THE INFLUENCE OF 3,5-DIETHYLHYDANTOIN UPON SURVIVAL DURING ACUTE AND CHRONIC HYPOXIA

SCHOOL OF AEROSPACE MEDICINE

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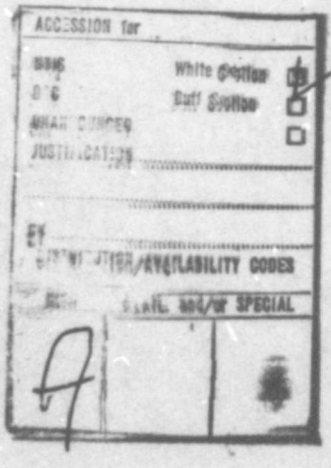

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The median survival times of rats decompressed within 5 minutes to a barometric pressure of 160 mm Hg (33.5 mm Hg P_{O_2}) were significantly increased ($P < .05$ and $P < .02$) in 2 groups of 15 rats each pretreated with 150 mg/kg body wt 3,5-diethylhydantoin (DH); rats pretreated with 50 mg/kg of the drug did not differ from controls. Maximum swimming times of 15 rats exposed to a P_{O_2} of 77 mm Hg and a water temperature of 35° C tended to be longer following pretreatment with 50 mg/kg DH ($P < .10$). Swimming times, however, did not differ

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from controls in rats pretreated with 150 mg/kg of the drug and exposed to a water temperature of 35° C or in rats pretreated with 50 mg/kg and swimming in water at 25° C. Sixteen of 20 saline-injected controls survived 11 days of continuous exposure to a P_{O_2} ranging from 37 to 49 mm Hg, compared with 10 of 20 animals treated daily with 50 mg/kg and 7 of 20 treated with 150 mg/kg DH. The drug-treated animals showed more abnormal pathology than controls. It was concluded that although DH treatment may result in an increase in survival time during acute hypoxia, its apparent toxicity with repeated use in a hypoxic environment precludes its consideration in U.S. Air Force operations.

THE INFLUENCE OF 3,5-DIETHYLHYDANTOIN UPON SURVIVAL DURING ACUTE AND CHRONIC HYPOXIA

INTRODUCTION

Hypoxia is a potential hazard faced by the Air Force flyer, and any drug providing protection against hypoxia would be of considerable interest. Pretreatment of mice and rats with 3,5-diethylhydantoin was reported to increase both exercise capability and survival time during hypoxia. Blood lactate increases (an estimate of tissue hypoxia) were also less (1). However, hypoxia was induced by placing animals in a closed environment with no CO_2 absorbent, so O_2 levels decreased, CO_2 increased.

The present experiment was designed to determine (a) if 3,5-diethylhydantoin exerted a protective effect during exposure to a hypoxic normocapnic environment, and (b) if the drug produced toxic effects with repeated daily treatment during chronic hypoxia.

METHODS

Young-adult male albino rats were used as experimental animals. Weights varied from 250 to 400 grams and were balanced between groups. Two dose levels of 3,5-diethylhydantoin (DH), 50 and 150 mg/kg body weight, were used for all experiments. The drug was prepared so that the concentration was either 50 or 150 mg/ml for a volume per dose of 1 ml/kg body wt. Controls received an equal volume of isotonic saline. The drug was given by intraperitoneal injection during chronic hypoxia, and intravenously in the swimming experiments and acute hypoxia exposures.

Swimming Experiments

Swimming tests were conducted in two glass chromatography jars, 12" in diameter and filled with water to within 5" of the top. Fifty ml of a photographic film wetting agent was put in each jar to eliminate bubble formation on the animal's fur. Each jar was covered with a plexiglass lid fitted with a gas inlet and outlet. Oxygen mixtures were administered via the gas inlet of each jar through rubber tubing connected to a flow regulator attached to a pressurized gas cylinder.

