Ultrastructural Study of Binucleation in Cells of the Rat Adrenal Glomerular Zone after a Prolonged Low-Sodium Diet

G. Palacios, M. Lafarga and R. Perez

Department of Histology, Faculty of Medicine, Autonomous University, Bellaterra, Barcelona (Spain), 19 January 1976.

Summary. Binucleate cells have been found in the glomerular zone of the adrenal cortex in rats subjected to low-sodium diets. By considering the various possibilities for their production, both the findings of nuclei in process of constriction and nuclei identical in form, confronted and smaller in size than those of neighbour cells, are in agreement with an amitotic nuclear division as the possible mechanism for the formation of these cells.

The presence of binucleate cells in the parenchyma of several organs is a verified fact. The process of production of these cells remains rather obscure. Recent studies upon the subject support amitotic nuclear division as the most probable mechanism of binucleation, although other possibilities have been considered[1–4]. In our work, we demonstrate the appearance of binucleate cells in the glomerular zone during periods of adrenal stimulation through low-sodium diets.

Material and methods. 60 adult male Wistar rats, weighing between 250–300 g were divided into 3 groups: a) A 1st group of 6 rats received a normal diet and served as a control. b) A 2nd group of 48 rats was given a low-sodium diet for 12 weeks. This diet followed a formula similar to that of Hartroft and Eisenstein[5]. Each week of the experiment 4 animals of this group were sacrificed. c) A 3rd group of 6 rats also serving as a control, which for 12 weeks received the same diet as the 2nd group but with a normal proportion of sodium. These animals were sacrificed as the end of the 12th week.

All animals were subjected to perfusion fixation with 3% glutaraldehyde in a 0.12 *M* phosphate buffer at pH 7.4 for 15 min. The adrenal glands were extracted, sectioned to fine slices, submerged in the same fixative for 2 h, and finally post-fixed in 1% osmium tetroxide in the same buffer. After dehydration in increasing concentrations of acetone, the material was included in Durcupan ACM (Fluka). Semi-thin sections of 1 or 2 µm were stained with 1% toluidine blue. Ultra-thin sections were stained with lead citrate and examined with a Hitachi HU-12 electron microscope.

Results. The glomerular zone of the adrenal glands of the 2 control groups of animals was composed of clusters of cells forming pericapillary cords or glomeruli (Figure 1a). Among their subcellular characteristics, a mitochondrial polymorphism with platelike or tubular and parallel cristae adopting pseudo-cristalline position was noted (Figure 1b). Other representative organelles were the

[1] F.-W. Pehlemann, Z. Zellforsch. *84*, 516 (1968).
[2] J. Boddingius, Z. Zellforsch. *108*, 59 (1970).
[3] D. N. Wheatley, Expl Cell Res. *74*, 455 (1972).
[4] H. P. Philipsen, O. Fejerskov and J. Theilade, Cell. Tissue Res. *150*, 113 (1974).
[5] P. M. Hartroft and A. B. Eisenstein, Endocrinology *60*, 641 (1957).

