Electrophoretic Patterns of Serum Proteins of Neotropical Bats (Chiroptera)

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DARIO VALDIVIESO is Research Associate in the Department of Mammalogy, Royal Ontario Museum.

J. R. TAMSITT is Curator in the Department of Mammalogy, Royal Ontario Museum, and Associate Professor in the Department of Zoology, University of Toronto.

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Abstract

Serum proteins of 18 species of neotropical Chiroptera were separated by cellulose polyacetate electrophoresis. Except for differences between non-parous, adult, and gravid females in relative quantities of fractions in the alpha globulin region, resulting electropherograms were independent of sex and age. Albumin and gamma globulin fractions were monomorphic, but polymorphism in fractions of the alpha and beta globulin regions occurred in six species of Colombian and Venezuelan bats and in one species of Puerto Rican bat. Of the species studied, the insular Artibeus jamaicensis presented the most intraspecific variation. Species differed in numbers and mobilities of protein fractions. The electropherogram of Pteronotus paradoxus (Mormoopidae) was not unique, whereas Phyllostomus hastatus and P. discolor (Phyllostomatinae) were not only distinct from other Phyllostomatidae but differed significantly from each other. Electropherograms of bats of the families Vespertilionidae and Molossidae were similar to each other and also, among the Phyllostomatidae, to two species of Glossophagidae, three species of Stenoderminae, and one species of Phyllonycterinae. Electrophoretic properties of serum proteins are taxonomically important but may have limited value as indicators of phylogenetic relationships. [Chiroptera; serum proteins; electrophoresis; systematics.]

Introduction

Previous work with bats has demonstrated the feasibility of correlating biochemical traits with systematic relationship. Manwell and Kerst (1966), Valdivieso et al. (1969), Tamsitt and Valdivieso (1969), and Mitchell (1970) found hemoglobin differences among species of bats to be minor but useful in estimating phylogeny at the subfamilial and familial levels. Manwell and Kerst (1966) and Valdivieso et al. (1968) found a number of differences at the generic level in lactate dehydrogenase isoenzymes, esterases, and tissue proteins, as well as ontogenetic and sexual differences and some polymorphism. Few researchers, however, have considered electrophoretic properties of bat sera as an adjunct to chiropteran classification. In a survey of serum proteins among many species of mammals, including bats of the families Vespertilionidae and Molossidae (number and taxa unspecified), Johnson and Wicks (1959, 1964) found that standard
patterns for species could be established and that most significant differences occurred at the generic and specific levels. Comparisons of total protein patterns, although less valid than comparisons of single, homologous proteins, provide reliable taxonomic data for amphibians (Coates, 1967), for example, and may be useful for taxonomic classification of mammals (Feeney and Allison, 1969).

Our major objectives were to compare species by a priori taxonomic arrangement (Koopman and Jones, 1970) and to evaluate the potential of these proteins as a tool for systematic analysis. Other objectives were to determine, where possible, individual variation within samples from single populations of a species, geographic variation between populations of the same species from different localities, and to determine the effect of age, sex, and reproductive condition on electrophoretic patterns of serum proteins.

A major limitation of this study was variability of sample size for most taxa. As the acquisition of specimens more often than not was fortuitous, some species were represented by one, others by only a few specimens. Although a single sample or a low number of individuals from a population may not reveal polymorphisms, we nonetheless present all data for comparison on the premise that limited data are preferable to none. In no way do we attempt here to “characterize” a species from only one or a few samples to eschew the stigma of the typological concept (Rasmussen, 1969).

Materials and Methods
Sera were obtained from 75 bats of 18 species collected in Colombia, Puerto Rico, and Venezuela in November and December 1968, January 1969, and March and April 1970 (Table I). Bats were captured in culverts, from caves, and in “mist” nets set across trails, streams, or other flight pathways. Shortly after capture, bats were weighed, and biological data, including species, sex, reproductive condition, and age, were recorded. State of maturity was assessed from the measurement of the forearm (Handley, 1959: 98), from characteristics of pelage, and by degree of epiphysial closure of wing bones. None of the bats collected displayed gross abnormalities, and all were included in the study. After blood was taken, bats were preserved to verify field identifications. All specimens were deposited in the Department of Mammalogy, Royal Ontario Museum.

Satisfactory samples of bat sera for electrophoretic separation of protein fractions were often not easy to obtain. Moreover, although serum was obtained easily from some bats, others that were handled identically yielded blood that hemolyzed. Hemolysis of the blood of one group, the free-tailed bats of the family Molossidae, was so rapid that it was extremely difficult to obtain usable sera. In one sample of Molossus fortis from Puerto Rico, for example, blood drawn from 13 bats yielded only four usable serum samples. As the electrophoretic pattern of hemolyzed samples typically resulted in the presence of an extra, heavily-stained fraction in the beta globulin region, pinkish or reddish sera were discarded.
Whole blood, obtained by heart puncture with sterile syringes from lightly etherized animals, was transferred to 5 ml centrifuge tubes and left at room temperature for 2 hours to allow erythrocytes to clot. Blood was then centrifuged at 3,000 rpm for 10-15 minutes to separate serum from the clotted erythrocytes. Sera were analyzed immediately after separation or frozen at -20°C. Before electrophoresis, frozen samples were thawed, shaken with half volume of ether, and centrifuged at 3,000 rpm for 20 minutes to extract fats and improve electrophoretic resolution. Characteristic mobilities of serum proteins after electrophoresis were stable after cold storage, and patterns were reproducible after a month or longer.

