrophoresis with cellulose acetate (Knight and Leek, 1973). By cellulose acetate electrophoresis, 6 protein fractions were separated in serum of lambs (Dobson, 1966).

For rabbits, the reported results in the present study (Table 1) revealed that the absolute concentrations (g/l) of total serum protein, albumin and globulin were 61.73, 28.56 and 33.17 respectively. In Table 2, the relative values (%) of different globulin fractions were α_1 (6.90), α_2 (5.13), β_1 (8.75), β_2 (6.08) and γ (26.87) respectively. A/G ratio was 0.86. On the absolute basis (g/l) these globulin fractions (α_1, α_2, β_1, β_2 and γ) were 4.62, 3.17, 5.40, 3.75 and 16.59 respectively. In comparison with the results obtained by Wesibroth *et al.* (1974), our results showed some decrease concentrations (g/l) of total protein, albumin and globulin (68, 32.2 and 35.8 respectively). The globulin fractions concentrations (g/l) were α_1 (4.9), α_2 (3.2), β (12.4) and γ (15.8), while A/G ratio was 0.90.

In the present study (Table 1and 3), the absolute concentrations (g/l) of total serum protein, albumin and globulin were 69.75, 31.96 and 37.79 for horse, 52.93, 25.62 and 27.31 for donkey, and 56.19, 26.23 and 29.96 for mules. The relative concentrations (%) of globulin fractions (α_1, α_2, β_1, β_2 and γ) were 3.96, 9.99, 13.85, 8.59 and 17.79 for horse, 2.42, 10.17, 11.60, 8.50 and 18.91 for donkey, and 3.59, 10. 45, 14.40, 6.25 and 18.63 for mules. A/G ratios were 0.85, 0.94 and 0.88 in horses, donkeys and mules respectively. On the absolute basis (g/l) these globulin fractions (α_1, α_2, β_1, β_2 and γ) in horse were 2.76, 6.97, 9.66, 5.99 and 12.41 respectively.

On the percentage basis (%), normal values for albumin and globulin fractions in horses varied as following according to Coffman (1968): albumin (43-47.3), α_1-globulin (5.34-14), α_2-globulin (9.9-18.41), β-globulin (9-22.6) and γ-globulin (14.28-19). On the absolute basis (g/L), normal values for albumin and globulin fractions varied as following according to Pierce (1975): albumin (22.7-31.4), α_1-globulin (1.9-7.3), α_2-globulin (6.5-10.9), β-globulin (12.8-20.9) and γ-globulin (8.2-17.6). Kaneko *et al.* (1997) reported that the total protein was 63.5 g/l and the absolute concentration and the range (g/l) of serum protein and its fractions in horse by using cellulose acetate electrophoresis

technique were: albumin (30.9), total globulin (33.3), α_1-globulin (1.9), α_2-globulin (6.5), β_1-globulin (9.2), β_2-globulin (5.7), γ-globulin (10), and A/G ratio was 0.91.

In the present study (Table 1and 3), the results revealed that total serum protein in chicken was 53.2 g/l and the concentrations of protein fractions were (g/l): pre-albumin (5.41), albumin (21.28), total globulin (26.51) and A/G ratio was 0.80. On the relative basis (%), the values were: pre-albumin (10.16), albumin (40.01), total globulin (49.83), α_1 (6.89), α_2 (8.22), β_1(6.43), β_2(9.19), and γ (19.10). The results of the present study are in-agreement with that reported by Fukata *et al.* (1997), Kaneko *et al.* (1997), and dis-agreement with Sells (1976) who used the same technique of electrophoresis.

Sells (1976) reported that the concentration of total serum protein was 31 g/l and the relative value (%) of its fractions in chickens by using cellulose acetate electrophoresis technique were: albumin (53.1), α-globulin (4.6), β-globulin (13.1), γ-globulin (29.2), and A/G ratio was 1.14. Fukata *et al.* (1997) found that total serum protein was 43.5 g/l with a range of 42.4 to 44.6 g/l, while albumin was 18.2 with a range of 17.4 to 19 g/l, total globulin was 25.3 g/l and A/G ratio was 0.72. Kaneko *et al.* (1997) found that total protein was 56 g/l, albumin was 25 g/l, globulin was 31 g/l and A/G ratio was 0.81.

