Hemoglobin Electrophoresis in the Systematics of Bats (Microchiroptera)

by J. R. Tamsitt¹ and Darío Valdivieso²

Electrophoresis is a most useful procedure for systematically comparing proteins of different organisms, and data from electrophoretic studies of blood proteins have aided in the clarification of phylogenetic relationships and in grouping higher taxonomic categories (Foreman, 1960; Dessauer, 1966; Johnson, 1968). Hemoglobins, unlike some serum proteins, are not affected by diet, age, reproductive state, temperature or other variables. There are, however, few electrophoretic data on bat hemoglobin. Manwell and Kerst (1966) and Mitchell (1966) found considerable individual variation and only minor differences between species in the hemoglobins of temperate bats of the family Vespertilionidae. Valdivieso et al. (1969) compared electrophoretic properties of hemoglobin from Puerto Rican bats of the families Phyllostomatidae, Vespertilionidae and Molossidae and found differences and similarities correlated with taxonomic affinity. Although hemoglobins of closely related species were indistinguishable, Valdivieso et al. (op. cit.) concluded that hemoglobin electrophoresis may be used to add additional evidence to estimates of relationships derived from traditional criteria.

Two distinct electrophoretic phenotypes of hemoglobin occur in bats of the family Vespertilionidae (Manwell and Kerst, op. cit.; Mitchell, op. cit.; Valdivieso et al., op. cit.), but only one phenotype is known in bats of the families Phyllostomatidae and Molossidae (Valdivieso et al., op. cit.). Extensive sampling of additional taxa should indicate whether Neotropical phyllostomatid and molossid bats possess the considerable hemoglobin polymorphism found in temperate vespertilionid bats, whether there are geographic differences in hemoglobins of disjunct populations of the same taxa, and should help determine the worth of hemoglobin electrophoretic properties in systematic studies of microchiropteran bats. We therefore describe here results of new electrophoretic studies on bat hemoglobin which confirm and extend previous observations.

Materials and Methods—Hemoglobin samples were obtained from 109 bats of 13 species collected in Colombia, Venezuela and Ontario in November and December, 1968, and January, 1969 (Table I). Bats were taken in “mist” nets or from culverts in Colombia and Venezuela and from a mine in Ontario. Identifications were made by R. L. Peterson and the senior author (JRT), and specimens are deposited in the mammal collection of the Royal Ontario Museum. The large Artibeus which occurs sympatrically with A. lituratus in west central Colombia was referred by Tamsitt and Valdivieso (1963: 175) to A. jamaicensis jamaicensis, but specimens (ROM 48959, 48981–48986) are indistinguishable from examples from Trinidad described by

¹. Department of Mammalogy, ROM.
². Seneca College, Willowdale, Ontario; Research Associate, Department of Mammalogy, ROM.
Andersen (1906: 420) as *A. planirostris trinitatis*. Although Hershkovitz (1949) considered *A. planirostris* to be synonymous with *A. jamaicensis*, the allocation of this large fruit bat to the species *A. jamaicensis* is questionable (R. L. Peterson, personal communication). Until the taxonomic status of the large *Artibeus* from northern South America is clarified, we therefore only tentatively refer our specimens from the Magdalena River Valley of Colombia to *A. j. trinitatis*.

Blood was obtained by heart puncture with sterile syringes rinsed with heparin, transferred to 0.5 ml tubes, and centrifuged at 3,000 rpm for 20 minutes to separate plasma from the packed cells. The Buffy coat was removed by aspiration with a micropipette. The cells were washed once with cold 0.85 per cent saline and then centrifuged. The hemolysate was prepared by adding 1.5 volumes of distilled water to the packed cells. After thorough mixing, the solution was frozen and thawed three times. The hemolysates were then centrifuged at 4,000 rpm for five minutes, and the supernatant was stored at —5°C. Samples were run as oxyhemoglobin. Before electrophoresis the samples were thawed, shaken with half volume of chloroform, and centrifuged in a Beckman Microfuge for two minutes at 14,000—17,000 rpm to extract methemoglobin and to improve electrophoretic resolution. Electrophoretic mobility characteristics of hemoglobins did not change during cold storage, and patterns were reproducible after two months. Although stabilizing procedures, e.g., derivitization of oxyhemoglobins to carbomonoxy- or cyanomet-forms, have been employed for hemoglobins, these methods were not conveniently adaptable to field conditions and were the motive for our decision to base electropherograms on mobility characteristics of oxyhemoglobins.

