late third instar larvae grown at 21°C or at 31°C were transferred to 37°C for 1 h (heat shock) and then allowed to recover from the heat shock at 24°C for 1 h following which their gut and associated structures were processed for X-gal staining as above.

The X-gal staining patterns (reflecting activity of the hsp70 promoter in this case) in non-heat shocked and heat-shocked $cSOD^{n108}/cSOD^{n108}$ homozygous (non-tubby) and $cSOD^{n108}/TM6B$ heterozygous (tubby) larvae grown at 21°C or at 31°C are shown in Figure 1. The absence of any significant X-gal staining in non-heat shocked tubby as well as non-tubby larvae showed that the hsp70 promoter was not constitutively activated in $cSOD^{n108}$ homozygous larvae, neither in those grown at 21°C nor in those grown at 31°C. After exposure to 37°C, the X-gal staining in $cSOD^{n108}$ homo- as well as heterozygotes (see Figure 1) indicated a typical induction of the hsp70 promoter. It may be noted here that the *Bg9*-dependent X-gal staining patterns (in non-heat shocked as well as heat shock conditions) seen in the present study with $cSOD^{n108}$ homo- and heterozygotes did not differ from those seen with *Bg9* in wild type genetic background (not shown, but see Figure 2 reference 14). These results thus showed that the expression pattern of hsp70 promoter (absence of constitutive expression but strongly inducible by TS) was not affected by SOD deficiency.

It may be inferred from these results that although H_2O_2 is known to induce heat shock genes[9–11], the excess of O_2^- ions that accumulate in $cSOD^{n108}$ null mutant larvae[13] does not induce heat shock genes; nor does this excess of O_2^- ions affect the inducibility of heat shock genes by TS. Therefore, the observed thermosensitivity of the $cSOD^{n108}$ mutant larvae is not because of a failure of the heat shock response but probably due to an increased oxygen toxicity at elevated temperature, analogous to the paraquat sensitivity of the cSOD null larvae[13].

1. Phillips, J. P. and Hilliker, A. J., *Adv. Genet*, 1990, **28**, 43–71.
2. Drummond, I. A. S. and Steinhardt, R. A., *Exp. Cell Res*, 1987, **173**, 439–449.
3. Kapoor, M. and Lewis, J., *Biochem. Biophys. Res. Commun*, 1987, **147**, 904–910.
4 Ropp, Courgeon, A-M, Calvayrac, R. and Best-Belpomme, M., *Can. J. Biochem Cell. Biol*, 1983, **61**, 456–461.
5. Hass, M. A. and Massaro, D., *J. Biol. Chem.*, 1988, **263**, 776–781
6. Privalle, C. and Fridovich, I., *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 2723–2726.
7. Ferreira, M., Costa, P., Guerreiro, P. and Rodrigues, P. C., *Cell Biol. Int Rep*, 1992, **16**, 19–26
8 Kapoor, M. and Sreenivasan, G. M., *Biochem. Biophys. Res. Commun*, 1988, **156**, 1097–1102.
9. Courgeon, A-M, Becker, J., Maingourd, M., Marsonhaute, C. and Best-Belpomme, M, *Free Rad Res. Comms*, 1990, **9**, 147–155.
10. Becker, J., Merger, V., Courgeon, A-M. and Best-Belpomme, M, *Eur. J. Biochem*, 1990, **189**, 553–558.
11. Courgeon, A-M, Rollet, E., Marsonhaute, C. and Best-Belpomme, M, *Eur J. Biochem*, 1988, **171**, 163–170.
12 Campbell, S. D., Hilliker, A. J. and Phillips, J. P., *Genetics*, 1986, **112**, 205–215
13 Phillips, J. P., Campbell, S D., Michaud, D., Charbonneau, M and Hilliker, A. J, *Proc. Natl Acad. Sci. USA*, 1989, **86**, 2761–2765.
14 Lakhotia, S. C. and Singh, A. K, *J. Genet.*, 1989, **68**, 129–137.
15. Lis, J. T., Simon, J. A. and Sutton, C. A., *Cell*, 1983, **35**, 403–410.
16. Lindsley, D. L. and Zimm, G. G, *The Genome of Drosophila melanogaster*, Academic Press, San Diego, 1992

ACKNOWLDGEMENT. I thank Dr John P. Phillips, Univ. Guelph,, Ontario, Canada, for kindly providing the $cSOD^{n108}$ *red/TM3* stock.

