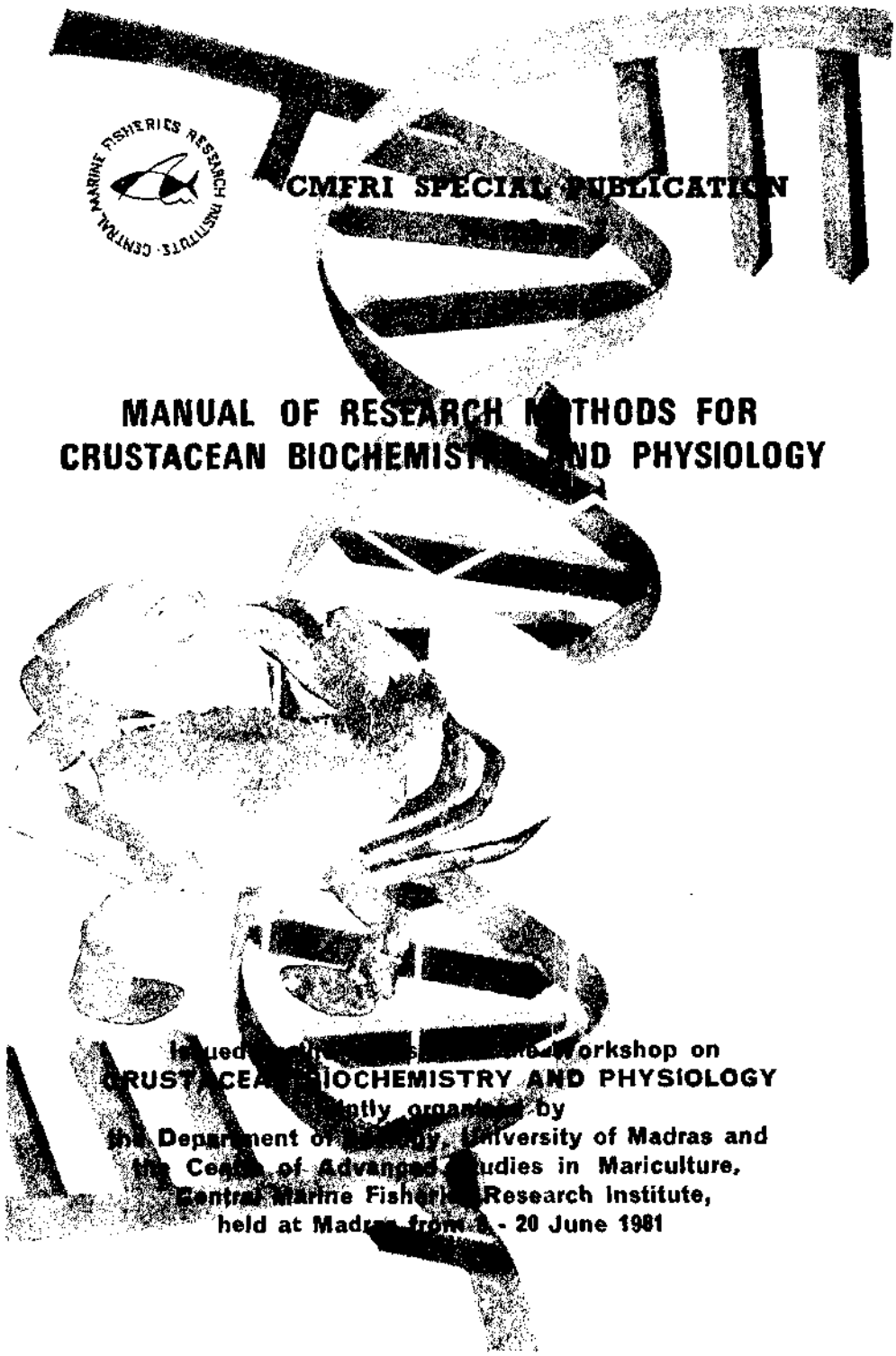
CMFRI SPECIAL PUBLICATION
Number 7

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

EDITED BY

M. H. RAVINDRANATH

School of Pathobiology, Department of Zoology, University of Madras, Madras 600 005

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7 FREE AMINO ACIDS *

7.1. PRINCIPLE

Ninhydrin deaminates amino acids liberating ammonia and gets reduced to hydrindantin. The liberated ammonia condenses with hydrindantin to form a violet coloured compound diketohydrindylidenediketohydrindamine (DYDA) at pH 5.0. Potassium cyanide prevents the oxidation of the reduced hydrindantin. The intensity of violet colour is directly proportional to the amount of amino acid (Yemm & Cocking, 1955).