Hemolysates were analyzed by electrophoresis in the Model R-101 Microzone Cell (Beckman Instrument Co., Fullerton, California) with Sepaphore III polyacrylamide 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 for 12 seconds, and separation was accomplished at 200 V for 20 minutes. Human hemoglobins A, C and S were run simultaneously with bat hemoglobin as controls. After electrophoresis, hemoglobins were stained with Ponceau S.

**Results**—Only one electropherogram pattern, a single anodal band, was seen in the 13 species at pH 8.6 (Figs. 1–3). The homogeneous hemoglobin components of *Phyllostomus discolor*, *P. hastatus*, *Glossophaga soricina*, *Lonchophylla robusta*, *Carollia perspicillata*, *Sturnira lilium*, *Artibeus phaeotis*, *A. cf. jamaicensis*, *A. lituratus* and *Desmodus rotundus* were identical and indistinguishable from human S. Hemoglobins of *Myotis lucifugus* and *M. subulatus* (Fig. 3, A and C) were identical and had mobilities corresponding to human C. The hemoglobin of *Mo1ossus molossus* (Figs. 2, E, and 3, C) migrated the least and had a mobility slightly more cathodal than human C. Neither clear genetic polymorphism nor intrapopulation or interspecific variation was found in any of the taxa, but only a few individuals of some species were tested, and further sampling may reveal variant patterns. Moreover, hemoglobins of *G. soricina* (Fig. 2, D), *C. perspicillata* (Fig. 1, C) and *A. lituratus* (Fig. 1, D and E), collected in Colombia and Venezuela from localities separated by the Eastern Andean Cordillera and approximately 1,000 airline kilometers apart, were indistinguishable, as were those of *P. discolor* and *A. lituratus* from Colombian localities 55 and 85 airline kilometers distant in the Magdalena River Valley and the western slopes of the East Andean Cordillera. No relationship was evident between pattern types and sex, age (neonate, juvenile, young adult or adult) or in female reproductive condition (nonparous, pregnant, lactating or post-lactating). Although fetal hemoglobin distinct from adult hemoglobin occurs in some mammals (reviewed by Manwell, 1960), patterns for juvenile and adult *D. rotundus* (Fig. 2, A) and for fetal and adult *P. discolor* (Fig. 2, B) do not differ.*

*The reduced amount and lower concentration of fetal hemoglobin account for the differences seen in Fig. 2, B in the width of the hemoglobin bands of adult and fetal *P. discolor*. 
Discussion—We encountered three electrophoretically different hemoglobins in this study. With the exception of the vampire bat (*D. rotundus*), there was in general a correlation of hemoglobin pattern with taxonomic relationship. One electrophoretic pattern, an anodal band corresponding to human S, was found in genera and species of the subfamilies Phyllostomatinae, Glossophaginae, Carollininae, Sturnirinae and Stenoderminae of the family Phyllostomidae (Table II, Fig. 4). This hemoglobin electrophoretic pattern is identical to that reported by Valdivieso et al. (1969) for the phyllostomatid genera *Monophyllus* (subfamily Glossophaginae), *Artibeus, Stenodermna* (subfamily Stenoderminae) and *Erophylla* (subfamily Phyllonycterinae) from Puerto Rico. Excluding *Chilonycteris* (subfamily Chilonycterinae), whose hemoglobin is different (Valdivieso et al., op. cit.) and whose status as a subfamily of the Phyllostomatidae has been questioned (Machado-Allison, 1967), the hemoglobins of the 13 species of phyllostomatids analyzed to date are invariably the same (Table II). We therefore find a reasonable amount of support from “classical” sources (Miller, 1907) for a close relationship among the subfamilies of the phyllostomatid complex as defined by hemoglobin electropherograms.

