

Nuclear bodies in the normal and hyperfunctional human adrenal cortex

M.M. Magalhães¹, D. Carvalho², M.A. Oliveira¹ and M.C. Magalhães¹

¹Institute of Histology and Embryology and ²Department of Medicine 4, Faculty of Medicine of Porto, and Center of Experimental Morphology of the University of Porto (INIC), Porto, Portugal

Summary. Adrenal pieces obtained from six female patients, three without increased adrenocortical function and three with Cushing's disease, showed, in all adrenal cortex zones, cells containing simple and complex nuclear bodies. The simple nuclear bodies were spherical or ovoid and had a filamentous structure surrounded by a clear halo. Complex nuclear bodies were more numerous and heterogeneous in patients with adrenal pathology, and they were spherical with a proteinaceous filamentous capsule surrounding a core; the core was granular, filamentous or a mixture of granular and filamentous material, sometimes with a reticular or concentric arrangement. Some bodies showed vacuolar or multilocular aspect, and others had a close relationship with the nucleolus or appeared near the interchromatin granules.

The meaning of adrenal nuclear bodies is discussed as well as their relationship with ACTH stimulation.

Key words: Adrenal, Ultrastructure, Nuclear bodies

Introduction

Nuclear bodies were first reported by De The et al. (1960) and thereafter they have been found in several tissues of different animal species in both normal and pathological conditions (Bouteille et al., 1974). In the last years numerous morphological and cytochemical studies have permitted a better knowledge of nuclear bodies (Sobrinho-Simões and Gonçalves, 1974; Dupuy-Coin and Bouteille, 1975; Le Goascogne and Baulieu, 1977; Doyle, 1980; Paula-Barbosa et al., 1980; Vagner-Capodano et al., 1980, 1982; Padykula et al., 1981; Fitzgerald and Padykula, 1983; Padykula and Pockwinse, 1983; Chegini and Rao, 1984; Cidadão and David-Ferreira, 1984; Masurovsky and Fields, 1984;

Echeverria et al., 1985); however, the origin and the functional meaning of these nuclear structures are not yet well clarified.

In adrenal cortex, nuclear bodies were observed in calf (Weber et al., 1964) and in domestic fowl (Kjaerheim, 1968a, b), but in human cortex, to the best of our knowledge, they have not been described so far. During a study of human adrenal cortex obtained from patients with Cushing's disease we were impressed by the presence of numerous nuclear bodies. The present report concerns some ultrastructural features of nuclear bodies of human adrenal cortex and their possible functional role.

Materials and methods

Adrenal fragments were obtained from six female patients ranging from 18 to 29 years old, three without increased adrenocortical function (cyst, acrocyanosis, and a female patient with polycystic ovary that underwent adrenal surgery because she had an adrenal pseudo tumor on the TAC) and three with Cushing's disease. The disease was diagnosed according to clinical and hormonal criteria, namely the glandular suppression following the administration of 2 mg of dexamethasone every 6 hours during three days - the large dose of dexamethasone suppression test (LDDT), which is not observed with a small dose (SDDT). The patients received thiopentone (Pentothal), succinylcholine (Scoline), diallyl-nor-toxiferine (Alloferine), and pethidine as anaesthetic drugs. After removal, the adrenals were quickly sectioned into small pieces and immersed in the fixative. The fragments were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.2, at 4° C for two hours, rinsed in buffer with 7% sucrose overnight, postfixed in 1% osmium tetroxide in veronal-acetate buffer, pH 7.3, at 4° C for 2 hours, dehydrated in a graded series of ethanol and Epon embedded. Semithin sections 1 or 2 µm in thickness were stained with methylene blue-azur II (Richardson et al., 1960). For

electron microscopy, ultrathin sections were stained with alcoholic uranyl acetate for 15 minutes and lead citrate for 10 minutes. The regressive EDTA staining procedure (Bernhard, 1969) was performed on ultrathin sections of pieces fixed in glutaraldehyde and osmium tetroxide according to Bendayan and Zollinger (1983). The sections were treated with sodium metaperiodate in distilled water for 60 minutes at room temperature, stained in 5% aqueous uranyl acetate 1 min, 0.2M EDTA in distilled water 10 min, and lead citrate 1 min.