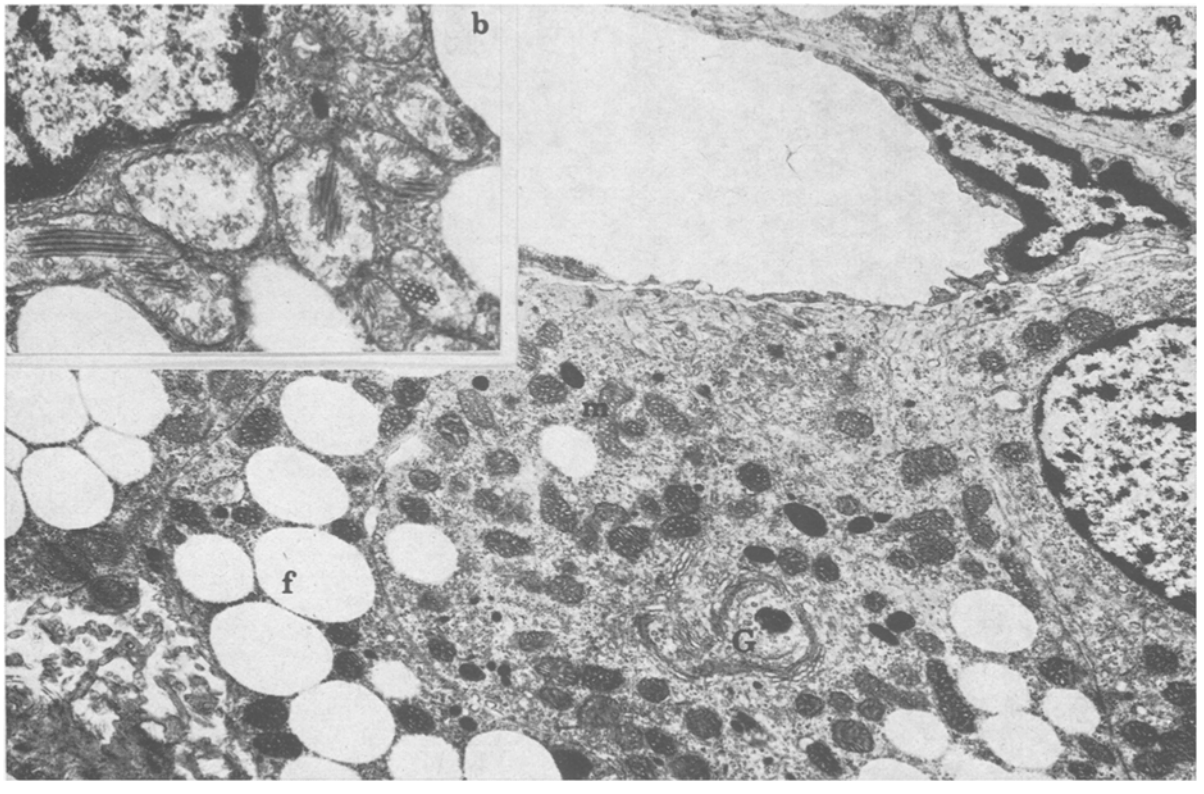

Fig. 1. Control animals. a) Glomerular zone cells in pericapillary arrangement. Mitochondria (m), fat droplets (f), and some Golgi apparatus (G) can be seen in their cytoplasm. ×4000. b) Characteristic pseudo-crystalline arrangement in some mitochondria. ×10,000.

smooth endoplasmic reticulum, free polyribosomes, numerous fat droplets, and a well developed Golgi apparatus (Figure 1b).

In the group of animals maintained on a low-sodium diets, the glomerular zone underwent a perceptible hypertrophy beginning in the 2nd week and continuing through the whole length of the experiment. The expansion of the zone occurred initially through an increase in the mitotic activity of its cells, and afterwards through an increase in cell volume due to a increase in some organelles, principally mitochondria, smooth endoplasmic reticulum, and fat droplets. In the 7th and 8th weeks of the experiments, images were observed in semi-thin sections of the glomerular zone which enable us to presume the existence of nuclear constriction and duplication (Figures 2a, b and c). Ultrastructural examination confirms the presence of binucleate cells (Figures 3, 4a, b c and d). The nuclei of these cells were generally smaller than those of neighbouring mononucleate cells. Nuclei were faced and separated by a space, sometimes narrow (Figures 4a, b and d) and in other cases wide and occupied by cytoplasmic organelles (Figures 3 and 4d). Their structure was similar to that of mononucleate cells with a double membrane containing nuclear pores, masses of heterochromatin attached to the membrane, and euchromatin occupying the remainder of the nuclear space (Figures 3, 4a, b, c and d). The nucleoli were seen usually in only 1 nucleus, and were composed of well developed fibrillar and granular parts and compact masses of satellite heterochromatin (Figures 4b, c and d).

