heating, are denatured, or coagulated.

The pigmented epithelium absorbs approximately 70 percent of the light at 6943 Å. However, its abosrption coefficient changes with wavelength; at 10,600 Å, approximately 20 percent of the light is absorbed. The absorption of the retina itself is slightly higher at 10,600 Å than at 6943 Å, so that at 10,600 Å the neural layers of the retina would be more highly absorbing than the pigment epithelium (*6*). The neodymium laser radiates at 10,600 Å. It therefore seemed likely that the retinal pathology of a chorioretinal burn from a neodymium laser would differ from that of a ruby laser burn. The pathology of an injury in the foveal region resulting from a pulse from a ruby laser is shown in Fig. 1*A*. A large patch of vesicular swelling (probably a gas pocket) is formed in the pigment epithelium, which displaces the rest of the retina. This lesion is accompanied by some degeneration of the adjacent choroid layer. In Fig. 1*B* is shown the result of a burn in the foveal region produced by pulse from a neodymium laser. Little of the energy seems to have been absorbed by the pigment layer, there is no vesiculation in the pigment layer, and there is relatively much greater destruction in all other layers of the retina than in Fig. 1*A*. The amount of energy necessary to cause such an injury is larger than that in the case of the ruby laser because the total absorption at this wavelength is smaller.

These results suggest that the biological hazards and uses of the laser may be a function of the wavelength of the radiating light. For example, the neodymium laser would seem to be almost worthless for photocoagulation, as inflammation of the pigment epithelium seems to be necessary to produce adhesions to hold the retina down. However, the neodymium laser would be more likely to cause involvement of the nerve fiber layer, especially in the region of the optic disk; large portions of the eye could be rendered functionally useless by the destruction of the nerve tracts leading from the disk. Thus the neodymium laser is essentially a greater source of ocular hazard than the ruby laser, when energy necessary to produce lesions of comparable size is present.

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16 August 1965

Hemoglobin and Transferrin Electrophoresis and Relationships of Island Populations of Anolis Lizards

Abstract. *Electrophoretic differences in hemoglobins and transferrins serve to differentiate* Anolis *of the same species populating the Lesser Antilles. When based on protein analyses, identifications of individuals may be less equivocal than identification based on descriptions of skin color and markings. Protein differences among island populations appear to be associated with long periods of geographic isolation. Such evidence often confirms estimates of relationship derived from more traditional criteria and in some cases provides a basis for formulating new taxonomic conclusions.*

The lizard genus *Anolis* occurs in large numbers and in a variety of forms throughout the islands of the West Indies (*1*). Many forms have differentiated into definitive species; others represent populations in intermediate stages of species formation. One way of estimating the degrees of divergence of such closely related organisms is by comparison of similarities and differences between tissue proteins (*2*). In our study the electrophoretic behavior of transferrins and hemoglobins was used to gage relationships of certain lizards of the eastern Caribbean, *Anolis roquet* from five islands and British Guiana (Fig. 1) and *Anolis richardi* from four islands (Fig. 2).

Live lizards were shipped to Louisiana State University School of Medicine. Blood, collected after decapitation or by cardiac puncture, was heparinized and centrifuged, and the red cells were separated from the plasma. Cells were washed three times with 1 percent saline, exposed to carbon monoxide to form the CO-hemoglobin derivative, and hemolyzed by alternate freezing and thawing. Plasma and red-cell proteins were analyzed by vertical starch-gel electrophoresis in 0.02*M* sodium borate buffer, *p*H 8.6. Transferrin, the iron-binding protein of plasma, was localized on gel electropherograms by autoradiography (*3*).

