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Chemical Protection Against Early Radiation Mortality of the Young Chicken.* (28536)

J. L. MONTOUR AND C. J. SHELLABARGER
Zoology Department and Kresge Radioisotope Unit, Medical School, University of Michigan, Ann Arbor

The acute radiation syndrome that follows exposure to lethal amounts of X- or gamma-radiation has been studied extensively in mammals where death occurs 5 to 15 days post exposure largely from damage to the gastrointestinal tract and the bone marrow (1). Acutely irradiated birds die in a comparable period and, although the exact mechanisms involved are not clearly understood, fluid imbalance caused by gastrointestinal damage, and hematopoietic failure are likely involved(2). In the young chicken, however, additional lethal processes occur following acute irradiation. Jacquez and Karnofsky(3) showed that with exposure rates above 15 roentgens per minute the majority of the animals died within 48 hours of exposure to 1000 r. At very large radiation doses mammals also die within 48 hours of exposure due to interference with nervous system function. Stearner *et al.*(4), however, have excluded central nervous system damage as the cause of death in chickens at radiation doses below 1000 r. Renal failure, hypotension and circulatory failure have been the major characteristics associated with the early death in the chicken(5).

Since 2-aminoethylisothiouronium bromide (AET) and similar compounds have been shown to protect mammals against gastrointestinal and bone marrow mortality(6,7), it was decided to investigate the protective capacity of AET against the early mortality of the young chicken. During the course of these studies it was noted that the early (0-48 hr) deaths fell into 2 separate periods, each with a different time interval between radiation exposure and death.

Materials and methods. Three-day old, White Leghorn cockerels were exposed to 0 r, 750 r, 850 r, or 950 r of X-rays, with or without prior AET treatment. Fifteen minutes before exposure half of the animals were injected subcutaneously with 10.5 mg (approximately 300 mg/kg of body weight) of AET in 0.1 cc of 0.1 *N* NaOH. These animals were then combined for exposure with a similar number injected with saline so that in all cases the AET-treated chicks and their controls were irradiated under identical conditions. The animals were exposed in groups of 20 in a cylindrical polyethylene chamber 9¾″ in diameter and 1¾″ in height which was rotated in the X-ray field at approximately 2/3 rpm.

The conditions of irradiation were as follows. X-rays were obtained from a conventional therapy X-ray machine operated at 250 kv and 15 ma with 0.5 mm Cu and 1.0 mm Al filters. The dose rate of 103 r per minute at a target distance of 55 cm was held constant throughout the exposure periods, the various doses being obtained by changing the total exposure time. The X-ray dose was measured in air with a commercially-calibrated Victoreen condenser r-meter placed at the midpoint of the rotating exposure chamber. A decrease in dose of less than 10% was found at the perimeter of the exposure field.

Throughout the experiment, the chicks

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TABLE I. Effects of AET Administration on 48-Hour Mortality in X-irradiated Chicks. Percent mortality of irradiated animals given in parentheses.

Treatment	Dose	N	3-9 hr	10-16 hr	17-30 hr	31-48 hr	Total 48 hr
Saline	0r	10	0	0	0	0	0
AET	"	10	0	0	0	0	0
Saline	750r	40	3(8%)	0	19(48%)	0	22(55%)
AET	"	40	2(5%)	0	2(5%)	0	4(10%)
Saline	850r	30	8(27%)	0	13(43%)	1(3%)	22(73%)
AET	"	30	0	0	3(10%)	0	3(10%)
Saline	950r	30	16(53%)	1(3%)	12(40%)	0	29(97%)
AET	"	30	4(13%)	0	11(37%)	0	15(50%)
Total		220	33	1	60	1	95

were maintained at room temperature without food or water, and deaths were recorded hourly for the 48 hours following exposure. Preliminary experiments indicated no difference in X-ray sensitivity, survival, or AET effects whether or not food and water were withheld in chicks of this age through the 48-hour experimental period.