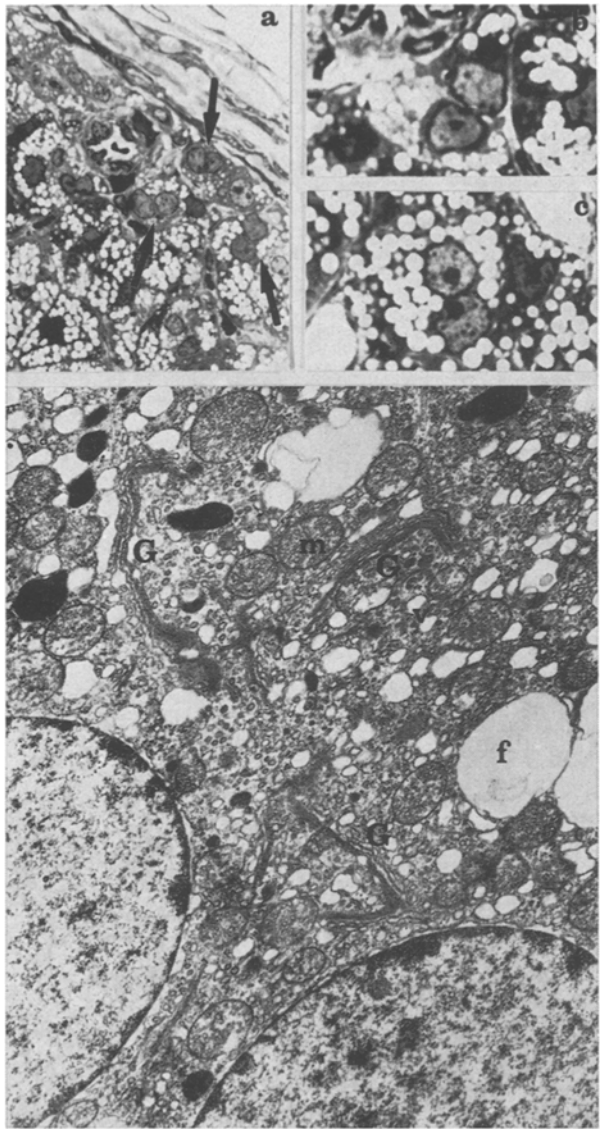

Fig. 2. Light micrograph of semi-thin sections of the glomerular zone, stained with toluidine blue. a) Several apparently binucleate cells can be seen (arrows). ×400. b) An image of nuclear constriction. ×1000. c) Detail of an apparently binucleate cell. ×1000.

Fig. 3. Electron micrograph of a binucleate cell, its extensive cytoplasm containing mitochondria (m), fat droplets (f), vacuolar elements of the smooth endoplasmic reticulum (v), and a dissociated Golgi apparatus (G). ×6000.

As a whole, the binucleate cells were generally of larger size than their neighbours. Their cytoplasm was extensive, containing numerous mitochondria, vacuolar elements of the smooth endoplasmic reticulum, fat droplets, and well developed dissociated Golgi complexes, presenting dense bodies and coated vesicles in their area (Figure 3). The majority of the binucleate cells had a subcapsular location, although others were located in the external part of the zone, in the sudanophobic region, and had characteristics similar to those of the underlying fascicular zone, containing abundant mitochondria with tubular-vesicular cristae and a few fat droplets, some with cholesterol crystals (Figure 4d).

Discussion. PHILIPSEN et al.[4] consider 3 mechanisms for the production of binucleate cells: 1. mitotic division without cytokinesis, a process commonly found in the liver[3]; 2. nuclear fusion; 3. amitotic nuclear division which is postulated for the production of binucleate cells in the palatal mucosa[4].

We have evaluated these mechanisms discounting mitotic division without cytokinesis since at this stage of the experiment the capacity for division is already very reduced, and mitotic figures are rare. Neither have we found indication of cytoplasmic segmentation in the binucleate cells. It also seems improbable that these cells are produced by the simple fusion of neighbour cells since their nuclei are smaller, and the cytoplasmic organization does not appear to develop through a simple fusion.

Two principal findings support an amitotic division mechanism for the production of binucleate cells 1. the indications of nuclear constrictions seen in semi-thin sections which, although scarce, must be evaluated since the process of constriction may occur at a speed that makes its observation difficult[4]; 2. the smaller size of both nuclei in binucleate cells in comparison with mononucleate cells.

In the process of amitotic division, we could not demonstrate the participation of centrioles or a fibrillar system, as has been reported to occur in adrenal cortical cells of *Rana temporaria* L.[1] and in chromophobe cells of the pars intermedia of *Salmo irideus*[2].

In studies performed on mammalian adrenal glands using specific stimuli for the glomerular zone[6–11] or fascicular zone[12, 13], the presence of binucleate cells has not been demonstrated. However the phenomena of amitosis has been related with the functioning of the adrenal cortex. PEHLEMANN[1] notes an increase in the amitotic divisions of the adrenal cortex in the frog after stimulation with ACTH. Therefore this form of division can be regarded as important in the ensemble of morphological signs indicating hyperfunction of the glomerular zone. At this stage, the amitosis is a proliferative process which does not disturb the normal functioning of cellular biosynthetic processes in states of hyperactivity, like those produced by these diets[5–11].