The rats were tested in pairs, with one control and one DH-treated animal tested simultaneously in the two jars. Control and experimental animals were alternated between jars in succeeding runs. DH was administered 10 minutes prior to testing. The rats were placed in the jars, the lids replaced, and flushing with a gas mixture containing 10.3% O_2 and 89.7% N_2 started immediately. The gas flow was initially adjusted so that washout of the jars was greater than 99% complete in 1 minute. Swimming times were recorded from the time the lids were replaced and gas washout started. The rats were considered to be exhausted when they sank and were unable to surface for 20 seconds, after which they were rescued.

Experiments were conducted at a water temperature of 35°C. Fifteen animals received 50 mg/kg DH; 15 received 150 mg/kg DH; and 30 received saline. The animals were untrained for swimming. Another experiment was performed at a water temperature of 25°C. Thirty animals were randomly selected from those used in the previous experiment. Each animal, therefore, had one previous swimming trial. Fifteen were given 50 mg/kg DH and 15 saline. Differences in swimming times were compared using the Mann-Whitney U Test (2). To use this test, it was assumed that each experimental value was independent of the control; that is, a paired control and experimental animal did not tend to be more alike than a control and experimental animal from different pairs.

Acute Hypoxia

Survival times during acute hypoxia were tested by decompressing the rats in a 1.2-m^3 altitude chamber. The chamber was divided into two areas with equal floor space for experimental and control animals. A full-width transparent door allowed close observation of the animals. The animals were placed in the chamber and decompression was begun 30 minutes after treatment with DH. Decompression required 5 minutes to a final pressure of 160 mm Hg (37,500-ft equivalent altitude). Timing was begun when the pressure reached 160 mm Hg. Death was recorded as the time at the last inspiration. Each trial involved 5 control and 5 experimental animals; 3 trials were conducted using a dose level of 50 mg/kg and 3 using 150 mg/kg--for a total of 15 controls and 15 experimentals at each dose level. An additional group of 30 animals were randomly selected from the rats used in the swimming experiments; 15 were used as controls and 15 given 150 mg/kg DH. Differences were compared using the Mann-Whitney U Test (2). It was assumed that the 15 animals given like treatment were a homogeneous group.

Chronic Hypoxia

Chronic exposure studies were conducted in an altitude chamber having an interior volume of 350 ft^3 and equipped with a man lock to facilitate entry to the chamber with minimal disturbance to the environment. Total barometric pressure, temperature, relative humidity, oxygen, and CO_2 partial pressures were automatically controlled and continuously monitored. In addition, gas samples were collected daily and analyzed for oxygen using a Scholander apparatus. These values are shown in Figure 1. The chamber was maintained at a total barometric pressure of 380 mm Hg during the course of the experiment. The oxygen percentage was initially adjusted to 13%, with the balance nitrogen. The oxygen percentage was decreased to 11% on day 4, 10.5% on day 7, and 10% on day 10. Carbon dioxide levels were negligible.

Three groups of 20 animals were maintained in the chamber 11 days or until death occurred. Drug or saline injections were given daily starting the day prior to placement in the chamber. One group

