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A SIMPLE AND INEXPENSIVE METHOD OF MASS SCREENING FOR ABNORMAL HAEMOGLOBINS

S. CHANDRA, A. RAO, A. DUTTA and D. K. BHATTACHARYA

The Society For Research On Haematology and Blood Transfusion, Futnani Chambers, Corporation Place, Calcutta 700 087, India.

ABNORMAL haemoglobins are detected by electrophoresis in different media and buffer systems[1–4]. Electrophoresis on cellulose acetate, although extensively used, is expensive and thus limits mass survey of carrier traits for haemoglobinopathies. A rapid, simple and relatively inexpensive method for haemoglobin electrophoresis is described here.

Red cell haemolysate (10g/dl) was prepared by the standard method[1]. 1% Agarose (Gibco Laboratories, USA) in tris-EDTA-borate(TEB) buffer, pH 8.9 (tris-hydroxymethyl aminoethane 14.4 g; EDTA 1.5 g; boric acid 0.9 g; and water to 1 l) was prepared by heating until the solution becomes clear. This solution (18 ml) was poured on a glass plate (17 cm × 8 cm × 0.1 cm) fitted to a perspex frame of 1–2 mm thickness and covered by a glass plate of the same size and allowed to set for 30 min. The covering plate and the frame are removed and the plate with gel side up is placed in an electrophoresis trough (Shandon, UK). The buffer tanks are filled with TEB buffer pH 8.9 and contact is established with filter paper strips and a constant current of 24 m Amp at 150V was passed for 15 min (Vokam stabilized DC power supply, Shandon, UK). Haemolysates are applied to the gel plate using a multi-applicator (Shandon, UK) and the same current was passed for 60–90 min. The plate was removed, fixed in 5% acetic acid in 85% ethyl alcohol for 15 min, stained with Nigrosine Black (0.5 Nigrosine Black in 1% acetic acid) for 10 min and dried overnight on a filter paper. A permanent record may be kept by a photograph (figure 1) or a photocopy.

Figure 1 shows the preparation of haemoglobins on agarose gel plate. Samples 1,6,9 and 16 are from normal control, samples 2,7, 11 and 15 from patients E B-thalassaemia showing haemoglobin E and B bands, samples 3,8,10 and 12 are from haemoglobin

