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ABSTRACT

Serum from three species of water snakes *(Nerodia rhombifera, N. erythrogaster* and *N. fasciata)* from one geographic region was analyzed electrophoretically on cellulose acetate, and anodic mobility and relative concentration of the fractions were determined by a recording densitometer with an automatic integrator. Classification of fractions was based on mobility (R_f values), and for identification purposes, bands were labeled in order of decreasing mobility (albumin and alpha$_1$, alpha$_2$, alpha$_3$, beta$_1$, beta$_2$, gamma$_1$ and gamma$_2$ globulins). Seven fractions were identified in each species with alpha$_3$ being absent from *N. rhombifera* and *N. erythrogaster*, and only one gamma fraction was observed in *N. fasciata*. In the three species, gamma globulin was the predominant protein (42-46%), and albumin levels were characteristically low; however, a distinct difference was observed in albumin concentration *(N. fasciata*, 7%; *N. rhombifera* and *N. erythrogaster*, 16-18%). The R_f values and relative concentrations of other globulins showed heterogeneity in the three species, with the protein pattern of *N. fasciata* being distinct from the other two species.

INTRODUCTION

According to present dogma, proteins are the products of gene action; thus, the analyses of proteins from different organisms have provided a means for studying the genetic similarities between various groups. Electrophoretic analysis of blood proteins has been a useful tool for demonstrating the distinctness of taxonomic groups of reptiles. The electrophoretic patterns of blood proteins of members of the family Colubridae have been compared by Voris (1967), Dessauer (1975, 1974), Dessauer and Fox (1956, 1958, 1964) and Deutsch and McShan (1949). These studies include generalized descriptions of the electrophoretic patterns of blood proteins for taxonomic comparison most often at the family level. The most inclusive description of species of *Nerodia* was provided by Dessauer and Fox (1964) with emphasis on the variations of the electrophoretic pattern of transferrins at different taxonomic levels. Seniow (1963) and Dessauer and Pough (1975) described the electrophoretic pattern of certain blood proteins in the grass snake *(Natrix natrix)* and the kingsnake *(Lampropeltis getulus)*, respectively.

Taxonomic studies of snakes including species of *Nerodia* based on electrophoretic analysis of blood proteins have limitations due to variations in number, mobility, and/or concentration of some fractions that have been reported based on geographic separation, seasonal changes, and electrophoretic technique (Seniow, 1963; Voris, 1967; Dessauer and Fox, 1956, 1958, 1964). Electrophoretic heterogeneity of some blood proteins (especially transferrins) has been noted in individuals of the same population, and in members of a species from different geographic populations (Dessauer and Fox, 1958, 1964); however, the heterogeneity increased as the geographic range was expanded. Variation in the number, mobility, and quantity of fractions parallels the degree of taxonomic separation of species and, thus, can be used for estimating degrees of divergence in taxonomic categories.

The purpose of the study was to describe and analyze the electrophoretic pattern and relative concentrations of serum proteins in three species of *Nerodia*, *N. erythrogaster flavigaster* (9 specimens), *N. fasciata confluens* (5 specimens) and *N. rhombifera rhombifera* (15 specimens) from a localized population. Both males and females were included in the study.

MATERIALS AND METHODS

Snakes were captured from minnow ponds near Lonoke, Lonoke County, Arkansas in June and transported to the University of Arkansas at Little Rock where they were maintained on a minimum diet of minnows. Starvation preceded collection of blood samples which was made from July through January. Snakes were anesthetized with chloroform, a ventral incision made to expose the heart and major vessels, and bled with a puncture of the aorta. Blood samples were allowed to clot at room temperature for one hour, then centrifuged, and sera collected. Fresh samples were subjected to electrophoresis immediately; the remaining serum was frozen and later analyzed again by electrophoresis. Seven to nine samples (1.6 µl each) were applied across a wide cellulose acetate strip (78 x 150 mm) and subjected to electrophoresis in a Shandon U77 Apparatus. A barbital buffer (pH 8.6; ionic strength 0.075) was used in the tank. Separation was carried out toward the anode for 1½ hr. at a constant current of 5 ma. and a mean of 150 v. Strips were than fixed in 5% trichloroacetic acid, stained with Amido Black 10B, and washed in a solution of methyl alcohol/acetic acid. After visual analysis, strips were cleared with Sepra Clear (Gelman Company) and analyzed using a Helena recording densitometer with an automatic integrator. The R_f values for each fraction were determined by a ratio of the distance from the origin to the peak of the band and the distance from the origin to the anodic edge of the fastest migrating fraction. Relative concentrations of protein fractions were determined by optical density of each band.

Serum from each individual was subjected to several electrophoretic separations with only the clearest separations being used for comparisons. When several clear separations were available for a given individual, the mean R_f value and relative concentration of each component were recorded.

