tions were discontinued and ARS determined at 36 h following the last dose.

Results. A linear treatment duration-response relationship was demonstrated for the barbiturate-withdrawn animals, seizure susceptibility (percent responding) increasing with treatment duration (figure 1). No change in seizure severity accompanied the increased susceptibility (figure 2), nor was any spontaneous seizure activity observed. With the exception of 1 rat in the 10-day control group, which had an ARS of 3, control animals exhibited no response to sound stimulus.

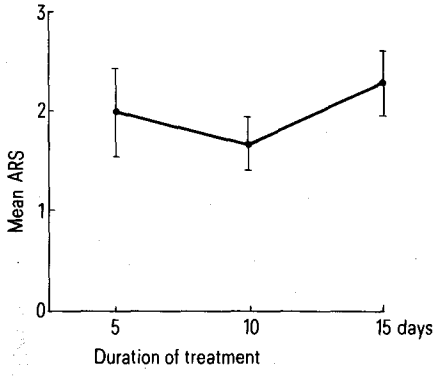

Fig. 2. Effect of barbiturate treatment duration on audiogenic response score (ARS) during withdrawal. Vertical bars represent SE of the mean.

These results provide evidence in agreement with the suggestion of others[5-7] that the physiological changes responsible for barbiturate dependence begin after only a short period of drug treatment. However, even at a sustained high dose level (i.e., the regimen used in this study) a time-dependent process is involved. This partially clarifies the results of a previous report from this laboratory[4], in which it was not possible to state whether the increased seizure response that accompanied progressive weekly increases in barbital dosage was a result of longer treatment or larger daily doses of barbital.

In addition, these results suggest the possibility that the lengthy and variable oral dosing regimens which have been widely employed in the past to produce barbiturate dependence may be an unnecessary complicating factor in investigating the phenomenon.

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Centrally acting hypotensive fraction in the venom of *Dendroaspis angusticeps*

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Summary. I.v. administration of 100 µg/kg of *Dendroaspis angusticeps* venom produced a biphasic vasodepressor response. The first fall in blood pressure is due to a cholinergic component, whereas the second fall may be due to central depressant effect.

In our previous work on *Dendroaspis jamesoni* venom, a central locus of action for the hypotensive response was suuggested[4,5]. This paper deals with the identifaction and mechanism of a hypotensive fraction in *Dendroaspis angusticeps* venom.

Materials and method. 32 cats (2.5-3.5 kg) were anaesthetized with ether followed by i.v. chloralose (80 mg/kg). The blood pressure was recorded from the right common carotid artery by a Statham transducer (P23D) and the heart rate on a Grass polygraph (Model 79-8P-40). The cats were artificially ventilated with an electronic ventilator (SRI, England) at a pressure of 15 cm of water per kg and a rate of 20 per min. The rectal temperature was maintained between 36 and 37 °C throughout the experiment. The efferent pathways of hypotensive response were determined in bilateral cervical vagotomized and spinalized (C-2) cats. The drugs and snake venom were injected through the right femoral vein. Electrophoresis was carried out on starch gel[5] and the protein content of the eluted fractions was determined by the method of Lowry et al.[6].

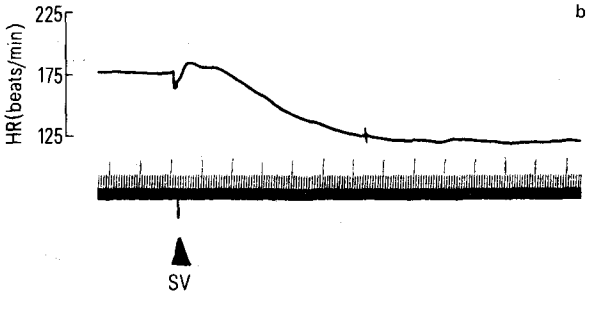

Fig. 1. Effect of i.v. injection of snake venom (100 µg/kg) on the blood pressure in chloralose anaesthetized (80 mg/kg) cat. Cat 2.8 kg, record of blood pressure after i.v. injection of snake venom. Note the initial quick fall, the partial recovery and the sustained fall.

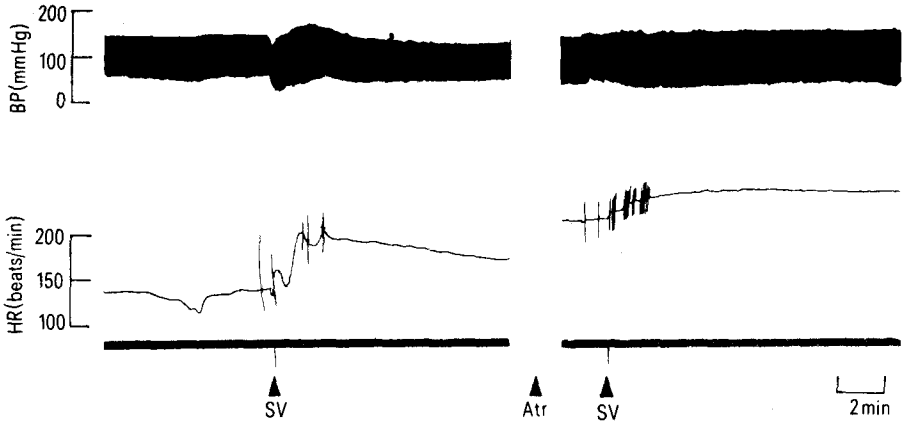

Fig. 2. Effect of spinal cord transection (C_2) and atropinization on the blood pressure response produced by i.v. injection of snake venom (100 µg/kg) in the cat.