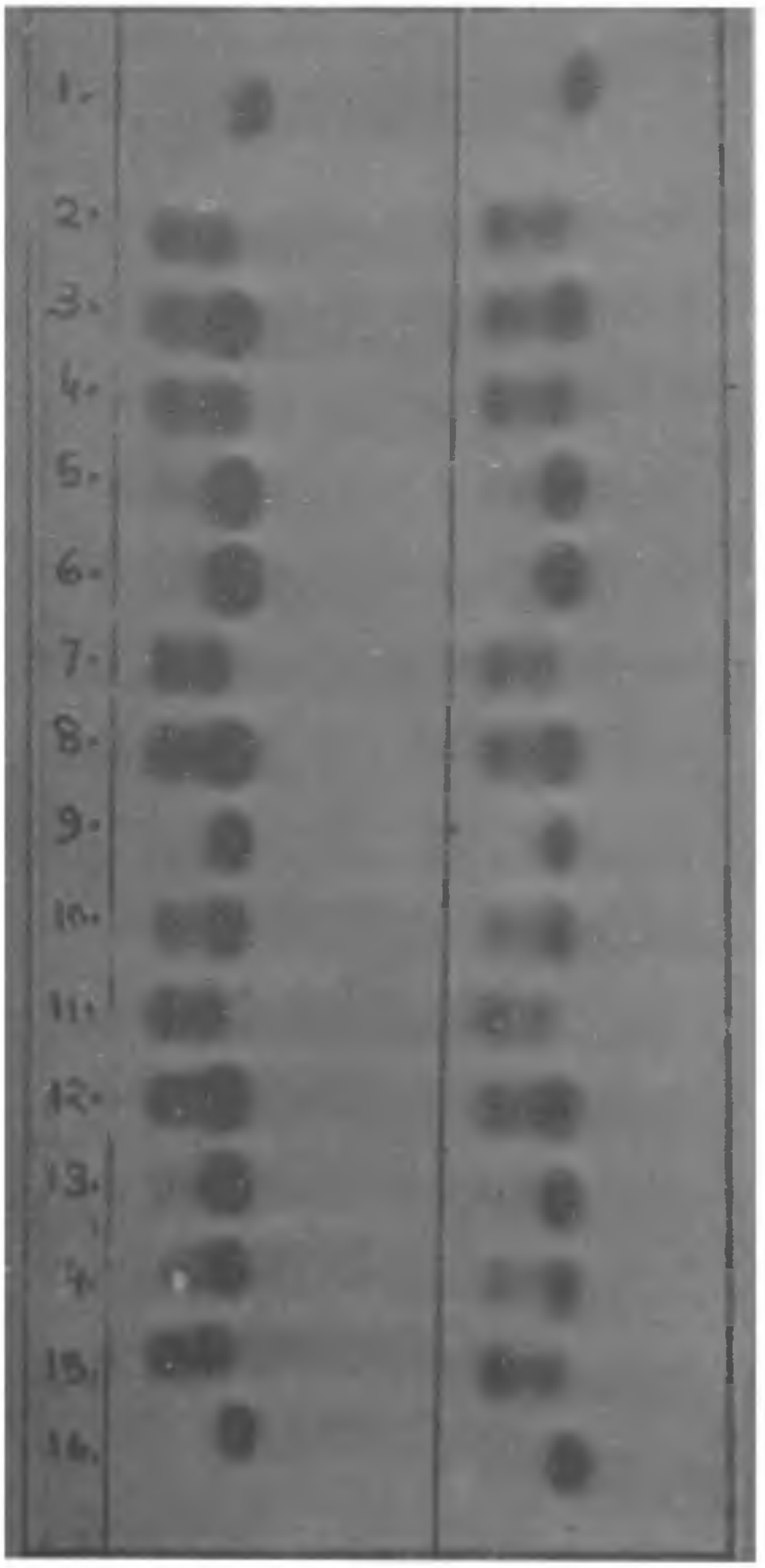

Figure 1. Agarose gel plate electrophoresis showing normal haemoglobin, beta-thalassaemia carrier state, haemoglobin E carrier state and E-Beta thalassaemia.

E carriers showing haemoglobin E and haemoglobin A bands while samples 5 and 13 are from B-thalassaemia carriers showing bands of haemoglobin A and faint bands of haemoglobin A_2.

The number of samples that can be processed per run is 32 in a single gel plate and with mulitple gel plates, a larger number of samples can be processed in a single working day. The gel plates are easy to handle, inexpensive and give excellent separation of haemoglobins. The method is recommended for screening of carriers for haemoglobinopathies in mass survey work.

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MASCULINIZATION OF *OREOCHROMIS MOSSAMBICUS* BY ADMINISTRATION OF 17 α-METHYL-5-ANDROSTEN-3β-17β-DIOL THROUGH REARING WATER

K. VARADARAJ and T. J. PANDIAN
School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India.

TILAPIAS are extensively cultured as food fish in warm waters. Elimination of uncontrolled reproduction is desirable to channelise the available energy for efficient growth and to quickly harvest marketable sized tilapias. Many approaches for controlling reproduction have been attempted and one of the most promising techniques is hormone induction of monosex population[1]. Besides mitigating unwanted reproduction, monosex male populations have greater growth potential[2].

Functional masculinization of genetic females of the cichlid *Oreochromis mossambicus* have been achieved[3,4] by supplementing the diet with steroids. However, the problems with the oral administration method are: (i) consumption of androgen-treated food will reduce in proportion to the amount of natural food consumed[5]; and (ii) high densities of try in treatment tanks cause competition and establishment of size hierarchy, resulting in differential consumption of the androgen-treated food and incomplete masculinization[6]. The desired masculinizing effect of the steroid could be realized by administering it in aquarium water[7,8]. Dissolved in ethanol, steroids like 17α-methyl-4-androsten-17β-ol-3-one or testosterone propionate become soluble in water. Paradoxically, exposure of *O. mossambicus*, *Tilapia heudeloti* and *Cichlasoma biocellatum* to water treated with one or the other steroid induces a feminizing potency[9]. Survey of literature shows that very little work has been done on sex-reversal in tilapias by exposing the fry to hormone dissolved in water and 100% masculinization[6,10,11] has not been achieved.

The present study was undertaken: (i) to determine the effect of 17α-methyl-5-androsten-3β-17β-diol on the gonad of genetic females reared in hormone dissolved water; (ii) to determine the optimum dosage and minimum duration required to ensure 100% sex reversal; and (iii) to estimate the growth efficiency of the treated tilapia. Hormone dissolved in 95% ethanol was added to rearing water at concentrations of 5 and 10 μg/litre water. The experimental fry were divided into series I: 7-day-old and series II: 10-day-old fry. Each series was further divided into two groups and each of these groups was treated with 5 or 10 μg steroid/litre until the 15, 20 or 25th day of hatching. The control and treated fry were examined for sex reversal following standard squash techniques[12].

Data presented in table 1 indicate that the female and male ratio of controls is 0.42:0.58. The administration of 17α-methyl-5-androsten-3β-17β-diol through the medium to 7 or 10-day-old *O. mossambicus* fry for periods of 11, 14, 16 or 19 days induced 100% masculinization. Testes of the sex-reversed males were identical in appearance with those of controls. When the treatment was terminated after 6 or 9 days, it failed to induce 100% sex-reversal, it resulted in the appearance of a few intersexes and female fry. It would appear that treatment between 10 and 20th days following hatching and a minimum dose of 5 μg steroid/litre water are the critical minimum requirements to ensure 100% sex reversal in *O. mossambicus*. Yamamoto[13] emphasized that the sex hormone should be administered during the