Results. At each of the 3 radiation doses employed, a statistically significant reduction in mortality was seen in the birds pretreated with AET, (X^2 $p<.001$). The $LD_{50}/2$ days was 950 r for the AET-treated animals compared to 750 r for the controls, an effective dose reduction of approximately 200 r (Table I).

300 mg/kg was very nearly the maximum tolerable dose of AET for the irradiated chick. In preliminary studies, unirradiated chicks were able to survive following injection of as much as 350 mg AET/kg body weight, but this amount of AET in combination with even low doses of radiation (650 r) was found to be lethal in most cases. Some protection was afforded by as little as 150 mg/kg but as has generally been found with radioprotective compounds, maximum protection was only realized with near toxic concentrations(7). Beta-mercaptoethylamine (MEA) was also found effective in protecting against the 0-2 day deaths in the chicken, but to a lesser degree than AET (unpublished observations).

Analysis of the 48-hour mortality discloses that of 95 deaths in all groups, 33 occurred between 3 and 9 hours post-irradiation, 60 occurred between 17 and 30 hours, and only 1 in each of the intervals of 10-17 and 31-48 hours post-irradiation (Fig. 1). This suggests the existence of two different injury

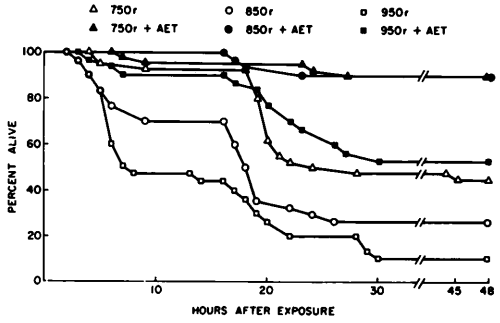

FIG. 1

processes. In all of the groups which showed a significant degree of mortality, approximately 40% of the irradiated animals died in the 17-30 hour period, while the number of deaths in the 3-9 hour period increased in an approximately linear fashion with increasing dose (Table II).

Discussion. Pretreatment with AET effectively protected the acutely irradiated young chicken from death in the period 0-48 hours post-exposure at all radiation doses used in this experiment. There does not appear to be preferential protection in either the 3-9 hour or the 17-30 hour mortality period, but instead the AET acts to produce an effective dose reduction of 200 r. This is clearly illustrated by comparison of the 950 r + AET and the 750 r curves in Fig. 1. The total mortality in each case is approximately 50%, 10% in the 3-9 hour period and 40% in the 17-30 hour period.

The 2 separate periods of mortality seen in the chick within 30 hours of acute X-irradiation, suggest that 2 distinct lethal mechanisms may be involved. Not only are the periods of mortality separated in time but the dose-mortality response above 750 r appears to be quite different in the 2 time

TABLE II. Hourly Tabulation of Deaths Following Exposure to 750r, 850r, or 950r of X-rays, With and Without Prior AET Administration.

Hr post-irradiation		Time of deaths in 48-hour mortality period of acutely irradiated chick																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
950r	N=30			1	2	2	7	3	1						1			1	1	2	1		2						2	1
950r + AET	N=30				1		1	1		1								1		1	2		2	1			2	1		1
850r	N=30			1	2	2	2			2								3	3	4			1		1		1				
850r + AET	N=30																	1	1					1							
750r	N=40					2				1										5	8	2	1		1	1			1		
750r + AET	N=40							1		1															1			1			
0r	N=10																														
0r + AET	N=10																														

periods. The number of animals dead in the first period increases in an approximately linear manner from 750 r to 950 r. Other data (unpublished), indicate that the mortality in the first period will continue to increase in this fashion for doses greater than 950 r. In groups of chicks exposed to 1100-1200 r at similar dose rates, all deaths occurred 3-9 hours post-irradiation. On the other hand, approximately 40% of the animals die in the second period regardless of the amount by which the dose exceeds a certain minimum, in this case 750 r.

