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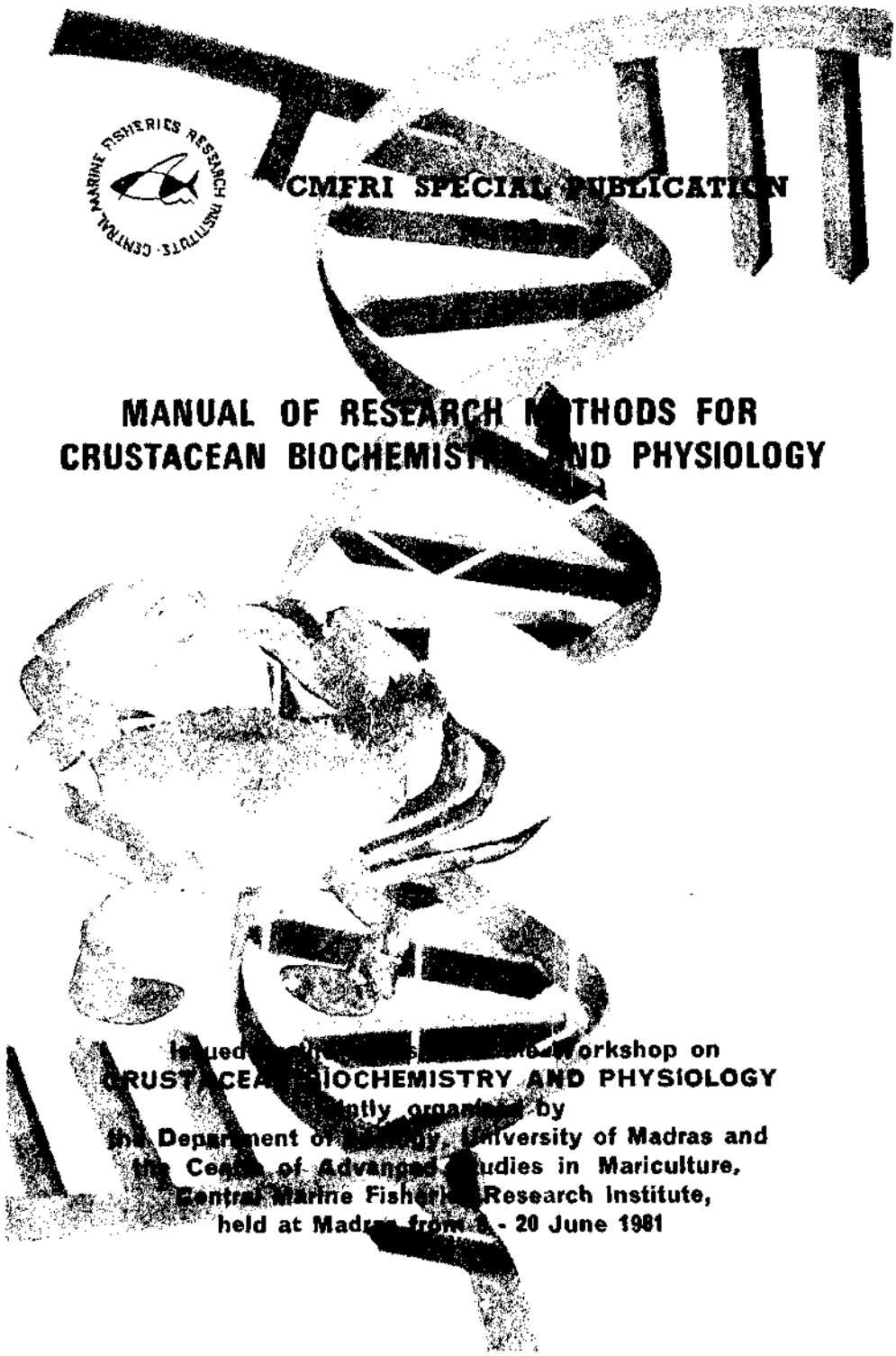
Number 7

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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PHOSPHATASES * 10

