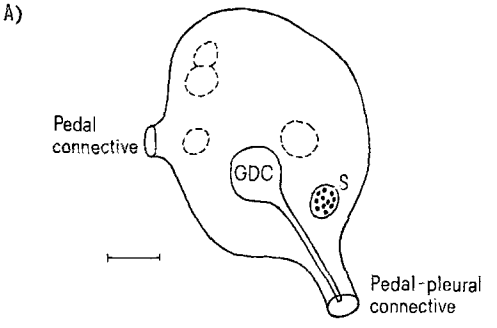
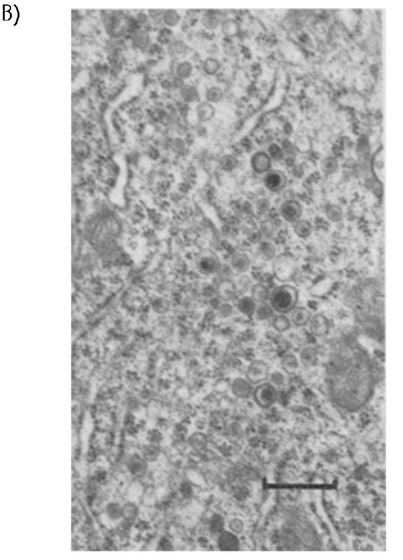
Ultrastructure of a Giant Dopamine-Containing Neurone in *Planorbis corneus*

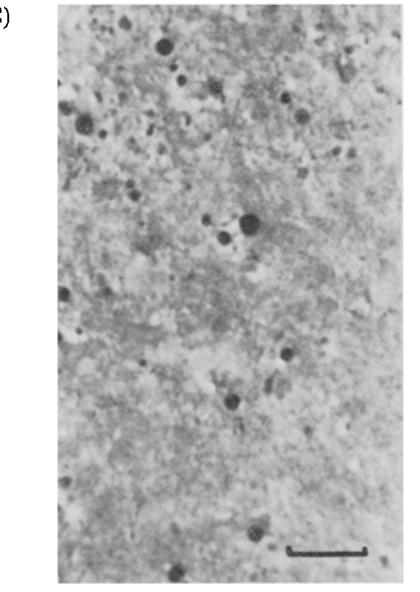
Dopamine probably acts as a neurotransmitter in the central nervous system of gastropod molluscs. The substance is localized in some neurone somata and their axons, and in certein varicosities present in areas of synaptic contact[1–5]. Many neurones undergo permeability changes when exposed to low concentrations of dopamine[6–8]. Electrical stimulation of nerve trunks produces inhibitory potentials in many neurones which appear to be mediated by dopamine because of their responses to drugs which antagonize dopamine receptors or modify levels of brain dopamine[7–9]. BERRY and COTTRELL[10] have demonstrated that an identified dopamine-containing neurone in *Planorbis corneus*, forms excitatory, inhibitory and dual excitatory-inhibitory synaptic links with other neurones in the central nervous system of the snail. Pharmacological evidence[10] suggests that dopamine is the transmitter released at these synapses. We have examined this neurone (termed giant dopamine cell, GDC[10]) in order to obtain information on the subcellular localization of dopamine. Results of the experiments suggest that dopamine is associated with granulated vesicles of 50–250 nm diameter.

The GDC is located in the left pedal ganglion of *Planorbis corneus*. MARSDEN and KERKUT[11] first noted that the cell fluoresced green after treatment by conventional fluorescence histochemical techniques, and concluded that it contained dopamine since dopamine, but no noradrenaline, was detected in the ganglion. Microspectrofluorimetric analysis of the identified neurone provides direct evidence for the presence of dopamine (communication by B. FALCK). Reserpine injected into snails in amounts of 0.1 to 1 mg during 24 to 48 h greatly reduced or abolished green fluorescence in the GDC.

The GDC soma varies in size (range 80–240 µm) in different animals, but is usually the largest in the ganglion. The large size and constant position of the cell on the surface of the ganglion made the cell readily identifiable (Figure A). Electron microscopy of the cell showed the presence of vesicles with granular cores (Figure B), together with mitochondria, particles resembling lysosomes







The diagram (A) shows the position of the giant dopamine cell (GDC) on the ventral surface of the isolated left pedal ganglion of *Planorbis corneus*. The diagram has been reproduced to scale from the photograph of a preparation. The statocyst (S) aids identification of the GDC. The dotted circles indicate other neurone somata whose sizes approach that of the GDC. The axon of the GDC leaves the left pedal ganglion via the left pedal-pleural connective. The scale is 200 µm. B) A group of dense-cored vesicles in the cytoplasm of a GDC fixed in veronal buffered osmium solution and stained with lead citrate and uranyl acetate. The scale is 0.5 µm. C) Electron dense deposits, which represent sites of amine localization, in a GDC processed by WOOD's method[15]. The scale is 0.5 µm.

