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.

V. W. PENTREATH and G. A. COTTRELL[19]

*Wellcome Laboratories of Pharmacology, Gatty Marine Laboratory, University of St. Andrews Fife (Scotland), 10 September 1973.*

[12] R. E. COGGESHALL, J. Neurophysiol. *30*, 1263 (1967).
[13] J. ROSENBLUTH, Z. Zellforsch. *60*, 213 (1963).
[14] J. G. WOOD, Nature, Lond. 209, 1131 (1966).
[15] G. A. COTTRELL and N. N. OSBORNE, in *Comparative Physiology of the Heart: Recent Trends* (Ed. F. V. MCCANN, Experientia Suppl. 15 (Birkhäuser Verlag, Basel 1969).
[16] I. ZS-NAGY, in *Neurobiology of Invertebrates* (Ed. J. SALANKI; Akadémiai Kiadó, Budapest, 1968), p. 69.
[17] G. A. COTTRELL, in *Neurobiology of Invertebrates* (Ed. J. SALANKI; Akadémiai Kiadó, Budapest, 1968), p. 353.
[18] A. DAHLSTRÖM, Phil. Trans. R. Soc. Lond. B, *261*, 325 (1971).
[19] We thank the Medical Research Council for financial support.

## 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].

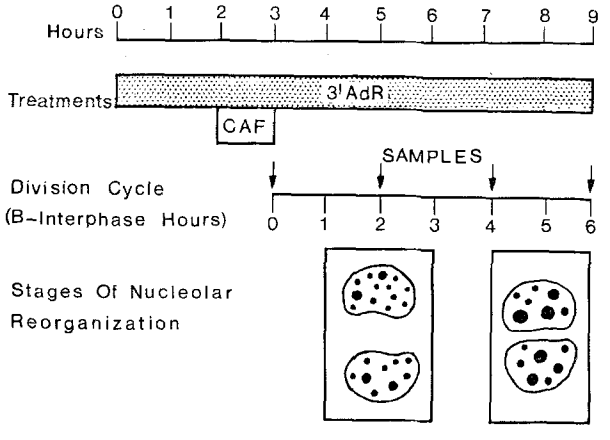


Fig. 1. Schematic diagram of the treatments with caffeine and cordycepin. There is no reorganization of the nucleolus.

*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

[1] J. C. STOCKERT, M. E. FERNANDEZ-GOMEZ, G. GIMENEZ-MARTÍN and J. F. LOPEZ-SAEZ, Protoplasma *69*, 265 (1970).
[2] G. GIMENEZ-MARTÍN, C. DE LA TORRE, M. E. FERNANDEZ-GOMEZ and A. GONZALEZ-FERNANDEZ, Caryologia *25*, 43 (1972).
[3] B. A. KIHLMAN and G. ODMARK, Hereditas *56*, 71 (1966).
[4] B. A. NEWTON, in *Metabolic Inhibitors* (Eds. R. M. HOCHSTER and J. H. QUASTEL; Academic Press, New York 1963 ),vol. 2, p. 285.
[5] M. SIEV, R. WEINBERG and S. PENMAN, J. Cell Biol. *41*, 510 (1969).
[6] G. GIMENEZ-MARTÍN, A. GONZALEZ-FERNANDEZ and J. F. LOPEZ-SAEZ, J. Cell Biol. *26*, 305 (1965).
[7] W. BERNHARD, J. Ultrastruct. Res. *27*, 250 (1969).
[8] J. C. STOCKERT, O. D. COLMAN, M. E. FERNANDEZ-GOMEZ and G. GIMENEZ-MARTÍN, Experientia *25*, 1306 (1969).

