ELECTROPHORETIC ANALYSIS OF SERUM PROTEINS OF BIRDS AND MAMMALS

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ABSTRACT: The electrophoretic patterns of the blood serum of birds such as pigeons and chickens and mammals such as Guinea pigs, cats, dogs and rabbits were checked. Various protein glycoprotein and lipoprotein bands were separated by both native and SDS methods. Chickens showed 13 bands while pigeons showed 12 bands by the native electrophoretic method for total proteins. On the other hand, both cats and dogs showed 7 bands, while Guinea pigs and rabbits showed 10 and 13 bands respectively. Total proteins by SDS methods for birds achieved 8 and 13 bands for both pigeons and chickens respectively. The SDS method revealed 13 bands for Guinea pigs and rabbits and showed 10 and 18 bands for cats and dogs, respectively. Native glycoproteins serum showed 8 bands for both pigeons and chickens. Rabbits and Guinea pigs showed 8 bands, while cats showed 10 and dogs showed 7 bands. SDS glycoproteins were significantly different between pigeons and chickens showing 4 and 10 bands respectively. The serum of Guinea pigs showed 8 bands but that of dogs and cats showed 11 and 16 bands respectively. Prestaining lipoprotein showed 2 bands for cats, dogs and rabbits. Four bands were separated for both chickens and Guinea pigs and 5 bands for pigeons. The basic data reported here represents a fingerprint peculiar specific to each animal useful in classification and evolution studies and it may be of a great importance in forensic science.

KEY WORDS: Electrophoresis; Glycoproteins; Lipoproteins.

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INTRODUCTION

The technique of disc electrophoresis is the most valuable addition to the many variations of electrophoresis in current, routine use. It is so named because the bands or zones stack up to form a series of concentrated discs at the beginning of the procedure or because the proteins are located as a series of discs at the end of the experiment.

Determination of the molecular weights of polypeptide chains in oligomeric proteins is an important problem.

Sharpio, Vinuela and Maizel [5] reported that the separation of proteins by polyacrylamide electrophoresis in the presence of the anionic detergent sodium dodecyl

sulfate is dependent on the molecular weights of their polypeptide chains. We used both native and SDS gel electrophoresis during this study to illustrate any differentiation of their protein structure. Apparently, not only are protein-protein interaction are eliminated but protein-carbohydrate and protein-lipid interactions as well. With these factors in mind we employed acrylamide gels at a concentration of 7.5% in columns for electrophoretic separation of animal proteins. Therefore, the present study is an attempt towards at establishing comprehensive base line data on the susceptibility of some birds and mammals based on conventional biochemical methods.

MATERIAL AND METHODS

Five animals from each of the following species Guinea pigs, rabbits, dogs, cats, chickens and pigeons were kept in cages with food and water supplied ad libitum. Animals were acclimatized for one week in the laboratory. Blood was collected by heart puncture in clean centrifuge tubes and allowed to coagulate at room temperature, then centrifuged at 3000 rpm for 15 minutes to separate serum. Serum was separated in clean plastic vials and stored at 20°C. Electrophoresis techniques were carried out for native protein according to Davis[1] and SDS system according to Laemmli [3]. Total proteins were stained with comassie brilliant blue R-250 stain (Fox, 1980), glycoproteins were stained by periodic acid schiff's [6]. And lipoproteins were prestained by using acetylated sudan black according to the method modified by westfallard Culp [7].

RESULTS AND DISCUSSION

Native total proteins show 12 bands for pigeon serum with different Rf, as shown in Table I. There were at least two patterns of proteins found in the serum of chickens (Table I).

In one of them the gamma globulin, which is characterized generally by its least mobility, migrated markedly from the start of the run. In the second the globulins behaved normally and the gamma type was confined at the start of the run. In both cases the albumin pattern was more or less similar and it did not show a faster migration comparable with the fast globulin migration. The electrophoretic serum native protein pattern of the chicken under the present investigation ranged between 10 and 13 bands, of these 6–8 constituted the globulin fractions and 4 to 6 bands constituted the albumin ones. Through these two patterns were sounding differences between the comparable bands. This made it hard to compare the patterns or the individual bands of the two patterns. These variations may be genetic ones present in the breed of the animals, or they may be due to antibiotics and drug treatments usually applied to the animals during the growing period. These results were in agreement with Helal [2]. On the other hand, Prosky et al.