For turkey the concentrations (g/l) of total serum protein, albumin and globulin (Table 1) were 51.73, 22.57 and 25.01. In the Table 3, the relative concentration (%) of different globulin fractions (α_1, α_2, β_1, β_2 and γ) were 7.12, 9.03, 8.27, 10.21 and 13.72 respectively. A/G ratio was 0.90. On the absolute basis (g/l) these globulin fractions (α_1, α_2, β_1, β_2 and γ) were 3.68, 4.67, 4.28, 5.28 and 7.10 respectively. By using cellulose acetate paper strips electrophoresis, Al-Heeti *et al.* (1985) found that total serum protein in turkeys was 62.6 g/l , and protein fractions were (g/l): albumin (32.3), globulin (30.1), α_1 (4.1), α_2 (8.2), β (9), γ (8.8), and A/G ratio was 1.06. Our results showed some depletion in total protein which reflected by decrease in both albumin and globulin concentrations when compared with the results of Al-Heeti *et al.* (1985). On the other hand pre-albumin zone is not detected by him.

The recorded results in the present study for pigeons (Table 1) showed that total serum protein was 48.35 g/l and the concentrations of its fractions were (g/l): pre-albumin (8.2), albumin (16.48) and total globulin (23.67). On the relative basis (%) in Table 3, the value of different globulin fractions (α_1, α_2, β_1, β_2 and γ) were 5.83, 6.52, 7.10,

12.14 and 17.37 respectively. A/G ratio was 0.70. On the absolute basis (g/l) these globulin fractions (α_1, α_2, β_1, β_2 and γ) were 2.82, 3.15, 3.43, 5.87 and 8.4 respectively. These results were higher than that observed by Lumei and de Bruijne (1985) and Kaneko *et al.* (1997). Lumei and de Bruijne (1985) reported that the total protein (27), and the absolute concentration (g/L) of serum protein fractions of the normal pigeons by using cellulose acetate membrane electrophoresis technique as: pre-albumin (2.8), albumin (17.5), α-globulin (2.29), β-globulin (4.3), γ-globulin (1.9), and A/G ratio was 1.84. And, Kaneko *et al.* (1997) which reported that the total protein concentration was 21 g/l and A/G ratio was from 1.5.

The results obtained in this study (Table 1) for electrophoretic pattern of normal dogs revealed that the concentration (g/l) of total serum protein was 56.82, albumin (26.7) and total globulin (30.12). In Table 4, the relative values (%) of different globulin fractions (α_1, α_2, β_1, β_2, γ_1 and γ_2) were 4.51, 7.97, 6.46, 11.12, 13.27 and 9.68 respectively. A/G ratio was 0.87. The obtained results in the present study are in agreement with that recorded by Pickrell *et al.* (1974) and Kaneko *et al.* (1997). Pickrell *et al.* (1974) found that the relative value of serum protein fractions as: albumin (57.1), α_1 (8.4), α_2 (9.0), β_1 (7.5), β_2 (9.0), γ (9.3), and A/G ratio was graduated from 0.9 to 1.9. Kaneko *et al.* (1997) reported that the concentration (g/L) of serum protein and its fractions of the normal dogs by using cellulose acetate electrophoresis technique were: total protein (61), albumin (29.1), globulin (31.9), and A/G ratio was 0.91.

Serum electrophoresis is being used with increasing frequency in veterinary medicine as an aid patient evaluation. The ability to define protein fractions in greater detail than the division into albumin and globulin adds further description to many disease processes. Feline serum has been evaluated previously by agar gel , cellulose acetate, and paper electrophoretic techniques. Agar gel electrophoresis has been reported to result in a good resolution of 6 to 7 major bands, as well as definition of 1 to 3 minor bands (Gotz & Balogh , 1967) . However, these reports did not name or define these fractions. Both paper and cellulose acetate electrophoresis have resulted in 6 major bands in some reports (Causse- Vaills *et al.*, 1961; Osbaldiston , 1972). Normal concentrations of serum protein fractions vary somewhat according to electrophoretic technique and the samples.