Enzymatic digestions

Ultrathin sections on uncoated copper grids were treated with H_2O_2 for 15 minutes, washed in distilled water and immersed in 0.5% pronase (TAAB Laboratories, Emmer Green, Reading, England) in distilled water pH 7.4 for 30 and 90 minutes at 40° C; 0.5% pepsin (Worthington Biochemical Corp., Freehold, N.J.-USA) in 0.1M HCl for 60 and 240 minutes at 38° C; 0.5% ribonuclease (Koch - Light Laboratories Ltd., Colnbrook, England) in distilled water, pH 6.8, for 60, 120 minutes and 5 hours at 38° C (Monneron and Bernhard, 1966). The sections were washed with distilled water and stained with uranyl acetate and lead citrate.

Results

Nuclear bodies were observed in cells of all adrenal zones from all patients referred to in the material and methods. They could be separated into two major populations: simple and complex nuclear bodies. In patients without increased adrenocortical function simple bodies were predominant, while in patients with adrenal pathology complex bodies appeared in larger number (2-3

per nucleus). Sometimes, both simple and complex nuclear bodies were seen in the same nucleus (Figs. 1, 3).

Simple nuclear bodies were spherical or ovoid, measured $\sim 0.5 \mu m$ in diameter and had a filamentous structure; a clear halo surrounded them (Figs. 1, 3).

Complex bodies were spherical, structurally heterogeneous and measured 0.9-1.8 μm in diameter (Figs. 1-7). They consisted of a spherical filamentous capsule that surrounded a core which contained granular, filamentous, or a mixture of granular and filamentous material (Figs. 1-7), sometimes with a reticular or concentric arrangement (Figs. 2, 4, 5). Surrounding the capsule there was often a clear zone in which some filaments were in apparent connection with the capsule. The most frequent bodies presented a filamentous capsule and a core composed of dense granules of variable size (Figs. 2, 6, 7); they could be classified as type III or IV according to the classification of Bouteille et al. (1967). A few bodies had a close relationship with the nucleolus, but not with the nuclear envelope. Sometimes clusters of interchromatin granules occurred near the nuclear bodies (Figs. 1, 4).

The treatment with pronase and pepsin showed a digestion of the simple bodies and of the capsule of the complex ones (Figs. 8, 9). Ribonuclease treatment did not affect either the simple or the complex nuclear bodies (Fig. 10).

The EDTA technique produced an overall decrease in the electron density of chromatin areas, while structures which contained ribonucleoproteins, such as the nucleolus, the peri- and interchromatin granules, appeared well contrasted. The majority of nuclear bodies showed no loss of contrast with this technique. Interesting was the maintenance of contrast by the granular components of the complex bodies.