Although a variety of electrophoretic techniques exist to analyze serum proteins, we chose cellulose polyacetate (Nerenberg, 1966) rather than disc gel, acrylamide gel, or comparable high-resolution techniques because it is less affected by physiological and environmental variables and consequently is suited for survey purposes (see, for example, Dessauer and Fox, 1964; Huntsman, 1970). Sera were analyzed by electrophoresis in the Model R-101 Microzone Cell (Beckman Instrument Co., Fullerton, California) with Sepaphore III polyacetate membranes (Gelman Instrument Co., Ann Arbor, Michigan) and barbital buffer pH 8.6, ionic strength 0.075. The sample (0.25 μl) was applied to the membrane with a Beckman applicator, and separation was accomplished at 250 V for 20 minutes. Normal human serum (NHS) was run simultaneously with bat sera as a standard. After electrophoresis, serum proteins were stained with Ponceau S (Gelman Instrument Co.). Membranes were cleared in glacial acetic acid and absolute methanol as recommended by the Gelman Instrument Co. (1968). Transparent membranes were scanned optically in a Beckman Microzone Densitometer, Model R-110, or a Spinco Analytrol, Model RB (Beckman Instrument Co.), for the integration of protein fractions. Percentages of serum proteins were calculated from resultant densitometric tracings (Beckman Instrument Co., 1967). When sufficient sera were available, total protein concentrations were determined by an adaptation of the methods of Kingsley (1939) and Gornall et al. (1949) for small quantities as suggested by the Beckman Instrument Co. (1962). Relative mobilities of albumin were calculated as the distance on the densitometric tracing (in centimetres) from the origin to the centre of the peak divided by the distance (in centimetres) from the origin to the centre of the peak of human albumin (15.0 ± 1.0 cm). Relative mobilities of globulins were not calculated because of difficulties in differentiating between proximal or juxtaposed fractions. The nomenclature of globulins was established in relation to their positions as compared to those of human serum globulins. Means (X), standard errors (SE), and Student's t-tests were performed on an Olivetti-Underwood Programma 101 (Olivetti Underwood Co., New York, N.Y.) using programs devised for small samples, n-1 (Sokal and Rohlf, 1969). The sequence and nomenclature of taxa given here follow that of Koopman and Jones (1970) and Smith (1972).
Table I. Relative concentrations of serum proteins (X ± SE, extremes).

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<tr>
<th>Species</th>
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<th>3</th>
<th>4</th>
<th>1</th>
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<td>Age and sex</td>
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<td>2</td>
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<td>—</td>
<td>17.3</td>
</tr>
<tr>
<td><em>Erophylla bombifrons</em></td>
<td>5 4</td>
<td>adult ♂♂ 0.90 ± 0.01 (0.87-0.93)</td>
<td>61.9</td>
<td>1.8</td>
<td>2.6</td>
<td>2.9</td>
<td>3.4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>8 1</td>
<td>lactating ♀</td>
<td>59.4</td>
<td>1.5</td>
<td>2.5</td>
<td>2.5</td>
<td>3.0</td>
<td>15.8</td>
<td>15.3</td>
<td>11.9</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td><em>Desmodustrotundus</em></td>
<td>5 1</td>
<td>gravid ♀</td>
<td>64.8</td>
<td>3.1</td>
<td>3.1</td>
<td>4.4</td>
<td>3.8</td>
<td>11.9</td>
<td>8.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 1</td>
<td>adult ♂♂ 0.97 ± 0.01 (0.94 -1.01)</td>
<td>65.4</td>
<td>4.5</td>
<td>5.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>8.1</td>
<td>9.3</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 2</td>
<td>adult ♀♀</td>
<td>(66.3)</td>
<td>4.1</td>
<td>5.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.9</td>
<td>7.9</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 2</td>
<td>gravid ♀♀</td>
<td>(60.2, 72.3)</td>
<td>5.5</td>
<td>6.4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.2</td>
<td>8.3</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td><em>Eptesicus fuscus</em></td>
<td>5 1</td>
<td>gravid ♀</td>
<td>0.93 (54.2)</td>
<td>6.0</td>
<td>6.4</td>
<td>10.8</td>
<td>—</td>
<td>—</td>
<td>6.4</td>
<td>8.8</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>adult δδ 0.92 ± 0.02 (0.86-0.95)</td>
<td>68.5</td>
<td>5.8</td>
<td>7.4</td>
<td>—</td>
<td>—</td>
<td>7.3</td>
<td>5.4</td>
<td>5.8</td>
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<td>---</td>
<td>-----</td>
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</tr>
<tr>
<td><strong>Molossus</strong></td>
<td>1</td>
<td>1</td>
<td>adult ♀ 69.7</td>
<td>62.2</td>
<td>5.5</td>
<td>6.0</td>
<td>(6.8, 8.0)</td>
<td>—</td>
<td>—</td>
<td>(5.3, 9.2)</td>
<td>(5.2, 5.5)</td>
<td>(3.0, 8.6)</td>
</tr>
<tr>
<td><strong>molossus</strong></td>
<td>1</td>
<td>1</td>
<td>lactating ♀ 69.4</td>
<td>6.7</td>
<td>6.7</td>
<td>6.7</td>
<td>—</td>
<td>—</td>
<td>8.2</td>
<td>6.7</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td><strong>Molossus</strong></td>
<td>7</td>
<td>1</td>
<td>adult δ 0.91 ± 0.01 (0.91-0.94)</td>
<td>56.7</td>
<td>7.2</td>
<td>4.2</td>
<td>4.1</td>
<td>—</td>
<td>—</td>
<td>10.5</td>
<td>10.6</td>
<td>6.7</td>
</tr>
<tr>
<td><strong>fortis</strong></td>
<td>7</td>
<td>3</td>
<td>gravid ♀♀ 55.8</td>
<td>5.8</td>
<td>5.1</td>
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<td>—</td>
<td>—</td>
<td>10.2</td>
<td>10.2</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td><strong>Normal human</strong></td>
<td>14</td>
<td></td>
<td>1.00</td>
<td>65.4 ± 1.6</td>
<td>3.8 ± 0.15</td>
<td>10.1 ± 0.22</td>
<td>—</td>
<td>—</td>
<td>7.8 ± 0.29</td>
<td>—</td>
<td>13.0 ± 1.48</td>
<td></td>
</tr>
<tr>
<td>serum (NHS)</td>
<td></td>
<td></td>
<td>(60.0-69.0) (3.3-4.2) (9.6-11.0)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>(7.3-9.2)</td>
<td>—</td>
<td>—</td>
<td>(8.9-17.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*COLOMBIA: 1, Cundinamarca: Mesitas del Colegio (1210 m; 4°35' N, 74°27' W), December 1968; 2, Cundinamarca: Villleta (804 m; 5°01' N, 74°28' W), December 1968; 3, Tolima: Melgar (430 m; 4°15' N, 74°35' W), January 1969; 4, Tolima: 9 km NW Melgar (approx. 430 m), January 1969. PUERTO RICO: 5, Cuevas de Aguas Buenas (260 m; 18°16' N, 66°10' W), April 1970; 6, Cuevas de Corozal (95 m; 18°21' N, 66°19' W), April 1970; 7, Isla Verde (approx. 3 m; 18°29' N, 66°08' W), April 1970; 8, Luquillo National Forest (approx. 457 m; 18°19' N, 65°45' W), April 1970. VENEZUELA: 9, Aragua: Portachuelo Pass, Rancho Grande (1100 m; 10°22' N, 67°41' W), November 1968.