The present study (Table 1) revealed that the concentration (g/l) of total serum protein in examined cats was 62.36, albumin (25.41) and total globulin (36.95). In Table 4, the relative values (%) of different globulin fractions (α_1, α_2, β_1, β_2, γ_1 and γ_2) were 6.31, 7.46, 8.60, 10.61, 11.47 and 14.80 respectively. A/G ratio was 0.69. On the absolute basis (g/l) these globulin fractions (α_1, α_2, β_1, β_2, γ_1 and γ) were 3.94, 4.65, 5.26, 6.62, 7.15 and 9.23 respectively. The present results are nearly similar to that previously reported for normal cats (Osbaldiston, 1972; Barsanti & Hubbell, 1980; Kaneko *et al.*, 1997). Osbaldiston (1972) reported that the absolute concentration (g/L) of serum protein and its fractions of the normal cats by using cellulose acetate electrophoresis technique were: total protein (69), albumin (30), α_1-globulin (6), α_2-globulin (8), β_1-globulin (4), β_2-globulin (4) and γ-globulin (16.5). Barsanti and Hubbell (1980) reported that the relative and absolute concentration of serum protein and its fractions of the normal cats by using cellulose acetate electrophoresis technique were: total protein (72.1 g/L), albumin (41.3 %), α_1-globulin (2.9 %), α_2-globulin (16.3 %), β_1-globulin (4.9 %), β_2-globulin (6.8 %) and γ-globulin (27.6 %). Kaneko *et al.* (1997) found that total serum protein was 66 g/l, albumin 27 g/l, globulin 39 g/l, and A/G ratio was 0.71. Our results in this study are in agreement with Kaneko *et al.* (1997) who recorded that globulin was resoluted into six zones as α_1, α_2, β_1, β_2, γ_1 and γ_2 as we found.

The absolute concentrations (g/l) of total serum protein, albumin and globulin of albino mice (Table 1) were 64.31, 26.72 and 37.59 respectively. The relative value (%) in Table 4 for globulin fractions (α_1, α_2, β and γ) were 10.45, 15.60, 18.58 and 13.82. On the absolute basis (g/l) these globulin fractions (α_1, α_2, β and γ) were 6.72, 10.03, 11.95 and 8.89. A/G ratio was 0.71. These results showed some differences than that reported by Finch and Foster (1973), and Kaneko *et al.* (1997). Finch and Foster (1973) who recorded that total serum protein was 62 g/l, and that for protein fractions were (g/l): albumin (31.4), α_1 (5.3), α_2 (9.1), β (10.4), and γ (6.1), while A/G ratio was 1.02, and Kaneko *et al.* (1997) which found that total serum protein was 62 g/l and the absolute concentration (g/L) of protein fractions of the normal albino mice by using cellulose acetate electrophoresis technique were: albumin (34), globulin (28), and A/G ratio was 1.21.

In Table 1, the concentrations (g/l) of total serum protein, albumin and globulin of albino rats were 76.23, 33.86 and 42.37. The relative

value (%) for globulin fractions (α_1, α_2, β and γ) were 14.08, 10.51, 14.39 and 16.60 respectively (Table 4). On the absolute basis (g/l) these globulin fractions (α_1, α_2, β and γ) were 10.73, 8.01, 10.97 and 12.66. A/G ratio was 0.80. The results showed some similarity when compared with the results of Nomura *et al.* (1975), Marino *et al.* (1976) and Kaneko *et al.* (1997). Nomura *et al.* (1975) found that total protein was 76.1 g/l and the protein fractions in normal albino rat were (g/l): albumin (37.3), α_1-globulin (10.3), α_2-globulin (7.1), β-globulin (10.7), γ-globulin (10.5) and A/G ratio was 0.96 with a range of 0.72 to 1.21. Marino *et al.* (1976) reported that the relative values (%) of serum protein fractions of the Sprague Dawley rat by using cellulose acetate electrophoresis as: albumin (49.1), α-globulin (27.2), β-globulin (14), γ-globulin (9.6) and A/G ratio was 0.96. Kaneko *et al.* (1997) reported that total serum protein was 75.2 g/l and its fractions of the normal albino rat by using cellulose acetate electrophoresis technique were (g/l): albumin (41.7), globulin (33.5), and A/G ratio was 1.24.

Normal values of biochemical constituents of blood and serum in animals are of academic as well as clinical importance. Extensive research revealed that these values vary from region to region. It is therefore, necessary to establish the normal levels of biochemical constituents of blood and serum in animals of particular region. The obvious difference of total serum protein, albumin, and globulin recorded in the present study, is considered a helpful index in differentiation between different animal species.

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Table 2: Electrophoretic patterns of serum proteins of healthy cows, buffaloes, camels, sheep, goats and rabbits (relative value, %) (mean ± S.E.).

