The Occurrence of Peripherical Molluscan Neurons (*Cryptomphallus aspersa*)

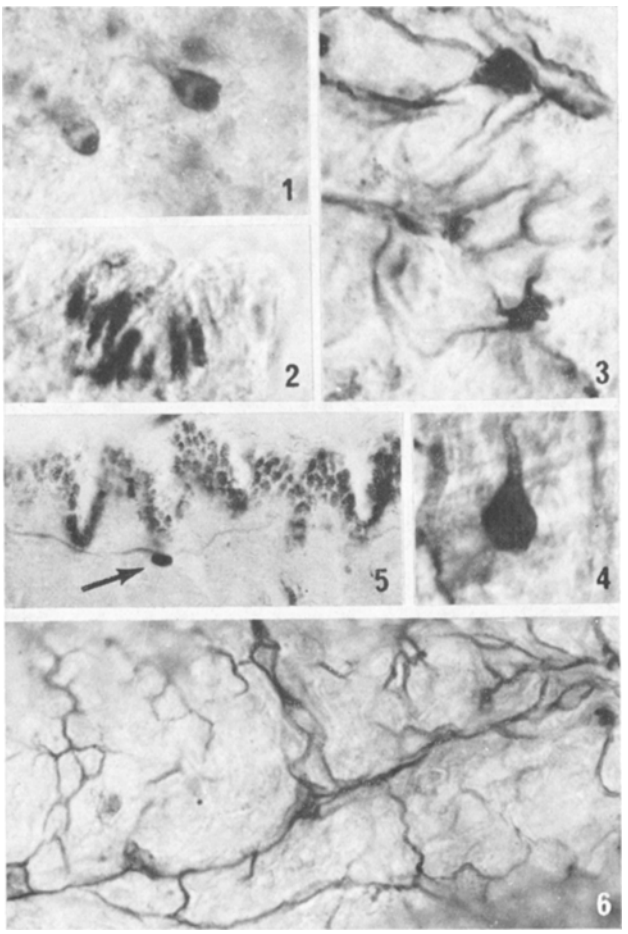
In the course of neurophysiological studies on the bioelectrical activity of the giant neurons of the land snail *Cryptomphallus aspersa*, IZQUIERDO[1] found that the mantle region, mainly the respiratory pore, was very sensitive to mechanical stimulation. This was evidenced by the appearance of evoked potentials in impalled giant neurons of the ventral ganglionic mass. The cells and plexuses found in the mantle collar by using methylene blue staining at the light microscopic level will be reported here.

The mantle membrane and a block of tissue which included a part of the mantle collar and the respiratory pore were dissected free in 17 adult specimens of *C. aspersa* (Gasteropoda, Pulmonata) and studied in different ways. A solution of 0.30 mg of methylene blue in 100 ml of Ringer solution for snails[2] was vitally perfused in all cases by intracardiac injection. The preparations were dissected and fixed by inmersion in ammonia molibdate 4% in the same Ringer solution. Sections from the tissue blocks and the mantle membrane were both squashed after making thin manual sections with a razor blade. The second group, quantitatively the most numerous, was dehydrated, passed through butanol[3] and xylol, embedded in paraffin and sectioned to 14 μ thickness. The third group was cut on the freezing microtome. Controls of this zone were made by staining with Nissl-thionine and hematoxylin-eosin.

Cells with different shapes and other distinctive morphological features may be recognized. Type I consists of a monopolar oval cell (about 8×15 μ) of irregular shape and with a deeply stained nucleus. This type occurs both in the mantle membrane (Figure 1) and in the mantle collar (Figure 4) and their appearance resembles the neurons of the central nervous system[4]. Type II are multipolar cells, triangular or pentagonal in shape, about 8×9 μ, with processes which run among the colourless neighbouring cells (Figure 3). The cytoplasm is rather scant and the nuclei is large, sometimes pale blue, showing 1 or 2 nucleoli. In the mantle membrane, at low magnification, it may be observed that Type II cells are more numerous than Type I, and they form there mixed groups spred in the non-pigmented zones of the mantle membrane. By the other hand, in the mantle collar, this type is located between the surface and the pigment layer of the epithelium, showing slight shape variations by the arrangement of their processes, generally all going toward the surface. Type III cells are more numerous bipolar cells with a small soma, about 4×7 μ in diameter (Figure 5). These cells are situated near the epithelial surface of the mantle collar and their processes extended either parallel or perpendicular to the surface. In the last case, the distal process is shorter than the proximal, which runs down and makes contact with a plexus, at the level containing the pigment layer. Type IV consists of little cell bodies without visible processes, arranged in clusters of about 33×42 μ, near the epithelial surface of the mantle collar (Figure 2). In the squash made with the mantle membrane, as in the sections of the mantle collar, a rich plexus of fibres and some Type II cells intensely stained with the methylene blue against a colourless background are observed (Figure 6). This plexus is particularly rich in the vicinity of the respiratory pore.