**The use of the name Artibeus cf. jamaicensis for the large species that occurs sympatrically with A. lituratus in Colombia was discussed elsewhere (Tamsitt and Valdivieso, 1969).**
Results

ELECTROPHEROGRAMS OF SERA

Four to eight fractions were visible in electrophoretic patterns of chiropteran serum proteins (Figs. 1 and 2), and patterns of 18 species are summarized in Fig. 3. Quantitative aspects of profiles are compared in Table 1. When polymorphism occurred, it involved fractions in the alpha and beta globulins. Interspecific differences were observed in the total number of fractions and in their relative mobilities (Fig. 3). Although varying in electrophoretic mobility among species, albumin, the most intense and rapidly migrating zone, and the gamma globulin fraction were monomorphic; consequently, although included in the total number of fractions, they are not discussed here.

Intraspecific variation in the number of fractions in the alpha and beta globulin regions occurred in seven species (Table 1; Fig. 3). In *Phyllostomus hastatus* (Pallas) sera separated into five or six fractions (Fig. 1B,C). In the alpha region two fractions were present in a male and a female, whereas in another male and female three fractions were present. Five fractions, including two in the alpha and one in the beta region, were seen in adult *P. discolor* (Wagner). Serum of a male fetus, however, showed four fractions, with only one in the alpha region (Fig. 1D). Four or five fractions were present in *Glossophaga soricina* (Pallas). An adult, non-parous female possessed a single alpha globulin and a single beta globulin, whereas in a gravid female an additional alpha fraction was present (Fig. 1C).

In *Artibeus jamaicensis* Leach serum protein fractions varied from five to eight (Figs. 1A,E; 2A). Adult males had the greatest number (eight), with four fractions in the alpha region and two in the beta globulin region. In two adult females fractions numbered seven (three alpha and two beta). A lactating female had only five fractions, with two alphas and a single beta, a pattern similar to that of three young adult males in which the third and fourth alpha fractions were absent. Of the age groups, young adult males were the most variable; two had seven fractions (three alpha and two beta), one had six (two alpha and two beta), and three had five (two alpha and one beta fractions). One young adult female had six fractions, two alpha and two beta globulins that were comparable to those of one of the young adult males.

Five or six fractions were found in sera of *Artibeus cf. jamaicensis* (Fig. 2B). Six fractions were found in an adult male and a gravid female, with two in the alpha region and two in the beta region. Only five fractions were seen in a lactating female, whose electropherogram was identical to that of other adults except for the absence of one of the beta globulins.

Serum fractions of *Artibeus lituratus* Ofers from Colombia and Venezuela varied in number from four to six (Figs. 1C; 2B,C). Adult males possessed five or six fractions; those with five fractions had either two fractions in the alpha region and one in the beta region (one male from Colombia, one male from Venezuela) or one fraction in the alpha region.
and two fractions in the beta region (one male from Colombia). Males with six fractions possessed two in the alpha and two in the beta regions (four males from Colombia, five from Venezuela). Serum fractions in females varied from four to six. One adult female (Venezuela) had only four fractions, including a single alpha and a single beta globulin. One adult, non-parous female (Venezuela) had six fractions, with two in the alpha and two in the beta regions. One gravid female (Colombia) and one adult, non-parous female (Venezuela) possessed five fractions, including one in the alpha and two in the beta regions. Samples from Colombia and Venezuela showed the same degree of variability, and numbers of serum fractions varied in individuals of each of the disjunct populations.

In *Carollia perspicillata* Linnaeus five or six serum fractions were present (Fig. 1c,f). Patterns in two males and two gravid females were identical, consisting of three fractions in the alpha and one in the beta region. In one gravid female, however, only two fractions were present in the alpha region. Comparison of percentage composition of serum proteins by fraction according to sex and age without specific allocation resulted in only one statistically significant difference. The percentage (13.8) of fractions in the alpha region was significantly less in 14 adult, non-parous females than that (29.8) in 13 gravid females (*P* ≤ 0.025), although no other differences were found. Nor were significant differences (*P* < 0.05) found between adult and young adult males, between adult males and adult females, between adult and lactating females, or between adult and young adult females.