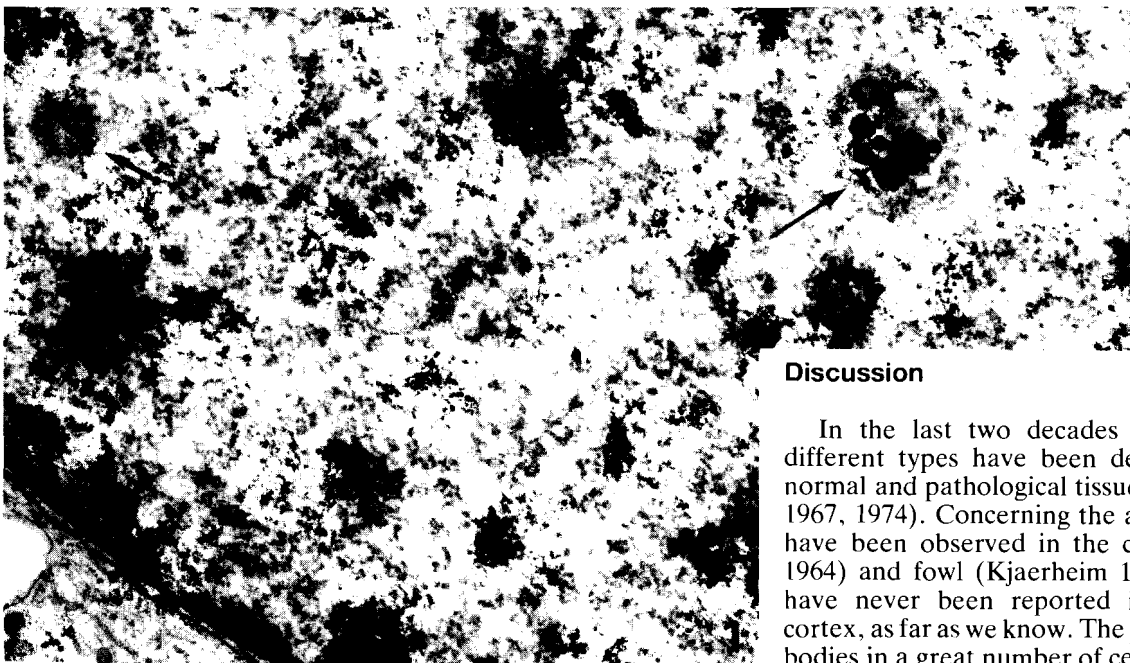


Fig. 1. Patient with acrocyanosis. Adrenal zona glomerulosa cell. In the nucleus one simple nuclear body (short arrow) and a complex nuclear body (long arrow) are observed. Fixation: glutaraldehyde and osmium tetroxide; staining: uranyl acetate and lead citrate. $\times 18,000$

Discussion

In the last two decades nuclear bodies of different types have been described in several normal and pathological tissues (Bouteille et al., 1967, 1974). Concerning the adrenal cortex they have been observed in the calf (Weber et al., 1964) and fowl (Kjaerheim 1968a, b), but they have never been reported in human adrenal cortex, as far as we know. The presence of nuclear bodies in a great number of cells from all adrenal

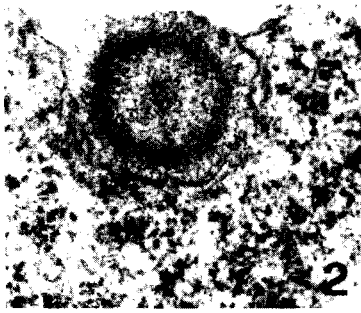


Fig. 2. Complex nuclear body in an adrenal zona fasciculata cell from the patient with acrocyanosis. Fixation and staining as for Fig. 1. $\times 36,000$

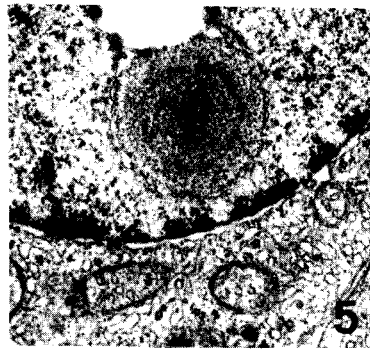


Fig. 5. Patient with Cushing's disease. Zona fasciculata cell. Complex nuclear body with the filamentous material exhibiting a concentric arrangement. Fixation and staining as for Fig. 1. $\times 18,000$

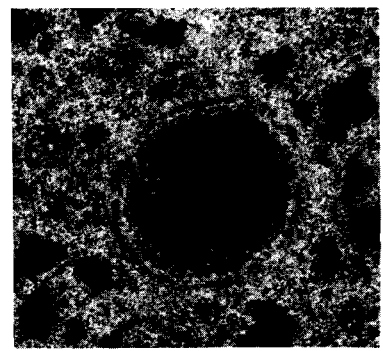


Fig. 6. Patient with Cushing's disease. Zona fasciculata cell. Complex nuclear body whose core presents a granular material. Fixation and staining as for Fig. 1. $\times 18,000$