The different serum fractions were named according to the classification of human fractions applied by Tiselius (1973): albumin, alpha$_1$, alpha$_2$, alpha$_3$, beta$_1$, beta$_2$, gamma$_1$, gamma$_2$ globulin in order of decreasing mobility. Investigators using the mobility and names of human fractions as a frame of reference in describing blood proteins in snakes include Dessauer and Fox (1964), Dessauer (1974), and Seniow (1963). Writers have used letters (Voris, 1967) or Arabic numerals (Dessauer and Fox, 1958; Deutsch and McShan, 1949) in identifying protein fractions or have in some cases simply identified the fastest migrating fraction as albumin and the slower ones as globulins. Simultaneous separation of human and *Nerodia* sera indicated that the mobilities of albumin, alpha, beta and gamma fractions of *Nerodia* are within the same range as equally named human fractions. Thus, this nomenclature is appropriate for serum proteins of *Nerodia*. Since the classification is based solely on mobility, one cannot assume that the serum fractions of snakes have the same physiological properties of equally named fractions of human serum. However, Baril et al. (1961) identified the fastest migrating fraction of alligator serum as albumin and the major component as alpha globulin based on physiochemical comparison to mammalian sera. In a review of the literature, Dessauer (1974) reported that reptilian immunoglobulins are similar to antibodies of the G and M classes in mammals and that the physiochemical properties of reptilian albumin are similar to human albumin.

RESULTS AND DISCUSSION

The serum proteins of *Nerodia* separated into eight electrophoretic bands: albumin (A1) and alpha$_1$ (α_1), alpha$_2$ (α_2), alpha$_3$ (α_3), beta$_1$ (β_1), beta$_2$ (β_2), gamma$_1$ (γ_1), gamma$_2$ (γ_2) globulins (Fig. 1). Gamma$_2$ was absent from *N. fasciata*, and alpha$_3$ was absent in *N. rhombifera* and *N. erythrogaster*. The R_f values and relative concentration of each fraction in the three species are given in Table 1 and Table 2. No distinct variations were noted among individuals of a species. Hemoglobin, which was present in some samples due to hemolysis, added an additional band which was the slowest in migration. The mobility of isolated fractions of hemoglobin verified that the band nearest the origin was due to hemoglobin contamination. Further study of this protein was not pursued.

Table 1. Relative mobility of serum proteins in three species of *Nerodia*.

	N. rhombifera (15)[1]		*N. erythrogaster* (9)		*N. fasciata* (5)	
	R_f[2]	SD[3]	R_f	SD	R_f	SD
gamma$_2$	.248	.024	.274	.014		
gamma$_1$	.313	.031	.326	.015	.323	.031
beta$_2$	.420	.029	.404	.023	.426	.023
beta$_1$	.549	.031	.541	.029	.549	.029
alpha$_3$					.678	.014
alpha$_2$	.749	.032	.685	.025	.773	.009
alpha$_1$	.842	.020	.814	.025	.855	.002
albumin	.926	.015	.928	.009	.925	.008

1. Number of individuals.
2. Mean R_f value defined as the ratio of the distance from the origin to the fraction peak to the total distance of the run.
3. Mean Standard Deviation.

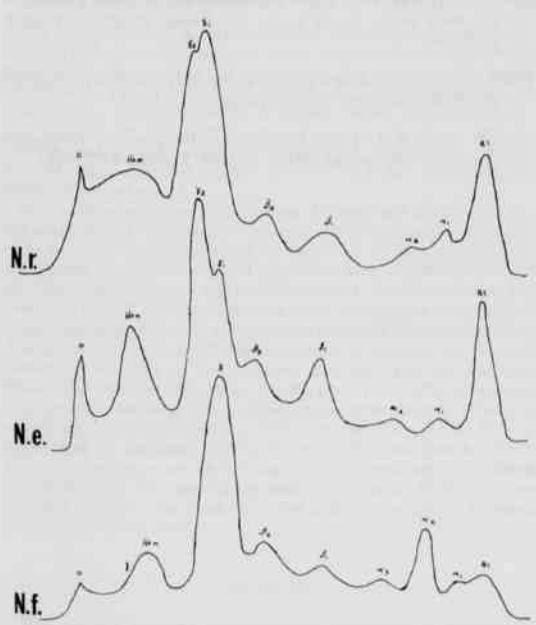

Figure 1. Electrophoretic patterns of serum proteins in *N. r. (Nerodia rhombifera), N. e. (Nerodia erythrogaster), N. f. (Nerodia fasciata).* [O (origin); Hem. (hemoglobin), Al (albumin).]

Table 2. Relative concentration of serum proteins in three species of *Nerodia*.

	N. rhombifera (15)[1]		*N. erythrogaster* (9)		*N. fasciata* (5)	
	Relative Concentration[2]	SD[3]	Relative Concentration	SD	Relative Concentration	SD
gamma$_2$	16.33	5.56	29.80	4.29		
gamma$_1$	30.19	4.91	12.73	5.92	42.71	7.10
beta$_2$	10.75	1.98	11.50	2.00	11.94	4.04
beta$_1$	12.38	3.10	16.05	1.78	13.39	2.92
alpha$_3$					5.91	0.89
alpha$_2$	8.74	2.31	8.27	1.75	12.30	1.60
alpha$_1$	5.96	1.69	3.56	1.02	4.29	0.47
albumin	15.93	5.14	18.14	2.98	7.03	0.58

1. Number of individuals.
2. Mean percentage of fraction to total serum proteins determined by optical density of the bands.
3. Mean Standard Deviation.

