# Induction of nucleolar segregation in adrenal fasciculata cells by actinomycin D

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*Summary.* Actinomycin D administration to young rats provokes segregation of the nucleoli of adrenal fasciculata cells into 5 distinct zones (granular, fibrillar and vacuolar zones, a fibrillar center, and a dense fibrillar or "contrasted" zone); in the cytoplasm, there were dilations of Golgi cisternae. In spite of the nucleolar segregation, the cytoplasmic alterations suggest the maintenance of protein synthesis.

Actinomycin D is an antibiotic which prevents RNA synthesis catalyzed by RNA polymerase, by binding to the DNA helix which contains the rRNA cistrons[2]. In addition, actinomycin D, in vivo, blocks the steroidogenic effect of ACTH which is mediated by newly-synthesized specific proteins[3]. Since it is well known that in the young rat there is a high rate of adrenal protein synthesis[4], as well as a good steroidogenic capacity[5] related to the typical vesicular morphology of the mitochondrial cristae, it seemed interesting to study the nuclear and cytoplasmic effects of actinomycin D administration on adrenal fasciculata cells.

*Material and methods.* 8 young rats, 20 g in weight, from the Colony of the Gulbenkian Institute of Science, Oeiras, Portugal, were injected i.p., between 9.00 and 10.00 h., with a single dose of 1.25 µg/g/b.wt of actinomycin D (Dactinomycin, Merck Sharp & Dohme, Rahway, New Jersey) in 0.1 ml of distilled water[6]. 4 control animals were injected with 0.1 ml of saline. Under light ether anesthesia test and control animals were sacrificed 1 h (4 animals) or 2 h (4 animals) thereafter. Adrenals were fixed for 2 h at 4 °C in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate buffer, washed overnight and post-fixed for 2 h at 4 °C in 1% osmium tetroxide in veronal-acetate buffer, pH 7.3. Some samples were only fixed in osmium tetroxide.

*Results and discussion.* In control rats, nucleoli were single or double, irregularly outlined and of the reticular type. The coarse threads of the nucleolonema had the usual granular component with particles 148–180 Å in diameter and the fibrillar component made up of fibrils 38–75 Å in diameter. Among the twisting nucleolonema there were nucleolar vacuoles. 0.17–0.25 µm across, as well as fibrillar centers measuring 0.3–0.45 µm in diameter. The nucleoli were often surrounded by associated condensed chromatin (figure 1).

60 min after actinomycin D administration the nucleolar appearance was greatly changed while dense patches of chromatin occurred in the nucleoplasm. Nucleoli showed a compact texture and a regular spherical or oval contour. Within the nucleolar mass, the components were totally segregated from one another. The granular component was concentrated at one end, the fibrillar component occupied the center of the organelle while a large fibrillar center occurred at the other end. A central or paracentral large nucleolar vacuole, filled with material similar to that observed in nucleolar vacuoles of control animals, was often observed (figure 2). In addition, a dense fibrillar plaque or contrasted zone[7] was frequent at the periphery of the nucleolus (figure 2). No nucleolar associated chromatin was noted. Cytoplasmic alterations were absent at 60 min.

At 120 min after actinomycin D administration nuclear and nucleolar changes were similar to those at 60 min. In the cytoplasm, however, vacuolization of the mitochondrial matrix and dilation of Golgi cisternae occurred.

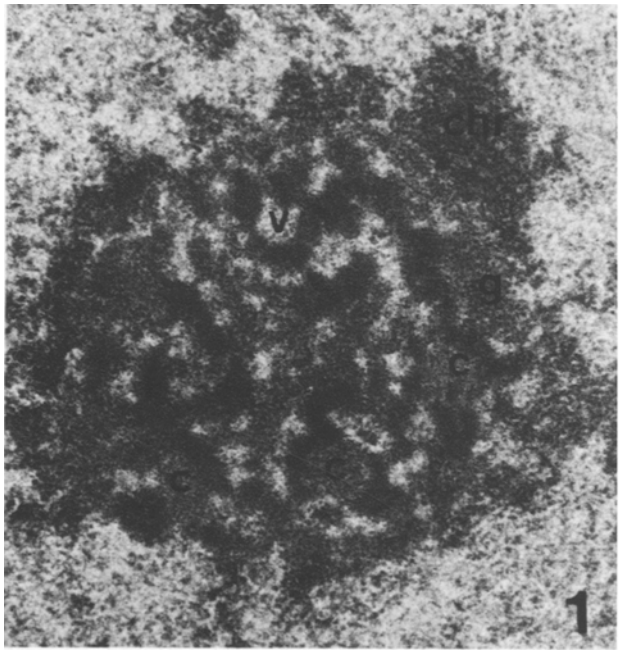


Fig. 1. Nucleolus of a normal adrenal fasciculata cell, showing the granular (g) and fibrillar (f) components of nucleolonema, the nucleolar vacuoles (v) and the fibrillar centers (c); chr – associated chromatin. Fixation, glutaraldehyde and osmium tetroxide; staining, uranyl acetate and lead citrate. ×27,000.

