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Haemoglobin Heterogeneity in Two Species of the Indian Carp & Their Fertile Hybrids

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Results of haemoglobin electrophoretic studies on the Indian carps *Labeo rohita* and *L. calbasu* and their fertile hybrids are presented. Presence of a new haemoglobin fraction found in the hybrids has been confirmed by *in vitro* dissociation—recombination studies. A simple paper electrophoretic technique gave results that compared favourably with those obtained with conventional techniques employing starch gel and agar gel.

HETEROGENEITY in fish haemoglobins have been reported earlier[1–5]. Presence of electrophoretically distinguishable haemoglobin variants were described in few Indian fishes which included, among others, the carps *Labeo rohita* and *L. calbasu*[6]. During a genetic study on these Indian carps and their fertile hybrids *'Calbahu'* (*L. calbasu* ♀. × *L. rohita* ♂) and *'Rosu'* (*L. rohita* ♀. × *L. calbasu* ♂) an attempt was made to study their haemoglobin patterns by electrophoresis, using filter paper, cellulose acetate, starch and agar gel. The present paper reports the findings on haemoglobin profile of the species studied, stressing the usefulness of paper electrophoretic technique in such investigations. The presence of a new haemoglobin fraction detected in the hybrid specimens could be confirmed by *in vitro* dissociation-recombination studies.

Materials used for the study included *L. rohita* and *L. calbasu*, collected from a freshwater lake near Bombay and the fertile hybrids were those produced and maintained in the laboratories of Central Institute of Fisheries Education, Bombay[7].

Haemoglobin solution was prepared according to the method described earlier[8]. Fresh haemoglobin samples were used for all investigations. Whenever samples were to be stored, the haemoglobin was converted to cyan-methaemoglobin by addition of 2% KCN (1 drop / 1 ml of Hb solution).

Vertical paper electrophoresis was carried out in a discontinuous buffer system using filter paper (Whatman No. 3, size 3½″ × 14″). Stock TEB buffer (Tris 50.4 g, EDTA 5.0 g and boric acid 3.8 g/litre, *p*H 8.9) diluted 1 in 5 with distilled water was used for wetting the paper and veronal buffer (sodium diethyl barbiturate 10.3 g, diethyl barbituric acid 1.84 g/litre, *p*H 8.6) was used for the chamber. Haemoglobin solution was applied on the middle of the paper strip (which was previously soaked and blotted to remove excess of the buffer) and was run overnight (14 to 16 hr, current 1–1 .5 mA/strip/about 140 V). After the run the strips were dried under hot air blower.

Cellulose acetate electrophoresis was carried out in Kohn's type apparatus, using cellulose acetate membrane (Sepraphore III, Gelmen Instrument Co., USA). Tris buffer *p*H 8.4 (ref. 9) was employed.

Agar (Difco) gel electrophoresis, using veronal buffer *p*H 8.6 (ref. 6) was carried out. Gels after the run, were stained with amido black.

Starch gel electrophoresis was employed using hydrolysed starch (Connaught laboratories, Toronto, Canada) in a discontinuous buffer system, Tris citrate *p*H 8.8 for gel and borate buffer *p*H 8.3 for electrolyte compartments as described earlier[8]. Gels were stained with *O*-dianisidine.

In vitro hybridization experiment was carried out according to the method already described[10].

On paper electrophoresis *L. rohita* revealed only a single anodic fraction while *L. calbasu* showed a fast moving major anodic fraction along with a minor fraction (8–10%) moving cathodically. Interestingly both the hybrid fishes showed 3 major fractions, one each, corresponding to the parent species and a third one with an intermediate mobility between the two, besides the minor fraction (Fig. 1). The figure under reference does not show these minor bands because, being too faint, the bands could not be photographically reproduced from an unstained haemoglobin pattern. Patterns obtained on starch gel as well as cellulose acetate were similar to the one obtained by paper electrophoresis (Fig. 2 and 3). Agar gel electrophoresis, revealed patterns similar to those reported earlier[6] on the two species; however, haemoglobins from hybrid fishes did not reveal satisfactory separation. *In vitro* hybridization showed patterns similar to the one obtained in the hybrid fishes while the artificial mixture (unhybrid) was devoid of this fraction, lending support to the fact that this was formed as a result of dissociation and recombination at random. The above experiment is suggestive of structural differences in the similar polypeptide chains of the parental haemoglobins.

