were recrystallized from benzene; m.p. 97-99°; yield 2·2 g. (68 per cent). (Found: C, 67·93; H, 8·19; N, 4·33. $C_{18}H_{27}NO_4$ requires C, 67·24; H, 8·46; N, 4·35%.)

N-(β-3,4-*Dimethoxyphenethyl*)-4-*hydroxycyclohexyl ethylamine* (VII) — The amide (V) (1·5 g.) was reduced with lithium aluminium hydride (2 g.) in tetrahydrofuran (30 ml.) in a three-necked flask (250 ml.) as before. In this case the mixture was refluxed for 40 hr and the excess lithium aluminium hydride destroyed by the drop-wise addition of aqueous tetrahydrofuran and the solution filtered. The residue was washed thoroughly with warm tetrahydrofuran and the mixed tetrahydrofuran extracts dried over anhydrous sodium sulphate and the solvent removed; b.p. 190-200°/0·1 mm.; n_D^{22}, 1·5297; yield 0·74 g. (53 per cent). (Found: C, 70·32; H, 9·46; N, 4·55. $C_{18}H_{29}NO_3$ requires C, 70·35; H, 9·44; N, 4·56%.)

References

1. Karim, M. A., Linnell, W. H. & Sharp, L. K., *J. Pharm., Lond.*, **12** (1960), 82-86.
2. Miller, F. M. & Weinberg, M. S., *Chem. Engng News*, **34** (40) (1956), 4760.
3. Vejdelek, Z. I. & Trcka, V., *Chem. Listy*, **51** (1957), 2109.
4. Sastry, B. V. & Lasslo, A., *J. org. Chem.*, **23** (1958), 1577.
5. Karim, M. A., *J. sci. industr. Res.*, **19B** (1960), 272-5.

On the Qualitative Distribution of Free Amino Acids in Different Species of Prawns

N. K. VELANKAR & K. MAHADEVA IYER

Central Fisheries Technological Research Station, Ernakulam

Manuscript received 14 *November* 1960

The free amino acids present in the muscle of several species of prawns and in lobster (*Panulirus dassypus*) and crab (*Neptunus pelagicus*) have been examined qualitatively by paper chromatography. Six among the eleven spots present have been identified as lysine, arginine, glycine, proline, valine and leucine. An overall similarity in the chromatographic pattern among the several species has been observed. The implications of the results in the application of paper chromatography to species differentiation in the Crustacea have been indicated.

THE presence of free amino acids in prawns and other crustaceans in concentrations about ten times as high as in the fishes has been reported by Velankar and Govindan[1]. The free amino acids contribute to the flavour of the meat and probably serve as substrate for bacteria in the initial stage of spoilage. An investigation of the free amino acids of prawns, crabs and lobsters was, therefore, undertaken by us. The amino acids present in the free condition in prawn muscle were examined by paper chromatography using the one-dimensional descending technique. The preliminary results, which appear to be of interest from the standpoint of application of paper chromatography to species differentiation in the crustaceans, are reported in this note.

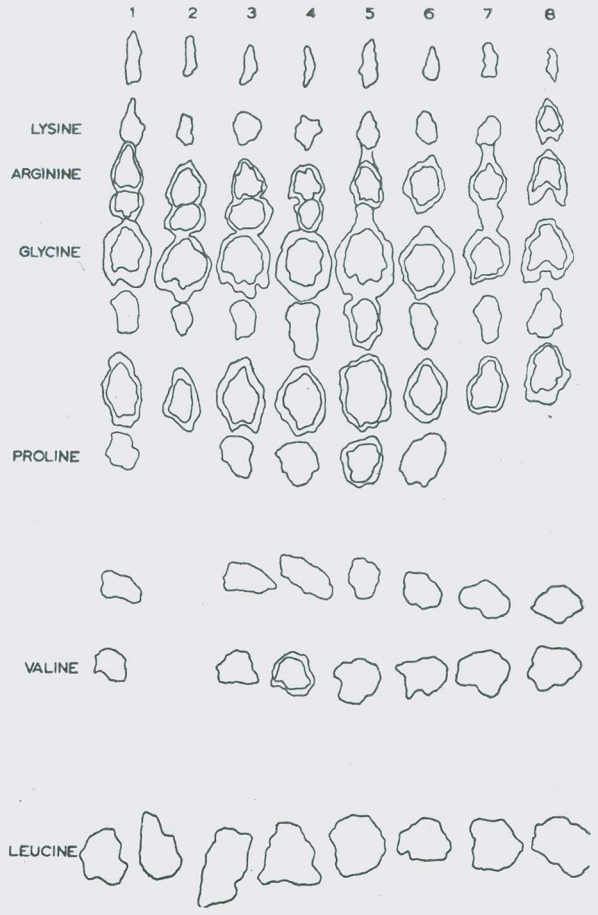

Fig. 1 — Chromatograms of the muscle of prawn, lobster and crab [(1) *Metapenaeus affinis*; (2) *Penaeus carinatus*; (3) *Metapenaeus dobsoni*; (4) *Penaeus indicus*; (5) *Metapenaeus monoceros*; (6) *Neptunus pelagicus* (crab); (7) *Palemon carcinus*; (8) *Panulirus dassypus* (lobster). Paper: Whatman No. 1, size 10·5 × 22 in. Colour reagent: 0·5 per cent nihydrin in water-saturated butanol. Solvent: *n*-butanol-glacial acetic acid-water (25 : 6 : 25), vol./vol. Time of run: 40 hr]

The chromatograms obtained with different species of prawns, crab and lobster are shown in Fig. 1.