Anolis roquet. We have examined blood from each of five named subspecies of *Anolis roquet* (*4, 5*): 35 specimens of *A. r. roquet* from Martinique; 21 *A. r. extremus* from Barbados; 12 *A. r. gentilis* from Bequia; 18 *A. r. aeneus* from Trinidad; and 13 of the same subspecies from British Guiana. Color and pattern differences of this medium-sized lizard have been the major characters used for distinguishing subspecies; however, identification based on these criteria may be equivocal. Even on a single island color and pattern variation, independent of chromatophoric activity, can be startling. On Martinique, with an area of only 975 km^2, ground color of lizards may be deep green, pale yellow, or gray brown. Markings may be white dots or black chevrons. Such variation appears strongly correlated with ecological conditions. Green forms are found in wet montane areas; gray-brown forms occur in the dry areas. A similar situation prevails in Grenada.

Electrophoretic properties of blood proteins are less variable than color and markings. Animals, representing color extremes from four disjunct populations from Martinique, have identical transferrins, hemoglobins, and patterns of plasma proteins. *Anolis r. extremus* of Barbados, likewise, exhibits no intra-island variation. Specimens from the southernmost area of the range of *Anolis roquet*, described as three subspecies (*4, 5*), are electrophoretically indistinguishable. We can not differentiate Trinidad and British Guiana *A. r. aeneus*, Grenada *A. r. cinereus*, and Bequia *A. r. gentilis*

(Fig. 3, Nos. 1, 2, and 3). For convenience we shall refer to these populations as the "*aeneus* complex." In contrast, *A. r. roquet, A. r. extremus,*

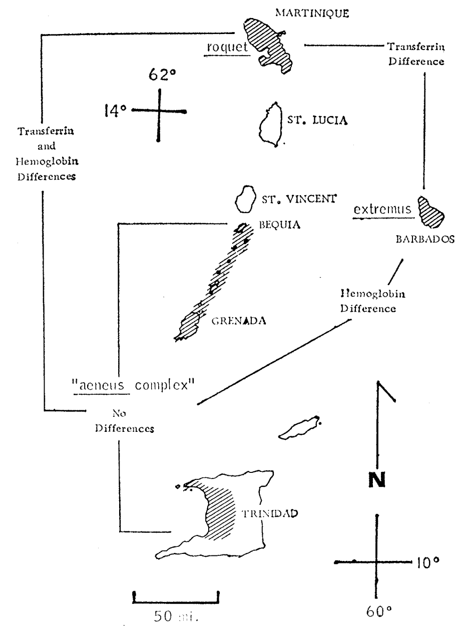

Fig. 1. Hemoglobin and transferrin distribution among island populations of *Anolis roquet*. Shaded areas denote range of the species.

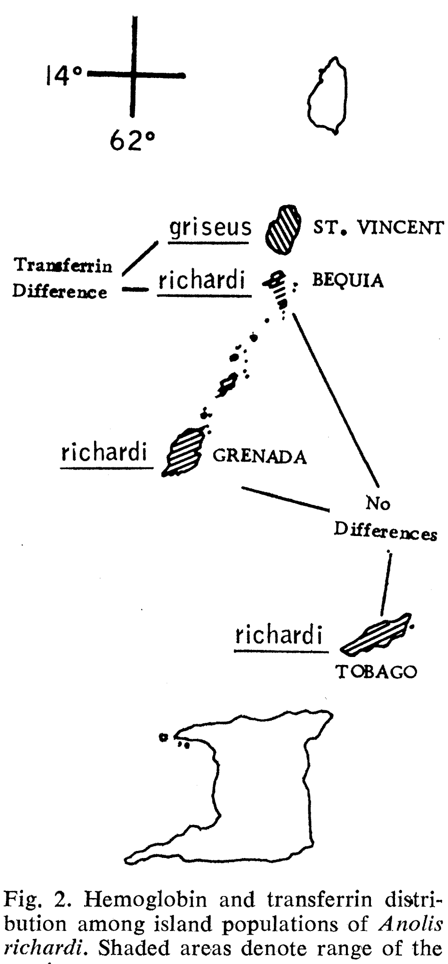

Fig. 2. Hemoglobin and transferrin distribution among island populations of *Anolis richardi*. Shaded areas denote range of the species.

and the "*aeneus* complex" are readily differentiated from each other. Plasma of *A. r. extremus* differs in transferrin pattern from that of *A. r. roquet* and has a different hemoglobin from the "*aeneus* complex." Both hemoglobin and the transferrins of the "*aeneus* complex" distinguish it from *A. r. roquet*, the northernmost subspecies (Fig. 1; Fig. 3, Nos. 1 to 5).