Protein fraction	Cows	Buffaloes	Camels	Sheep	Goats	Rabbits
Albumin	45.93 ± 0.72	49.75 ± 0.66	48.62 ± 0.22	42.82 ± 0.42	47.33 ± 0.51	46.27 ± 0.17
Total L	54.07 ± 0.84	50.25 ± 0.67	51.38 ± 0.24	57.18 ± 0.73	52.67 ± 0.56	53.73 ± 0.19
α	14.93 ± 0.85	12.76 ± 0.17	.....	.....	9.48 ± 0.10	.....
α_1	.....	.....	5.31 ± 0.02	7.11 ± 0.09	.....	6.90 ± 0.02
α_2	.....	.....	7.53 ± 0.03	8.89 ± 0.11	.....	5.13 ± 0.01
Globulin β	17.72 ± 0.27	15.95 ± 0.21	.....	.....	.....	.....
β_1	.....	.....	6.69 ± 0.03	10.25 ± 0.13	11.96 ± 0.12	8.75 ± 0.03
β_2	.....	.....	7.82 ± 0.03	11.13 ± 0.14	8.56 ± 0.09	6.08 ± 0.02
γ	21.42 ± 0.33	21.54 ± 0.28	24.03 ± 0.11	.....	22.67 ± 0.24	26.87 ± 0.09
γ_1	.....	.....	.....	14.72 ± 0.18	.....	.....
γ_2	.....	.....	.....	5.08 ± 0.06	.....	.....
A/G ratio	0.85	0.99	0.95	0.75	0.90	0.86

Table 3: Electrophoretic patterns of serum proteins of healthy horses, donkeys, mules, chickens, turkeys and pigeons (relative value, %) (mean ± S.E.).

Protein fraction		Horses	Donkeys	Mules	Chicken	Turkeys	Pigeons
Pre-albumin		---------	---------	---------	10.16 ± 0.03	8.01 ± 0.02	16.96 ± 0.21
Albumin		45.82 ± 0.50	48.40 ± 0.34	46.68 ± 0.51	40.01 ± 0.12	43.64 ± 0.14	34.08 ± 0.43
Globulin	Total	54.18 ± 0.60	51.60 ± 0.36	53.32 ± 0.58	49.83 ± 0.15	48.35 ± 0.16	48.96 ± 0.62
	α_1	3.96 ± 0.04	2.42 ± 0.01	3.59 ± 0.03	6.89 ± 0.02	7.12 ± 0.02	5.83 ± 0.07
	α_2	9.99 ± 0.11	10.17 = 0.07	10.45 ± 0.11	8.22 ± 0.02	9.03 ± 0.03	6.52 ± 0.08
	β_1	13.85 ± 0.15	11.60 ± 0.08	14.40 ± 0.15	6.43 ± 0.02	8.27 ± 0.02	7.10 ± 0.09
	β_2	8.59 ± 0.09	8.50 ± 0.06	6.25 ± 0.06	9.19 ± 0.02	10.21 ± 0.03	12.14 ± 0.15
	γ	17.79 ± 0.19	18.91 ± 0.13	18.63 ± 0.20	19.10 ± 0.06	13.72 ± 0.04	17.37 ± 0.22
A/G ratio		0.85	0.94	0.88	0.80	0.90	0.70

Table 4: Electrophoretic patterns of serum proteins of healthy dogs, cats, mice and rats (relative value, %) (mean ± S.E.).

Protein fraction		Dogs	Cats	Mice	Rats
Albumin		46.99 ± 0.51	40.75 ± 0.73	41.55 ± 0.21	44.42 ± 0.64
Globulin	Total	53.01 ± 0.57	59.25 ± 1.07	58.45 ± 0.29	55.58 ± 0.81
	α_1	4.51 ± 0.05	6.31 ± 0.11	10.45 ± 0.05	14.08 ± 0.20
	α_2	7.97 ± 0.08	7.46 ± 0.13	15.60 ± 0.07	10.51 ± 0.15
	β	------	------	18.58 ± 0.09	14.39 ± 0.21
	β_1	6.46 ± 0.07	8.60 ± 0.15	------	------
	β_2	11.12 ± 0.11	10.61 ± 0.19	------	------
	γ	------	------	13.82 ± 0.07	16.60 ± 0.24
	γ_1	13.27 ± 0.14	11.47 ± 0.20	------	------
	γ_2	9.68 ± 0.11	14.80 ± 0.26	------	------
A/Globulin ratio		0.87	0.69	0.71	0.80