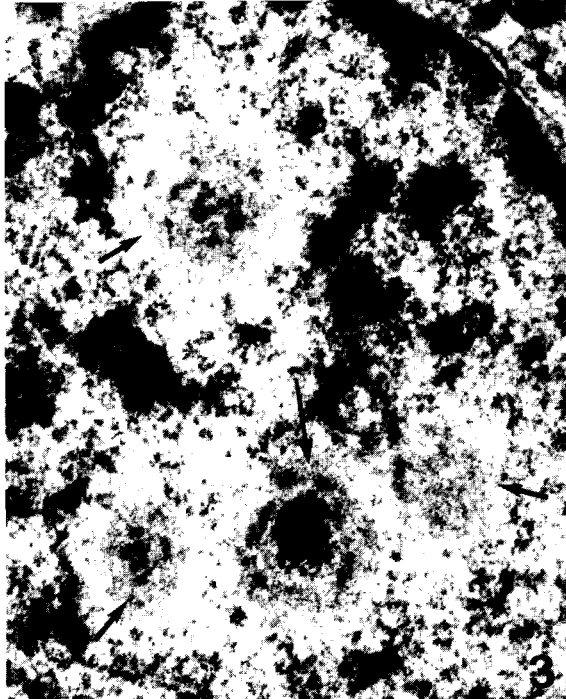


Fig. 3. Adrenal zona fasciculata cell from a patient with Cushing's disease. Three simple nuclear bodies (short arrows) and one complex body (long arrow) are clearly evident. Fixation and staining as for Fig. 1. $\times 18,000$

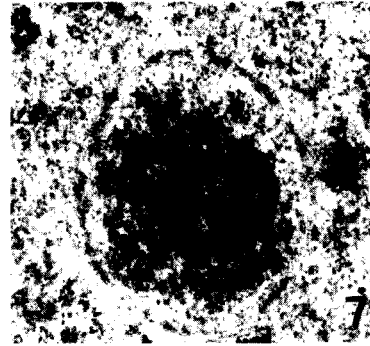


Fig. 7. Patient with Cushing's disease. Complex nuclear body in a zona fasciculata cell. The core shows a fine structure similar to that of some nucleolar components. Fixation and staining as for Fig. 1. $\times 27,000$

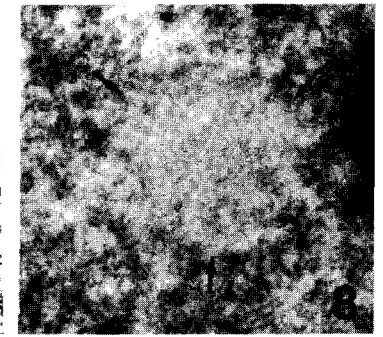


Fig. 8. Patient with Cushing's disease. Zona fasciculata cell. Treatment with pronase. A simple nuclear body appears digested by pronase (arrows). Fixation and staining as for Fig. 1. $\times 30,000$