The methylene blue staining, scantly used in recent years, gave in this case good pictures. The use of the perfusion method and of a special Ringer solution[2] instead a 'standard' one seems to be the decisive factors in the success of this staining. The specificity of the methylene blue to the nervous system, for a long time under discussion[5], has recently been confirmed, including the possibility of discriminating cholinergic from adrenergic axons at the electron microscope level[6,7].

Early authors pointed out the rich nerve supply of the body wall in gastropods[8–11] and attempted a morpho-



1. Type I cells of the mantle membrane ($\times$ 630). 2. Type IV cells of the mantle collar ($\times$ 630). 3. Type II cells of the mantle membrane ($\times$ 1150). 4. Type I cell of the mantle collar ($\times$ 1150). 5. Type III cell (arrow) of the mantle collar ($\times$ 630). 6. Plexuses and Type II cells in the mantle membrane ($\times$ 630).

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physiological correlation, but the physiological implication was not demonstrated. BULLOCK and HORRIDGE[12] point out that much evidence exists on the presence of clusters of peripheric sensory cells in gastropods. The possibility that plexuses play a convergence way role has also been postulated[12], and this would be their probable meaning. The recording of evoked potentials in central neurons after mechanical stimulation of the mantle collar, the amount of free ending processes and the presence of several cellular types leads us to assume the sensory role played by any of these elements in the mantle collar. However, more detailed physiological studies are necessary to support further conclusions[13].

Resumen. Se describen en la membrana y collar del manto próximos al poro respiratorio del molusco *Cryptomphallus aspersa* (Gasteropoda, Pulmonata) la presencia de varios tipos de neuronas y plexos. Estas observaciones morfológicas se discuten en relación a hallazgos previos con técnicas electrofisiólogicas obtenidos por otros autores.

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Delayed Cutaneous Hypersensitivity Reaction to Tumor-specific Antigens of Fibrosarcoma in Sensitized Mice

Homograft immunological reaction in animals sensitized to grafts of allogeneic tissue can be manifested as a cutaneous hypersensitivity reaction of the delayed type[1–3]. Tumor-specific antigens of some experimentally induced tumors produce delayed hypersensitivity reactions[4–8]. These reactions to tumor-specific antigens can be detected by the foot pad test[7,8], by inhibition of macrophage migration from capillary tubes[6], and by the passive transfer reaction[5]. Concomitant with the cutaneous reactions of the delayed hypersensitivity is a local increase of vascular permeability[3,9]. In this report we describe such an increase evoked in mice by tumor-specific antigen(s) of a fibrosarcoma induced by methylcholanthrene.

Studies were performed on C57BL mice with the third isotransplant generation of the methylcholanthrene-induced fibrosarcoma. Male C57BL mice, 3 months old, were immunized to the tumor. Immunization was performed by 3 consecutive injections of 2×10^7 irradiated tumor cells at 7-day intervals. Tumor cells were irradiated with 10,000 R with a 250 KV Phillips X-ray machine. For the first injection, cells were mixed with an equal volume of the complete Freund's adjuvant (Difco, Detroit) and then injected s.c. into both axillar and inguinal regions. The second and third injections were administered i.p. without adjuvant.

A suspension of single tumor cells was prepared from non-necrotic tumor pieces minced in physiological saline and passed through nylon gauze. The suspended cells were washed 3 times by centrifugation and finally resuspended in a desired volume of physiological saline. 7 days after the last immunizing injection, the mice were clipped on both flanks and injected intradermally with syngeneic spleen and liver cells mixed together and tumor cells. Each mouse received the tumor cells on the left side and the spleen and liver cells on the right side. The number of injected cells was 3×10^6 suspended in 0.05 ml of physiological saline. Spleen and liver cells from C57BL male mice were prepared in the same manner as tumor cells. Controls were normal C57BL mice injected intradermally with tumor and syngeneic spleen and liver cells.

24 h after the intradermal inoculation of cells, when the increase of vascular permeability due to delayed

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Delayed cutaneous hypersensitivity reactions in C57BL mice injected intradermally with syngeneic spleen and liver or fibrosarcoma cells

Recipients	Challenge (i.d.)	No. of mice with positive reaction over number of injected mice	Mean surface of blue areas at the site of injection ($mm^2 \pm$ S.E.)	
Immunized to tumor	Tumor cells	13/16	2.57 ± 0.86	$P < 0.01$
	Spleen and liver cells	4/16	0.19 ± 0.08	$P < 0.01$
Normal	Tumor cells	1/8	0.09	$P < 0.01$
	Spleen and liver cells	1/8	0.09	