**RELATIVE MOBILITY OF ALBUMIN**

Among the species studied, none of the albumins migrated anodal to the NHS control (Table I; Figs. 1-3). *Artibeus* cf. *jamaicensis*, *A. lituratus*, *A. phaeotis* (Miller), *Stenoderma rufum* St. Hilaire, *Carollia perspicillata*, and *Desmodus rotundus* (Geoffroy) possessed albumins that had the same mobility as the NHS control. *Glossophaga soricina*, *Monophyllus redmani* Leach, *Sturnira lillium* (Geoffroy), *Brachyphylla cavernarum* Gray, *Artibeus jamaicensis*, *Erophylla bombifrons* (Miller) (Phyllostomatidae), *Eptesicus fuscus* Palisot de Beauvois (Vespertilionidae), *Molossus molossus* (Pallas), and *M. fortis* Miller (Molossidae) possessed albumins with similar mobilities that were cathodic to the NHS control. *Phyllostomus discolor* and *P. hastatus* possessed albumin mobilities that differed from other species by being the most cathodic (Fig. 3). Likewise, the mobility of the albumin of *P. discolor* was more cathodic than that of *P. hastatus*. The mobility of the albumin of *Pteronotus parnellii* (Gray) (Mormoopidae) was slightly cathodic to albumins of the species of *Phyllostomus* studied.

**PROTEIN CONCENTRATION**

Values of total protein content (g/100 ml) of sera of adult bats ranged from 4.08 in *Monophyllus redmani* to 6.55 in *Phyllostomus discolor* and *Artibeus jamaicensis*, but most values were between 5 and 6 (Table II).
Fig. 1 Electrophoretic patterns of serum proteins of bats. The arrow indicates the point of application.


B. *Phyllostomus hastatus* (Venezuela): 1, 6, NHS; 2, 3, ♀; 4, 5, ♂♂.

C. 1, *Phyllostomus hastatus* ♀ (Venezuela); 2, *Glossophaga soricina* ♀ (Colombia); 3, *Carollia perspicillata* ♂ (Colombia); 4, *Sturnira lilium* ♂ (Colombia); 5, *Artibeus lituratus* ♂ (Colombia); 6, *Desmodus rotundus* ♀ (Colombia); 7, *Molossus molossus* ♂ (Colombia); 8, NHS.

D. *Phyllostomus discolor* (Colombia): 1, 6, NHS; 2, fetus ♂; 3, young adult ♂; 4, ♂; 5, ♂♀.
Differences in our samples unequivocally attributable to age were negligible except in *P. discolor*, in which values for an adult were greater than those of a fetus. Differences in protein concentration in young adult and adult *A. jamaicensis* were insignificant ($P \geq 0.05$), as were these values for adult males and females.

The relative proportions of protein (g%) of the serum fractions were variable and apparently influenced to some extent by age or reproductive condition. Adult and young adult *A. jamaicensis* did not differ significantly (0.10 $< P > 0.05$) in percentages of beta and gamma globulins, nor did other protein fractions differ significantly between age groups or between sexes. In *P. discolor* differences between the adult and fetus were primarily in the reduced percentages of albumin and alpha fractions of the latter, although per cent protein of the gamma of adult and fetus were comparable. Young adult and adult *A. jamaicensis* were only near-significantly different ($P \leq 0.05$) in per cent protein of the gamma fraction. The greatest percentage of protein in the gamma region was found in a lactating *Brachyphylla cavernarum* and the least in a young adult male *A. jamaicensis*, an adult male *Desmodus rotundus*, and an adult male *Molossus fortis* (Table II). Percentages of protein in the alpha and beta fractions varied remarkably and could not be correlated with age, sex, or taxon.

**Discussion**

Species similarities established by serum protein electropherograms (Fig. 3) do not agree with existing taxonomic arrangements. Bats of the family Phyllostomatidae have a particularly wide range of variation in serum protein patterns similar to that reported for karyotypes by Baker (1970).

Although no albumin polymorphism was observed in populations of species studied, differences and similarities were noted among several groups of species (Table I; Fig. 3). None of the albumins studied migrated anodically to the NHS control; in all species albumin had the same mobility as the control or was cathodic to it. According to mobility of albumin, four groups could be distinguished: (1) *Pteronotus parnellii* (Mormoopidae); (2) the Phyllostomatinae represented by *Phyllostomus hastatus* and *P. discolor*, which, although differing in mobilities, had the most cathodic albumins of the bats studied; (3) a group of phyllostomatid bats whose albumin mobilities were identical with NHS albumin: three species of *Artibeus* (*A. cf. jamaicensis*, *A. lituratus*, and *A. phaeotis*), the single species of *Stenoderma*, one species of *Carollia*, and the vampire bat *Desmodus rotundus*; and (4) a larger group of bats representing three families whose albumins were slightly cathodic to that of the control and similar to that of *P. parnellii*: the glossophagine bats *Glossophaga soricina* and *Monophyllus redmani*, the stenodermine bats *Sturnira lilium*, *A. jamaicen-
Fig. 2  Electrophoretic patterns of serum proteins of bats. The arrow indicates the point of application.

a. *Artibeus jamaicensis* (Puerto Rico): 1, 2, ♀ ♀; 3, 4, 6-8, ♂♂; 5, normal human serum (NHS).


c. 1, 8, NHS; 2-4, *Artibeus lituratus* ♂♂ (Colombia); 5-7, *A. lituratus* ♂♂ (Venezuela).

d. 1, 5, NHS; 2, 3, 6-8, *Erophylla bombifrons* (2, ♀; 3, 6-8, ♂♂); 4, *Pteronotus pennelli* ♂♂. All from Puerto Rico.

e. *Desmodus rotundus* (Colombia): 1, NHS; 2, 3, ♀ ♀; 4, 5, gravid ♀ ♀; 6, 7, ♂♂.

f. *Molossus molossus* (Colombia): 1, 6, NHS; 2, 3, ♂♂; 4, ♀ ♀; 5, lactating ♀.
sis, and *Brachyphylla cavernarum*, the phyllonycterine bat *Erophylla bombifrons* (Phyllostomatidae), *Eptesicus fuscus* (Vespertilionidae), and *Molossus molossus* and *M. fortes* (Molossidae).

