Exposures was to 400 R X-rays delivered at 70 R/min from a RUM therapeutic unit (180 kV, 15 mA, 3 mm Al filtration, distance 0.50 m). The animals were irradiated 11 at a time in round plexiglass boxes with 12 compartments, one of which contained a VA-J-18 dosimeter VA-K-251 ionization chamber in paraffin.

Protectants were administered 8 min prior to exposure, by slow i.p. injection of 1-ml ex tempore aqueous solutions of mechanical mixtures of: ATP, adenosine-5-triphosphoric acid disodium salt, Reanal, Budapest, 360 mg/kg b.wt; AET, 2-(2-aminoäthyl)-2-thiopseudoharnstoff dihydrobromid, Schuchardt, München, 24 mg/kg b.wt; serotonin, serotonin creatinine sulphate, Reanal, Budapest, 8 mg/kg b.wt. Doses were calculated for an average 25-g mouse.

The animals were sacrificed 100–120 days following treatment to prepare testis slides for cytogenetic analysis by the method of Evans et al.[9]. Translocation induction in spermatogonia was assayed by sampling diakinesis-metaphase I spermatocytes for ring or chain multivalents.

Heterogeneity testing of experimental findings showed that all animals investigated could be included in the analysis. The data were statistically treated by analysis of variance and the χ^2 criterion. To compare results from nonirradiated groups, use was made of Fisher's formula since expected results fell within constraints for applying the χ^2 criterion.

Results and discussion. Following spermatogonial exposure to 400 R X-rays, reciprocal translocation recovered in spermatocytes amounted to 7.65%. In mice pre-treated with the optimal-dose-ratio triple combination, translocation frequency after similar exposure was reduced to 3.70% ($\chi_1^2 = 29.17 > 10.83$; $p < 0.001$). The effect of the pair combination without ATP (AET + serotonin, at the same dosage) was considerably smaller, with no statistical significance ($\chi_1^2 = 3.50 > 3.84$; $p > 0.05$).

Our previous work, where protective and toxicologic characteristics of the 3 agents were examined in detail, has shown ATP to contribute insignificantly to total protection afforded by the triple combination in terms of animal survival[8]. Experimental evidence was obtained that in mice exposed to $LD_{100/11}$ the extent of protection provided is similar for the AET + serotonin pair and for the triple combination. It is thus evident that, with the triple mixture, the part played by ATP in combined effect varies with the parameter of damage considered. Based on survival after lethal irradiation, the role of ATP is minimal, whereas for the genetic measure of damage produced by a lower level of radiation exposure (400 R X-rays), the role of ATP is essential. One possible explanation may lie in a difference in the severity of radiation damage. In the case of highlevel exposure, the proportion of irreversible damage produced is larger and there is less repair activity, hence a smaller chance for ATP to intervene. At lower radiation doses, there is more reparable damage, and thus a better opportunity for ATP to exert its favorable influence on repair systems.

Under our experimental conditions, the spontaneous translocation rate was of 0.05% (1 translocation in 1967 cells sampled from 10 animals). An optimal-dose-ratio triple combination administered without radiation induced 2 translocations in 2 animals; a pair combination, 4 translocations in 3 animals. In the 1st case, the sensitive Fisher test indicated only a slight suspicion of mutagenicity; in the 2nd case, the difference from spontaneous rate was of marginal significance ($p\ 0.0495 > 0.01$, but < 0.05). Because of the relatively low sensitivity of the measure used in the mutagenicity test, these results should be regarded as tentative. It is clear, however, that ATP tends to exhibit antimutagenic activity against chemical mutagens given without radiation. Our findings indicate that the triple combination proposed has good antimutagenic properties, and emphasize that ATP should be considered as an essential component in selecting agent combinations intended to protect from genetic radiation injuries.

1 M. Pomerantzeva and G. Vilkina, Genetika *10*, 55 (1974).
2 M. Ashwood-Smith, E. Evans and A. Searle, Mut. Res. *2*, 544 (1965).
3 A. Leonard and Gh. Deknudt, Radiat. Res. *50*, 120 (1972).
4 N. Savcovic and J. Pecevski, Can. J. Genet. Cytol. *16*, 85 (1974).
5 A. Bayrakova, T. Pantev, I. Nikolov and N. Bokova, Mut. Res. *25*, 377 (1974).
6 D. Benova and I. Baev, Int. J. Radiat. Biol. *26*, 47 (1974).
7 A. Leonard and Gh. Deknudt, Strahlentherapie *145*, 174 (1973).
8 D. Benova, in press.
9 E. Evans, G. Breckon and C. Ford, Cytogenetics *3*, 289 (1964).