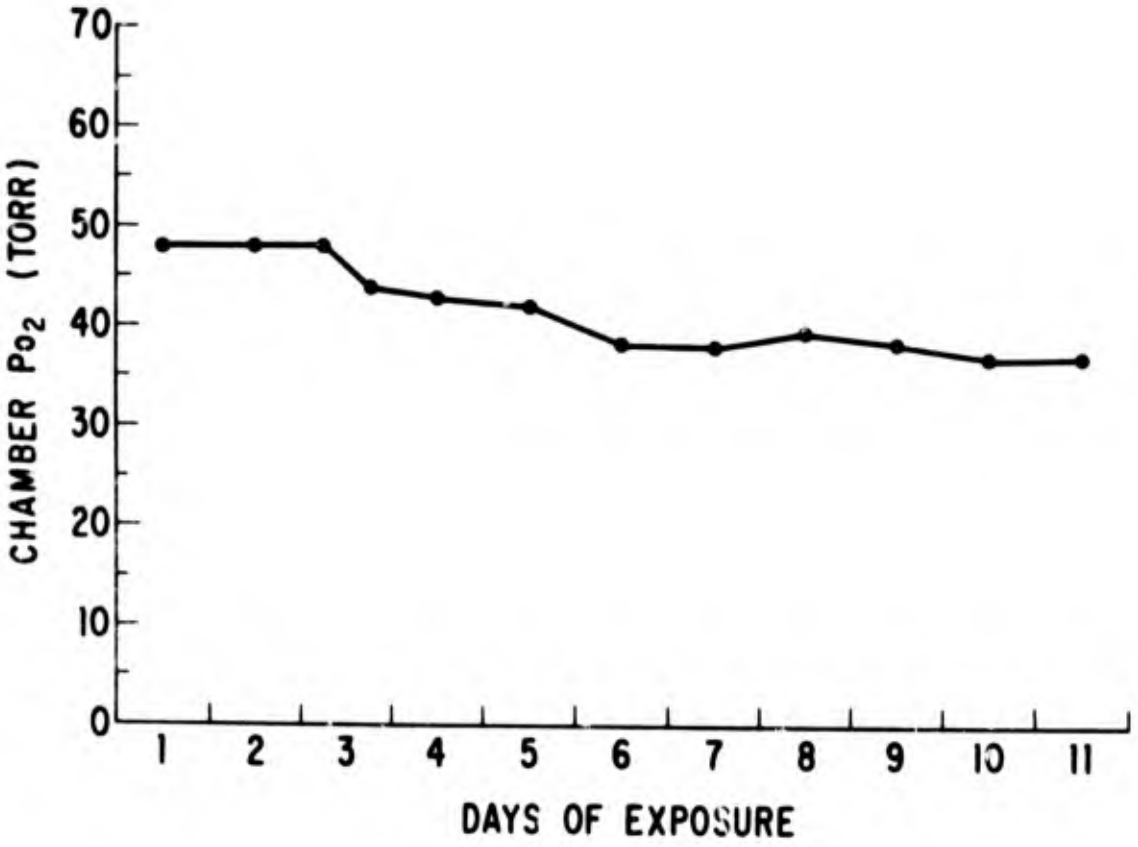

Figure 1. Altitude chamber P_{O_2} during 11-day exposure to hypoxia. Total barometric pressure was maintained at 380 mm Hg.

received 50 mg/kg DH, one group 150 mg/kg, and a third group saline. The chamber was entered daily in order to weigh and inject the rats and to remove dead animals. Three survivors from each group were examined pathologically for evidence of injury. The number of survivors in each group were compared using a Chi-square test (2).

RESULTS

Swimming Experiments

For swimming trials performed at a water temperature of 35°C, the median swimming time after a 50 mg/kg dose of DH was 3 min. 5 sec. compared with 2 min. 45 sec. for controls (Table 1). Differences were of borderline significance ($P < .10$). At the 150 mg/kg dose

level, the median swimming time was 3 min. 30 sec. for the drug-treated animals compared with 3 min. 41 sec. for control animals: a significant difference between these two values could not be detected. With the water temperature at 25°C, the median swimming time after treatment with 50 mg/kg of DH was 7 min. 15 sec. compared with 6 min. 9 sec. for controls and not statistically different.

Survival During Acute Hypoxia

Results are listed in Table 2. The median survival time at 160 mm Hg total pressure was 9 min. 32 sec. for the rats pretreated with 50 mg/kg DH, compared to 6 min. for control animals. A significant difference between these two values could not be detected. The median survival time after a 150 mg/kg dose of drug was 11 min. compared to 6 min. 30 sec. for controls. This difference was significant ($P < .05$). In a final experiment repeating the 150 mg/kg dose level, the median survival time was 12 min. 30 sec. compared with 5 min. 25 sec. for controls. These differences again were significant ($P < .02$).