The fastest migrating fraction, albumin, which is considered to have equal mobility in all three species, migrates slightly faster than human albumin. Seniow (1963) reported that the albumin of *Natrix natrix* migrated slightly slower than human albumin. The relative concentrations of albumin in *N. rhombifera* and *N. erythrogaster* are similar (16-18%), but the albumin concentration in *N. fasciata* is distinctly lower (7%), representing a minor component.

The concentration and R_f values of alpha$_1$ and alpha$_2$ in *N. erythrogaster* and *N. rhombifera* are very similar, the notable difference being that alpha$_1$ and alpha$_2$ fractions migrate slightly faster in *N. rhombifera*, thus serving as a distinguishable characteristic. Three alpha subfractions are present in *N. fasciata* with alpha$_1$ and alpha$_2$ having higher R_f values than in the other species; the relative concentration of alpha$_2$ was also higher. A fraction, named alpha$_3$ due to its mobility between alpha$_2$ and beta$_1$ is unique for *N. fasciata* and serves as a clearly distinguishable trait for this species.

The ranges of R_f values and relative concentration of the beta subfractions overlap between the species and thus are not considered to be distinguishing characteristics.

Gamma globulin clearly spearates into two fractions with similar mobilities in *N. erythrogaster* and *N. rhombifera*. However, the relative concentration of the two gamma fractions in these two species is approximately inversely proportional, gamma$_1$ subfraction (30%) being predominant to gamma$_2$ (16%) in *N. rhombifera*, and gamma$_2$ (30%) being predominant to gamma$_1$ (13%) in *N. erythrogaster*. There is no distinct difference in relative concentration of the total gamma fraction between these two species. Only one gamma fraction was observed in *N. fasciata* even when the time of electrophoretic separation was greatly increased. The mobility is similar to that of gamma$_1$, and the relative concentration (43%) of this one gamma fraction is not distinct from the total relative concentration of gamma globulin in the other two species.

The three species resemble each other in that the major component is the slowest fraction, gamma globulin. The ratio of globulin/albumin concentration is approximately 3:1 in *N. rhombifera* and slightly lower in *N. erythrogaster* but 6:1 in *N. fasciata*. A high ratio of globulin to albumin agrees with published reports for other species of snakes (Deutsch and McShan, 1949; Seniow, 1963). Kahil (1963) compared the concentration of albumin in several species of snakes and found that the blood of aquatic snakes had a lower albumin concentration than that found in semidesert or desert species. Dessauer (1974) reported that albumin averaged 32% of the total blood protein in terrestrial snakes and only 11% in water snakes.

The number of electrophoretic studies of serum proteins in snakes is limited, and most reports are of a survey nature comparing higher taxonomic groups or geographic variations. Some studies include species of water snakes but only within collective groups. Furthermore, it is difficult to compare the electrophoretic mobility and concentration of serum proteins of *Nerodia* described in this study to other reports of snakes due to differences in buffers and supporting

media, nomenclature, and methods of reporting mobility. The best comparison can be made to the report of Seniow (1963) who compared the globulin subfractions and albumin of the grass snake, *N. natrix* to the mobility and concentration of human fractions. Using cellulose acetate, he described seven protein bands which he named pre-albumin, albumin, and alpha$_1$, alpha$_2$, alpha$_3$, beta, and gamma globulins. The number of fractions corresponds to the number per species reported in this study, but the naming differs. Without physiological descriptions it is difficult to make comparisons. In *N. natrix*, beta globulin was the most prevalent with alpha globulin second in concentration, whereas gamma globulin was the fraction of highest concentraton in each of the three species of *Nerodia* described. Gamma globulin is also the most prevalent globulin in human serum.

The electrophoretic pattern and relative concentrations of serum proteins in *N. rhombifera*, *N. erythrogaster*, and *N. fasciata* from a localized population are distinct. The patterns of the first two species are more similar to each other than that of *N. fasciata* is to either of them. *N. rhombifera* and *N. erythrogaster* can be distinguished by differences in: (1) mobility of alpha$_1$ and alpha$_2$ which are faster in *N. rhombifera* and (2) concentration of gamma$_1$ and gamma$_2$ fractions which are roughly inversely proportional. The uniqueness of the serum protein pattern in *N. fasciata* compared with the other two species is noted: (1) there is only one gamma fraction, (2) there are three alpha subfractions, (3) alpha$_1$ and alpha$_2$ are faster migrating, and (4) the gamma globulin in relative concentration is higher and the albumin concentration is considerably lower.

The three species of *Nerodia* have examples of electrophoretic uniformity; however, the degree of electrophoretic variability allows identity of each species. Thus, the electrophoretic analysis of the serum proteins can be used for grouping and measuring the degree of taxonomic kinship between species of *Nerodia* from a given geographic location.

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