[4] separated chick plasma proteins into 15 fractions. However, these authors referred to changes in the serum protein after hatching.

TABLE I. Rf VALUES OF SERUM NATIVE PROTEIN BANDS OF SOME BIRDS AND MAMMALS

No. of bands	Pigeon	Chicken	Rabbit	Guinea pig	Cat	Dog
1	0	0.04	0	0	0	0
2	0.08	0.08	0.02	0.08	0.65	0.02
3	0.11	0.18	0.04	0.16	0.68	0.07
4	0.18	0.26	0.06	0.42	0.70	0.08
5	0.28	0.03	0.11	0.52	0.74	0.45
6	0.03	0.34	0.15	0.68	0.82	0.68
7	0.04	0.38	0.28	0.79	0.93	0.81
8	0.46	0.69	0.04	0.86	–	–
9	0.58	0.72	0.46	0.92	–	–
10	0.67	0.79	0.54	–	–	–
11	0.08	0.89	0.67	–	–	–
12	0.89	0.92	0.72	–	–	–
13	–	0.95	0.86	–	–	–

Native protein of rabbit's serum, however, reveals 13 bands as shown in Table I. The electrophoretic pattern of native protein of Guinea pig, cat and dog show 10 bands for Guinea pig and 7 bands for both cat and dog (Table I). It was clear that birds and mammals share some bands, but differ in others (Table II).

With regard to the serum, a band with Rf (0.02) was present in both rabbit and dog. A band with Rf (0.08) was present in pigeon, chicken, Guinea pig and dog. A band with Rf (0.11) was present in both pigeon and rabbit.

While bands with Rf (0.18, 0.3 and 0.089) were only present in birds. A bands with Rf (0.28, 0.4 and 0.67) were present in pigeon and rabbit, band with Rf (0.68) was found in both Guinea pig and dog. A bands with Rf (0.79 and 0.92) were present in chicken and Guinea pig.

Total protein with SDS-system showed 8 bands for pigeon, 13 bands for both chicken and rabbit, 12 bands for Guinea pig, 10 bands for cat and 18 bands for dog, with different Rf as shown in Table II. This method illustrated and confirmed the previous results. As regards these results, there are proteins with high molecular weight Rf(0) in Guinea pig, rabbit and cat which cann't be separated with this method and need less concentration of gel. A bands with Rf (0.02 and 0.36) were present in serum of rabbit and dog. A band with Rf (0.04) was present in pigeon and Guinea pig, while a band with Rf (0.1) was found in chicken and rabbit. A band with Rf (0.13) was clear in chicken, Guinea pig and rabbit. A band with Rf (0.28) was present in Guinea pig and dog. A band with Rf (0.64) was present in chicken and dog and band with Rf (0.66) was found in Guinea pig and rabbit. A band with Rf (0.7) was in rabbit and cat and a band with Rf (0.76) was in pigeon, Guinea pig and rabbit. A band (Rf 0.85) was in pigeon, chicken and

cat. A band no. 12 in both chicken and Guinea pig has Rf (0.89). A band which has Rf (0.9) was in rabbit and cat, while a band which has Rf (0.97) was in cat and dog.

