ruling out the possibility of kinin mediation of the depressor response[5]. Prior atropinization abolishes the immediate fall, showing cholinergic mediation of the first component of depressor response probably due to the large amount of acetylcholine present in the venom[9]. The abolition of secondary sustained fall in spinalized cats points towards central involvement in production of the sustained fall in blood pressure, confirmed by the absence of cardiovascular response in atropinized spinal cats. A similar sustained hypotensive response was observed on i.v. administration of C_{14} fraction obtained by starch gel-electrophoresis. No such response was seen in spinal cats.

1 The work reported here was undertaken in partial fulfilment of the requirements for the degree of PhD of the University of Nairobi.
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3 Acknowledgment. We are grateful to the University of Nairobi for the research grant (No. 670-052) which supported this work. We also thank Merck, Sharp & Dohme Ltd for a liberal supply of cyproheptadine and Mr E. Njogu (chief technician, Dept of Veterinary Anatomy) for photographic assistance.
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Protection of the mouse from genetic radiation damage by an optimal-dose-ratio combination of ATP, AET, and serotonin

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Summary. The study concerned antiradiation effects in germ-cell genetic structures produced by a combination of ATP, AET, and serotonin at dose ratio optimal for lethality namely, 45:3:1, as arrived at in our previous work. Such a combination was found to reduce by a factor of 2 the translocation yields observed after 400 R X-rays to mouse spermatogonia. In terms of animal survival, ATP has been shown to contribute little to total protection achieved by the same combination; in terms of genetic damage; however, the role of ATP proved essential. Removal of ATP from the combination led to a significant reduction in protective effect.

In assessment of genetic radiation hazards, mutation induction in spermatogonia are of primary importance. Mutations induced in this cell type persist throughout the reproductive life span of the male and are responsible for much of the heritable damage to offspring. In recent years, attempts have been made to modify such damage by use of chemicals known to produce a good effect in terms of animal survival. The limited amount of experimental evidence obtained in this area is conflicting[1-4]. Favorable effects have mostly been observed with combinations of protective agents, either as mechanical mixtures or as molecular combinations[5-7].

Our previous work[8], using the parameter of mouse survival after lethal radiation exposure, has shown the optimal-dose-ratio for a mixture of ATP, AET, and serotonin to be 45:3:1. It was demonstrated that on a survival basis combined protection is essentially due to AET and serotonin, with a minimal contribution by ATP. The latter, however, has the property to reduce overall toxicity of the combination.

The objective of the present study was to find out how this optimal-dose-ratio triple combination would affect reciprocal translocation induction in spermatogonia by 400 R X-rays, and to define the contribution of ATP to total effect in terms of this genetic measure of damage.

Materials and methods. The experiments used 12-14-week-old C57BL mouse males weighing 22-26 g. There were 6 treatment groups: 1. irradiation without protection; 2. irradiation and protection by the triple ATP + AET + serotonin combination; 3. irradiation and protection by the pair AET + serotonin combination; 4. and 5. administration of either the triple or the pair combination at the same doses without irradiation, for mutagenicity testing and 6. biological control group receiving neither radiation nor chemicals (table).

Effect of the combination ATP-AET-serotonin on reciprocal translocations induced in mouse spermatogonia by 400 R X-rays

Treatment groups (protectant doses in mg/kg)	No. of animals	Cells analyzed	Metaphases with translocations 1	2	3	%	Translocations per cell (%)
400 R	10	2000	135	16	2	7.65	8.65 ± 1.2
400 R + ATP 360 + AET 24 + 5-HT-8	10	2000	67	7	-	3.70	4.05 ± 0.6
400 R + AET 24 + 5-HT-8	10	2000	117	6	-	6.15	6.45 ± 0.6
Controls	10	1967	1	-	-	0.05	0.05 ± 0.05
ATP 360 + AET 24 + 5-HT-8	10	1989	2	-	-	0.10	0.10 ± 0.06
AET 24 + 5-HT-8	10	2000	4	-	-	0.20	0.20 ± 0.10

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.

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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.