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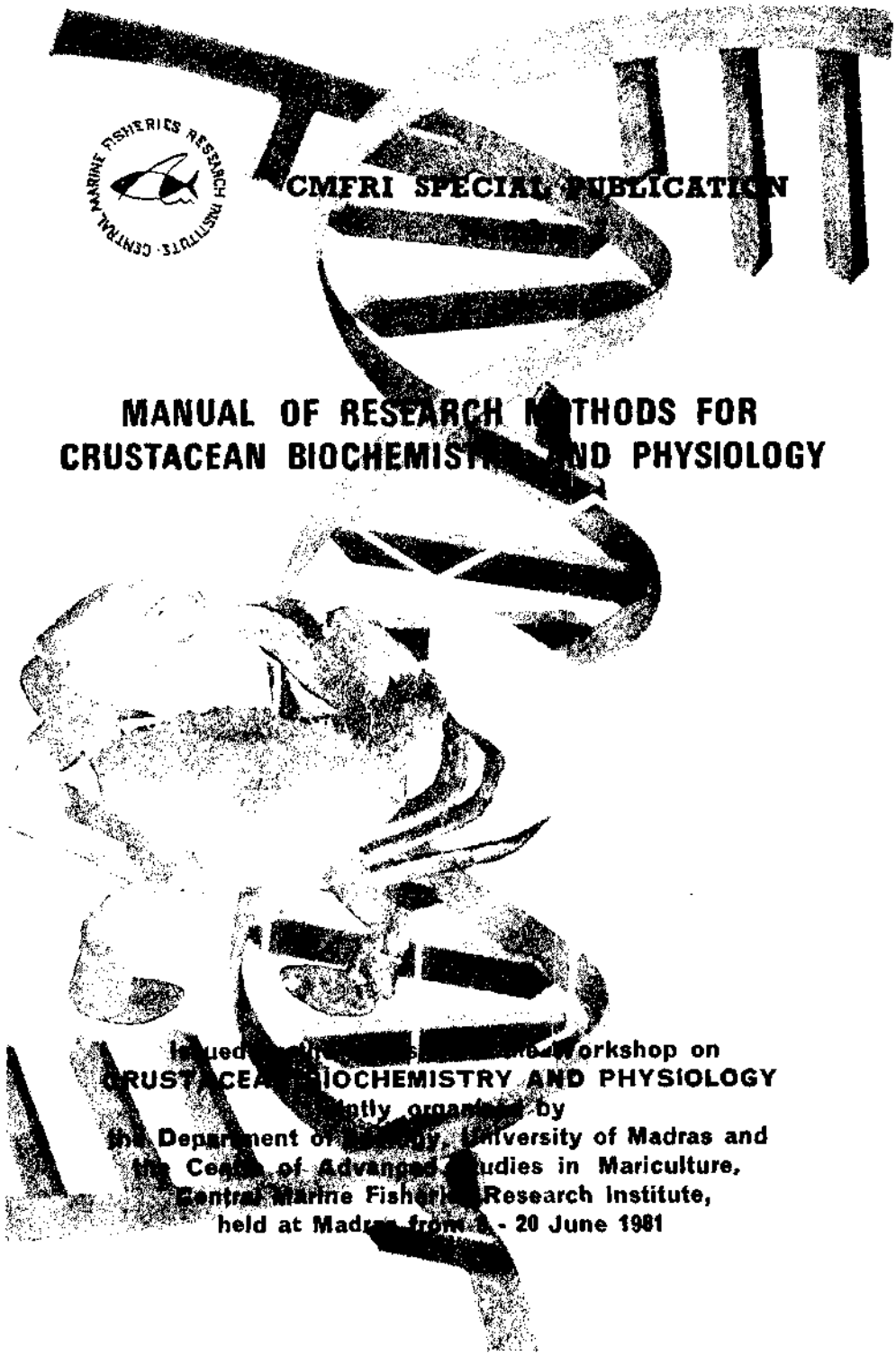
Number 7

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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THIN LAYER CHROMATOGRAPHIC SEPARATION OF LIPIDS * 18

18.1. Principle

A chromatographic separation in general is a technique in which a mobile phase while passing over a stationary phase transports different substances with different velocities in the direction of flow. In the case of thin layer chromatography the stationary phase (an adsorbent such as silica gel, silicic acid or cellulose) is placed on a glass support. A processed sample is spotted onto the stationary phase and then placed into a chromatographic chamber containing a relevant solvent system (mobile phase). As the solvent rises through the adsorbent by absorption and capillary action, it tends to resolve the components of the sample. Electrostatic forces of the stationary phase act to retard the component in the sample as the mobile phase rises. This and the fact that the components have different solubilities in the mobile phase cause the individual components to move at different rates below the solvent front (Stahl, 1958).

18.2. Reagents

1. *Chloroform* : *Methanol* (2 : 1) Mix 2 volumes of chloroform to one volume of methanol.
2. *Eluting solvent* : Mix benzene : diethyl ether : ethyl acetate : acetic acid in the ratio of 80 : 10 : 10 : 0.2.
3. *Iodine crystals.*

18.3. Procedure

18.3.1. Extraction of Lipid (Johnson & Davenport, 1971)

1. Take out 300-400 mg of hepatopancreas.
2. Add 15 ml of chloroform : methanol (2 : 1) and homogenize it well for 15 minutes.

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* Prepared and verified by S. Gunasekaran, School of Membrane Biology, Department of Zoology, University of Madras, Madras-600 005.

3. Mix with water in the ratio of 1 vol. of the mixture to 0.8 vol. of water shaking vigorously.
4. Allow the whole sample to settle in a separating funnel. (The chloroform rich layer settles down which contains all the lipids).
5. Separate the chloroform layer and evaporate the solvent at room temperature.
6. Find out the weight of the lipid gravimetrically.
7. Calculate the % lipid in hepatopancreas using the known weights of the tissue taken and the lipid extracted.
8. Dissolve the lipid in minimal quantity of chloroform and make it ready for spotting.

18.3.2. Thin Layer Chromotography

18.3.2.1. *Preparation of Plates and Activation*

1. Weigh 25 gm of silica gel G.
2. Grind it in a mortar using pestle in 50 ml of water and make a slurry.
3. Transfer it to a TLC spreader.
4. Adjust the slit width to 0.35 mm.
5. Arrange TLC plates (20 cm × 20 cm) on a TLC Board so that they are continuous.
6. Spread the slurry on TLC plates.
7. Dry the plates with slurry in air.
8. Activate the plates at 120°C for 30 minutes in an oven.

18.3.2.2. *Spotting, Elution and Identification*

1. Spot the lipid extract (known quantity) in TLC plates using a micropipette. Spots are to be 1" above the base line, small but concentrated.
2. Keep the plates vertically in eluting chamber which has approximately 150-180 ml of eluting solvent and close the lid to make it air-tight.

3. Allow the solvent to run on the plate till it reaches 1/2" below the top edge of the plate.
4. Air dry the plate and transfer it to another chamber containing iodine vapour.
5. After few minutes take out the plate and mark the spots of sublimated iodine using a needle and calculate the Rf values.
6. The spots could be identified by using known Rf values of phospholipids standard, eluted under identical conditions.

$$Rf = \frac{\text{Distance travelled by the lipid spot from the origin}}{\text{Distance travelled by the solvent from the origin}}$$

18.4 References

Johnson, A. R. & J. B. Davenport, 1971. *Biochemistry and methodology of lipids.* Wiley Interscience, pp. 578.

Stahl, E. 1958. *Thin layer chromotography.* Academic Press, New York, pp. 835.

For your own notes

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