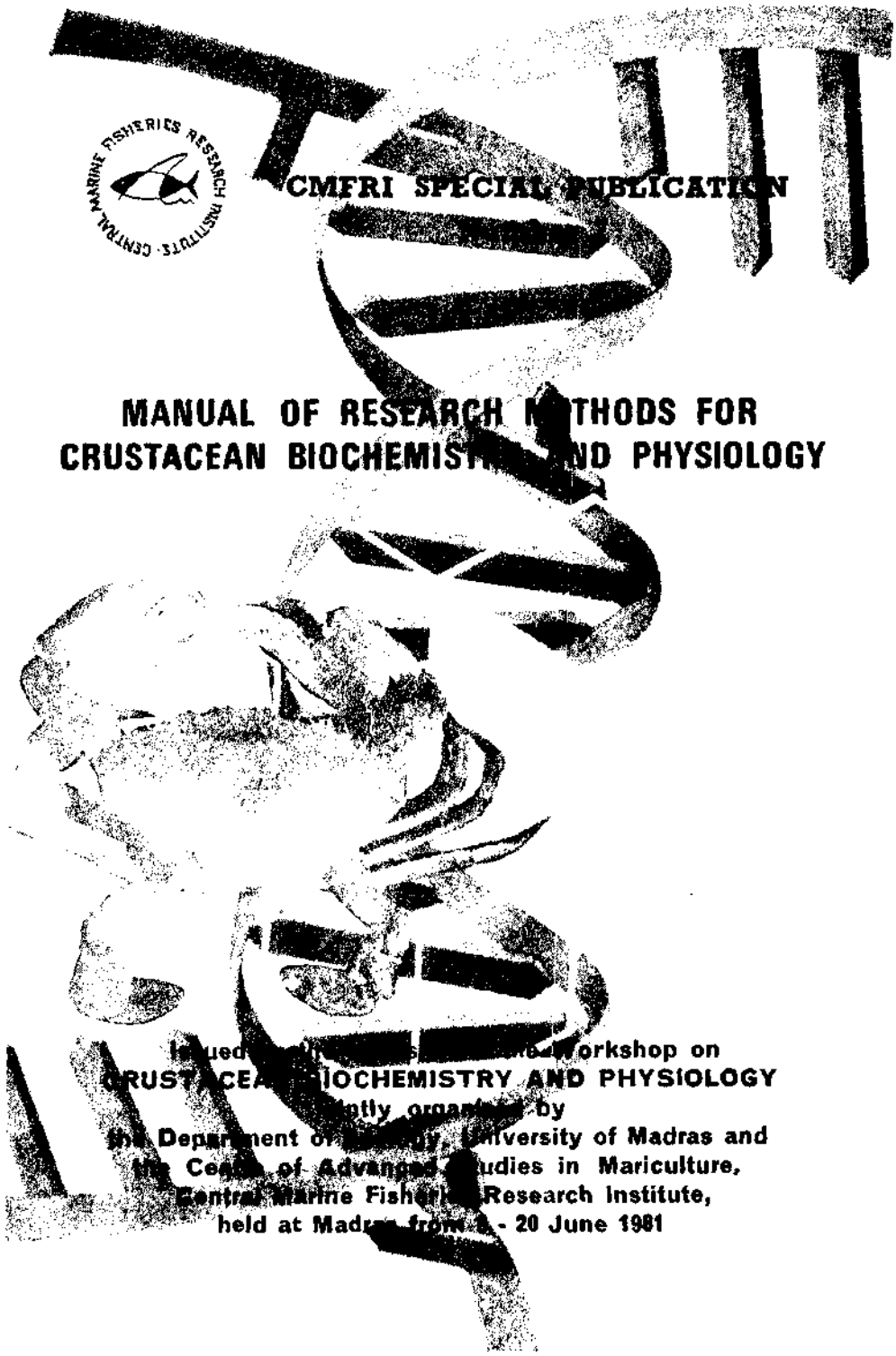
CMFRI SPECIAL PUBLICATION
Number 7

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

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CHROMATOGRAPHIC SEPARATION OF SUGARS * 16

16.1. PRINCIPLE

The principle of chromatography involves separation of a mixture on the basis of specific differences in physical and chemical properties, which result from the structural differences of the chemically related groups of compounds which are under investigation. They therefore have differential affinity for both the mobile and stationary phases of the chromatographic systems. This chromatographic separation is the resultant of propelling (mobile phase) and retarding forces (stationary phase). The stationary phase in strict sense includes the medium (paper) together with the polar solvent (water). The mobile phase or propelling force includes both polar and non-polar solvent.

The separation is brought about by continuous partition between the mobile phase (solvent flowing along the paper) and the water held in the paper and paper *per se*. Paper together with water acts as an adsorbent ; it has a strong affinity for polar molecules which are held by hydrogen bonding and vander Waals' forces (Smith & Seakins, 1976).

16.2. REAGENTS

1. 5% *TCA* : Prepare by dissolving 5 gm of TCA in 100 ml of distilled water.
2. **Pyridine undiluted.**
3. **10% Iso-Propyl alcohol :** Prepare by diluting 10 ml of isopropyl alcohol in to 100 ml with distilled water.
4. **Solvent system (Butanol : Pyridine : water) :** Prepare by mixing Butanol : Pyridine : water in the ratio of 2 : 2 : 1 (Smith & Seakins, 1976).

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

5. Alkaline silver oxide.

(*a*) *Saturated Silver nitrate in distilled water*—0.1 vol.

(*b*) *Sodium hydroxide :* Dissolve 0.5 gm NaOH in 5 ml of distilled water and dilute to 100 ml with ethanol—100 vol.

16.3. PROCEDURE

16.3.1. Preparation of sample for separation of sugars :

1. Take 0.1 ml of blood in 1 ml of 5% TCA and centrifuge at 2500 rpm for 5 minutes.
2. With 1 ml of supernatant, add 3 ml of pyridine and heat it over a boiling water bath, till the solution gets evaporated completely.
3. Dissolve the residue again in 3 ml of pyridine and evaporate it over a boiling water bath. Repeat this procedure for 4-5 times.
4. Dissolve the salt-free residue in 1 ml of 10% isopropyl alcohol.

16.3.2. Separation

1. Take a Whatman No. 1 chromatogram paper (23 × 18 cm) and note down the flow direction.
2. Draw a line two inches above the lower margin and make two spots.
3. Spot the sample and the standard on the points separately and dry it using a hair dryer.
4. Fold the paper into a hollow cylinder and join the ends with cellophane tape.
5. Take 40 ml of solvent in a glass container (1500 ml) and keep the paper inside, (care should be taken to avoid any contamination with paper and the paper should not touch the sides of the glass container) and allow it to run.
6. After the completion of the run, take out the paper carefully and dry it in air.

16.3.3. Localisation and Identification of spots

1. Dip the dried paper in silver nitrate.
2. When dried dip it in Sodium hydroxide.
3. Excess reagents to be removed by dipping in 2 M Ammonia.
4. Make out the spot and determine the Rg values and identify the different sugars.

16.4 References

SMITH, I. & J. W. T. SEAKINS, 1976. *Chromatographic and electrophoretic techniques.* Volume I. Paper and thin layer chromatography. pp. 465. William Heinemann Medical Book Ltd., London.

For your own notes

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