Morphological effects of estrogen on the female rat liver nucleolus

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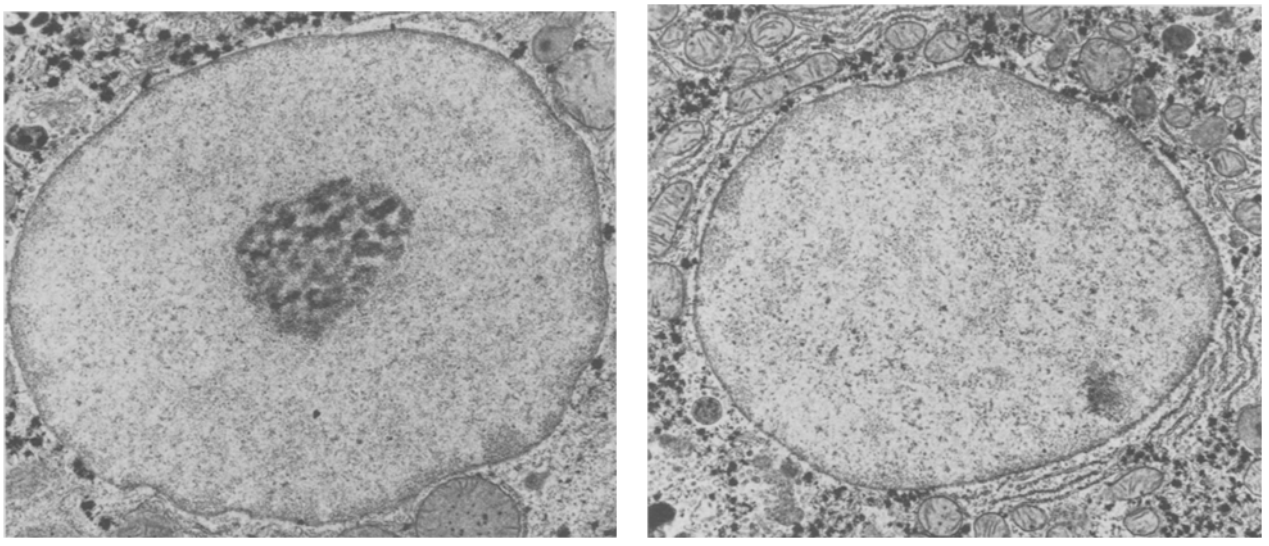
Summary. Prolonged administration of nonphysiological amounts of estrogen induced markedly enlarged nucleoli volumes in rat hepatocytes, indicative of increased nucleolar RNA synthesis. Physiological amounts of drug had no apparent morphological effects on the hepatocytes.

It recently has been demonstrated that female rat liver cytosol contains specific estradiol-binding proteins which can translocate to the nucleus and bind to chromatin after formation of the hormone:receptor complex[2]. These data correlate with previous experimental results which demonstrated an estrogen-induced increase in the template activity of rat liver chromatin[3], an estrogen-induced increase in plasma proteins of hepatic origin[4], an estrogen-mediated enhancement of rat liver tRNA methylase activity[5,6], and an activation of a liver protein kinase activity resulting in an increase in the activity of some of the aminoacyl synthetases[7]. Also observed have been increases in the synthesis of the blood-clotting factors[8,9], renin substrate[10], and pre-beta-lipoproteins[11,12] in the liver of human females taking estrogen-containing oral contraceptives. All of these findings are consistent with the hypothesis that mammalian liver can function as a target organ for estrogen. In the present communication this proposal is validated only for nonphysiological doses of estrogen. Evidence is presented to show that only prolonged administration of large amounts of estrogen can induce female rat liver nucleolar RNA synthesis, one of the first metabolic events following translocation of the cytosol receptor:hormone complex to chromatin.



a b

Nucleolar/nuclear region of a hepatocyte derived from a female rat which was given: *a* daily s.c. injections of 2 mg mestranol dispersed in 0.5 ml sesame oil for 4 days; and *b* daily s.c. injections of 0.5 ml sesame oil for 4 days. A Siemens Elminkop I electron microscope was employed; magnification × 6000.