The radioprotective action of AET against early mortality in the chick is consistent with its action in numerous other systems although Beaumariage(8) found cystamine ineffective in protecting the chicken against radiation death, possibly because of the very large radiation doses employed by this investigator. Stearner *et al.*(9), however, have demonstrated that epinephrine and low environmental oxygen tension, particularly when used in combination, protected the young chicken from death in the 48-hour period. Since the effective dose reduction noted in the present study with AET was of the same order of magnitude as that found with exposure in 7% oxygen(9), hypoxia may be involved in the chemical protection of chicks. In mammals, however, even though chemical protection is seldom more effective than hypoxia in reducing radiation effects, chemical protective agents do not appear to exert their entire effect through hypoxia(7). It is believed that the radiation injuries which lead to early death in the chicken may also occur in mammals but without such obvious effects. If this assumption is correct, then it is possible that a portion of the protection afforded by AET and similar compounds against otherwise lethal doses of ionizing radiation may be due to modification of damage to tissues in addition to only the bone marrow and the gastrointestinal tract.

Summary. AET given fifteen minutes prior to radiation exposure acts to protect the acutely irradiated chick from injuries leading to death in the early (0-48 hour) period, producing an effective dose reduction of approximately 200 r. Two distinct phases of

mortality are seen within 48 hours of exposure. The first period, 3-9 hours post-exposure, is characterized by an approximately linear dose-mortality response above 750 r. In the second phase (17-30 hours) approximately 40% of the animals irradiated with 750 r died, no increase being observed with higher radiation doses.

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Action of Ouabain on Isometric Twitch Tension in Striated Muscle.* (28537)

LORENZ M. HOFMANN AND THEODORE R. SHERROD (Introduced by K. R. Unna)
Department of Pharmacology, University of Illinois College of Medicine, Chicago

While the cardinal effects of the cardiac glycosides are manifested on heart muscle in congestive heart failure, there is evidence that these agents also affect extracardiac organs(1). The purpose of this study was to determine the action of g-strophanthidin (ouabain) on the isometric twitch tension of non-cardiac striated muscle. The rat diaphragm was chosen as a convenient preparation since it lends itself well to *in vitro* studies of drugs affecting muscle tension. This may be determined either by direct muscle stimulation or by indirect muscle stimulation *via* the phrenic nerve.

It was proposed to study the inotropic effects of ouabain on the isometric twitch tension of the indirectly stimulated rat phrenic nerve-diaphragm preparation and to correlate the results with the electrolyte (Na, K, Ca) changes in the diaphragm.

Methods. Adult male albino rats weighing 150 to 200 g were used. Isolated phrenic nerve-diaphragm preparations, according to the method of Bülbring(2), were immersed in 50 ml of oxygenated Tyrode solution at 27°C, containing 0.2% glucose. Tissue electrolyte determinations were made on both treated and control segments of diaphragmatic muscle.

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The nerve-diaphragm segment was firmly fixed by 2 metal hooks to a muscle holder at the costal margin. The phrenic nerve was placed in a chamber containing 2 platinum electrodes. A constant tension of 2 g was allowed to act on the diaphragm. For isometric twitch tension experiments, the tendinous end of the diaphragm segment was immobilized and attached to a Grass force-displacement transducer, model FT 03. The isometric twitch tension was recorded on a polygraph with a direct current drive amplifier. The median values of the developed tension in 30 consecutive isometric twitches were selected to determine dose-response relationships. For isotonic contractions the tendinous end of the muscle was attached to a light writing lever and the contractions recorded on smoked paper. The diaphragm segment was indirectly stimulated *via* the phrenic nerve every 10 seconds for a period of 5 minutes with single supra-maximal (10 Volt) direct current square wave stimuli of 0.25 millisecond duration.

A Beckman DU spectrophotometer with flame attachment was used for the electrolyte analyses. The tissue cations were expressed as mg % of dried weight. The paired *t* test was employed to determine the signifi-