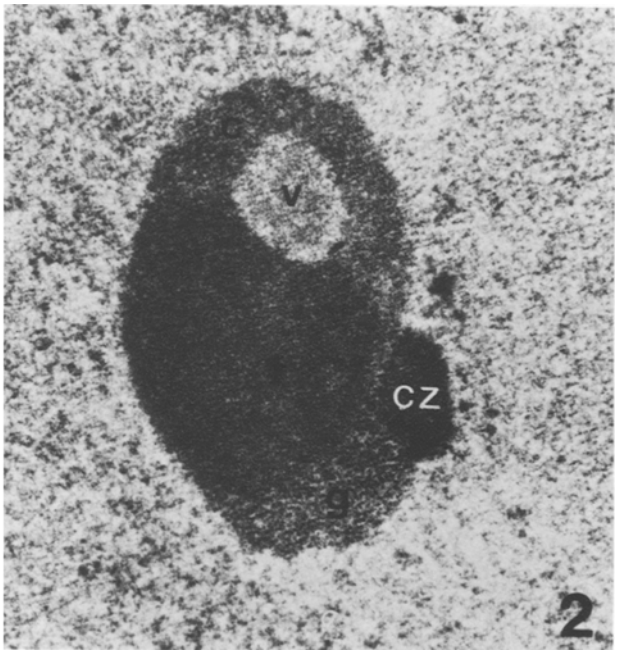


Fig. 2. Segregation of nucleolar components 1 h after the injection of actinomycin D. 5 zones can be easily identified: granular (g), fibrillar (f), vacuolar (v), fibrillar center (c) and contrasted zone (cz). Fixation, osmium tetroxide; staining as for figure 1. ×27,000.

The morphologic nuclear changes reported here were similar to those observed in the liver[8], pancreas[7,9], parotid[9] and embryonic cells[11] after actinomycin D-treatment and they are likely to be caused by blockade of the nucleolar transcription of 45 S ribosomal RNA precursors[12]. In fact, nucleolar ribosomal DNA contains a high percentage of deoxyguanosine and deoxycytosine bases, and it is known that actinomycin D selectively binds deoxyguanosine[13]. Since actinomycin D also inhibits phospholipid synthesis[14], mitochondrial changes may be ascribed to this mechanism, phospholipids being the main component of the extensive internal membrane system of adrenal mitochondria.

As to the dilation of the Golgi, we have no explanation yet for its mode of formation. In the pancreas of animals subjected to actinomycin D-treatment, similar changes were interpreted as due to a continuing protein synthesis in spite of the arrested RNA formation[7]. Further work is needed to elucidate this point.

1 Acknowledgments. The authors are indebted to Prof. A. Coimbra for critical reading of manuscript. Dactinomycin was kindly supplied by the Merck Sharp & Dohme Research Laboratories, USA.

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## Enhancement by caffeine of the frequency of anaphase-telophase chromatin bridges induced by triethylenemelamine (TEM)[1]

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*Summary.* Treatment of BHK cells for 8 h with triethylenemelamine (TEM) followed by caffeine for 4 or 8 h, increased the frequency of anaphase-telophase chromatin bridges in relation to controls and TEM-treated cells. These results indicate that TEM-induced chromosome lesions detected as chromatin bridges at anaphase-telophase could be potentiated by caffeine.

The ability of caffeine to potentiate the frequency of chromosome aberrations produced by different agents in animal and plant cells is well known[2]. In mammalian cells this effect is apparently due to the inhibition by caffeine of the gap-filling step in post-replication repair processes[2]. When cells are treated with a chromosome-breaking agent, only the unrepaired primary DNA lesions can result in structrual chromosome aberrations. Post-treatment with caffeine enhances the number of unrepaired lesions and, consequently, the frequency of derived chromosomal aberrations.

Analysis of anaphase-telophase aberrations in mitotic cells has been proposed as an ancillary test-system for chemical mutagens[3,4]. Primary lesions induced by several compounds can be detected as chromosome aberrations at the 1st mitotic metaphase after treatment. Other chemicals do not produce chromosome aberrations at the 1st mitosis, but they induce instead chromosome stickiness (detected at anaphase-telophase as chromatin bridges) due to the entanglement of chromatin fibrils[4].

Polyfunctional alkylating compounds are very efficient chromosome-breaking agents. The primary lesions induced by these chemicals in the DNA are both interstrand and intrastrand cross-links[5,6]. Triethylenemelamine (TEM), a trifunctional alkylating agent, is able to induce chromosomal alterations in both metaphase and anaphase of the 1st mitosis after treatment[7,8]. The experiment described below was performed in order to investigate the effect of caffeine on the frequency of anaphase-telophase chromatin bridges induced by TEM.

*Material and methods.* Baby hamster kidney (BHK) cells were cultured in Leighton tubes with Eagle Minimal Essential Medium plus 10% fetal calf serum and antibiotics. Treatments were carried out when cells were in the logarithmic growth phase.

Cultures were treated according to the following procedure: a) 200 µg of caffeine (BDH, product No.27574) per ml of tissue culture medium for 4 h; b) 200 µg/ml of caffeine for 8 h; c) 0.2 µg/ml of TEM for 8 h; d) 0.2 µg/ml of TEM for 8 h plus 200 µg/ml of caffeine for the last 4 h; e) 0.2 µg/ml of TEM plus 200 µg/ml of caffeine for 8 h. 5 Leighton tubes were used for each treatment. Another 5 untreated cultures were used as controls.

At the moment of harvesting, the culture medium was discarded and the cells were washed twice with methanol/acetic acid 3:1 and fixed for no less than 5 min with the methanol/acetic acid mixture. Coverslides were stained

| Treatment | Number of cells recorded | Chromatin bridges per 100 cells | Mitotic index |
|---|---|---|---|
| Control | 500 | 6.8 | 2.28 |
| Caffeine 4 h | 500 | 6.6 | 2.80 |
| Caffeine 8 h | 500 | 6.8 | 2.11 |
| TEM 8 h | 500 | 11.6 | 1.20 |
| TEM 8 h + caffeine 4 h | 500 | 16.2 | 1.55 |
| TEM 8 h + caffeine 8 h | 500 | 17.0 | 1.36 |