The relationship of one genus, *Sturnira*, to other Phyllostomatidae is still not clear. Although Miller (op. cit.) placed the genus in a separate subfamily because of its highly specialized tooth structure, de la Torre (1961) contended that *Sturnira* should be placed with the Stenoderminae. On the basis of chromosome similarities, Baker (1967) and Gardner and O’Neill (1969) concurred with de la Torre, as did Wenzel et al. (1966) after a study of parasitic flies (Strebliidae) and their hosts. The morphology of sturnirine spermatozoa, however, differs from that of other phyllostomatids (Forman, 1968), and certain mites (Spinturniciidae) parasitizing *Sturnira* are unique to the group (Machado-Allison, 1965). Although our limited hemoglobin data do not distinguish *Sturnira* from other phyllostomatids, the relationship of the sturnirines with the carollines (see Walton and Walton, 1968), on the one hand, and with the glossoptagines (see Baker, 1967), on the other, needs clarification before subfamilial status is disregarded and the group is allied with the stenodermines.

An interesting finding was that the hemoglobin of the vampire bat (*Desmodus rotundus*) was electrophoretically indistinguishable from those of bats of the family Phyllostomatidae. Although extensive modifications, reflecting adaptations to sanguinorous feeding habits, indicate considerable temporal isolation of the desmodontids from the phyllostomatids, evidence is accumulating to suggest that vampire bats are phylogenetically more closely allied to members of the family Phyllostomatidae than is implied by current classification. Miller (1907), and subsequently Simpson (1945), Cabrera (1958), Hall and Kelson (1959) and Anderson and Jones (1967), recognized the Desmodontidae, but Dobson (1875), Winge (1941-42) and Bourlière (1955) considered the desmodontids as a subfamily of the Phyllostomatidae (cf. Walton and Walton, 1968, for a recent summary of early nomenclatorial history and synonymy). Data from host-ectoparasite relationships (Machado-Allison, 1967; Wenzel et al., 1966), spermatozoa morphology (Forman et al., 1968), and karyotypes (Hsu and Benirschke, 1967; Forman et al., op. cit.) indicate a close relationship between the desmodontids and the Phyllostomatidae. Moreover, the degree of development of the neocortex of *Desmodus* is comparable to phyllostomatids (Mann, 1963), as are orientation sounds and behaviour (Griffin and Novick, 1955; Novick, 1963), palate and wing structure (Miller, op. cit.) and post-cranial skeletal elements (Walton and Walton, op. cit.). Although the hemoglobin morphs of *Desmodus* and nine genera of six subfamilies of the Phyllostomatidae are identical (Table II; Fig. 4), peptide mapping might show that these specific hemoglobins differ in primary structure, as Foreman (1964) has shown in rodents with nearly identical hemoglobin ionograms. In our opinion a detailed study of hemoglobin and additional biochemical characters of the involved genera (*Desmodus, Diaemus, Diphylla*) is needed before vampire bats are assigned as a subfamily of the family Phyllostomatidae.
A second electrophoretic pattern was seen in bats of the family Vespertilionidae. In *Myotis lucifugus* and *M. subulatus* from Ontario, hemoglobin migration was the same and approximately equal to human C (Table II, Fig. 4). A comparable hemoglobin was found by Mitchell (1966) in *M. lucifugus* and *M. griseus* from Missouri. Manwell and Kerst (1966), on the other hand, found a slowly moving major and a more rapidly moving anodal zone in both *M. lucifugus* and *M. keenii* from Illinois. Populations of *M. lucifugus* from Missouri, Illinois and Ontario represent the same subspecies (*M. l. lucifugus*), and in the absence of intrapopulational variation, these geographically different phenotypes are not easily interpreted unless hemoglobin is polymorphic in this species. Comparable geographic differences have been found in *Eptesicus fuscus*. Heterogeneous hemoglobins were found by Manwell and Kerst (1966) in *E. f. fuscus* from Illinois and by Valdivieso et al. (1969) in *E. f. wetmorei* from Puerto Rico, but Mitchell (op. cit.) found a homogeneous hemoglobin in Missouri *E. f. fuscus*. In other vespertilionids, e.g., Pipistrellus subflavus and Plecotus townsendii, however, similar heterogeneous hemoglobins have been found in both Illinois and Missouri. Differences in electrophoretic technique could account for these differences, but this is unlikely as Mitchell (op. cit.) and Valdivieso et al. (op. cit.) used the same electrophoretic procedure (cellulose polyacetate) but obtained different results. A more logical explanation to account for the presence of more than one adult hemoglobin in *M. lucifugus* and *E. fuscus* is polymorphism. But the numbers of *Eptesicus* and *Myotis* and the geographic areas sampled to date have been small, and extensive sampling throughout the range of these and other vespertilionids is needed to determine the degree of allelic variation and also to determine whether we are correct in attributing these differences to polymorphism.