Received 27 January 1993; revised accepted 4 September 1993

Oviducal gland of planktonic copepod, *Heliodiaptomus viduus* Gurney—a new report

K. Altaff and M. R. Chandran*

Department of Zoology, The New College, Madras 600 014, India
*School of Life Sciences, Bharathidasan University, Tiruchirapalli 620 024, India

The occurrence of a distinct oviducal gland at the posterior end of the oviduct in the freshwater planktonic copepod, *Heliodiaptomus viduus* is reported for the first time. This gland forms an elastic sac within itself, which is filled with secretory materials. The elastic sac and its secretion subsequently form the ovisac. This paper reports on the histology and histochemistry of this gland. The oviducal gland of *H. viduus* resembles that of cirripedes in its structure and function. This gland performs the functions of a crustacean spermatheca by holding the released eggs for internal fertilization as well as the embryonic development till naupliar stage.

THE secretory epithelial cells lining the oviduct as well as accessory sex glands provide materials for the extracellular envelops of the released eggs to serve a protective and nutritive role to the female gametes. Among female crustaceans information on the occurrence of such glands, their physiological and biochemical aspects is still scarce. Occurrence of such a gland is reported so far only in some cirripedes[1]. We report here, for the first time, the presence of an oviducal gland in the freshwater planktonic copepod *Heliodiaptomus viduus*. The histology and histochemistry of this gland have also been investigated in detail.

In *H. viduus* a pair of oviducts originate from the anterolateral aspects of the ovary and after giving rise to anterior diverticulae, run posteriorly and at the end of the prosome lead into oviducal glands (Figure 1). This gland which occupies the lateral aspects of the posterior segment of the prosome measures 126 ± 7 and $76 \pm 3\,\mu$ in length and width respectively and continues as the distal part of the oviduct to open to the exterior by the common female reproductive pore.