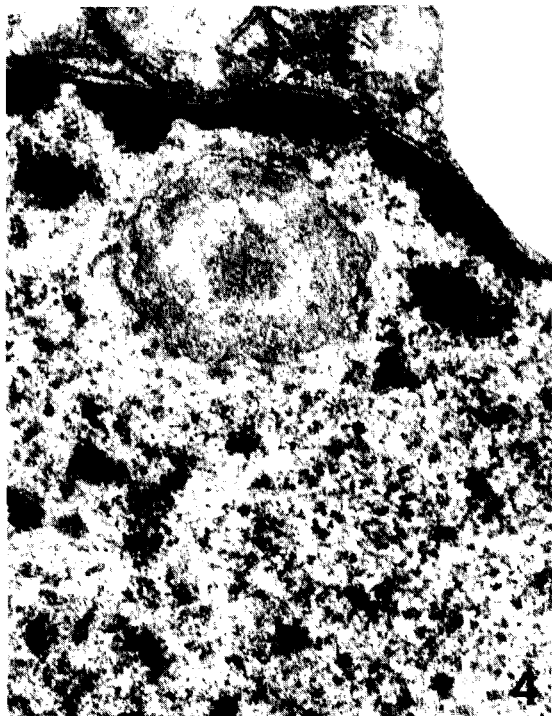


Fig. 4. Complex nuclear body in an adrenal zona fasciculata cell from the patient referred to in Fig. 3. Fixation and staining as for Fig. 1. $\times 27,000$

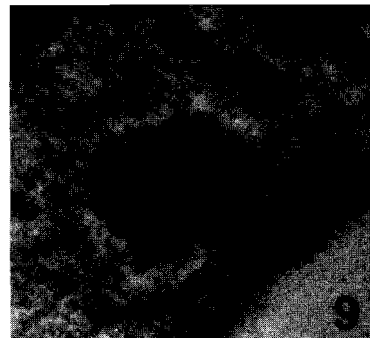


Fig. 9. Patient with Cushing's disease. Zona fasciculata cell. Treatment with pronase. The capsule of the complex nuclear body appears digested by the enzyme. Fixation and staining as for Fig. 1. $\times 36,000$

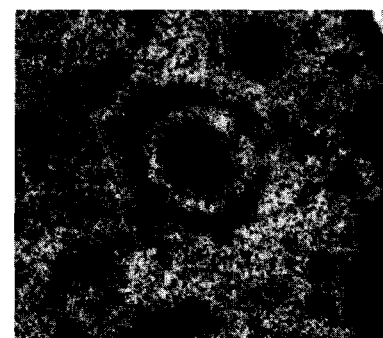


Fig. 10. Patient with Cushing's disease. Zona fasciculata. Treatment with RNase. The complex nuclear body present in the picture was not affected by the enzymatic treatment. Fixation and staining as for Fig. 1. $\times 27,000$

cortex zones in patients with and without an increased adrenocortical function, is highly suggestive that adrenal nuclear bodies are normal organelles, at least in women.

The digestion of the simple bodies and of the capsule of complex ones by pepsin and pronase confirmed the proteinaceous nature of these structures. Concerning the nature of the cores of complex bodies the data were not conclusive, since RNase treatment did not affect the area; however, the staining of the core with the EDTA technique suggests that it contains ribonucleoproteins. This has also been advanced for the nuclear bodies of other tissues (Dupuy-Coin et al., 1972; Paula-Barbosa et al., 1980; Vagner-Capodano et al., 1982).

Data from experimental endocrinology have shown a close relationship between hormonal stimulation and the occurrence of nuclear bodies. In uterine luminal epithelial cells the frequency of nuclear bodies has been associated with estrogenic stimulation

(Le Goascogne and Baulieu, 1977; Fitzgerald and Padykula, 1983; Padykula and Pockwinse, 1983). On the other hand, cultured thyroid cells stimulated by thyrotropin, were shown to present nuclear bodies (Vagner-Capodano et al., 1978, 1980, 1982). In the adrenal cortex, again hormonal stimulation appears to reflect on the nuclear bodies, since Weber et al. (1964) and Kjaerheim (1968a) showed that ACTH administration increased the number and modified the morphology of nuclear bodies. Our observations are in accordance with that suggestion, since the patients with Cushing's disease, which represents an adrenal hyperfunctional situation, presented an increased number of heterogeneous complex nuclear bodies. Therefore, it can be assumed that nuclear bodies are related to the functional state of the gland.