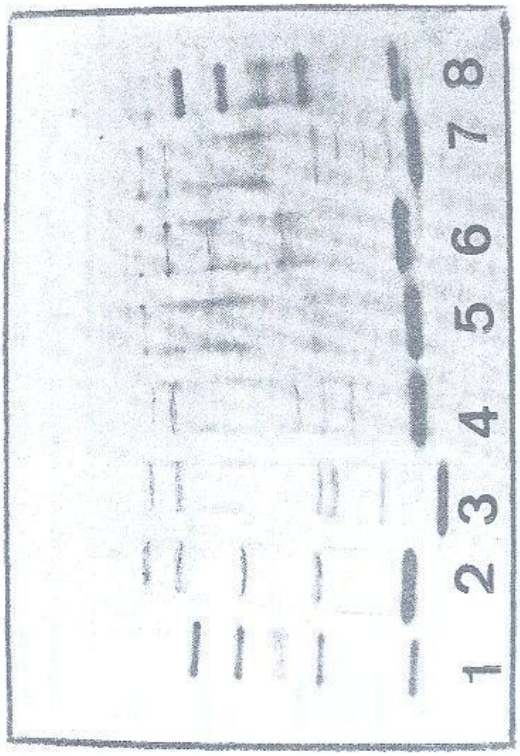

Fig. 1: Electrophoretic patterns of serum proteins of some healthy animals. 1- donkey, 2- mule, 3- cat, 4- horse, 5- cow, 6- buffalo, 7- dog, 8- camel.

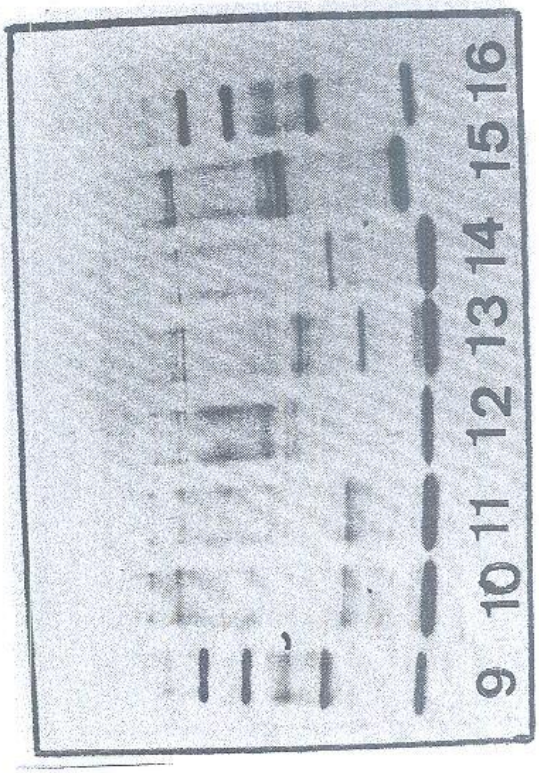

Fig. 2: Electrophoretic patterns of serum proteins of some healthy animals. 9- pigeon, 10- goat, 11- sheep, 12- chicken, 13- albino mouse, 14- rabbit, 15- albino rat, 16- turkey.