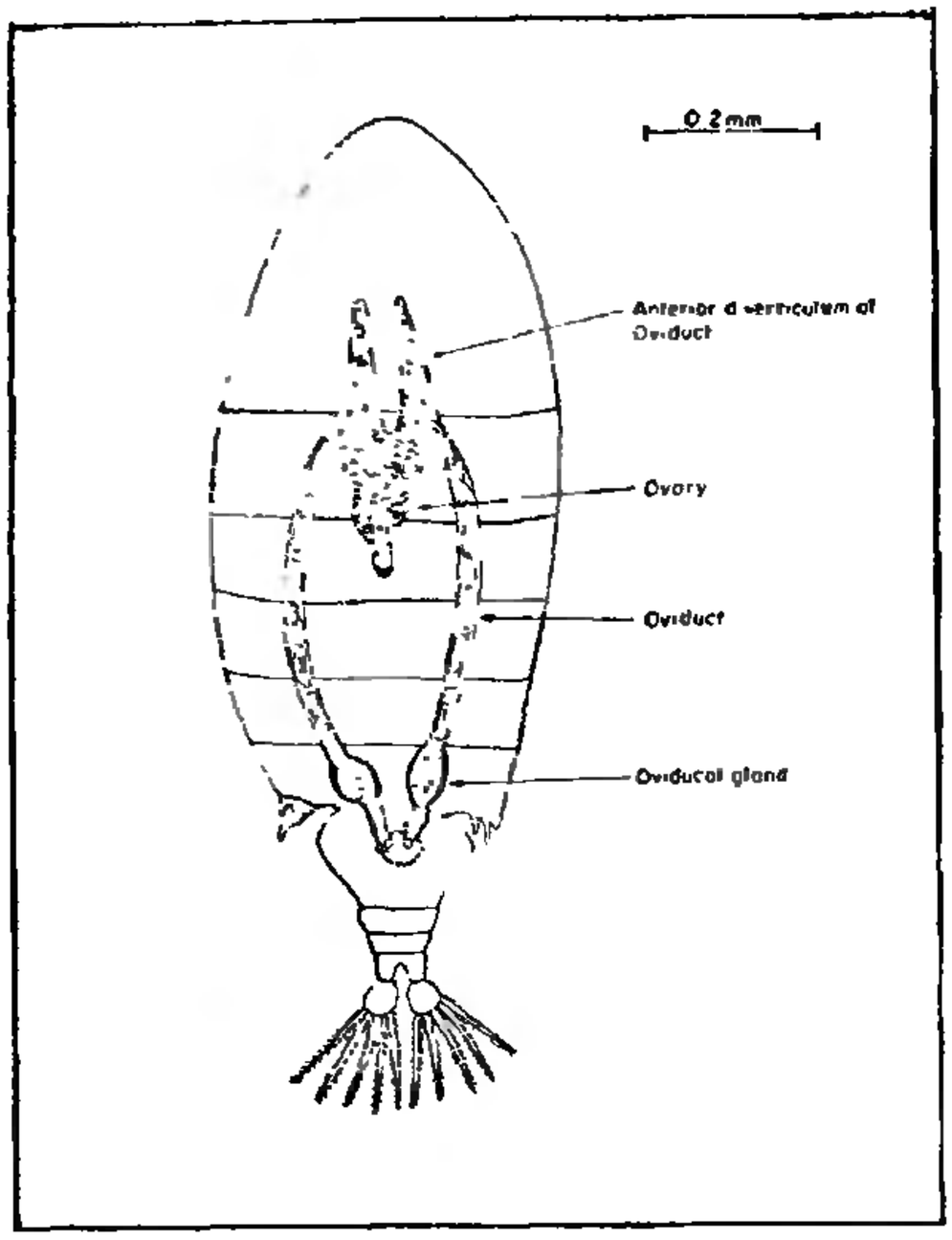

Figure 1. Diagram to show the position of component parts of reproductive system of adult female *Heliodiaptomus viduus* from the dorsal side.