7.2. REAGENTS

1. *Citrate Buffer pH* 5.0 (0.2 *M*) : Dissolve 21.008 gm of citric acid $C_6H_8O_7.H_2O$ in 200 ml of distilled water, add 200 ml of N sodium hydroxide and dilute to 500 ml ; store in the cold with little thymol.
2. *Potassium cyanide* (0.01 *M*) : Dissolve 0.1628 gm of potassium cyanide in distilled water and dilute to 250 ml. This solution is stable for at least 3 months at room temperature.
3. 60% *ethanol* : Dilute 60 ml of absolute ethanol to 100 ml with distilled water.
4. *Amino acid standard solutions* :

   Standard A : *Glycine standard* :

   Dissolve 0.268 gm of pure dry glycine in 5 ml of distilled water. Add 35 ml of N hydrochloric acid and 1 gm of sodium benzoate, dilute to 500 ml with distilled water.

* Prepared and verified by M. H. Subhashini, School of Pathobiology, Department of Zoology, University of Madras, Madras-600 005.

Standard B : Glutamic acid standard :

Dissolve 0.525 gm of pure dry glutamic acid in 5 ml of water. Add 35 ml of N hydrochloric acid and 1 gm of sodium benzoate and dilute to 500 ml with distilled water.

Standard C :

Mix 3 ml of standard *A* and 3 ml of standard *B* and dilute to 100 ml with distilled water. 1 ml of this solution contains 0.006 mg of amino acid nitrogen. It is stable for 1 week if kept in the cold.

5. *Solution A* : Dilute 5 ml of 0.01 M potassium cyanide to 250 ml with methyl cellosolve. This solution is stable for at least 1 month at room temperature.
6. *Solution B* : Dilute 500 mg of ninhydrin in 10 ml of methyl cellosolve. This solution is stable for at least 6 months at room temperature.
7. *Solution C* : Mix 50 ml of solution *B* with 250 ml of solution *A*. The resulting solution is first red, but soon becomes yellow. It is stable for at least one week when kept in a stoppered flask at room temperature.
8. *Deproteinizing agent* :

   80% *Ethanol* : As mentioned in 4.2.2.

7.3. PROCEDURE

1. To 0.05 ml of blood add 2 ml of deproteinizing agent.
2. Centrifuge at 5000 rpm for 5 minutes and collect the supernatant (sample).
3. Add 0.5 ml of citrate buffer pH 5.0 (0.2 M) to 1 ml of supernatant (sample), 1 ml of standard amino acid solution (standard) and 1 ml of distilled water (blank).
4. Add to each solution 1.2 ml of solution C.
5. Heat the solutions for 15 minutes at 100°C.
6. Cool in running tap water for 5 minutes.
7. Add 2.3 ml of 60% ethanol to each tube.
8. Determine the optical density at 570 nm in a spectrophotometer.

7.4. Calculation

$$\text{Amount of amino acid nitrogen present in the sample} = \frac{\text{O.D of sample}}{\text{O.D. of standard}} \times \frac{0.006}{0.025} \times 100 = \text{mg\%}$$

Where 0.006 refers to the amount of nitrogen present in the standard, 0.025 refers to the amount of blood present in one ml of sample (Oser, 1971). (0.05 ml of blood was diluted to make 2 ml with deproteinizing agent and 1 ml of this is taken for analysis.)

7.5 References

Oser, B. L. 1971. *Hawk's physiological chemistry.* Fourteenth edition. pp. 1472. Tata McGraw-Hill Publishing Company, New Delhi.

Yemm, E. W. & E. C. Cocking 1955. The determination of amino acids with ninhydrin. *Analyst*, **80** : 209-213.

For your own notes

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