TABLE II. Rf-VALUES OF TOTAL PROTEIN BY SDS-METHOD FOR SOME BIRDS AND MAMMALS

No. of bands	Pigeon	Chicken	Rabbit	Guinea pig	Cat	Dog
1	0.04	0	0.05	0	0	0.02
2	0.07	0.04	0.08	0.02	0.03	0.06
3	0.25	0.09	0.01	0.06	0.28	0.09
4	0.53	0.13	0.13	0.10	0.67	0.13
5	0.58	0.17	0.39	0.36	0.07	0.16
6	0.76	0.28	0.59	0.38	0.75	0.21
7	0.80	0.65	0.64	0.42	0.78	0.28
8	0.85	0.66	0.69	0.56	0.85	0.31
9	–	0.72	0.74	0.62	0.95	0.36
10	–	0.76	0.81	0.66	0.97	0.04
11	–	0.79	0.85	0.07	–	0.43
12	–	0.89	0.89	0.76	–	0.46
13	–	–	0.95	0.90	–	0.64
14	–	–	–	–	–	0.68
15	–	–	–	–	–	0.73
16	–	–	–	–	–	0.88
17	–	–	–	–	–	0.92
18	–	–	–	–	–	0.97

Different glycoprotein bands of different migration ratios were separated in either the serum of pigeon or the serum of chickens. The migration ratios were 0.01, .24, .27 and .67 for the chicken. This was in contrast to what was found by Prosky et al. [4], who found 2 distinct glycoprotein bands in serum chicken – as many as four were sometimes visible. The two most common ones migrate to a position not as far from the origin. The glicoprotein pattern in the native state for Guinea pig, rabbit, cats and dogs migration ratios of (0, .03, .13, .44, .49, .57, .6 and .63) for Guinea pig (0, .02, .08, .18, .22, .62, .84 and 1) for rabbit (0.05, .11, .16, .2, .27, .45, .53, .73 and 1) for cat and (0, .003, .08, .2, .29, .32 and .44) for dog.

TABLE III. Rf-VALUES OF NATIVE GLYCOPROTEIN FOR SOME BIRDS AND MAMMALS

No. of bands	Pigeon	Chicken	Rabbit	Guinea pig	Cat	Dog
1	0	0	0	0	0	0
2	0.01	0.03	0.04	0.02	0.05	0.01
3	0.24	0.13	0.08	0.08	0.11	0.08

4	0.27	0.41	0.01	0.18	0.16	0.20
5	0.36	0.44	0.15	0.22	0.02	0.29
6	0.47	0.49	0.18	0.62	0.27	0.32
7	0.52	0.57	0.27	0.84	0.45	0.44
8	0.57	0.60	0.67	1.00	0.53	–
9	–	–	–	–	0.73	–
10	–	–	–	–	1.00	–

Pre-stained lipoprotein gave 4 bands for pigeon with M.R. (.01, .24, .98 and 1). The electrophoretic pattern of fresh serum lipoproteins of chickens revealed the presence of six bands. Three of these bands had a slow migrating rate. They may belong to the chylomicrons which are characterised by slow mobility. They were given the numbers 1, 2 and 3. They were thin and sharp bands. The other three bands (4, 5 and 6) migrated much faster in the gel. Band (4) which seemed to belong to the beta lipoprotein was a dense band. The other two bands: band 5 and band 6 may be prebeta and alpha lipoproteins. They were also present as thin band. The alpha lipoprotein band was the fastest one while the prebeta was more closer to the beta band.

SDS-electrophoresis for glycoprotein showed 4 bands for both pigeon and rabbit, 10 bands for both chicken and dog, 6 bands for Guinea pig and 14 bands for cat. A band with Rf (0.16) was present in chicken and Guinea pig, while chicken and dog were shared a band with Rf (0.68). On the other hand, both cat and dog were only shared a band with Rf (0.25) as shown in Table IV.

Lipoprotein of Guinea pig serum, however, showed three bands with Rf (0.5, .4 and 1). The band with Rf(1), was the fastest and thickest, it resembled the beta lipoprotein. The other two bands were thin and sharp and they resembled the chylomicrons.