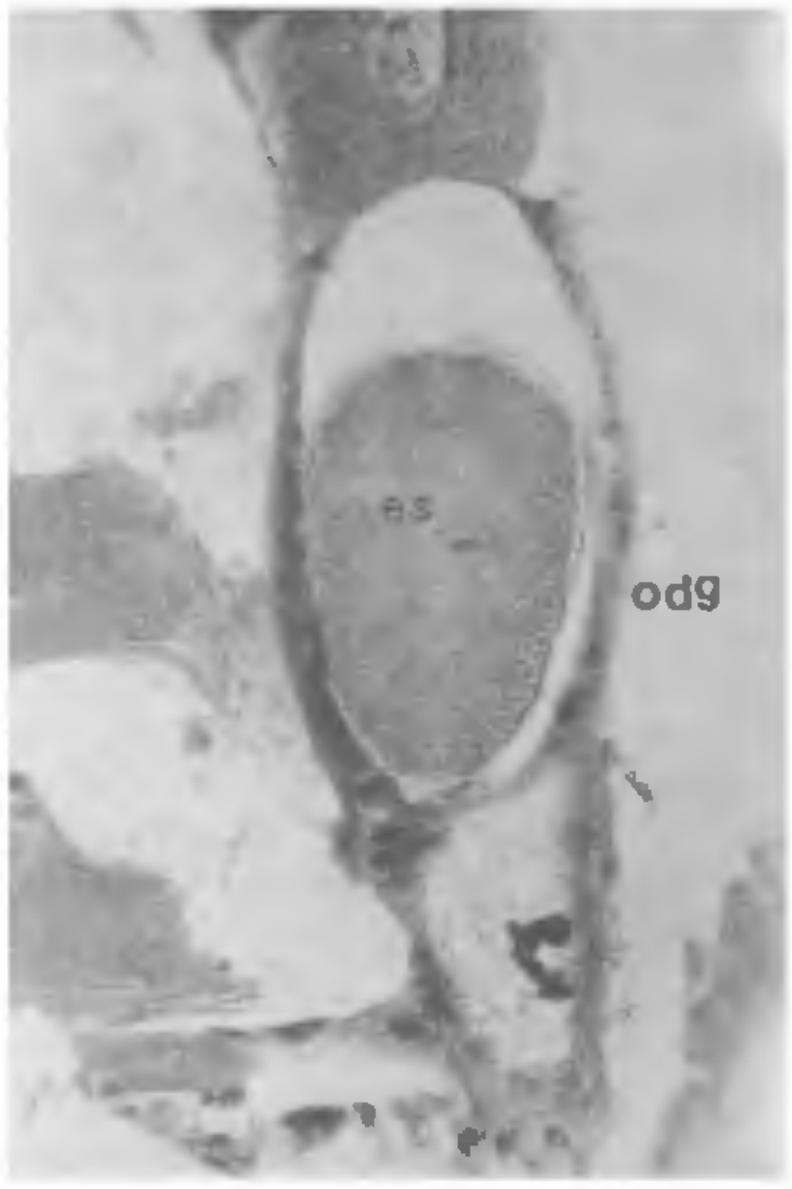

Figure 2. Vertical section of oviducal gland (× 160). es, elastic sac; Odg, oviducal gland.

Histological studies reveal that the wall of this gland is multicellular and thick (12.5 μ) when compared to the other parts of the oviduct; the cells are cuboidal in shape, highly glandular and arranged in 3 to 4 tiers. Secretion of this gland produces an elastic sac within itself which is elongate or oblong (Figure 2). It is observed that when the oviduct contains previtellogenic oocytes, the elastic sac is found attached to the inner wall of the oviducal gland all over its surface. As the oocytes get fully developed the elastic sac detaches from the wall of the oviduct and released into the distal part of the oviduct, located in the genital segment, where it holds the eggs for internal fertilization. After fertilization of eggs, the elastic sac transforms into ovisac in which fertilized eggs are enclosed (Figure 3).

The results of the histochemical tests are presented in Table 1. The wall and content of the oviducal gland give positive reaction to acidic and basic proteins besides showing positive reaction to Millon's test DMAB and PFS tests indicating the presence of tyrosine, tryptophan and disulphide groups. However, reaction to ferric-ferricyanide test is negative suggesting the absence of SH group. Though glycogen and mucopolysaccharides occur both in the wall and content of the oviducal gland, as revealed by the PAS test, intensity of the reaction is higher in the content than the wall. The acid mucopolysaccharides are characterized as phosphated and sulphated in the wall while the content also showed the presence of carboxyl group. Presence of acidic and neutral mucins is also indicated in the gland by the positive reaction to alcian blue PAS test. Tests for lipid on the wall of the oviducal gland indicate negativity whereas the content of the gland shows positive reaction to acidic and phospholipids.

There appears to be no counterpart for the oviducal gland and the elastic sac among other copepods studied so far. Histochemical tests indicate the direct involvement of the elastic sac secretion in the formation of ovisac. In some copepods such as *Calanus finmarchicus*[2] and *Epilabidocera amphitrites*[3] there is no ovisac formation and eggs are released directly into the water. In *Diarthrodes cystoecus*[4] an ovisac is released from the oviduct; however, the presence of an oviducal gland, as reported in the present study has not been found. The oviducal gland of *H. viduus* resembles that of cirripedes[5-7]. In the cirripedes *Pollicipes pollicipes*, *Balanus balanus* and *Semibalanus balanoides* the elastic sac formed by the oviducal gland holds the eggs in the mantle until they get fertilized. In *Semibalanus balanoides* the females become receptive for mating only after the formation of elastic sac.

In *H. viduus* mating may stimulate the release of elastic sac from the oviducal gland into the distal part of oviduct, where fertilization of the eggs takes place. It also appears that mating precedes all subsequent clutch formation in this species. Embryonic development up

Table 1. Histochemical reactions for proteins, carbohydrates and lipids in the oviducal gland of *H. viduus*