Although we have no evident morphological or cytochemical data to relate adrenal nuclear bodies to the other nuclear components, the following data must be noted: a) the proximity of the nuclear bodies to clusters of interchromatin granules and the nucleolus; b) the resemblance of some nuclear bodies with the nucleolus (Fig. 7); c) the proteinaceous nature of simple nuclear bodies, the capsule of complex ones, and some components of the nuclear matrix. On the other hand, Brasch and Peters (1985) observed that nuclear body residues were visible in isolated nuclear matrices, suggesting that they might be structurally related to the internal protein framework. These observations, taken on the whole, suggest that adrenal nuclear bodies may be related to a general nuclear compartment participating in ribonucleoprotein metabolism (Padykula and Clark 1981), where a rapid transcriptional activity will take place, all the more since, ACTH stimulation of steroidogenesis is mediated by a specific labile protein (Garren et al., 1965; Pon and Orme-Johnson, 1984). Other morphological, cytochemical and biochemical studies will however be necessary to clarify whether adrenal nuclear bodies represent, or not, hormonal receptors, transcriptional sites or reorganized nuclear structures.

Acknowledgements. We wish to thank Prof. A. Coimbra for critical reading of the manuscript. We thank Produtos Sandoz Lda (Portugal) for their financial support.

References

- Bendayan M. and Zollinger M. (1983). Ultrastructural localization of antigenic sites on osmium-fixed tissues applying the protein A-gold technique. *J. Histochem. Cytochem.* 31, 101-109.
- Bernhard W. (1969). A new staining procedure for electron microscopical cytology. *J. Ultrastruct. Res.* 27, 250-265.
- Bouteille M., Kalifat S.R. and Delarue J. (1967). Ultrastructural variations of nuclear bodies in human diseases. *J. Ultrastruct. Res.* 19, 474-486.
- Bouteille M., Laval M. and Dupuy-Coin A.M. (1974). Localization of nuclear functions as revealed by ultrastructural autoradiography and cytochemistry. In: *The Cell Nucleus*. Vol. I. Bush H. (ed). Academic Press. New York. pp 3-71.
- Brasch K. and Peters K.E. (1985). Nuclear protein, matrix and structural changes in rooster liver after estrogenic induction of vitellogenesis. *Biol. Cell* 54, 109-122.
- Chegini N. and Rao Ch.V. (1984). The appearance of nuclear bodies in luteal slices incubated with human chorionic gonadotropin (hCG). *J. Cell Biol.* 99, 213a.
- Cidadão A.J. and David-Ferreira J.F. (1984). Testosterone-induced nuclear changes in the rat uterine luminal epithelium. A stereological study. *Biol. Cell* 52, 109-118.
- De Thè G., Rivière M. and Bernhard W. (1960). Examen au microscope électronique de la tumeur VX₂ du lapin domestique dérivée du papillome de Shope. *Bull. Cancer* 47, 570-584.
- Doyle D.G. (1980). The origin of nuclear bodies: a study of the undifferentiated epithelial cells of the equine small intestine. *Amer. J. Anat.* 157, 61-70.
- Dupuy-Coin A.M., Kalifat S.R. and Bouteille M. (1972). Nuclear bodies as proteinaceous structures containing ribonucleoproteins. *J. Ultrastruct. Res.* 38, 174-187.
- Dupuy-Coin A.M. and Bouteille M. (1975). Protein renewal in nuclear bodies, as studied by quantitative autoradiography. *Exp. Cell Res.* 90, 111-118.
- Echeverría O.M., Osuna J.D., Hernández I., Sánchez C., Parra R., Hernández R. and Vásquez-Nin G.H. (1985). Effects of progesterone on nuclear ribonucleoprotein constituents of endometrial cells of the rat. *Acta. Anat.* 112, 205-211.
- Fitzgerald M. and Padykula H.A. (1983). Differing functional responses of simple and complex nuclear bodies in uterine luminal epithelial cells following estrogenic stimuli. *Anat. Rec.* 205, 131-141.
- Garren L.D., Ney R.L. and Davis W.W. (1965). Studies on the role of protein synthesis in the regulation of corticosterone production by adrenocorticotrophic hormone *in vivo*. *Proc. Natl. Acad. Sci.* 53, 1443-1450.
- Kjaerheim A. (1968a). Studies of adrenocortical ultrastructure. 4-Effects of ACTH on interrenal cells of the domestic fowl. *J. Microsc.* 7, 715-738.
- Kjaerheim A. (1968b). Studies of adrenocortical ultrastructure. 5-Effects of metopiron on interrenal cells of the domestic fowl. *J. Microsc.* 7, 739-754.
- Le Goascogne C. and Baulieu E.E. (1977). Hormonally controlled «nuclear bodies» during the development of the prepuberal rat uterus. *Biol. Cell* 30, 195-206.
- Masurovsky E.B. and Fields K.L. (1984). Monoclonal antibodies binding to nuclear structures and axons in adult avian dorsal root ganglia. *Biol. Cell.* 51, 407-410.
- Monneron A. and Bernhard W. (1966). Action de certaines enzymes sur des tissus inclus en Epon. *J. Microsc.* 4, 697-713.
- Padykula H.A. and Clark J.H. (1981). Nuclear bodies as functional indicators in the target cells of sex steroid hormones. In *The Cell Nucleus*. Nuclear particles. Vol IX, part B. Busch H. (ed). Academic Press. New York. pp 309-339
- Padykula H.A., Fitzgerald M., Clark J.H. and Hardin J.W. (1981). Nuclear bodies as structural indicators of estrogenic stimulation in uterine luminal epithelial cells. *Anat. Rec.* 201, 679-686.
- Padykula H.A. and Pockwinse S.M. (1983). Uterine simple and complex nuclear bodies are separate structural entities. *Anat. Rec.* 205, 119-130.