A recent reexamination of more standard evidence also suggests that animals of the *aeneus* complex should be grouped into a single taxonomic unit. The range of color variation among populations of *A. r. cinereus* on Grenada, as observed by one of us (G.C.G.), virtually encompasses that of *A. r. gentilis* and *A. r. aeneus*, making these three named forms taxonomically inseparable. Trinidad and British Guiana populations may be recent introductions from Grenada. The populations are restricted to major port towns, whereas *Anolis roquet* in other parts of its range is found in all habitats, from sea level to 900 m, from very dry areas to forest, from wilderness to township. Further, most individuals are gray, even though they are in verdant areas, whereas *A. roquet* normally is green.

Anolis richardi. We have examined blood from both subspecies of a related, though much larger lizard, *Anolis richardi* (*4*): 13 specimens of *A. r. griseus* from St. Vincent; 9 *A. r. richardi* from Bequia, 9 from Grenada, and 18 from Tobago. In this species also, proteins of animals from a single island exhibit remarkably constant electrophoretic properties. Even populations of *A. r. richardi* from three widely separated islands are very similar. The two subspecies, however, are clearly distinguishable on the basis of their transferrins (Fig. 2; Fig. 3, Nos. 6 and 7). St. Vincent is only 11.2 km north of Bequia, yet populations of lizards on the two islands have different transferrins. In contrast, Bequia is 96 km north of Grenada but populations of *A. r. richardi* on the two islands have identical proteins. These findings may have biogeographical significance. St. Vincent has never been connected to islands to the south; however, Bequia and Grenada lie on the same bank and are known to have been connected during the Pleistocene (*5*). Thus *Anolis richardi* of Grenada and the Grenadines have had more recent opportunity for genetic exchange. Tobago *A. r. richardi*, having proteins electrophoretically identical with those

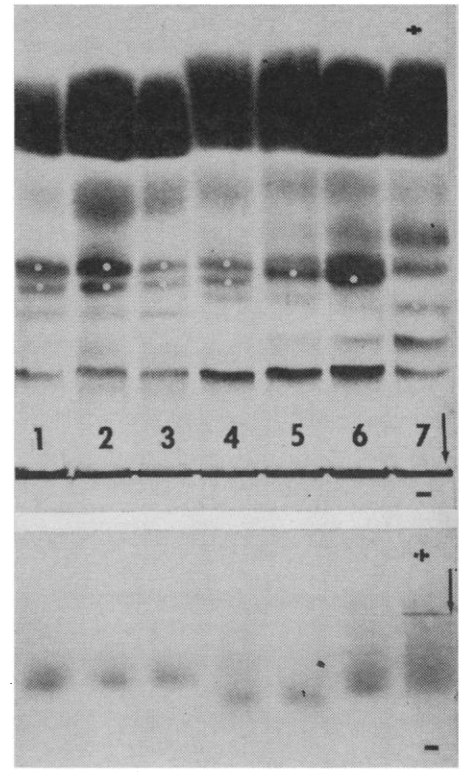

Fig. 3. Electrophoresis patterns of plasma proteins (top) and hemoglobins (bottom). Dots identify transferrins. Arrows indicate baselines of sample application. (1) *Anolis roquet aeneus* from Trinidad; (2) *A. roquet gentilis* from Bequia; (3) *A. roquet cinereus* from Grenada; (4) *A. roquet extremus* from Barbados; (5) *A. roquet roquet* from Martinique; (6) *A. richardi griseus* from St. Vincent; (7) *A. richardi richardi* from Bequia.

of animals from the Grenada bank, probably have not been on Tobago long enough to have become differentiated.

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