It would appear from these studies that hybrid fishes possess a characteristic haemoglobin pattern different from the parents, the identity of which could be confirmed by *in vitro* dissociation—recombination experiments. Thus electrophoretic studies on fish haemoglobins can be profitably used as an additional parameter in identifying both artificially produced hybrids as well as those found in nature. The paper electrophoretic technique described here is simple, economical and seems to compare well with the conventional starch gel and agar gel electrophoresis. It may be mentioned here that many of the mutant human haemoglobins were originally identified by their mobility on paper electrophoresis[11].

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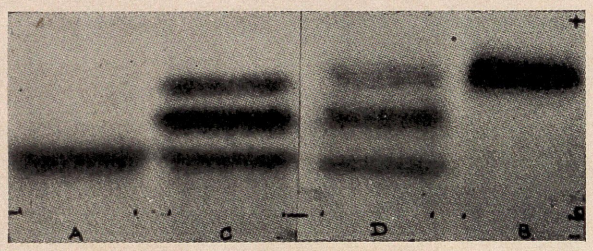

Fig. 1 — Haemoglobin paper electrophoretic patterns (unstained) of (A) *L. rohita* (B) *L. calbasu* (C) '*Calbahu*' and (D) '*Rosu*'

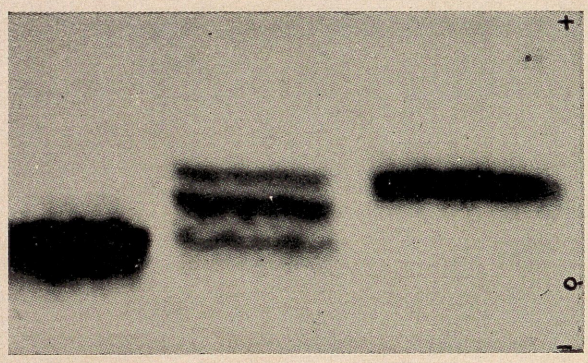

Fig. 2 — Haemoglobin cellulose acetate electrophoretic patterns (unstained) of (Left to right) *L. rohita*, '*Calbahu*', *L. calbasu*

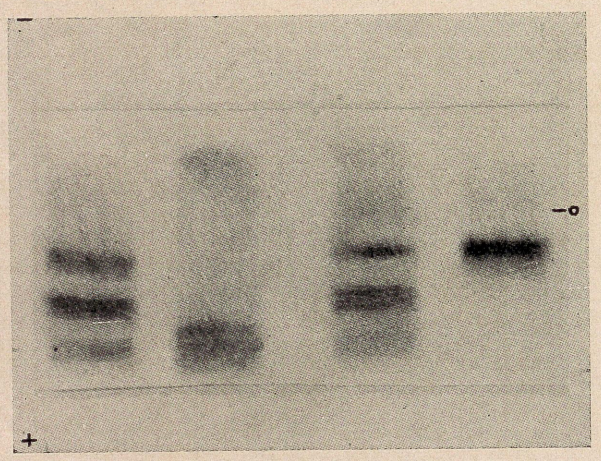

Fig. 3 — Starch gel electrophoresis of haemoglobins of (from left to right) *L. rohita*, '*Rosu*', *L. calbasu* and '*Calbahu*' (stained with 0-dianisidine)

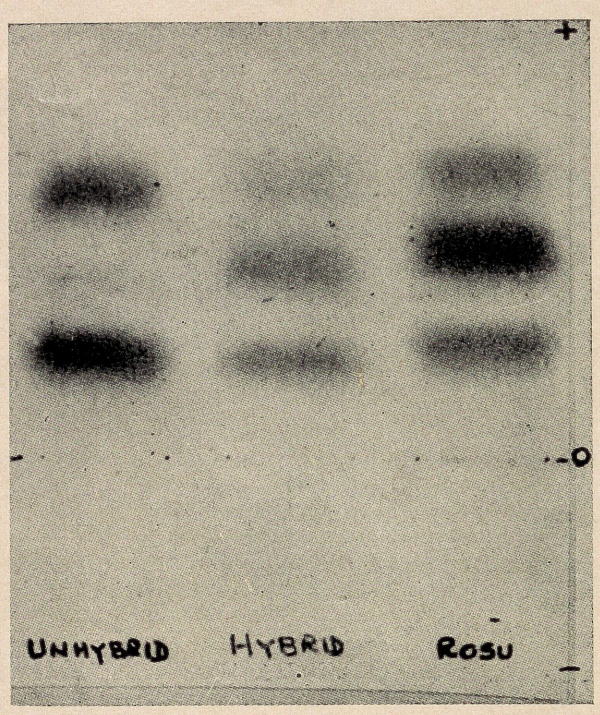

Fig. 4 — Hybridization of haemoglobins from *L. rohita* and *L. calbasu* (hybrid) along with unhybrid and '*Rosu*' (Paper electrophoresis—unstained)

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