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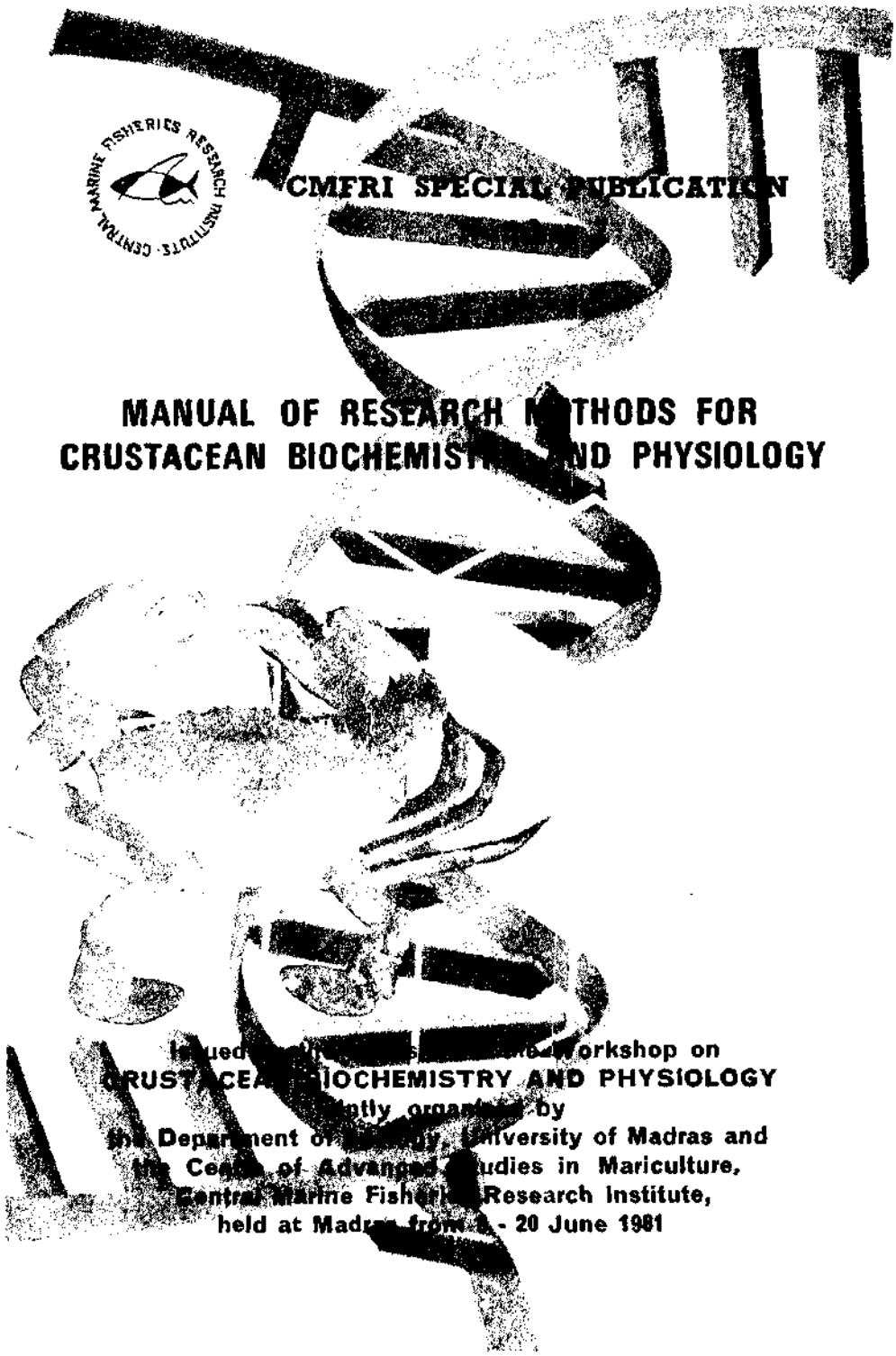
Number 7

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

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POLYSACCHARIDES * 5

5.1. Introduction

Polysaccharides in crustacean tissues occur both in free state as well as bound to proteins. The free polyaccharides are invariably glycogen. The protein bound sugars may be determined after precipitating the protein (Saravanan, 1979).