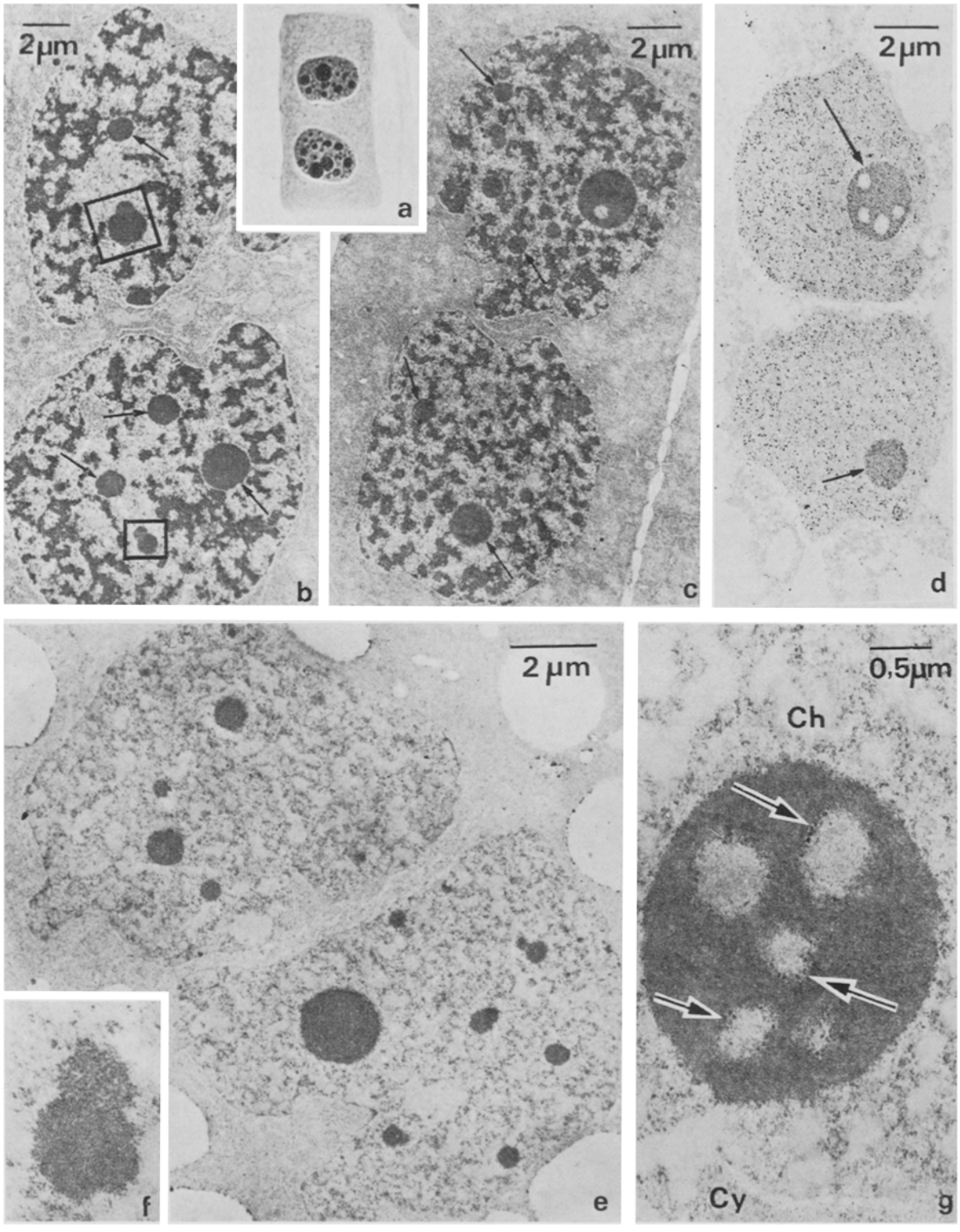


Fig. 2. a) Silver impregnated prenucleolar bodies in a binucleate meristematic cell treated with 3'AdR, observed under the light microscope. b) and c). Binucleate cells after 5 and 7 h treatment with 3'AdR respectively, showing prenucleolar bodies (arrows). Uranyl-lead staining. d) Binucleate cell after 9 h treatment with 3'AdR. The arrows indicate the prenucleolar bodies. Silver impregnation for electron microscopy. e) and g) With the use of the uranyl-EDTA-lead staining method, the prenucleolar bodies appear deeply stained, Cy, cytoplasm; ch, chromatin; arrows indicate granules of 400–500 Å in diameter. f) Prenucleolar body with 'cap'. Uranyl-EDTA lead staining method.

them under the electron microscope. The observations were realized with a Philips 300 EM[9].

*Results and discussion.* In the fine sections we observed the binucleate cells subjected to the action of the 3'AdR for 3, 5, 7 and 9 h (Figure 1). The presence of 3'AdR for 2 h before the caffeine treatment justifies us in assuming that the binucleate cells produced subsequently have gone through their anaphase and telophase under the effect of the 3'AdR.

The nuclei of these cells show numerous lobulations at the surface. The prenucleolar material can be observed in the interchromatinic spaces in the form of small round bodies of varying sizes (Figure 2b and c) and of argyrophilic nature (Figure 2 a). These prenucleolar bodies appear compact and consist of apparently fibrillar material, homogeneously distributed and possessing an electron density similar to that of the pars fibrosa of a normal nucleolus. In the first few hours of the interphase, the prenucleolar bodies generally do not show any vacuoles inside them, but in the course of the following hours, in addition to some small bodies, other larger bodies appear with vacuoles in them. Both the smaller and the larger ones generally show granules 400–500 Å in diameter on the periphery and on the inner surface of the vacuoles (Figure 2g). In some of the prenucleolar bodies observed, there is a small 'cap' of material that appears to be trabecular in nature.

With the uranyl-EDTA-lead method, which is selective for RNA staining[7], the prenucleolar bodies show up in strong contrast, while the chromatin remains unstained (Figure 2e). With this staining technique, the granules as well as the described 'caps' appear positive (Figure 2f–g).

With silver impregnation, the prenucleolar bodies stand out clearly in the nucleus (Figure 2d), appearing homogeneous in point of staining intensity and size of silver grains, which are generally smaller than those seen in the rest of the nucleus.

