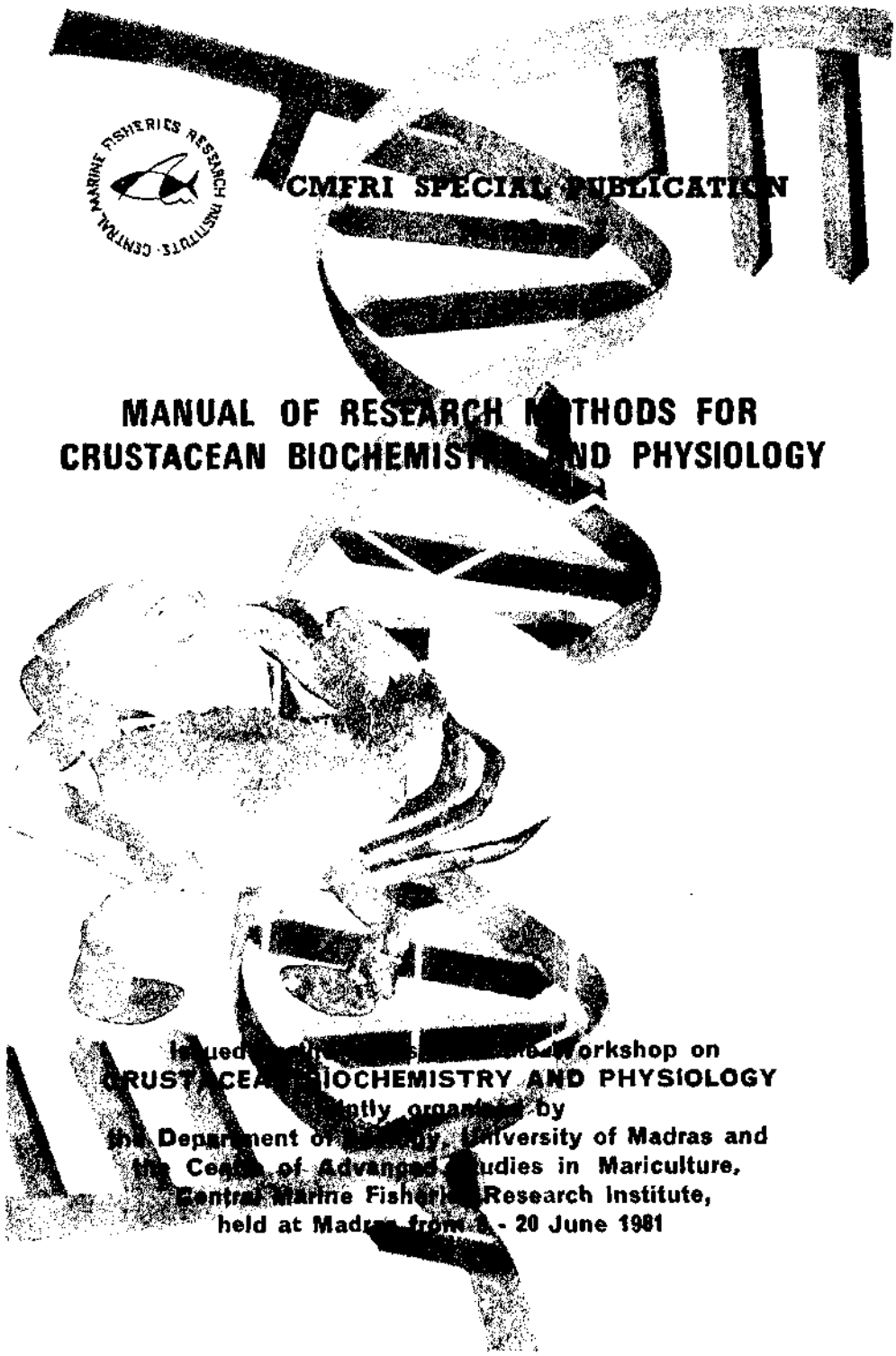
CMFRI SPECIAL PUBLICATION
Number 7

MANUAL OF RESEARCH METHODS FOR CRUSTACEAN BIOCHEMISTRY AND PHYSIOLOGY

Issued on the occasion of the Workshop on
CRUSTACEAN BIOCHEMISTRY AND PHYSIOLOGY
jointly organised by
the Department of Zoology, University of Madras and
the Centre of Advanced Studies in Mariculture,
Central Marine Fisheries Research Institute,
held at Madras from 8 - 20 June 1981