A third electrophoretic pattern, a slow anodal band, was seen in *Molossus molossus* of the family Molossidae (Table II, Fig. 4). This same pattern has been found as well in the molossid bats *Tadarida brasiliensis* (Johnson and Wicks, 1959) and in *M. m. fortes* from Puerto Rico (Valdivieso et al., 1969). Although serum proteins (Johnson and Wicks, op. cit.) and immunologic data (Forman et al., 1968) do not differentiate the Molossidae from the Vespertilionidae, hemoglobin and karyotype differences (Baker and Patton, 1967; Wainberg, 1966) support Miller's (1907) interpretation that the two groups of insectivorous bats are sufficiently distinct to merit familial separation. Furthermore, lactate dehydrogenase patterns of *Molossus* and *Eptesicus* tissues, although similar, are distinguishable and different from those of phyllostomatid bats (unpublished data).

The similarities and differences we have found among bat hemoglobins correspond in large part to the phylogeny presented by Simpson (1945). The hemoglobins of members of the superfamilies Phyllostomatoidea and Vespertilionoidea differ from each other more than the hemoglobins of bats within each of these groups differ. Except for the Chilonycterinae, evidence to date suggests that the hemoglobins of the Phyllostomatoidea are quite similar. Hemoglobins of the Vespertilionoidea, on the other hand, appear to differ among themselves far more than do the hemoglobins of the Phyllostomatoidea.

Recent publications (Thompson et al., 1966; Foreman, 1968; Rasmussen et al., 1968; Self A., 1968) have revealed not only hemoglobin variation between species of the same genus but also the occurrence of polymorphism within a given species in the order Rodentia. This type of variation, however, is not always the case, as there is a remarkable homogeneity between the hemoglobins of Microchiroptera (Manwell and Kerst, 1966; Valdivieso et al., 1969; this study). Because the rate of hemoglobin change is low, based on the amount of variation found within and between closely related genera, similarities in bat hemoglobin electrophoretic patterns, although of limited taxonomic value, may be indicative of phylogenetic relationships. Hemoglobin analysis certainly supports the validity of the common origin of the Phyllostomatidae and the Desmodontidae, whose genera have hemoglobins indistinguishable from one another but different from those of the Vespertilionidae and Molossidae.
Figure 1
Electrophoretic patterns of erythrocyte hemolysates at pH 8.6. Arrows indicate baselines of sample application.
A. Carollia perspicillata (Venezuela): 1-3, 5-8, δ δ; 4, Human A.
B. C. perspicillata (Venezuela): 1-3, 5-8, ♀ ♀; 4, Human S.
C. C. perspicillata; 1, 2, δ δ (Colombia); 3, δ (Venezuela); 4, Human S; 5-8, ♀ ♀ (Venezuela).
D. Artibeus lituratus: 1-4, δ δ (Colombia); 5-7, δ δ (Venezuela); 8, Human CA.
E. A. lituratus: 1, 2 (Colombia); 2-4, ♀ ♀ (Venezuela); 5-7, δ δ (Venezuela); 8, Human CA.
F. A. cf. jamaicensis: 1-3, ♀ ♀ (Colombia); 4, 5, δ δ (Colombia); A. phaeotis: 6, 7, δ δ (Colombia); 8, Human CA.