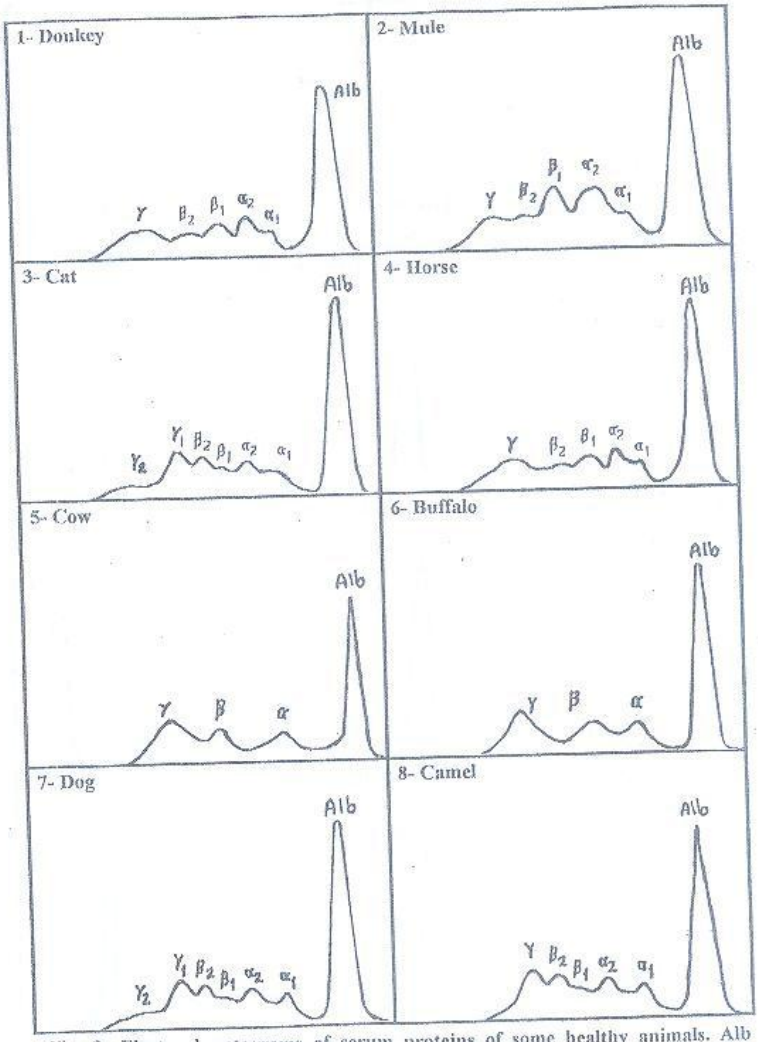

Fig. 3: Electrophoretograms of serum proteins of some healthy animals. Alb (albumin), α (alpha-globulin), α_1 (alpha $_1$-globulin), α_2 (alpha $_2$-globulin), β (beta- globulin), β_1 (beta $_1$- globulin), β_2 (beta $_2$- globulin), γ (gamma-globulin), γ_1 (gamma $_1$-globulin), γ_2 (gamma $_2$-globulin).

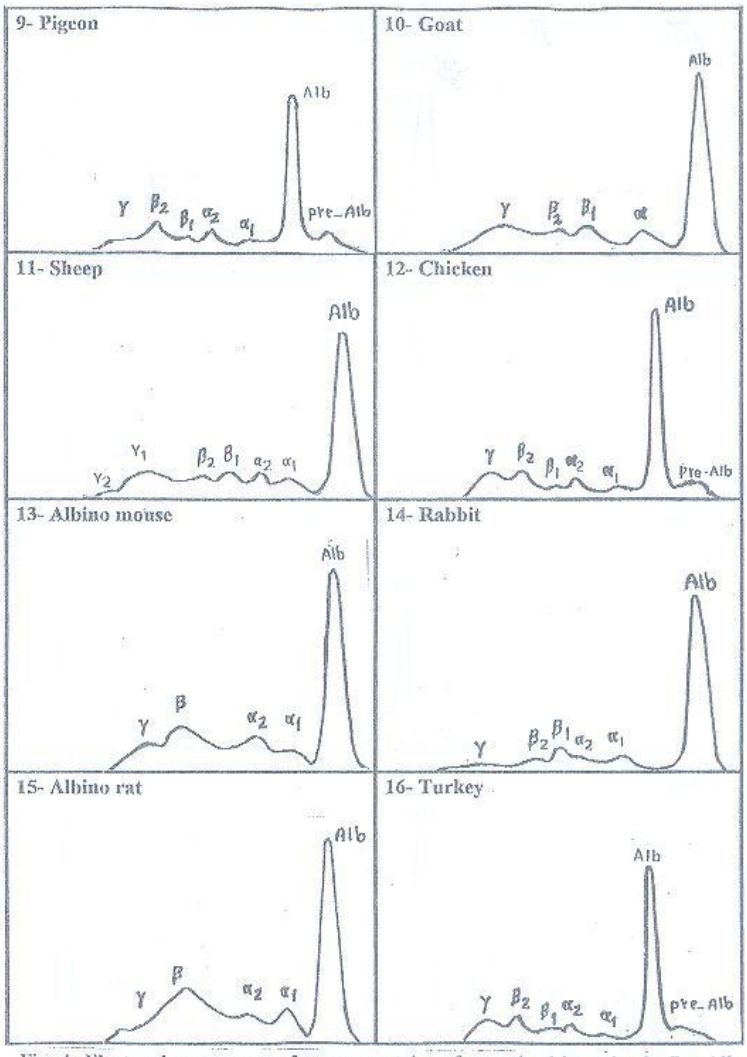

Fig. 4: Electrophoretograms of serum proteins of some healthy animals. Pre-Alb (Pre-albumin), Alb (albumin), α (alpha-globulin), α_1 (alpha $_1$-globulin), α_2 (alpha $_2$-globulin), β (beta- globulin), β_1 (beta $_1$- globulin), β_2 (beta $_2$- globulin), γ (gamma-globulin), γ_1 (gamma $_1$-globulin), γ_2 (gamma $_2$-globulin).