Materials and methods. Holtzman female rats (120–150 g) were used in all of the experiments. 2 mg of mestranol (17-beta ethynylestradiol-d-methyl ether) dispersed in 0.5 ml of sesame oil was administered daily for 6 days by s.c. injections or by intubate feeding. Sesame oil without estrogen was given to control animals, using the same protocol as with the test animals. Other animals were given 0.1–20 µg of mestranol in 1 dose by s.c. or i.p. injection. All of the animals were sacrificed 24 h after their last injection by a blow on the head, after which they were allowed to bleed freely by severing the jugular vein. A portion of the left lobe of the liver was rapidly excised, trimmed in 1-mm cubes, and fixed in: a) 1% osmic acid in 0.2 M phosphate buffer, pH 7.4, for 3 h at room temperature, washed twice with buffer for 30 min, dehydrated in graded alcohols and propylene oxide, then b) fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at room temperature for 3 h, washed twice with buffer, and subjected to osmic acid treatment as in step a. 40 µm nonfrozen sections were then prepared using an LKB Ultramicrotome. The sections were mounted on naked copper grids and were examined, either unstained or stained with lead citrate, in the Siemens Elminkop I electron microscope.

Results and discussion. Initial studies showed that 0.1–20 µg of mestranol had no apparent effects on rat liver nucleolar RNA synthesis[5]. However, as illustrated in the figure, an increase in the volume of the nucleolar region, indicative of increased nucleolar RNA synthesis, appeared in hepatocytes after 3 days of 2 mg daily of mestranol treatment by s.c. injections or by intubate feeding. After 3 days, 10–20% of the nucleoli were enlarged, while 60–70% were enlarged after 6 days. There were no other extraordinary changes in the morphology of the other hepatic cell organelles after mestranol treatment. These data correlate well with previous studies showing that increases in hepatic protein synthesis occurred only after s.c. injections of nonphysiological amounts of mestranol were administered for 3–6 days[5].

Although it is apparent that nonphysiological amounts of estrogen can increase mammalian liver nucleolar RNA, and thus protein synthesis, it appears unlikely that physiological amounts of the hormone can modulate liver protein synthesis. The use of the estrogen-containing oral contraceptives by humans provides a nonphysiological situation in which there is a steady supply of exogenous estrogen. This latter situation may conceivably mimic the effects of prolonged administration of the hormone to rodents, and result in an increase in hepatic nucleolar RNA and protein synthesis. This, in turn, may be responsible, as implied by Eisenfeld et al.[2], for a) the increase in the synthesis of the blood-clotting factors[8,9], which may play a role in thromboembolism; b) the increase in renin substrate[10], which may intensify an existing hypertensive or prehypertensive condition; c) the increase in lipoprotein synthesis[11,12], which may be involved in atherosclerosis; and d) liver adenomas[13,14], occurring in women taking the oral contraceptives.

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2 A.J. Eisenfeld, R. Aten, M. Weinberger, G. Haselbacher, K. Halpern and L. Krakoff, Science *191*, 862 (1976).
3 T.H. Hamilton, Science *161*, 649 (1968).
4 U.S. Seal and R.P. Doe, in: Metabolic effects of gonadal hormones and contraceptive steroids, p. 272. Ed. H.A. Salhanic, D.M. Kipnis and R.E. Vande Wiele. Plenum Press, New York 1969.
5 B. Sheid, E. Bilik and L. Biempica, Archs Biochem. Biophys. *140*, 437 (1970).
6 A.E. Pegg, Biochim. biophys. Acta *319*, 354 (1973).
7 B. Berg, Biochim. biophys. Acta *479*, 152 (1977).
8 J. Conrad, M. Samama and Y. Salamon, Lancet *2*, 1148 (1972).
9 M. Dugdale and A.T. Masi, J. chron. Dis. *23*, 775 (1970).
10 J.H. Laragh, L. Baer, H.R. Brunner, F.R. Buhler, J.E. Sealey and E.D. Vaugn, Jr Am. J. Med. *52*, 633 (1972).
11 T. Stokes and V. Wynn, Lancet *2*, 677 (1971).
12 S. Rossner, U. Larsson-Cohn, L.A. Carlson and J. Boberg, Acta med. scand. *190*, 301 (1971).
13 J.K. Baum, F. Holts, J.J. Bookstein and E.W. Klein, Lancet *2*, 926 (1973).
14 H.A. Edmondson, B. Henderson and B. Benton, New Engl. J. Med. *29*, 470 (1976).