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and other inclusions typical of molluscan neurones[12,13]. The axon of the GDC was traced for several hundred microns with semi-serial sections. Dense-cored vesicles similar to those present in the soma were seen at all levels in the axon. Some neurones adjacent to the GDC also contained dense-cored vesicles (diameters about 100 nm), but they were rarely observed in such large numbers as those in the GDC.

Isolated ganglia are fixed and processed by the method of WOOD[14] for detecting amines contained electron-dense reaction products in the cytoplasm of the GDC (Figure C). These reactive sites correspond in size and distribution to the cores of the dense-cored vesicles. The histochemical method has been shown to be specific for amines in molluscan nervous tissues[15]. We therefore conclude that at least a part of the dopamine within the GDC is located within the dense-cored vesicles. The vesicles have similar appearance to those which are thought to bind primary catecholamines in bivalve ganglia[16,17].

Some presynaptic endings of the GDC are thought to occur in the neuropile of the visceral and parietal ganglia[10]. Electron microscopic examination of these areas showed many axon profiles and presumed nerve endings which contained concentrations of dense-cored vesicles similar to those in the GDC soma. The presence of such vesicles in the GDC soma and in its axon, and within neuropile thought to contain some of its presynaptic endings is consistent with a transport of dopamine in intraneuronal vesicles. Thus it appears that there is a close parallel to the situation which occurs in the noradrenergic neurone[18].

Résumé. La microscopie électronique d'un neurone géant à dopamine dans le système nerveux central de *Planorbis corneus* à révélé la présence de vésicules sous-celluleuses granulaires d'un diamètre de 50–250 nm. Il est probable que ces vésicules retiennent la dopamine.

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Ultrastructural Observations on the Inhibition of Nucleolar Reorganization by 3'-Deoxyadenosine in *Allium cepa* Roots

The inhibition of nucleolar reorganization in the telophase in *Alliun cepa* meristematic cells was previously studied under the light microscope[1,2] by means of drugs which inhibit the synthesis of nucleic acids, such as 3'deoxyadenosine (3'AdR)[3] or ethidium bromide[4]. These drugs do not block the formation of the prenucleolar bodies, but the latter prove incapable of aggregating so as to form nucleoli. In this study we have tried to analyse the ultrastructure of the prenucleolar material while nucleolar reorganization is inhibited.

For this purpose we used binucleate cells produced by caffeine and treated with 3'AdR, an inhibitor of ribosomal RNA synthesis which is already known[5].

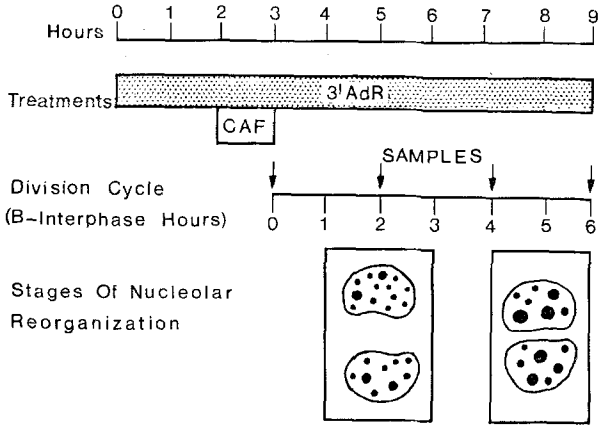

Fig. 1. Schematic diagram of the treatments with caffeine and cordycepin. There is no reorganization of the nucleolus.

Material and methods. The material used consisted of *Allium cepa* roots grown at 15° ± 0.5 °C in tap-water, which was renewed every 24 h, and with continuous aeration (10–20 cm³/min). The roots, still attached to the bulbs, were immersed in a 0.1% solution of caffeine for 1 h. This drug inhibits cytokinesis in cells passing through the telophase, so producing a binucleate meristematic population which enters the interphase and goes through the cell division synchronously[6].

The 3'AdR, 10^{-4} *M*, was used in continuous treatment for 9 h, beginning 2 h before the caffeine treatment (Figure 1). The roots were fixed as follows: a) with 3% glutaraldehyde in Millonig's buffer at pH 6.9 for 2 h, followed by 2% omium tetroxide for 1 h; b) with 3% glutaraldehyde in Millonig's buffer for 2 h, this material being destined for staining with uranyl-EDTA-lead[7]; c) in 5% formaldehyde-0.5% hydroquinone, for a silver impregnation technique[8] slightly modified. All the specimens were included in EPON 812. Thick sections (2 μm) were observed by phase contrast microscopy so as to locate the binucleate cells, with a view to studying

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