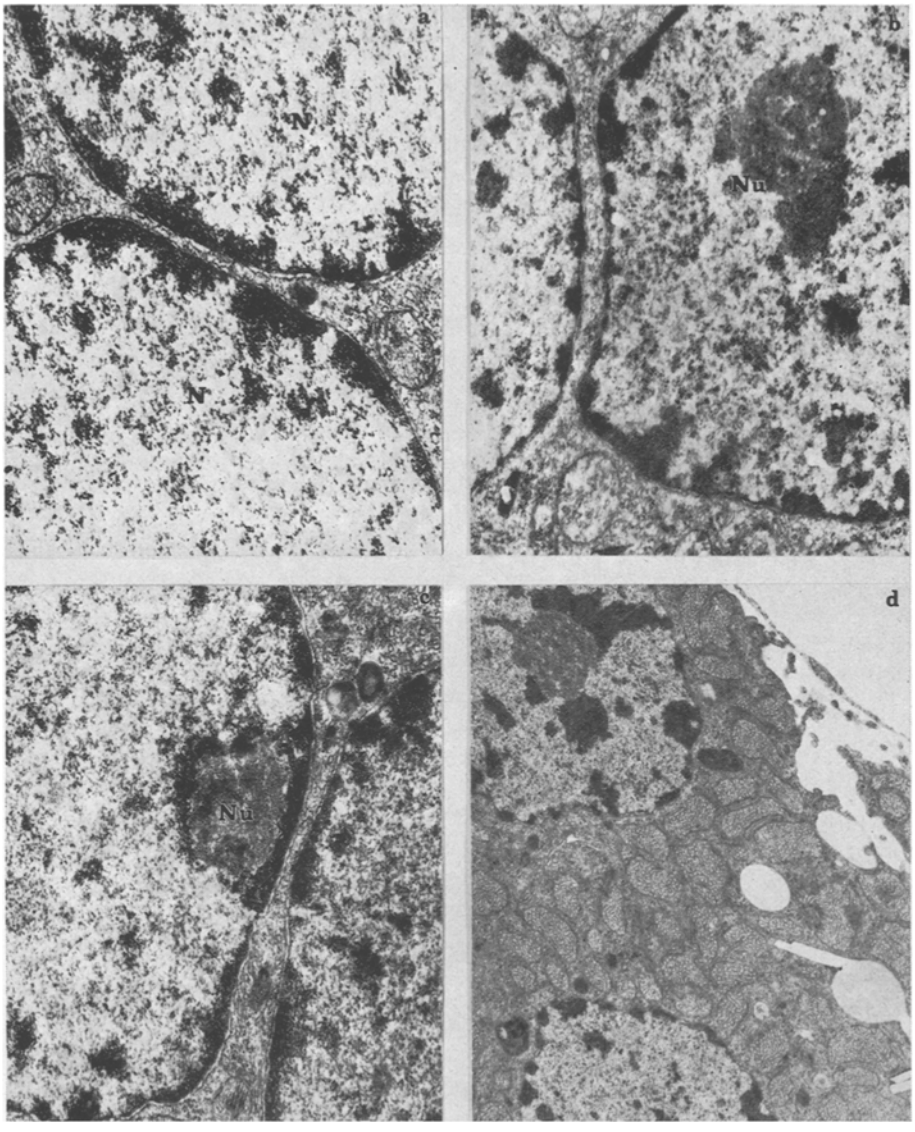

Fig. 4. a) Binucleate cell with confronted nuclei (N). ×15,000. b) and c) binucleate cells, the nucleolus (Nu) with the satellite heterochromatin can be seen in some of the nuclei. ×15,000. d) Binucleate cell in the sudanophobic region. ×6000.

[6] J. D. Lever, Endocrinology *58*, 163 (1956).
[7] F. Giacomelli, J. Weiner and D. Spiro, J. cell. Biol. *26*, 499 (1965).
[8] J. A. Long and A. L. Jones, Anat. Rec. *166*, 1 (1970).
[9] J. H. Shelton and A. L. Jones, Anat. Rec. *170*, 147 (1971).
[10] H. A. Smiciklas, R. L. Pike and H. Schraer, J. Nutr. *101*, 1045 (1971).
[11] D. T. Domoto, J. E. Boyd, P. J. Mulrow and M. Kashgarian, Am. J. Path. *72*, 433 (1973).
[12] G. C. Nussdorfer, G. Mazzochi and L. Rebonato, Z. Zellforsch. *115*, 30 (1971).
[13] J. A. G. Rhodin, J. Ultrastruct. Res. *34*, 23 (1971).

Mitostatic Action of 4,6-Dimethyl-2-Amino-3,4,5-Trimethoxyphenyl-Pyrimidine on Mammalian Cells[1]

Maria L. Schivo, Maria A. Marcialis, Ornella Flore, S. Dessy, A. Garzia and B. Loddo

Istituto di Microbiologia II dell'Università degli Studi di Cagliari, Via G.T. Porcell 12, Cagliari (Italy); and Istituto Chemioterapico Italiano, Lodi (Italy), 15 December 1975.

Summary. 4,6 dimethyl-2-amino-3,4,5-trimethoxyphenylpyrimidine arrests the mitotic cycle of mammalian cells in metaphase, both in vitro and in vivo. The mitostatic effect is promptly reverted by interruption of drug treatment.

In the course of studies on the toxic effects of pyrimidine derivatives on mammalian cells, the mitostatic activity of 4,6-dimethyl-2-amino-3,4,5-trimethoxyphenylpyrimidine (B 31) has been reavealed. Results from these experiments are reported here.

Material and methods. B 31 and other pyrimidine derivatives were synthesized by Istituto Chemioterapico Italiano (I.C.I.) Lodi; vinblastine (Lilly) and colchicine (Simes) were also used. In vitro tests were carried out in cells of the human aneuploid line HEp2 (American Type