It is noteworthy that albumin mobilities of the two congeneric species *Phyllostomus hastatus* and *P. discolor* were significantly different (0.005 < \( P \) > 0.001). Likewise, although mobilities of albumin of the three species of mainland *Artibeus* were similar or not significantly different (\( P > 0.05 \)), mobilities of albumin of *A. jamaicensis* from Puerto Rico and of mainland *A. lituratus* and *A. cf. jamaicensis* differed significantly (0.01 < \( P \) > 0.025).

The status of bats previously placed in the subfamily Chilonycterinae has been questioned (Koopman and Jones, 1970; Jones and Genoways, 1970), and species of the genera *Pteronotus* and *Mormoops* were recently placed by Smith (1972) in a distinct family, the Mormoopidae. Although *P. parnelli* was the only taxon of this family we studied, the mobility of the albumin of this species from Puerto Rico was intermediate between the phyllostomatid bats of the subfamily Phyllostomatinae (*P. hastatus* and *P. discolor*) on the one hand and the phyllostomatid bats of the subfamilies Stenoderminae (*G. soricina, M. redmani, S. lilium, B. cavernarum, A. jamaicensis*) and Phyllonycterinae (*E. bombifrons*), and of the families Vespertilionidae (*E. fuscus*) and Molossidae (*M. molossus* and *M. fortis*) on the other. *G. soricina, M. redmani*, and *S. lilium* have fewer globulin fractions than *P. parnelli*, whereas *B. cavernarum* and *A. jamaicensis* have a greater number. Hemoglobins (Valdivieso et al., 1969) and immunologic properties of serum proteins (Gerber and Leone, 1971) of species of *Pteronotus* differ from those of bats of the Phyllostomatidae. But the serum protein pattern of *P. parnelli* does not differ markedly from other bats studied and consequently does not support the contention of Smith (1972) that this group represents a distinct family.

The two species of the subfamily Phyllostomatinae, *Phyllostomus hastatus* and *P. discolor*, have the most cathodic albumin of all bats studied and differ from other taxa in the Phyllostomatidae in this character. In numbers and mobilities of protein fractions, *P. hastatus* is more similar to *Pteronotus parnelli* of the Mormoopidae than to either *P. discolor* of the same genus or to other taxa of the Phyllostomatidae. Adult *P. discolor* have one less fraction than *P. hastatus* and are unique among the bats studied. These two species differ in a number of morphological characters and food habits (Walker, 1968) and as well differ in susceptibility to dental disease (Phillips and Jones, 1970) and in thermoregulatory abilities (McNab, 1969). Likewise, although chromosomes of the two taxa are similar, they differ in one pair of autosomes, an exception to the undifferentiated karyotypes typically encountered among different species of the same genus (Kiblisky, 1969). Electrophoretic patterns of hemoglobins, however, do not differ between the two species or among bats of other subfamilies of the Phyllostomatidae (Tamsitt and Valdivieso, 1969).

*Sturnira lilium*, in albumin mobility, does not differ from *Brachyphylla cavernarum, Artibeus jamaicensis, Monophyllus redmani*, and *Erophylla*
Table II. Total protein and relative proportions of fractions of bat sera (X ± SE, extremes).

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>Age and sex</th>
<th>Total protein (g/100 ml)</th>
<th>Albumin</th>
<th>a1</th>
<th>g% total protein</th>
<th>a2</th>
<th>β</th>
<th>γ</th>
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</thead>
<tbody>
<tr>
<td><strong>Phyllostomus discolor</strong></td>
<td>1</td>
<td>adult ♂</td>
<td>6.55</td>
<td>3.70</td>
<td>0.47</td>
<td>0.64</td>
<td>0.83</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>fetus ♂</td>
<td>5.12</td>
<td>2.64</td>
<td>—</td>
<td>—</td>
<td>0.60</td>
<td>0.99</td>
<td>0.96</td>
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</tr>
<tr>
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<td>adult ♀</td>
<td>4.08</td>
<td>2.14</td>
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<td>0.18</td>
<td>0.68</td>
<td>0.79</td>
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<tr>
<td><strong>Brachyphylla cavernarum</strong></td>
<td>1</td>
<td>lactating ♀</td>
<td>6.03</td>
<td>3.09</td>
<td>0.42</td>
<td>0.80</td>
<td>0.55</td>
<td>1.17</td>
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</tr>
<tr>
<td><strong>Artibeus jamaicensis</strong></td>
<td>3</td>
<td>adult ♂</td>
<td>5.77 ± 0.41 (5.19-6.55)</td>
<td>2.91 ± 0.25 (2.62-3.41)</td>
<td>0.32 ± 0.04* (0.24-0.37)</td>
<td>0.56 ± 0.06* (0.43-0.62)</td>
<td>1.07 ± 0.03* (1.01-1.13)</td>
<td>0.91 ± 0.08 (0.74-1.00)</td>
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</tr>
<tr>
<td>6</td>
<td>young adult ♂</td>
<td>5.83 ± 0.22 (5.19-6.23)</td>
<td>3.18 ± 0.13 (2.68-3.70)</td>
<td>0.23 ± 0.03* (0.13-0.31)</td>
<td>0.59 ± 0.10** (0.31-0.87)</td>
<td>1.22 ± 0.07** (1.05-1.45)</td>
<td>0.62 ± 0.07 (0.36-0.84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>adult ♀</td>
<td>5.35 (5.33, 5.38)</td>
<td>3.00 (2.98, 3.02)</td>
<td>0.31* (0.29, 0.32)</td>
<td>0.36 (0.33, 0.38) (0.87, 0.97)</td>
<td>0.92* (0.87, 0.97)</td>
<td>0.77</td>
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<td>1</td>
<td>adult ♂</td>
<td>6.27</td>
<td>3.99</td>
<td>0.18*</td>
<td>0.22*</td>
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<td>0.77</td>
<td>0.52*</td>
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<td>adult ♂</td>
<td>6.47</td>
<td>3.67</td>
<td>0.47</td>
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<td>1.36*</td>
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<td><strong>Normal Human Serum (NHS)</strong></td>
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<td>0.24</td>
<td>0.63</td>
<td>0.49</td>
<td>1.07</td>
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*two fractions
**one or two fractions
In the number of fractions in the globulin regions, however, *S. lilium* is more similar to *Stenoderma rufum* and *Monophyllum redmani* than to other phyllostomatid bats. Although once considered to be a separate subfamily, the Sturnirinae (Miller, 1907), Koopman and Jones (1970) placed bats of the genus *Sturnira* in the Sternotherinae but in a separate tribe, the Sturini. In karyotypes (Gardner and O'Neill, 1969), electrophoretic properties of hemoglobins (Tamsitt and Valdivieso, 1969), and immunological properties of sera (Gerber and Leone, 1971), species of *Sturnira* have strong affinities with bats of the subfamily Stenoderminae. The serum protein pattern of *S. lilium* closely resembles that of *Carollia perspicillata* (Fig. 3), differing primarily in albumin mobility. Our data thus indicate a relationship with the carolline and glossoptahagine bats as well as with stenodermine bats.