5.2. Method For Glycogen

5.2.1. Principle

Sulphuric acid in the anthrone reagent hydrolyses the glycogen into glucose and then dehydrates it into furfurals. This compound reacts with anthrone to produce a complex coloured product, the intensity of which is proportional to the amount of glucose present in glycogen (Caroll *et al.*, 1956).

5.2.2. Reagents

1. *Anthrone reagent* : Dissolve 50 mg of Anthrone and 1gm of thiourea in 100 ml of 72% Conc. H_2SO_4.
2. *Glucose standard* : Dissolve 100 mg of glucose in 100 ml of water.
3. 5% *TCA* : As mentioned in 4.2.2.
4. *Absolute ethanol.*

5.2.3. Procedure

1. Add 0.1 ml of blood, to 1 ml of 5% TCA.
2. Centrifuge for few minutes at 2500 rpm and separate the supernatant.
3. Add 5 ml of absolute ethanol to 1 ml of the above supernatant.

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* Prepared and verified by T. S. Saravanan, School of Pathobiology, Department of Zoology, University of Madras, Madras-600 005.

4. Keep this set up undisturbed for overnight in refrigerator.
5. Then centrifuge for 15 minutes at 2500 rpm.
6. Decant the supernatant completely and then add 1 ml of water to dissolve the precipitated glycogen.
7. To 1 ml of the sample, 1 ml of standard solution and 1 ml of distilled water add 10 ml of anthrone reagent.
8. Keep them in boiling water for 10-15 minutes then cool at room temperature in dark.
9. Determine the optical density at 620 nm.

5.2.4. Calculation

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

= mg% of glycogen (in glucose equivalents)

5.3. Method for Protein Bound Sugars

5.3.1. Principle

Sulphuric acid hydrolyses polysaccharides bound to proteins into monosaccharides and dehydrates all monosaccharides into furfural or furfural derivatives. They combine with anthrone to form a coloured complex which is proportional to the amount of monosaccharides complexed with the proteins (Caroll *et al.*, 1956).

5.3.2. Reagents

1. *5% TCA* : As mentioned in 4.2.2.
2. *1N H_2SO_4* : Dilute 27.8 ml of Conc. H_2SO_4 to 100 ml with distilled water.
3. *Anthrone reagent* : As mentioned in 5.2.2.
4. *Glucose standard* : As mentioned in 5.2.2.

5.3.3. Procedure

1. Collect 0.1 ml of blood in 1 ml of 5% TCA and centrifuge at 2500 rpm for 5 minutes.

2. Decant the supernatant. Add 1.5 ml of 1N H_2SO_4 to the precipitate in the serum tubes.
3. Keep the tube closed with marble and place it in oven at 100°C for 12 to 14 hours for hydrolysis.
4. Add 10 ml of the anthrone reagent to the hydrolysate, 1 ml of standard glucose, containing 1 mg of glucose and 1 ml of water to be used as a blank respectively.
5. Heat the mixture in water bath for 10 to 15 minutes.
6. Cool in dark at room temperature for 30 minutes.
7. Read the optical density at 620 nm in a spectrophotometer.

5.3.4. Calculation

$$\frac{\text{O.D. of the unknown}}{\text{O.D. of the standard}} \times \text{Concentration of standard} \times \frac{100}{\text{Vol. of sample}} = \text{mg\% (in glucose equivalents)}$$

5.4. Interpretation

Observation made on *S. serrata* reveals that the glycogen occurs both in free state as well as bound with proteins (Saravanan, 1979). Electrophoretic analysis reveals that almost all protein fractions show diastase labile PAS positivity.

5.5. References

Caroll, W. V., R. W. Longley & J. H. Roe, 1956. The determination of glycogen in the liver and muscle by the use of anthrone reagent. *J. Biol. Chem.*, 220 : 583-593.

Saravanan, T. S. 1979. Studies on haemolymph carbohydrates of *Scylla serrata* Forsskal (Crustacea : Decapoda). Ph. D. Thesis, University of Madras. p. 251.

For your own notes

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