10.1. INTRODUCTION

The phosphatases are the group of enzymes of low substrate specificity and are characterised by the ability to hydrolyse a large variety of organic phosphate esters with the formation of an alcohol and phosphate ions. This group is composed of those enzymes which attack only monoesters of orthophosphoric acid. The alcohol esterified to the orthophosphoric acid, $(HO)_2 P{=}O$, may be a simple aliphatic alcohol, a polyhydric alcohol such as sugar or any one of a variety of aromatic hydroxyl compounds such as tyrosine. The phosphatases are not one enzyme but a group of related enzymes. In crustaceans in general, two types of enzymes are recognised : alkaline phosphatase and acid phosphatase. In *Scylla serrata*, the optimal activity of acid phosphatase is at pH 5.0 and that of alkaline phosphatase at pH 9.0 (Mercy, 1979). The probable function of the phosphatases is the transfer of the phosphate group from a donor substrate to an acceptor compound containing an (OH group). If the acceptor is water, the net effect is hydrolysis.

10.2. PRINCIPLE

The phosphatases activity is determined following the procedure of Barret (1972). The enzyme is allowed to hydrolyse an organic phosphate ester. The liberated phosphate combines with ammonium molybdate. The compound thus formed combines with elon giving a blue colour which is read at 650 nm in a spectrophotometer.

* Prepared and verified by Sr. P. D. Mercy & M. H. Ravindranath, School of Pathobiology, Department of Zoology, University of Madras, Madras-600 005.

10.3. Reagents

1. *Substrate B-glycerophosphate* (BGP) and *p-nitrophenyl-phosphate* (PNP) : 0.5% solution is prepared with citrate buffer at pH 5.0.
2. *Ammonium molybdate* : 2.5% of ammonium molybdate is prepared by dissolving 2.5 gm of salt in 100 ml of N H_2SO_4.
3. *Elon* : 0.5 gm of elon (P-methyl amino phenol sulphate) is dissolved in 10% sodium metabisulphate solution to make 100 ml.
4. *Standard phosphate solution* : A 30 mM stock solution of KH_2 PO_4 in 5N H_2SO_4 is used as a 1 : 100 dilution containing 0.30 μM/ml.
5. *Enzyme sample* : 2.5 ml of blood is diluted 10 times with double distilled water and stored immediately in a deep freezer at -7°c.

10.4. Procedure

10.4.1. Acid phosphatase

1. Add 2.0 ml of buffered β-glycerophosphate (BGP) or p-nitrophenylphosphate (PNP) to 1 ml of enzyme sample at 37°C.
2. After 30 minutes, stop the reaction by adding 3 ml of ice-cold 10% trichloroacetic acid which results in the formation of a precipitate.
3. After 15 minutes in the cold, filter the mixture.
4. Add 0.8 ml of ammonium molybdate and 0.3 ml of elon to 2.0 ml of the filtrate, 2 ml of distilled water and 2 ml of standard solution of potassium phosphate separately to serve as sample, blank and standard respectively.
5. Maintain a suitable control by adding 10% TCA to the substrate, before the addition of enzyme source.
6. After 15 minutes, measure the colour intensity at 660 nm in a spectrophotometer against the blank.

10.4.2. Alkaline phosphatase

For alkaline phosphatase, the substrates are dissolved in boric acid-borax buffer at pH 9.0. The buffer is prepared by mixing 0.2 M boric acid and 0.5 M borax solution. The procedure for the enzyme assay is same as that of acid phosphatase.

Determine the protein concentration in the sample by the biuret method as given in 6.2.

10.5. Calculation

1. $\frac{\text{O.D. of sample-O.D. of the control}}{\text{O.D. of standard phosphate}} \times \text{concentration of standard (in mg)} = \text{mg phosphate liberated.}$

2. $\frac{\text{mg phosphate liberated}}{\text{mg protein in sample} \times 15} = \text{mg phosphate libertaed/mg protein/minute.}$

10.6 References

Barret, A. J. 1972. Lysosomal enzyme. Chapter 2. *In*, '*Lysosomes : a laboratory handbook*' (Ed. J. T. Dingle) pp. 46-135, North Holland Publishing Company, Amsterdam.

Mercy, P. D. 1979. Haemolymph phosphatases of *Scylla serrata* Forskal (Crustacea : Decapoda). M. Phil. Dissertation, University of Madras, p. 71.

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