Species of the genus *Brachyphylla* were recently placed as a tribe of the subfamily Stenoderminae (Phyllostomatidae) by Koopman and Jones (1970) but as a member of the Phyllonycterinae by Silva and Pine (1969). In albumin mobility *B. cavernarum* is similar to phyllostomats from mainland South America (*G. soricina* and *S. lilium*) and from Puerto Rico (*M. redmani, A. jamaicensis, and E. bombifrons*). In globulin fractions, however, *B. cavernarum* differs primarily from the above in number and position except in those individuals of *A. jamaicensis* that possess six fractions. Although species of the genera *Erophylla* and *Brachyphylla* are similar in morphology and behaviour (Silva and Pine, 1969), karyotypes (Baker and Lopez, 1970), hair structure (Benedict, 1957), and possess in common host-specific spinturnicid mites (Dushábek, 1969), the serum protein pattern of *B. cavernarum* is surprisingly unlike that of *E. bombifrons* and more similar to that of the vespertilionid bat *Eptesicus fuscus*.

*Erophylla bombifrons* differs from other phyllostomatids studied in the same way that *B. cavernarum* differs. These two species are similar in albumin mobility but differ in mobilities and numbers of globulin fractions. Lactate dehydrogenase isoenzymes of *B. bombifrons* differ from those of Puerto Rican *A. jamaicensis* (Valdivieso et al., 1968), and although species of *Erophylla* unquestionably are taxonomically distinct from stenodermine bats, results from electrophoretic studies of serum proteins do not confirm the conclusion of Silva and Pine (1969).

Of the four species of *Artibeus*, the insular *A. jamaicensis* differs from the mainland *A. lituratus*, *A. cf. jamaicensis*, and *A. phaeotis* by having a slower-moving albumin and by a greater number of protein fractions (eight as opposed to 4-6). *A. lituratus* and *A. cf. jamaicensis* are similar in mobilities of protein fractions, but although the slower fraction in the beta region may or may not be present in individuals of both species, only in *A. cf. jamaicensis* is the fastest fraction in the alpha region always present. *A. phaeotis*, although having the same albumin mobility as *A. lituratus* and *A. cf. jamaicensis*, differs from these two taxa by the absence of the dimorphic, slower beta fraction and by the absence of the faster fraction in the alpha region.
<table>
<thead>
<tr>
<th>Alb.</th>
<th>a1</th>
<th>a2</th>
<th>β</th>
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</table>

- **NORMAL HUMAN SERUM**
- **FAMILY MORMOOPIDAE**
  - Pteronotus purnelli
- **FAMILY PHYLLOSTOMATIDAE**
  - Phyllostomus hastatus
  - Phyllostomus discolor
  - Glossophaga soricina
  - Monophyllum redmani
  - Stumira lilium
  - Brachyphylia cavernarum
- **FAMILY VESPERTILIONIDAE**
  - Artibeus jamaicensis
  - Artibeus cf. jamaicensis
  - Artibeus litoratus
  - Artibeus phaeotis
  - Stenoderma rufum
  - Carollia perspicillata
  - Erophylla bombifrons
  - Desmodus rotundus
- **FAMILY MOLOSSIDAE**
  - Eptesicus fuscus
  - Molossus molossus
  - Molossus fortis

**NORMAL HUMAN SERUM**

**16**
Comparison among these congeneric species presents striking differences that raise questions of functional necessity and diversity of serum proteins. In *A. phaeotis*, the limited number of fractions contrasts with the marked complexities of fractions of sera in all individuals of *A. lituratus* and *A. jamaicensis*. In many respects species of *Artibeus* are obviously diverse, e.g., *A. phaeotis* has higher immunological affinities with other phyllostomatid bats than with the related *A. jamaicensis* (Gerber and Leone, 1971) and moreover differs chromosomally from other species of *Artibeus* (Genoways and Baker, 1972). On the other hand, hemoglobins of all *Artibeus* species studied here are electrophoretically indistinguishable from each other and from other phyllostomatid bats (Valdivieso et al., 1969; Tamsitt and Valdivieso, 1969).

