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.

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2 E. Reich and I.H. Goldberg, Prog. Nucleic Acid Res. molec. Biol. *3*, 183 (1964).
3 J. Vernikos-Danellis and M. Hall, Nature *207*, 766 (1965).
4 M.C. Magalhães, M.M. Magalhães and A. Coimbra, Am. J. Anat. *132*, 109 (1971).
5 J.E. Philpott, M.X. Zarrow and V.H. Denenberg, Steroids *14*, 21 (1969).
6 P.J. Goldblatt, R.J. Sullivan and E. Farber, Lab. Invest. *20*, 283 (1969).
7 A.N. Jézéquel and W. Bernhard, J. Microsc. *3*, 279 (1964).
8 P.J. Goldblatt, R.J. Sullivan and E. Farber, Cancer Res. *29*, 124 (1969).
9 G.I. Schoefl, J. ultrastruct. Res. *10*, 224 (1964).
10 S.S. Han, Am. J. Anat. *120*, 161 (1967).
11 R. Simard and W. Bernhard, Int. J. Cancer *1*, 463 (1966).
12 R.P. Perry, Exp. Cell Res. *29*, 400 (1963).
13 R. Simard, Y. Langelier, R. Mandeville, N. Maestraci and A. Royal, in: The Cell Nucleus, vol. 3, p. 447. Ed. H. Busch. Academic Press, New York 1974.
14 I. Pastan and R.M. Friedman, Science *160*, 316 (1968).

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

with carbol-fuchsin and attached with Permount onto slides.

Preparations were coded and analyzed blind by 1 investigator. A total number of 100 anaphase-telophase cells per tube was scored for chromatin bridges. Mitotic index was determined by counting at least 5000 cells for each experiment. This value was expressed as the number of mitotic plates per 100 cells. Comparison between treated and untreated cells was made by the Student t-test.

Results. The table summarizes the results obtained from the different treatments and controls. In 4 and 8 h caffeine-treated cells, the frequency of chromatin bridges in anaphase-telophase was not statistically different from those of controls. On the other hand, treatments with TEM alone induced a significant increase in the frequency of chromatin bridges ($p < 0.01$). In TEM plus caffeine treatments the percentage of anaphase-telophase cells with chromatin bridges increased again, showing significant differences between controls ($p < 0.001$) and TEM-treated cells ($p < 0.05$).

The analysis of the mitotic index also revealed the effect of both chemicals. The 4 h caffeine treatments induced a little increase in the frequency of dividing cells, whereas in 8 h treatments the mitotic index was slightly lower. However, there were no statistical differences between caffeine-treated and control cells. TEM treatment induced a significant decrease of the mitotic index ($p < 0.001$). Combined treatments with TEM and caffeine exhibited a slight increment of dividing cells, but the mitotic index was statistically lower than that of controls ($p < 0.01$ and $p < 0.001$ respectively).

Discussion. Chromatin bridges at anaphase-telophase arise from: a) Chromosome stickiness; b) dicentric chromosomes showing criss-cross separation of chromatids; c) dicentric chromatids with centromeres oriented toward opposite poles[9,10]. However, the origin of chromatin bridges cannot be determined accurately in anaphase-telophase plates.

The increase in chromatin bridges in treated cultures could be a result of chromosome exchanges induced by TEM and enhanced by caffeine. This assumption is in godd agreement with the well known effects of caffeine in chromosome aberration potentiation[5,6] but it would imply that most of the TEM-induced lesions lead to chromosome exchanges which will be detected at the 1st mitosis after treatment. On the other hand, chromatin bridges at anaphase-telophase could arise from chromosome stickiness. In such a case, the potentiating effect of caffeine might be an indication that, like other subchromatid or chromatid aberrations, entangled chromatin fibrils are repaired by post-replication repair or another mechanism which can be inhibited by caffeine.

Our results do not decisively support any of these hypothesis. The influence of caffeine post-treatment on the frequency of chromatin bridges suggests, however, a relation between metaphase and anaphase-telophase chromosome aberrations and is a further evidence of the relevance of anaphase-telophase analysis for chemical mutagenesis.

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2 B.A. Kihlman, Caffeine and chromosomes. Elsevier, Amsterdam 1977.
3 Committee Report on Mutation Testing, Toxic. appl. Pharmac. *22*, 269 (1972).
4 T.C. Hsu, C.J. Collie, A.F. Lusby and D.A. Johnston, Mutation Res. *45*, 233 (1977).
5 B.M. Cattanach, C.E. Pollard and J.M. Isaacson. Mutation Res. *6*, 297 (1968).
6 A.T. Natarajan, Biol. Zbl. *95*, 139 (1976).
7 K.E. Hampel and N. Gerhartz, Exp. Cell Res. *37*, 251 (1965).
8 M.L. Larramendy and F.N. Dulout, Mendeliana *3*, 51 (1978).
9 D. Davidson, Chromosoma *9*, 39 (1957).
10 B.R. Brinkley and N. Hittelman, Int. Rev. Cytol. *12*, 42 (1975).

Synthesis of maleimide derivative of cortisol for enzyme coupling in cortisol enzyme immunoassay[1]

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Summary. A new cortisol derivative, cortisol-21-m-maleimidobenzoate (CMB), was synthesized and conjugated with sulfhydryl groups of β-galactosidase (BG). Both CMB-BG and CHS-BG conjugates have a high immunoreactivity to cortisol antibody, and although CHS-BG does not displace well with the added cortisol, CMB-BG does.

We have previously described the use of a novel m-maleimido-benzoyl derivative of thyroxine (T_4) methyl ester for the development of enzyme-labeled immunoassay (EIA)[3]. These studies showed a high efficiency of hapten coupling to sulfhydryl group of β-galactosidase without reduction in both enzyme activity and immunoreactivity, resulting in highly sensitive and reproducible EIA, and indicated the possibility that the same approach for enzyme-hapten conjugation could be applied to other haptens. This report describes the preparation of that m-maleimidobenzoyl derivative of cortisol and use of the compound for the development of EIA.

Materials and methods. Cortisol, cortisol-21-hemisuccinate, cortisone, corticosterone, cortisol-21-acetate and o-nitrophenyl-β-D-galacto-pyranoside were obtained from Sigma Chemical Co. Antiserum to cortisol-21-hemisuccinate (CHS, figure 1)-bovine serum albumin (BSA) was raised in rabbits following the procedure of Ruder et al.[4]. m-Maleimido-benzoic acid was prepared according to the method of Kitagawa and Aikawa[5]. Conversion to its carbonyl chloride (MBC) was done by the procedure described previously[3]. Synthesis of cortisol-21-m-maleimidobenzoate (CMB) was carried out as follows: 100 mg of MBC dissolved in 2 ml of tetrahydrofuran (THF) was added dropwise to 148 mg of cortisol dissolved in 2 ml of THF. The mixture was refluxed for 1 h and the synthesized product was monitored by TLC using Eastman chromatogram 13179 (Eastman Kodak Co.) as an eluting plate and ethyl acetate as an eluting solvent ($R_f = 0.67$). The product isolation was carried out by silica gel column chromatography (1.5 × 30 cm) using chloroform-ethyl acetate (3:1) as an eluting solvent. The isolated product gave a single fluorescent spot on TLC by sulfuric acid spray (10% concentration sulfuric acid in ethanol). Esterification at position 21 was