TABLE IV. Rf-VALUES OF GLYCOPROTEIN (BY SDS-METHOD) FOR SOME BIRDS AND MAMMALS

No. of bands	Pigeon	Chicken	Rabbit	Guinea pig	Cat	Dog
1	0.06	0.16	0.21	0.16	0.03	0.05
2	0.09	0.02	0.43	0.23	0.05	0.20
3	0.51	0.24	0.65	0.29	0.11	0.25
4	0.64	0.46	0.73	0.59	0.20	0.50
5	–	0.05	–	0.63	0.25	0.53
6	–	0.57	–	0.64	0.29	0.61
7	–	–	–	0.68	0.37	0.64
8	–	–	–	0.81	0.45	0.67
9	–	–	–	–	0.57	0.68
10	–	–	–	–	0.60	0.81
11	–	–	–	–	0.62	0.86
12	–	–	–	–	0.66	–
13	–	–	–	–	0.74	–
14	–	–	–	–	0.80	–
15	–	–	–	–	0.83	–
16	–	–	–	–	0.94	–

TABLE V. Rf-VALUES OF LIPOPROTEIN (PRE-STAINED METHOD) FOR SOME BIRDS AND MAMMALS

No. of bands	Pigeon	Chicken	Rabbit	Guinea pig	Cat	Dog
1	0.01	0.01	0.36	0.05	0	0
2	0.02	0.68	0.86	0.40	0.14	0.06
3	0.24	0.90	0.92	0.90	0.94	0.81
4	0.98	1.00	–	1.00	–	0.95
5	1.00	–	–	–	–	–

The electrophoretic pattern of pigeon serum showed 4 thin and sharp bands. Band (1) may belong to a chylomicron, band (2) belongs to beta lipoprotein, where as bands (3 and 4) resemble prebeta and alpha lipoprotein respectively. Lipoprotein pattern of chicken serum showed 3 bands. The slowest moving lipoprotein, probably a single protein fraction, is the sharp band near the top of the gel and is probably a β-lipoprotein. The other two bands may belong to the prebeta and alpha lipoprotein. This was in agreement with Prosky et al. [4].

In contrast to our results, Held (1989) stated that the electrophoretic pattern of fresh serum lipoproteins of the control chickens revealed the presence of six bands. Three of these bands were of slow migrating rate. They may belong to the chylomicrons which are characterised by slow mobility. They were given the numbers 1, 2 and 3. They were thin and sharp bands. The other three bands (4, 5 and 6) migrated much faster in the gel. Band (4) which seemed to belong to the beta lipoproteins was a dense band. The other two bands: band 5 and band 6 may be prebeta and alpha lipoproteins. They were also present as thin bands. The alpha lipoprotein band was the fastest one while the prebeta was closer to the beta band.

Rabbit, cat and dog showed 2 bands of lipoprotein. The first band may be betalipoprotein and the second one may be alpha lipoprotein.

It is well known that proteins are synthesized in the microsomes through the translation process. However, the induction and maturation of some specific proteins may occur in a post transitional event. In all cases, the translation process is still a very specific and genetic process closely related to the genetic information peculiar to the subject. That is to say the translation processes and protein induction is a process that expresses the already stored genetic codes. Thus we can utilize the electrophoretic pattern of any subject as a sort of fingerprint – useful in classification, recognization, differentiation and evolution study. The electrophoretic pattern, as revelated under our research conditions will form a cornerstone in identifying species and strains.

References:

1. Davis B. J., Disc electrophoresis – 11. Method and application of human serum proteins, *Annals of the New York Academy Sciences* 1964, vol. 121, pp. 404–420.
2. Helal E. G. E., Effect of some stimulatory and inhibitory agents on the mineralocorticoid activity of young chickens, Zoology Dep. Faculty of Science, Al-Azhar University (Girls), 1989.
3. Laemmli R. K., Cleavage of structutral proteins during the assembly of the head of bacteriophage T4, *Nature* 1970, vol. 227, p. 680.
4. Prosky R. G, O'Dell D., Libby A., Flick D. E., *Poultry Science* 1968, vol. 47, pp. 185–190.
5. Shapiro A. L., Vinuela E., Maizel J. V., *Biochemical and Biophysical Research Communication* 1974, vol. 28, pp. 815–820.
6. Smith L., Chromatographic and electrophoretic techniques, Williams Heinemann Medical Books, London 1976, vol. 2, pp. 211–249.
7. Westfall C. L., Clup T. W., Serum lipoprotein electrophoresis: An improved polyacrylamide procedure, *Biochemical Medicine* 1972, vol. 6, pp. 464–470.