Chronic Hypoxia

The number of rats surviving are shown in Figure 2. All control animals survived the first 3 days of exposure, and 16 of 20 survived the entire 11 days. Three animals in each experimental group died within the first day of exposure to hypoxia. Ten of 20 animals treated daily with 50 mg/kg DH survived the full 11-day exposure to hypoxia. This difference compared with controls was of borderline significance ($P < .10$). Seven of 20 treated with 150 mg/kg of the drug survived. This was significantly fewer than controls ($P < .05$).

Rats treated with either dose level of drug were diarrhetic throughout the experiment. A purulent drainage around the genitalia, and priapism, occurred in several experimental animals as well as necrosis of the tail and feet. The control animals appeared outwardly healthy, although near the end of the experiment they became quite emaciated. The small number of animals studied for pathology (3 per group) did not allow a quantitative comparison to be made between experimental and control animals. All groups showed chronic mild to moderately severe interstitial pneumonitis with purulent rhinitis, intravascular coagulation of blood, and infarction. Coagulative necrosis was found in the testicles of some animals, possibly resulting from clotting and infarction. The greater degree of change in the rats that received the drug appeared to be dose related.

DISCUSSION

Throughout the years many attempts have been made to find methods of increasing resistance to hypoxia. A common approach has been to endeavor to reduce metabolic rate and thereby oxygen demand. Examples of such studies include production of hypothyroidism (3), beta adrenergic blockage (4), and starvation (5). Other experiments have

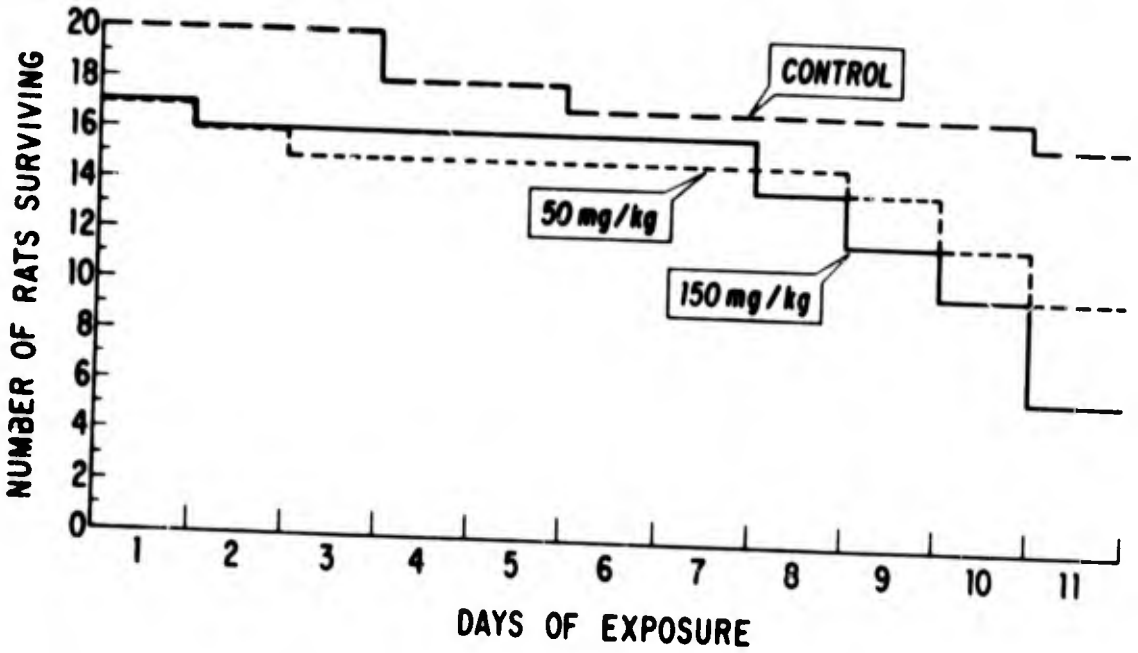

Figure 2. Influence of 3,5-diethylhydantoin upon survival during chronic hypoxia.

involved attempts to develop adaptive mechanisms prior to exposure to hypoxia. In one such study circulating red cell mass was artificially increased (6). Another area which was investigated in some detail by the U.S. Air Force was the use of carbonic anhydrase inhibitor (7). This inhibitor blocked bicarbonate formation, decreasing blood-buffering capacity, and allowed a greater minute-volume respiration without alkalosis.

