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CHARACTERISTIC ELECTROPHORETIC PATTERNS OF SERUM PROTEINS OF SEVERAL SPECIES OF SNAKES OF IRAN (*)

By

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Abstract

Paper and starch-gel electrophoresis of serum proteins of several species and subspecies of poisonous and nonpoisonous snakes of Iran have been investigated. The patterns obtained, especially by means of the starch-gel method, are characteristic for each species. Electrophoretic patterns of samples of serum from different individuals of the same species were very similar.

Introduction

Paper and starch-gel electrophoresis of serum proteins of seven species and subspecies of poisonous and nonpoisonous snakes captured in various geographic localities of Iran have been investigated. These snakes include *Natrix tessellata, Coluber ravergierii, Coluber jugularis erythrogaster*, and *Coluber jugularis caspicus*, which are nonpoisonous, and *Vipera lebetina turanica* and *Naja naja oxiana*, which are poisonous.

Materials and Methods

Blood was collected from adult animals by heart puncture or from the veins. The blood was allowed to stand at room temperature overnight and the serum was separated by mild centrifugation on the next day. The samples were examined on the same day or were frozen at −20 °C until used. Some of the sera were also stored in a refrigerator at 4 °C under sterile conditions.

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Paper electrophoresis of individual sera of each species and subspecies was performed using barbital-sulfate of pH 8.6 and an ionic strength of 0.1. All protein separations were carried out on a horizontal apparatus as described by Grassmann *et al.* (1). Whatman No. 1 filter paper was used.

Vertical starch-gel electrophoresis as described by Smithies (2) was also applied to the study of the serum proteins of these snakes. Hydrolyzed starch* was used to make the gels. Gels were prepared in 0.030 *M* borate buffer of pH 8.6 with the bridge solution being 0.30 *M*. Occasionally, the discontinuous system of Tris-borate buffer (3) was also used. Electrophoresis was run for 16 hours with a voltage gradient of 4-5 volts per centimeter.

Results

Sera from two to five different specimens of each form were examined. The forms belonging to the genus *Coluber* yielded nearly identical patterns on paper electrophoresis in having two albumins, two alpha-globulins, two beta-globulins, and a faintly staining diffused band corresponding to gamma-globulin of normal human serum. The nomenclature adopted for the protein zones of these forms is taken from other investigators working on some similar reptiles (4-6). Within a given species individual variations were insignificant on paper electrophoresis. The patterns given by *Vipera lebetina turanica* and *Naja naja oxiana* differed somewhat from the rest of the species in having fewer protein zones on paper electrophoresis. No attempt has been made to give the relative percentages of these various zones, because the number of specimens available was limited.

In Figs. 1 and 2, typical patterns of each species and subspecies of these three orders are shown, for paper as well as starch-gel electrophoresis. There were differences between the starch-gel patterns of the various species of these snakes and also differences between the three subspecies of the form *Coluber jugularis*. There was little individual qualitative variation among the specimens belonging to each form, except in the case of *Coluber jugularis erythrogaster* in which the two specimens tested were found to be different from each other in their starch-gel patterns. Unfortunately, no information was available about their sex, but these two individuals were captured from two geographically different areas of Iran.

Discussion

From the starch-gel patterns of these seven forms, it may be suggested that the further apart these forms are phylogenetically, the more the electrophoretic patterns of their sera differ. The overall patterns belonging to the three subspecies of *Coluber jugularis* are alike. *Naja naja oxiana* and *Vipera lebetina turanica* differ from each other and from the rest of the species in their starch-gel electrophoretic patterns.

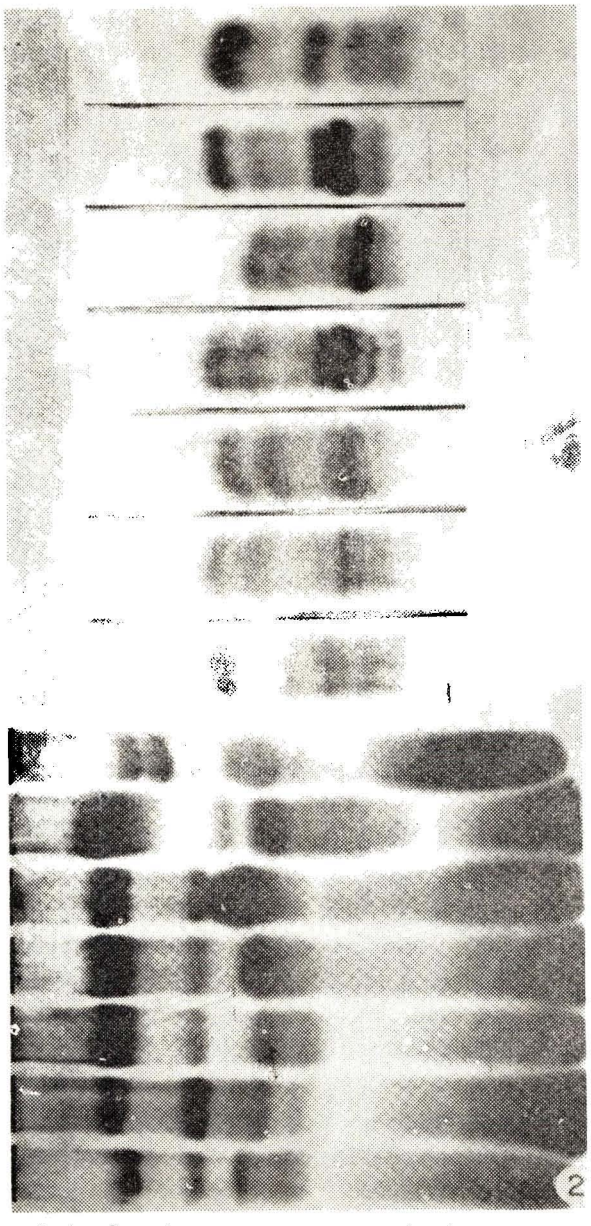

Fig. 1. Paper electrophoresis of seven species and subspecies of snakes captured in various geographic localities of Iran. The anode is to the left. The strips belong, from top to bottom, to *Natrix tessellata, Coluber ravergierii, Coluber jugularis asianus, C. jugularis erythrogaster, C. jugularis caspicus, Vipera lebetina turanica,* and *Naja naja oxiana.*

Fig. 2. Starch-gel electrophoresis of seven species and subspecies of snakes captured in various geographic localities of Iran. The anode is to the right. The strips belong, from top to bottom, to *Natrix tessellata, Coluber ravergierii, Coluber jugularis asianus, C. jugularis erythrogaster, C. jugularis caspicus, Vipera lebetina turanica,* and *Naja naja oxiana.*

Sera from all these forms were found to be toxic, when tested in mice being neurotoxic as well as hemolytic. To find any possible common antigenic determinants between the toxins found in snake serum and those present in venom, the antivenin produced against *Vipera lebetina turanica* in horse was added to the serum of this species and incubated at 37 °C for 30 minutes. No flocculation was produced, and the toxicity of the serum was unaffected when injected into mice, indicating that there is apparently no serological relationship between the toxins present in serum and those present in venom. When solutions of lyophilized venoms of *Vipera lebetina turanica* and *Naja naja oxiana* were examined by starch-gel electrophoresis along with the sera of these two species, the patterns of the venoms were found to be completely different from their corresponding sera belonging to the same species.

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