HEMOGLOBIN POLYMORPHISM IN INDIAN FROG (*RANA TIGRINA*)

By

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Hemoglobin of different species of frog has been studied by various workers (2,7,8,9,13). Review of literature failed to reveal similar reports on the Indian frog (Rana tigrina). In order to compare hemoglobin of this specie with that of other species of its family, a preliminary characterization of the hemoglobin of Rana tigrina was undertaken. Results of these studies have been presented in this communication.

MATERIALS AND METHODS

Adult frogs of either sex of the specie Rana tigrina were used in this study. Blood was obtained as described elsewhere(2). Hemoglobin solution was prepared by the usual method. To prevent autoxidation, the hemoglobin solution was immediately converted into cyanmethemoglobin and was used in all studies unless otherwise stated.

Horizontal starch gel electrophoresis was performed in Tris-EDTA-Boric acid buffer pH 8.6 (5), and the gels stained with O-dianisidine*. Column chromatography was performed on CM-Sephadex (Sigma) cation exchanger, 200-400 mesh, as described by Madlo and Sulo (10). A pH gradient of 0.01M phosphate buffers containing 100 *mg* KCN/1 lit. with a pH range from 6.5 to 8.5 was employed for elution purpose. The effluents—approximately 4 *ml.* in each tube—were read at 415 *mu* in a junior spectrophotometer. Alkali denaturation was done by the modified technique of Chernoff(4).

RESULTS AND DISCUSSION

Hemoglobin polymorphism in species other than Rana tigrina has been reported in literature (8, 9, 12, 13). Fig. 1 clearly shows dimorphism in the hemoglobin of Rana tigrina. The slower fraction is major while the one which moves faster is minor. There is an evident difference between the mobilities of native hemoglobin (Center slot) and cyanmethemoglobin (top and bottom slots), Fig. 1, as reported by Chernoff and Pettit (6) for human hemoglobins. Chieffi *et al.* (3) reported two fractions in the hemoglobin of Rana esculents L. This corroborates our findings for dimorphism in the hemoglobin of Rana tigrina. Five heme containing fractions in the hemoglobin of Rana esculenta L has been reported by Tentori *et al.* (12), whereas, Baglioni and

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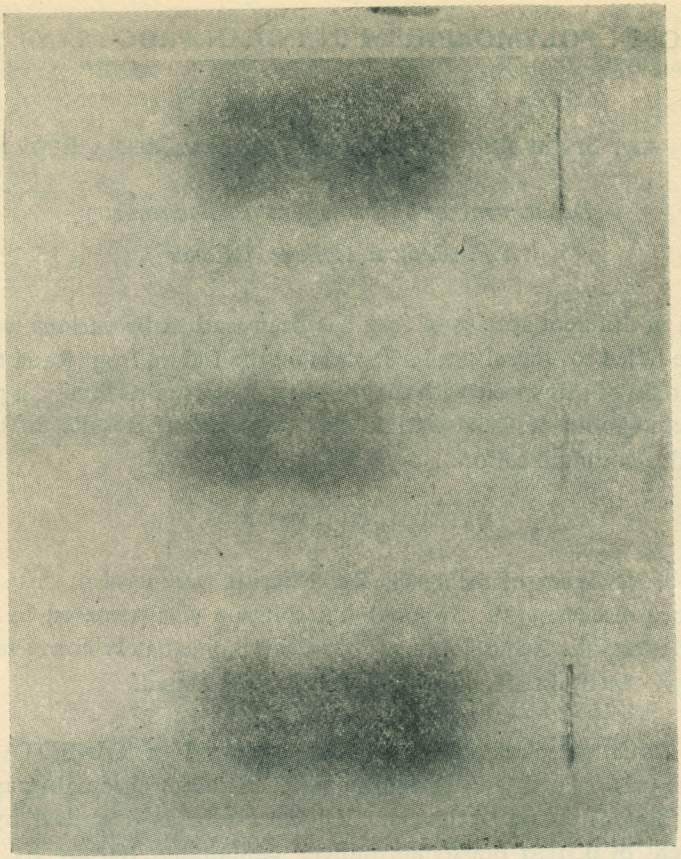

Fig. 1

Showing the starch gel electrophoresis of the hemoglobin o Rana tigrina.

Spark (1) mentioned only four fractions in the hemoglobin of Rana catesbeiana indicating that polymorphism in hemoglobin may differ within the same specie.

Fig. 2 shows a typical separation of hemoglobin of Rana tigrina on CM-Sephadex column. At the start of chromatography run, two fractions elute out in the first 80 *ml.* of the initial buffer pH 6.5. These two fractions resemble V_1 and V_2 of human hemoglobins and with the first non-heme fraction of Rana esculenta L (13). Before the column was connected to the gradient device about 250 *ml.* of initial buffer pH 6.5 was run through it. As seen in Fig. 2, only two fractions were resolved in addition to V_1 and V_2. The two main fractions which elutes out at pH 7.3 and 7.6 resembles with the fast minor and slow major fractions respectively on starch gel.

The values for alkali resistant hemoglobin is 3.3% which is in confirmation with those reported by other workers (11), for Rana tigrina but differs from those for Rana esculenta L (12).

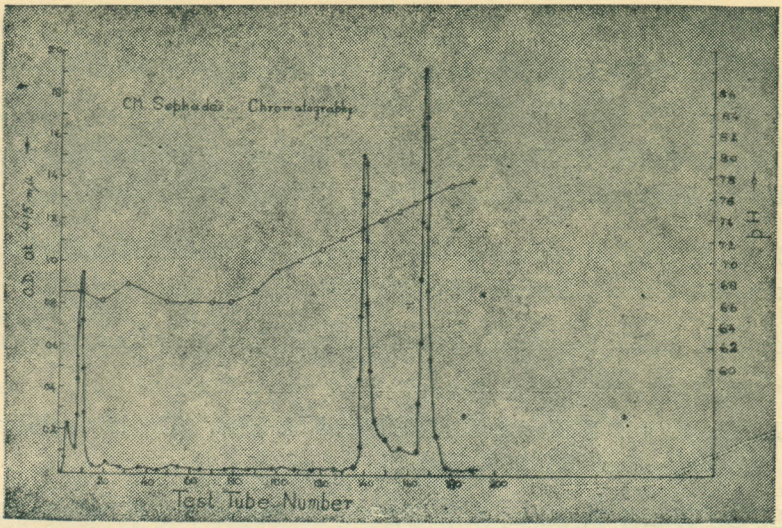

Fig. 2

Showing the elution pattern of the hemoglobin of Rana tigrina on CM-sephadex column.

These observations are highly suggestive of varying degree of polymorphism in the Rana family and that such polymorphism is not very uncommon in the animal kingdom.

SUMMARY

A preliminary report on the hemoglobin polymorphism in Rana tigrina has been presented. On the basis of starch gel and column chromatography, dimorphism in the hemoglobin of Rana tigrina has been established.

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