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Figure 2
Electrophoretic patterns of erythrocyte hemolysates at pH 8.6. Arrows indicate baselines of sample application.

A. Desmodus rotundus (Colombia): 1, juvenile δ; 2-4, ♀ ♂; 5, Human CA.
B. Phyllostomus discolor (Colombia): 1, δ fetus; 2-4, δ δ; 5, Human CA.
C. P. hastatus (Venezuela): 1, δ ♂; 2-4, ♀ ♀; 5, Human CA.
D. Glossophaga soricina: 1-3, δ δ (Venezuela); 4, ♀ ♀ (Venezuela); 5, ♀ (Colombia); 6, Human CA.
E. Molossus molossus (Colombia): 1, 2, δ δ; 3-5, ♀ ♀; 6, Human CA.

Summary—Hemoglobin of 109 examples of 13 species of bats from Colombia, Venezuela and Canada were compared by cellulose polyacetate electrophoresis. All bats exhibited a single-banded hemoglobin phenotype. The phyllostomatid bats Phyllostomus discolor, P. hastatus (subfamily Phyllostomatinae), Glossophaga soricina, Lonchophylla robusta (subfamily Glossophaginae), Carollia perspicillata (subfamily Carollininae), Sturnira lilium (subfamily Sturnirinae), Artibeus phaeotis, A. cf. jamaicensis and A. lituratus (subfamily Stenoderminae) had indistinguishable hemoglobin morphs. Hemoglobin electropherograms of the vampire bat, Desmodus rotundus (family Desmodontidae), and the phyllostomatid bats could not be differentiated, suggesting a closer relationship of the desmodontid to the phyllostomatid bats than is implied by current classification. The hemoglobins of the vespertilionid bats Myotis lucifugus and M. subulatus were identical but differed from the phyllostomatid and desmodontid bats by...
Electrophoretic patterns of erythrocyte hemolysates at pH 8.6. Arrows indicate the baselines of sample application.

A. 1, Lonchophylla robusta ♀ (Colombia); 2, Sturnira lilium ♂ (Colombia); 3, S. lilium ♀ (Colombia); 4, Artibeus cf. jamaicensis ♂ (Colombia); 5, Myotis subulatus ♂ (Ontario); 6, Human CA.

B. 1, Phyllostomus hastatus ♂ (Venezuela); 2, Glossophaga soricina ♂ (Venezuela); 3, Carollia perspicillata ♂ (Venezuela); 4, S. lilium ♂ (Colombia); 5, A. lituratus ♀ (Venezuela); 6, Human CA.

C. 1, A. lituratus ♂ (Venezuela); 2, Desmodus rotundus ♀ (Colombia); 3, Molossus molossus ♂ (Colombia); 4, Myotis lucifugus ♂ (Ontario); 5, M. molossus ♀ (Colombia); 6, Human CA.

Figure 3

A slower migration. A slow, almost isoelectric hemoglobin characterized the molossid bat Molossus molossus and distinguished it from bats of the families Phyllostomatidae, Desmodontidae and Vespertilionidae.

Electropherograms were independent of sex and age, and there was no variation in the electrophoretic patterns of individuals of a species from the same or different localities. Electrophoretic properties of bat hemoglobin have limited taxonomic value at lower levels of organization but are useful in phylogenetic analysis.