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Manual of Research Methods for Crustacean Biochemistry and Physiology

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POTASSIUM * 13

13.1. PRINCIPLE

Potassium in the blood is precipitated as potassium cobalt nitrite and when dissolved in sodium thiosulphate it liberates cobalt. The amount of cobalt liberated is equivalent to the amount of potassium present in the sample and this cobalt concentration is determined spectrophotometrically by choline chloride and potassium ferricyanide which impart green colour to the sample (Snell & Snell, 1959).

13.2. REAGENTS

1. *Silver cobaltinitrite reagent*

   Solution A : Dissolve 25 gm of cobaltinitrite in 150 ml of distilled water and then add 12.5 ml of glacial acetic acid.

   Solution B : Dissolve 120 gm of sodium nitrite in 180 ml of water (the final volume will be 210 ml).

   Then add 210 ml of B to A and remove the nitrous fumes using a pipette.

   Store the reagent at 0°C in a refrigerator. Before use, filter 20 ml of cobaltinitrite reagent and add 1 ml of 40% silver nitrate.

2. 1% *aqueous sodium thiosulphate* : Dissolve 1 gm of sodium thiosulphate in 100 ml of distilled water.

3. 1% *aqueous choline chloride* : Dissolve 1 gm of choline chloride in 100 ml of distilled water.

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* Prepared and verified by K. Kannan & M. Arumugam, School of Pathobiology, Department of Zoology, University of Madras, Madras-600 005.

4. 2% *aqueous potassium ferricyanide* : Dissolve 2 gm of potassium ferricyanide in 100 ml of distilled water.
5. *Washing agent* : Prepare by mixing 95% ethanol : ether : water in 2 : 1 : 2 V/V/V.
6. *Standard K^+ Solution* : Dissolve 0.191 gm of potassium chloride already oven-dried at 110°C for over night in 100 ml of distilled water. This solution contains 1 mg of K^+ per ml of the solution.
7. 40% *silver nitrate* : Dissolve 40 gm of silver nitrate in 100 ml of distilled water.
8. *Deproteinizing agent* : 80% ethanol.
9. 2.5% *silver nitrate* : Dissolve 2.5 gm of silver nitrate in 100 ml of distilled water.

13.3. PROCEDURE

1. Add 0.8 ml of distilled water to 0.2 ml of blood and 0.2 ml of K^+ standard solution.
2. Add 4 ml of 80% ethanol to all the tubes and centrifuge at 3500 rpm for 5 minutes.
3. Add 0.5 ml of 2.5% silver nitrate to the supernatant and centrifuge at 3500 rpm for 10 minutes.
4. Add 0.4 ml of silver cobaltinitrite to the supernatant and centrifuge at 3500 rpm for 10 minutes and decant the supernatant.
5. Wash the precipitate in 2 ml of 95% ethanol : ether : water mixture twice.
6. Dissolve the precipitate in 5 ml of 1% aqueous sodium thiosulphate.
7. Add 1 ml of 1% choline chloride and 2% potassium ferricyanide.
8. Read the colour intensity developed at 430 nm in a spectrophotometer.
9. 5 ml of 1% sodium thiosulphate serves as blank.

13.4. CALCULATION

$$\frac{\text{O.D. of sample}}{\text{O.D. of standard}} \times \frac{\text{Concentration of the standard} \times 100}{\text{Vol of sample}}$$

$$= \text{mg } K^+/100 \text{ ml of blood}$$

$$= \frac{\text{mg } K^+/100 \text{ ml of blood}}{\text{moulded weight of K (39)}}$$

$$= \text{mM/L or mEq/L}$$

13.5. PRECAUTIONS

1. The sample should be free of proteins and chlorides.
2. The washing of the precipitate should be complete. All the excess cobaltinitrite should be washed.

13.6 REFERENCE

SNELL, F. D. & C. T. SNELL, 1959. *Colorimetric methods of analysis*, Vol. II A. Von Nostrand Co. Inc., New York, pp. 463.

For your own notes