As morphological elements typical of telophase nuclei, the prenucleolar bodies have already been studied in previous papers[1,2], and it is worth calling attention to their argyrophilia[10,11] and to their staining affinity with uranyl-EDTA-lead[9,12]. The absence of nucleolar reorganization in the telophase, while the prenucleolar bodies are still present under the effect of RNA synthesis inhibitors, has been studied under the light microscope[1,2]. This paper confirms the results previously obtained, and the staining peculiarities of the prenucleolar material. The phenomenon of vacuolization observed in the larger prenucleolar bodies – probably due to the coalescence of smaller bodies – appears to be similar to that which is found in interphase nucleoli treated with 3'AdR[2]. In addition to the prenucleolar material of fibrillar character, and in close connexion with it, we can also observe other ultrastructural elements: the small caps of apparently trabecular material, similar to the 'coiled bodies'[13], and granules measuring 400–500 Å in diameter which seem to resemble those observed in *Allium cepa* interphase nucleoli under the action of 3'AdR[14].

*Resumen.* El estudio ultraestructural de los cuerpos prenucleolares en células meristematicas de *Allium cepa*, en las que se ha inhibido la reorganizacion del nucleolo por accion del 3'AdR, revela la naturaleza fibrilar de los mismos, la existencia sobre ellos de granulos de 400–500 Å de diámetro, así como formaciones a modo de «cap» de material con aspecto trabecular. Se observa a lo largo del tratamiento un fenómeno de vacuolación semejante al descrito en nucleolos interfasicos tratados con 3'AdR.

A. M. SACRISTAN-GARATE[15] and J. C. STOCKERT

*Departamento de Citología, Instituto de Biología Celular, C.S.I.C., Velázquez 144 Madrid-6 (Spain), 26 October 1973.*

[9] J. C. STOCKERT, O. D. COLMAN and P. ESPONDA, J. Microsc. *9*, 823 (1970).
[10] C. J. TANDLER, Expl. Cell Res. *17*, 560 (1959).
[11] N. K. DAS and M. ALFERT, Ann. Histochem. *8*, 109 (1963).
[12] J. L. DIEZ, M. F. MARIN, P. ESPONDA and J. C. STOCKERT, Experientia *27*, 266 (1971).
[13] W. BERNHARD, in *Advances in Cytopharmacology*, First International Symposium on Cell Biology and Cytopharmacology (Rave Press, New York 1971), vol. 1, p. 49.
[14] G. GIMENEZ-MARTÍN, M. C. RISUEÑO, M. E. FERNANDEZ-GOMEZ and P. AHMADIAN, Cytobiologie *7*, 181 (1973).
[15] The work reported in this paper was undertaken during the tenure of a Fellowship awarded by the Ministerio de Educación y Ciencia of Spain to one of us (A.S.G.).

## Neural Differentiation in Aggregates Containing Mixtures of Cell Types

One of the most striking aspects of neural development is the differentiation of several types of neurons which associate themselves by means of specialized junctions, the synapses. Very little is known about the cell interactions required for the development of the synaptic junction. The great complexity of the embryonic neural tube makes it very difficult to analyse these interactions in vivo, so that it is advisable to use simplified models. Reaggregation cultures[1,2] which have proved to be important tools for studying cell interactions, have been used to study various aspects of neural differentiation[3–9]. This culture technique is being used in our laboratory to study interactions between different types of neural cells or between neural and non-neural cells when they become associated in 'combined' aggregates. Some of the ultrastructural observations reported here indicate that this procedure can give valuable information on the mechanisms which regulate synapse formation.

Optic lobe (OL), telencephalon (T), neural retina (NR) and limb (LB) cells from 7-day-old White Leghorn chick embryos were used. These tissues were chosen because some of them (e.g.: neural retina and optic lobe) are known to interconnect selectively during development. Before dissociation, OL and T were freed from surrounding mesenchyme, and NR isolated from pigmented retina. Dissociation was carried out as previously described[4,5], following with some modifications the procedures developed by MOSCONA[1] and STEINBERG[2]. Cell suspensions were adjusted to a final concentration of 10–20 ×

[1] A. A. MOSCONA, in *Cells and Tissues in Culture* (Ed. E. N. WILLMER; Academic Press, New York 1965), vol.1, p. 489.
[2] M. S. STEINBERG, in *Methods in Developmental Biology*; (Eds. E. H. WILT and N. K. WESSELS; Crowell, New York 1967), p. 565.
[3] A. STEFANELLI, A. M. ZACCHEI, S. CARAVITA, A. CATALDI and L. A. IERADI, Experientia *23*, 199 (1967).
[4] R. ADLER, Devl. Biol. *21*, 403 (1970).
[5] R. ADLER, Expl. Cell Res. *68*, 395 (1971).
[6] R. ADLER, Expl. Cell Res. *77*, 367 (1973).
[7] G. R. DE LONG and R. L. SIDMAN, Devl. Biol. *22*, 584 (1970).
[8] J. B. SHEFFIELD and A. A. MOSCONA, Devl. Biol. *23*, 36 (1970).
[9] S. M. CRAIN and M. B. BORNSTEIN, Science *176*, 182 (1972).