*Stenoderma rufum*, one of the least known neotropical stenodermine bats (Jones et al., 1971), is similar to mainland South American phyllostomatids (*Carollia, Artibeus*, and *Desmodus*) but differs from other Puerto Rican phyllostomatid bats (*A. jamaicensis, B. cavernarum, M. redmani*, and *E. bombifrons*) in albumin mobility. In number and mobilities of globulin fractions, *S. rufum* differs from all phyllostomatids studied and is more similar to the glossophagine bats *M. redmani* and *G. soricina*. Chromosomally, however, *S. rufum* is similar to *Artibeus* species (Baker and Lopez, 1970; Genoways and Baker, 1972) and moreover shares a species of listrophorid mite in common with Puerto Rican *A. jamaicensis* (Cruz et al., 1974). The electrophoretic pattern of hemoglobin of *S. rufum* is indistinguishable from Puerto Rican or mainland phyllostomatids (Valdivieso et al., 1969; Tamsitt and Valdivieso, 1969). Why the serum protein fractions of *S. rufum* are more similar to glossophagine bats, both insular and mainland, than to phylogenetically-related stenodermine bats remains to be determined.

The genus *Carollia*, placed in the subfamily Carollinae of the Phyllostomatidae (Koopman and Jones, 1970), is similar to mainland *Artibeus* species, *S. rufum*, and *D. rotundus* in albumin mobility but differs from these taxa in number and mobilities of fractions in the globulin regions. Although electrophoretic properties of hemoglobins of *C. perspicillata* do not differ from other phyllostomatid bats (Tamsitt and Valdivieso, 1969), immunologically species of *Glossophaga* (Glossophaginae) and *Carollia* are more closely related than has been inferred from morphological evidence (Gerber and Leone, 1971). Consequently, the taxonomic status of the Glossophaginae and Carolininae warrants further study.

*Glossophaga soricina* from South America and *Monophyllus redmani* from Puerto Rico (Glossophaginae) are similar in albumin mobilities but differ in number and mobilities of globulin fractions. Although similarities in chromosomes between species of *Glossophaga* and *Phyllostomus* were reported by Baker (1970), electropherograms of these taxa are strikingly different (Fig. 3).

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Fig. 3 Schematic diagram of patterns of serum proteins of neotropical bats (cellulose polyacetate). The pattern of normal human serum is given as a reference. Fractions always present in the alpha and beta globulin regions are indicated by dark stippling; fractions that may be absent are represented by light stippling. O marks the origin; anode at left. Mobility of human albumin is indicated by a thin vertical line.
Desmodus rotundus is similar to S. rufum, C. perspicillata, and all species of Artibeus examined except the insular A. jamaicensis in albumin mobility. The pattern of the serum electropherogram of D. rotundus is indistinguishable from those individuals of A. lituratus that exhibit a complete set of five globulin fractions and all but indistinguishable from those A. cf. jamaicensis possessing the same number of globulin fractions. Although once considered to be a distinct family, Desmodontidae (Anderson and Jones, 1967), vampire bats are now considered to be a subfamily of the Phyllostomatidae (Koopman and Jones, 1970). Immunological results (Gerber and Leone, 1971), karyotypic information (Forman et al., 1968), and data from electrophoretic analyses of hemoglobin (Tamsitt and Valdivieso, 1969), as well as data presented here on serum proteins, support this taxonomic allocation.

Of the molossids, insular Molossus fortis is similar to mainland M. molossus in albumin mobility, but the former differs from the latter by a larger number of fractions in the globulin regions. Although the molossid bats and the vespertilionid bat Eptesicus fuscus are similar to certain phyllostomatid bats in albumin mobility, these species differ from all phyllostomatid bats studied to date in electrophoretic properties of hemoglobin (Tamsitt and Valdivieso, 1969) and lactate dehydrogenases (Valdivieso et al., 1968). E. fuscus (Vespertilionidae) is similar to Molossus species in albumin mobility and in mobility of the two fractions in the beta region but differs by mobilities of the fractions in the alpha region.

Data on serum protein concentrations (total protein) have been published on hedgehogs (Morris and Rudge, 1970), deer (Seal and Erickson, 1969), and other mammals, primarily domestic (Schalm, 1970). A wide range (6-11 g/100 ml) has been reported in total protein of mammals, and concentrations reported here (4.1-6.5 g/100 ml) for bats are toward the lower extreme of values (Table ii). Although differences among reports may result from analytical procedures, as most methods are influenced by variations in technique (King, 1964), reasons for reduced concentrations of protein in bat sera remain unexplained.

The sera or plasma of many different mammals including rodents (Nadler, 1968; Dalby and Lillevik, 1969), artiodactyls (Nadler et al., 1967; Seal and Erickson, 1969), and cetaceans (Gallien et al., 1970) have been studied electrophoretically, and data have been applied to the systematics of groups at various levels of the taxonomic hierarchy. In some groups serum protein patterns are useful to distinguish species (van Tets and McT. Cowan, 1966; Petersen, 1968; and others), whereas in others they are phylogenetically conservative (Gallien et al., 1970). Certain fractions of sera have been demonstrated to be polymorphic within a species, particularly albumins, transferrins, haptoglobins, and other post-albumins (see Manwell and Baker, 1970). Few data, however, are available to compare the amount of protein variation in tropical and temperate species; moreover, except for immunologic analyses of some phyllostomatids (Forman et al., 1968; Gerber and Leone, 1971), no data exist on serum proteins of tropical bats except those presented here.
Bats are a suitable group for studies in biochemical systematics, for much of their taxonomy has been well defined by morphological characters. There are nonetheless many interesting taxonomic problems in neotropical Chiroptera, and the recent application of criteria from biochemistry, serology, and particularly karyology (see Baker, 1970) have helped elucidate evolutionary relationships not revealed by conventional morphological characters. The Desmodontinae (vampire bats), once regarded as a distinct family, is now recognized as a subfamily of the Phyllostomatidae (Koopman and Jones, 1970); evidence from karyotypes (Hsu and Bernirshke, 1967) and immunologic analyses (Forman et al., 1968) was instrumental in determining this affinity. Bats of the genus Sturnira, previously considered a separate subfamily (Sturnirinae) of the Phyllostomatidae, were placed in the subfamily Stenoderminae (Koopman and Jones, 1970), an allocation supported by similarities of chromosomes (Gardiner and O'Neill, 1969), hemoglobins (Tamsitt and Valdivieso, 1969), and data presented here on serum proteins.

