ULTRASTRUCTURAL OBSERVATIONS OF THE PROTOCEREBRUM OF CHIROCEPHALUS DIAPHANUS PRÉVOST (BRANCHIOPODA, ANOSTRACA), WITH PARTICULAR REFERENCE TO NEUROSECRETION

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Electron microscope studies of neurosecretory elements in invertebrates, especially in insects, are now quite numerous. In the Crustacea, the malacostracan groups have attracted considerable attention. The majority of studies on malacostracans have been concerned with the fine structure of neurohaemal organs. The sinus gland of the crab *Gecarcinus lateralis* (Fréminville) was studied by Hodge & Chapman (1958) and more recently, Weitzman (1969) investigated the process of elementary granule release from the sinus gland of this crab. In the crab, *Carcinus maenas* (L.), Meusy (1968) investigated the ultrastructure of the sinus gland. In crayfish, Shivers (1967, 1969) and Bunt & Ashby (1967) have investigated the ultrastructure of the sinus glands, and Bunt & Ashby (1968) examined the effects of electrical stimulation of the sinus gland on the ultrastructure of this gland.

Other neurohaemal organs investigated with the electron microscope include the postcommissural organs (Knowles, 1958), the sinus glands (Knowles, 1959) and the pericardial organs of *Squilla mantis* (L.) (Knowles, 1962, 1964) and the pericardial organs of a number of Brachyura (Maynard & Maynard, 1962).

The only studies so far of the ultrastructure of the neurosecretory cells in the Malacostraca are those of Fingerman & Aoto (1959), Miyawaki (1960) and Shivers (1967). In the entomostracan groups, the only study so far published on the ultrastructure of the neurosecretory elements, is that of Bohm & Parker (1968) studying the supraoesophageal and optic ganglia of the cladoceran, *Daphnia schodleri* Sars.

The light microscopy of the neurosecretory system of the Anostraca has been studied by Lochhead & Resner (1958), Menon (1962), Hentschel (1963, 1965) and Lake (1969). In *Chirocephalus diaphanus*, Lake (1969) identified in the brain, four types of neurosecretory cells, three of which were paraldehyde-fuchsin positive and one type which was Light Green positive. These neurosecretory cells were predominantly confined to the protocerebrum and it appears that neurosecretory material, especially the paraldehyde-fuchsin positive type, is transported by axonal pathways from the protocerebrum to the optic neurohaemal organ in the eyestalk.
The present study was carried out in order to confirm the presence of neurosecretory cells in the protocerebrum of *C. diaphanous* and to examine their ultrastructure as well as the various non-secretory structures of this region of the brain.

**MATERIALS AND METHODS**

Mature specimens of *Chirocephalus diaphanous* were obtained from laboratory cultures. The animals were decapitated and the heads fixed in ice-cold 2% osmium tetroxide in veronal-acetate buffer at pH 7.2. Fixation was for a period of 2 hours at 4-5°C. The heads were then washed in two changes of cold 70% ethanol, and then left in 70% ethanol for one hour. They were then dehydrated through 80% ethanol, 90% ethanol and absolute ethanol. The heads were then cleared in propylene oxide and embedded in Araldite. Sections were examined with a Philips E.M. 75 electron microscope or an A.E.I. E.M. 6 electron microscope. Some sections were stained, prior to examination, with uranyl acetate and lead citrate.

**RESULTS**

The perineurium of the protocerebrum of *C. diaphanous* contains no well-differentiated connective tissue (pl. 1 fig. 1, pl. 2 fig. 1). The neural lamella consists of an outer osmiophilic membrane, 300 to 350 Å thick. Beneath this lamella, lies neuroglial tissue which is continuous with the glial processes surrounding the neurones. The perineurium is 2,000 to 3,000 Å thick.

The neurones of the protocerebrum are separated from one another and from the surrounding haemocoel by an enveloping sheath of neuroglial tissue (pl. 2 fig. 2). The neuroglial sheath is usually rather thin, of the order of 1,500 to 2,000 Å in thickness. In some cases, neuroglial processes may be invaginated into the cytoplasm of the neurones.

There appear to be two types of neuroglial cell nuclei. One type is small (2.5 μ in diameter), oval in outline and contains little electron-dense, chromatin material. The other type occurs in the neuroglial sheath of the brain and is generally elongated in shape and rich in electron-dense chromatin material (pl. 1 fig. 1).

The neurones of the protocerebrum of *C. diaphanous* are predominantly monopolar. They are orientated in a layer, 1 to 2 cells thick, around the neuropile of the protocerebrum (pl. 1 fig. 1). In outline, sections of the neurones are oval to polygonal, though the outline may be contorted by neuroglial invaginations. The nuclei of the cells are typically oval in shape and central in position in relation to the cytoplasm, though in occasional cells, the nuclei may be indented (pl. 2 fig. 1). Coarsely granular electron-dense, chromatin material is scattered throughout the nucleoplasm. The nucleoli are electron-dense and irregularly oval in outline (pl. 2 fig. 2).

In the neuropile, when viewed in transverse section, a range in the size of the diameters of the axons can be discerned (pl. 1 fig. 2). The largest axons of the neuropile are generally 0.4 to 0.6 μ in diameter and the smallest axons are between 0.1 to 0.2 μ in diameter. Neuroglial tissue surrounding the axons is not always easy to discern, though in the case of the larger axons it is usually present.