- Paula-Barbosa M.M., Ruela C., Gonçalves V. and Sobrinho-Simões M.A. (1980). The biological significance of granular nuclear bodies in subacute sclerosing panencephalitis (SSPE). An Ultrastructural and cytochemical study. *J. Submicrosc. Cytol.* 12, 655-663.
- Pon L.A. and Orme-Johnson N.R. (1984). Protein synthesis requirement for acute ACTH stimulation of adrenal corticosteroidogenesis. *Endocr. Res.* 10, 585-590.
- Richardson K.C., Jarret L. and Finke E.H. (1960). Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technol.* 35, 313-325.
- Sobrinho-Simões M.A. and Gonçalves V. (1974). Nuclear bodies in papillary carcinomas of the human thyroid gland. *Arch. Pathol.* 98, 94-99.
- Vagner-Capodano A.M., Mauchamp J., Delgrossi M.H., Stahl A. and Lissitzky S. (1978). Nuclear bodies in cultured thyroid cells stimulated by thyrotropin. *Biol. Cell* 33, 181-184.
- Vagner-Capodano A.M., Mauchamp J., Stahl A. and Lissitzky S. (1980). Nucleolar budding and formation of nuclear bodies in cultured thyroid cells stimulated by thyrotropin, dibutyryl cyclic AMP, and prostaglandin E₂. *J. Ultrastruct. Res.* 70, 37-51.
- Vagner-Capodano A.M., Bouteille M., Stahl A. and Lissitzky S. (1982). Nucleolar ribonucleoprotein release into the nucleoplasm as nuclear bodies in cultured thyrotropin-stimulated thyroid cells: autoradiographic kinetics. *J. Ultrastruct. Res.* 78, 13-25.
- Weber A., Whipp S., Usenik E. and Frommes S. (1964). Structural changes in the nuclear body in the adrenal zona fasciculata of the calf following the administration of ACTH. *J. Ultrastruct. Res.* 11, 564-576.

Accepted November 20, 1988