Speculation or correlation between electrophoretic patterns and developmental processes has been intentionally avoided until the separated proteins can be more fully characterized. But the normal electropherograms of certain species of neotropical bats have been established, and knowledge of such electrophoretic patterns may be useful in other studies. Likewise, cellulose polyacetate patterns of sera of bats may have taxonomic value, for species can be differentiated, and relationships between certain taxa are indicated. Here, for example, we pointed out potential problems concerning relationships among the Phyllostomatidae and the relationship of species of Phyllostomus to other phyllostomatids. Variations in patterns of individuals and populations were considerable but may provide information for intraspecific studies. A thorough analysis of quantitative differences within a single species is needed, as are immunological data to determine homologies of serum fractions among taxa.

Summary

Serum proteins of 75 bats of 18 species of the families Mormoopidae, Phyllostomatidae, Vespertilionidae, and Molossidae from Colombia, Puerto Rico, and Venezuela were compared by cellulose polyacetate electrophoresis. Number of fractions varied from four to eight in electrophoretic patterns. Interspecific differences were observed in the total number of fractions and in their relative mobilities. Although varying in mobility among species, albumin, the most intense and rapidly migrating zone, and the fraction in the gamma globulin region were monomorphic in all species. Polymorphism occurred in the number of fractions in the alpha and beta regions of Phyllostomus hastatus, P. discolor, Glossophaga soricina, Artibeus jamaicensis, A. cf. jamaicensis, A. lituratus, and Carollia perspicillata (Phyllostomatidae).

Comparison of percentage composition of fractions of serum proteins by sex and age without specific allocations resulted in statistically insignificant differences, except that the percentage of fractions in the alpha glob-
ulín region was significantly less in adult than in gravid females. Total protein content (g/100 ml) varied from 4.1-6.5, and differences by species or attributable to sex or age were negligible except that values for an adult *Phyllostomus discolor* were greater than those of a fetus.

Based on similarities of albumin mobilities, four groups were evident: (A) *Pteronotus parnellii* (Mormoopidae); (B) the phyllostomatid bats *Phyllostomus hastatus* and *P. discolor* (Phyllostomatinae); (C) the phyllostomatid bats *Glossophaga soricina* and *Monophyllus redmani* (Glossophilinae), *Sturnira lilium*, *Brachyphylla cavernarum*, *Artibeus jamaicensis* (Stenoderminae), *Erophylla bombifrons* (Phylonycterinae), the vespertilionid bat *Eptesicus fuscus* (Vespertilionidae), and the free-tailed bats *Molossus molossus* and *M. fortis* (Molossidae); and (D) the phyllostomatid bats *Artibeus* cf. *jamaicensis*, *A. lituratus*, *A. phaeotis*, *Stenoderma rufum* (Stenoderminae), *Carollia perspicillata* (Carollinae), and *Desmodus rotundus* (Desmodontinae).

**Resumen**

Se comparan las proteínas de sueros de 75 ejemplares correspondientes a 18 especies de quirópteros de las familias Mormoopidae, Phyllostomatidae, Vespertilionidae y Molossidae de Colombia, Puerto Rico y Venezuela por medio de electroforesis de poliacetato de celulosa. Cuatro a ocho fracciones son visibles en los electroferogramas de estos murciélagos. Similaridades y diferencias cuantitativas y cualitativas son bien aparentes. Diferencias interespecíficas se observan en el número total de fracciones, lo mismo que en sus mobilidades relativas.

Albúmina, la zona más intensa y rápida, varía en ciertas de las especies estudiadas siendo en todos los casos monomórfica así como lo es la fracción correspondiente a la región de la gama globulina.

Cuando se observa polimorfismo en las fracciones proteínicas del suero, éste ocurre únicamente en el número de bandas presentes en las regiones alfa y beta de *Phyllostomus hastatus*, *P. discolor*, *Glossophaga soricina*, *Artibeus jamaicensis*, *A. cf. jamaicensis*, *A. lituratus* y *Carollia perspicillata* (Phyllostomatidae).

Comparaciones en el porcentaje de la composición de las fracciones de estas proteínas por sexo o edad, sin tener en cuenta alocaciones específicas, demuestran solamente una diferencia estadística significante: el porcentaje de fracciones en la región alfa es menor que en hembras y varía de 4.1 a 6.5 sin observarse diferencias referentes a especies, sexo o edades excepto por los valores obtenidos en un *Phyllostomus discolor* adulto en el cual son mayores que aquéllos correspondientes a un feto de la misma especie.

Basándonos en similaridades de mobilidad de albúminas podemos distinguir cuatro grupos diferentes: (A) *Pteronotus parnellii* (Mormoopidae); (B) los murciélagos filostomátidos *Phyllostomus hastatus* y *P. discolor* (Phyllostomatinae); (C) los filostomátidos *Glossophaga soricina* y *Monophyllus redmani* (Glossophilinae), *Sturnira lilium*, *Brachyphylla cavernarum*, *Artibeus jamaicensis* (Stenoderminae), *Erophylla bombifrons*
(Phylloncyterinae), el vespertiliónido *Eptesicus fuscus* (Vespertilionidae) y los molósidos *Molossus molossus* y *M. fortis* (Molossidae); y (D) los filostomátidos *A. cf. jamaicensis*, *A. lituratus*, *A. phaeotis*, *Stenoderma rufum* (Stenoderminae), *Carollia perspicillata* (Carollinae) y el vampiro *Desmodus rotundus* (Desmodontinae).

Las propiedades electroforéticas de las proteínas del suero son taxonómicamente importantes pero pueden representar un valor limitado como indicadores de relaciones filogenéticas en estos mamíferos.

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