The following amino acids have been so far identified in the chromatograms: lysine, arginine, glycine, proline, valine and leucine.

Four species of marine prawns, viz. *Penaeus indicus*, *Metapenaeus affinis*, *M. dobsoni* and *M. monoceros*, give identical chromatograms; in the case of *Penaeus carinatus* (species of marine prawn) the characteristic

yellow spot (proline) and the spot corresponding to valine are absent. The chromatogram of the crab, *Neptunus pelagicus*, is identical with the chromatogram of the four marine prawns except that the small spot between glycine and arginine is not distinct in the crab. The chromatograms of *Palemon carcinus* (fresh-water prawn species) and of the lobster, *Panulirus dassypus*, are identical and differ from those of the four prawn species in the absence of proline.

No differences were observed in the chromatograms of juvenile specimen taken from the backwaters and adult specimen taken from the sea in the case of *P. indicus* and *M. monoceros*. Juvenile specimens were not available for study in the case of the other species.

An overall similarity in the chromatographic pattern of the crab, prawn and lobster may be expected since they all belong to the same class, i.e. crustacean. The absence of proline in the lobster, which occupies an intermediate position between the prawn and crab in the zoological order, is noteworthy since it is present in the crab and in several species of prawns.

The application of paper chromatography in differentiating closely related species and even races of fishes is receiving considerable attention in recent years. The technique is likely to be useful particularly for identifying the species in the larval stage. On the hypothesis that the free amino acids in the muscle tissue are hereditary, identification of the larval forms of fish species has been attempted[2]. Our observation that the juveniles from the backwaters, where the salinity is low, and adults from the sea give identical pattern, lends support to the view that the free amino acids have a fundamental significance since they are apparently not influenced by environmental differences and differences in the growth stages. However, the fact that several marine species of prawns produce identical chromatogram and also that the lobster, fresh-water species of prawn (*Palemon carcinus*) and marine species of prawn (*Penaeus carinatus*) give uniform chromatographic patterns indicates definite limitations in the possible application of the paper chromatographic technique to species differentiation in the Crustacea. Further investigations are in progress to ascertain whether solvent systems other than the one employed in the present study could differentiate the species which have shown identical patterns.

References

1. Velankar, N. K. & Govindan, T. K., *Proc. Indian Acad. Sci.*, **47B** (1958), 202.
2. Matsumoto, M., U.S. Department of the Interior, Fish and Wildlife Service, Washington, *Special Scientific Report Fisheries No.* 337, 1960.

Serum Histaminase Activity in Experimental Atherosclerosis

BALAKRISHNA & S. H. ZAIDI
Central Drug Research Institute, Lucknow

Manuscript received 30 *November* 1960

Serum histaminase activity has been shown to be inhibited in atherosclerotic rabbits. This inhibition may affect endothelial permeability of blood vessels and help in cholesterol and lipid deposition.

THE biochemical picture in experimental cholesterol atherosclerosis is associated with inhibition of certain enzymes such as lipoprotein lipase[1,2], pancreatic elastase[3] and hexokinase[4,5]. The changes in enzyme activity have been interpreted as adaptation to the cholesterol diet. In this report the serum histaminase activity in normal and atherosclerotic rabbits has been estimated in view of its relation to basic processes of intracellular metabolism and the transport of ions and molecules at cellular level[6-8].

Rabbits of the C.D.R.I. colony of average weight 1·4 kg. were fed, in addition to the stock diet, a daily dose of 0·5 g. cholesterol suspended in 5 ml. of water with a drop of serum for a period of three months. Animals on stock diet alone served as normal controls.

For the estimation of serum histaminase activity volumetric method of Keppler[9] has been used. The enzyme activity has been expressed in permanganate units (PU). One PU represents the amount of enzyme, which after incubation for 24 hr at 37°C. and *p*H 7·2 in an atmosphere of oxygen with 1 mg. of histamine hydrochloride as substrate and with an aqueous solution of indigo disulphonate, takes up 0·1 ml. of 0·002*N* $KMnO_4$. The amount of $KMnO_4$ utilized in the assay for the test was subtracted from the amount taken by blank. The difference indicated the amount of H_2O_2 formed by the action of histaminase on histamine and thus indicated the enzyme activity.

The normal rabbits with a serum cholesterol of 85·69 mg./100 ml. and lipid phosphorus of 5·53 mg./100 ml. (C/P ratio 15·7) showed an enzyme activity of 4·0 PU/ml. The atherosclerotic rabbits with serum cholesterol of 558·2 mg./100 ml. and lipid phosphorus of 14·21 mg./100 ml. (C/P ratio 38·17) showed an enzyme activity of 1·00 PU/ml. The decrease in enzyme activity in atherosclerotic rabbits is significant ($p < 0·01$) (Table 1).

Histaminase is transferred from the kidneys and intestinal mucosa via the lymphatics into the blood stream[10]. In experimental atherosclerosis, the inhibition of serum histaminase may indicate a change in the histamine-histaminase system. This may affect