Tests	Wall	Content	To detect
Mercuric bromophenol blue	++B	++B	General protein
Aqueous bromophenol blue (ABB)	++B	++B	Basic proteins
Deamination + ABB	–	–	Removal of amino group
Ninhydrin Schiff	++B	++B	Amino group
Toluidine blue (TB)	++B	++B	Acidic protein
Methylation + TB	–	–	Removal of acidic group
Millon's test	+B	++B	Tyrosine
Bromination + Millon's	–	–	To block OH group
P-Dimethyl amino-benzaldehyde (DMAB)	+B	+B	Tryptophan
40% Formalin + DMAB	–	–	To block tryptophenyl group
Methyl green pyronin G (MGP)	+G	–	RNA
10% cold perchloric acid + MGP	–	–	To extract RNA
Ferric-ferricyanide (FFC)	+PB	–	S H Group
Mercuric chloride + FFC	–	–	To block –SH group
Performic acid Schiff	+M	+M	–S–S group
Thioglycollate + PAS	–	–	To block –S–S group
Feulgen's	+M	–	DNA
Schiff alone	–	–	Free aldehyde
Periodic acid Schiff (PAS)	++M	++M	Glycogen, 1.2,glycol, Unsaturated fatty acid, and acid mucopolysaccharides
Diastase + PAS	+M	+M	Removal of glycogen
Acetylation + PAS	–	–	Removal of 1,2 Glycol
Deacetylation + PAS	+M	+M	1,2 glycol group
Best Carmine	+	+	Glycogen
Alcian blue critical electrolyte concentration (CEC)			
0.2 M $MgCl_2$	+	++B	Carboxylated mucosubstances
0.4 M $MgCl_2$	–	+	Phosphated mucosubstances
0.6 M $MgCl_2$	–	+	
0.8 M $MgCl_2$	–	–	Sulphated mucosubstances
Toluidine blue (TB) at			
pH 2	–	+	Sulphated mucopolysaccharides
pH 4	–	++B	Phosphated and carboxylated mucopolysaccharides
pH 6	+	++B	
Benzidine test (Bracco-curti)	–	± I	Sulphated group
Chitosan	–	–	Chitin
Sudan black B (SBB)	–	+Bb	General lipids
Chloroform/Methanol + SBB	–	–	Removal of lipids
Oil red 'O'	–	± I	Neutral lipids
Nile blue sulphate	–	+B	Acidic and neutral lipids
Acid haematin	–	+R	Phospholipids

B, blue, Bb, bluish black; G, green; M, magenta; R, red, –, absent, I, doubtful, +, ++, degrees of positive reaction intensity

to naupliar stage also takes place in the ovisac and then its wall ruptures to release the nauplii.

Figure 3. Vertical section of urosome and ovisac (× 100). Os, ovisac.

Thus the oviducal gland of *H. viduus* is a distinct part of the oviduct which shows changes in accordance with the oocyte maturation and ovisac formation. Presence of this gland in this species as well as its resemblance to the oviducal gland of cirripedes indicate that the freshwater diaptomids are more specialized than their marine counterparts in this regard.

1. Adiyodi, K. G. and Anil Kumar, in *Reproductive Biology of Invertebrates* (eds. Adiyodi, K. G. and Adiyodi, R. G.), Oxford and IBH Publishing Co., New Delhi, 1988, vol. III, pp. 261–318.
2. Marshall, S. M. and Orr, A. P., *The Biology of a Marine Copepod, Calanus finmarchicus* (Gunnerus), Oliver and Boyd, Edinburgh, 1955, p. 188
3. Park, T. S., *Cellule*, 1966, **66**, 129–251.
4. Fahrenbach, W. H., *Cellule*, 1962, **62**, 302–376.
5. Klepal, W. and Barnes, H., *J. Exp. Mar. Biol. Ecol.*, 1977, **27**, 261–287.
6. Barnes, H. and Blackstock, J., *J. Exp. Mar. Biol. Ecol.*, 1977, **27**, 251–259.
7. Adiyodi, R. G., in *The Biology of Crustacea* (eds. Bliss, D. E. and Mantel, L. H.), Academic Press, 1985, vol. IX, pp. 147–215

ACKNOWLEDGEMENTS. We thank Dr. H. Md. Mohideen for laboratory facilities and Dr. N. Munuswamy for help in histochemical studies. We also thank Prof. T. Subramoniam for useful discussion while preparing the manuscript.

Received 1 September 1993, accepted 29 October 1993