Resumen—Se comparan las hemoglobinas de 109 ejemplares correspondientes a 13 especies de quirópteros de Colombia, Venezuela y Canadá por medio de electroforesis de poliacetato de celulosa. El fenotipo exhibido por todos los murciélagos es de una banda simple de hemoglobina. Los quirópteros filostomátidos Phyllostomus discolor, P. hastatus (subfamilia Phyllostomatinae), Glossophaga soricina, Lonchophylla robusta (subfamilia Glossophaginae), Carollia perspicillata (subfamilia Carolliniae), Sturnira lilium (subfamilia Sturnirinae), Artibeus
Artibeus phaeotis, A. cf. jamaicensis and A. lituratus (subfamilia Stenoderminae) presentan morfos idénticos de hemoglobinas. Los electroferogramas del vampiro, Desmodus rotundus (familia Desmodontidae), y de los filostomátidos no pueden diferenciarse, lo cual sugiere un parentesco más cercano entre los desmodóntidos y filostomátidos de lo implicado en la clasificación actual. Las hemoglobinas de los vespertilionídos Myotis lucifugus y M. subulatus son idénticas pero diferen de las de los filostomátidos y de los desmodóntidos por presentar migraciones más lentas. Una hemoglobina lenta, casi isoelectría, caracteriza al molósido Molossus molossus distinguiéndolo de los filostomátidos, desmodóntidos y vespertilionídos.

Diferencias en sexo y edad no afectan los patrones de los electroferogramas. No existe variación en los modelos electroforeticos de ejemplares de una especie ya sea de la misma o de diferentes localidades. Las propiedades electroforeticas de la hemoglobina quiropteriana tienen valor taxonómico limitado en niveles de organización bajos pero son de utilidad en análisis filogenético.

Figure 4
Intergeneric and interspecific comparison of hemoglobin morphs of bats. The circle represents the origin.
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<td>8</td>
</tr>
<tr>
<td>Myotis subulatus</td>
<td>Adult ♂</td>
<td>1</td>
<td>&quot;</td>
<td>8</td>
</tr>
<tr>
<td>Molossus molossus</td>
<td>Adult ♂</td>
<td>2</td>
<td>December 22, 1968</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Adult ♀</td>
<td>2</td>
<td>&quot;</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Lactating ♀</td>
<td>1</td>
<td>&quot;</td>
<td>3</td>
</tr>
</tbody>
</table>

**KEY TO LOCALITIES**

**Colombia**
1. Cundinamarca: Mesitas del Colegio (1210 m).
2. " : Pacho (1859 m).
3. " : Villeta (804 m).
4. Tolima: Melgar (430 m).
5. " : 9 km NW Melgar (approx. 430 m).

**Venezuela**
7. Lara: 7 km E Barquisimeto (566 m).

**Canada**
TABLE II

Grouping of species of bats by hemoglobin electropherograms.* Heterogeneous hemoglobins are designated by the subscript “1” for the anodal band and “2” for the cathodal band.

<table>
<thead>
<tr>
<th>Origin Zone</th>
<th>Human Hb-C</th>
<th>Human Hb-S</th>
<th>Human Hb-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Pipistrellus subflavus₂</td>
<td>Myotis lucifugus</td>
<td>Chilonycteris parnellii</td>
</tr>
<tr>
<td>3</td>
<td>Eptesicus fuscus₂</td>
<td>Myotis grisescens</td>
<td>Myotis subulatus</td>
</tr>
<tr>
<td>4</td>
<td>Tadarida brasiliensis</td>
<td>Glossophaga soricina</td>
<td>Monophyllus redmani</td>
</tr>
<tr>
<td>5</td>
<td>Molossus molossus</td>
<td>Lonychophylla robusta</td>
<td>Artibeus phaetos</td>
</tr>
<tr>
<td></td>
<td>Pipistrellus subflavus₁</td>
<td>Artibeus jamaicensis</td>
<td>Artibeus cf. jamaicensis</td>
</tr>
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<td></td>
<td>Myotis subulatus</td>
<td>Artibeus lituratus</td>
<td>Artibeus lituratus</td>
</tr>
<tr>
<td></td>
<td>Myotis lucifugus</td>
<td>Myotis lucifugus</td>
<td>Myotis lucifugus</td>
</tr>
</tbody>
</table>

*Data from Johnson and Wicks (1959), Mitchell (1966), Valdivieso et al. (1969) and this study. Published information from species whose hemoglobins were subjected to electrophoresis with reference hemoglobins other than human are not included in the table.

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