Cat 3.5 kg spinalized, left hand record shows presence of quick fall and absence of sustained fall, the right hand record shows no change in blood pressure after prior atropinization (2 mg/kg) in the same spinalized cat.

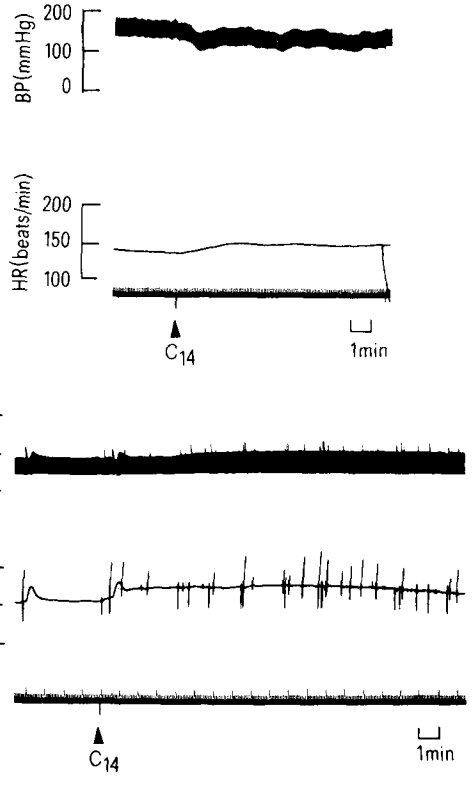

Fig. 3. Effect of C_{14} fraction (23.2 µg/kg) administered in i.v. in normal chloralose anaesthetized and spinalized cats. Upper record: Cat 2.5 kg, note only the sustained fall and absence of quick fall. Lower record: Cat 5.0 kg, spinalized, no change in blood pressure after i.v. injection of same dose of the fraction.

Effect of various agents administered i.v. on the vasodepressor response induced by i.v. administration of snake venom (100 µg/kg) in anaesthetized cats

Blocking agent	N	Blood pressure (mm Hg ± SE) Normal	Decrease after venom Immediate	Secondary sustained
Control group	5	139 ± 17.2	59.2 ± 8.7	38.2 ± 13.1
Atropine (2 mg/kg)	5	140 ± 29.4	2.5 ± 2.5*	30.0 ± 4.1
Hexamethonium (10 mg/kg)	5	120 ± 9.5	61.0 ± 4.0	10.0 ± 0.0*

N, denotes total number of cats used. * These values are significantly different ($p < 0.05$) from decreases in the control group.

Drugs used. Atropine sulphate (Sigma, London), mepyramine maleate (May & Baker, England), trasylol (Bayer, Federal Republic of Germany), hexamethonium bromide (Sigma, London), cyproheptadine hydrochloride (Merck, Sharp & Dohme, UK). Dessicated whole venom was obtained from Mr J.H. Leakey, Baringo Snake Farm, P.O. Box 1141, Nakuru, Kenya.

Results and discussion. I.v. injection of 100 µg/kg snake venom produced a biphasic vasodepressor response, an initial brief fall with partial recovery of blood pressure followed by a secondary sustained fall (figure 1). Repeated administration of same dose of whole venom did not exhibit the phenomenon of tachyphylaxis (N = 2).

Administration of atropine (2 mg/kg i.v.) 45 min before the venom abolished the initial brief fall, but not the sustained fall, whereas pretreatment with hexamethonium 10 µg/kg i.v., 1 h before the venom attenuated significantly the sustained fall (table). Prior administration of mepyramine (N = 2: 5 mg/kg), trasylol (N = 2: 5000 kIU)[7], cyproheptadine (N = 2: 100 µg/kg) produced both components of vasodepressor response. Spinal cord transection (C-2) did not affect the immediate fall in blood pressure abolished the sustained fall, whereas combination of spinal cord transection and atropinization abolished the immediate fall (N = 4: figure 2). No change in vasodepressor response was noticed in bilateral vagotomized cats (N = 3).

Fractionation of venom by starch gel-electrophoresis revealed 3 cathodic fractions (J. Wangai, unpublished communication). Administration of C_{14} fraction in a dose of 23.2 µg/kg produced a sustained fall in blood pressure, a fall that was not elicited in cats with a transsected spinal cord (figure 3).

The present investigation shows that i.v. administration of whole venom of *Dendroaspis angusticeps* causes a biphasic vasodepressor response. The failure of mepyramine and cyproheptadine to modify the components of hypotension shows that histamine may not play a role in the mediation of venom induced cardiovascular collapse[5,8].

Prior systemic administration of trasylol produced no change in the depressor response to i.v. injection of venom,

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.

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2 Department of Physiology, University of Nairobi.
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