While the mechanism of DH action is as yet uncertain, previous studies have suggested that it somehow improves the efficiency of oxygen utilization. This oxygen-sparing effect is not attributed to a stimulation of the pituitary-adrenal axis, an antithyroid effect, a hypothermic effect, or a nonspecific CNS depression (1). One effect

of DH, an increase in cardiac-phosphorylase-a activity, may be important in increasing the efficiency of oxygen utilization. This action is evidently mediated by beta adrenergic receptors since cardiac-phosphorylase-a activity is increased by beta agonists, while any enhancement of its activity by DH is inhibited by beta adrenergic blocking agents (8). The drug has also been shown to antagonize the calorigenic effect of epinephrine, suggesting a possible involvement of lipid metabolism (1).

In the present experiment, survival time during acute hypoxia was increased significantly by the higher dose level of the drug. The profile of this experiment was similar to that which might occur during loss of cabin pressurization while flying at 37,000 feet. Pretreatment of the flyer with such a drug might therefore be of potential value. In order to achieve this protection, however, the flyer would need to take the drug before each flight; this could be as often as daily, or even more. Additional evidence indicated that repeated doses during hypoxia were toxic. The potential toxicity to humans that is inferred may endanger health, and thus it is concluded that the drug would not be of value in USAF operations.

REFERENCES

1. RMI 36709 (3,5-Diethylhydantoin). Investigational brochure. William S. Merrell Company, Division of Richardson-Merrell Inc., Cincinnati, Ohio.

2. Siegel, S. Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill Book Co., Inc., 1956.

3. Zerrow, M. X., et al. Comparison of effects of experimental hyperthyroidism and hypothyroidism on resistance to anoxia in rats and mice. Am J Physiol 167:171-175 (1951).

4. Cain, S. M. Survival time of hypoxic dogs given epinephrine or propranolol. The Physiologist 15:98 (1972).

5. Smith, D. C., R. H. Oster, and J. E. P. Toman. The effect of thiamine deficiency and of reduced food intake on resistance to low oxygen tension in the cat Am J Physiol 140:603-608 (1944).

6. Burton, R. R., et al. Role of hematocrit, heart mass, and high altitude exposure in acute hypoxia tolerance. J Appl Physiol 27:49-52 (1969).

7. Cain, S. M. Respiratory effects of carbonic anhydrase inhibition. SAM-TR-60-48, June 1960.

8. Bilder, G. E., and M. E. Hess. The effect of 3,5-diethylhydantoin on cardiac phosphorylase activity. Eur J Pharmacol 12:245-248 (1970).

TABLE 1. EFFECT OF INTRAVENOUS INJECTION OF DIETHYL-HYDANTOIN UPON SWIMMING TIMES IN RATS BREATHING 10.3% O_2 (17,000-FT EQUIVALENT P_{O_2})

	Water Temperature 35°C				Water Temperature 25°C	
	50 mg/kg	saline	150 mg/kg	saline	50 mg/kg	saline
	2:55	3:50	3:37	3:57	10:50	10:25
	3:45	4:10	6:15	3:52	7:15	21:17
	35:45	2:20	3:52	3:10	11:14	4:37
	4:25	3:45	3:10	2:30	4:45	5:05
	4:10	3:50	2:57	4:07	8:54	3:30
	9:20	2:40	3:30	2:25	5:22	4:52
	3:05	3:30	3:10	2:05	4:25	6:09
	8:05	2:35	2:37	2:55	10:28	17:50
	2:20	2:20	2:55	4:21	9:47	10:44
	2:50	1:50	3:38	2:20	6:43	4:50
	2:55	1:30	2:58	3:45	15:07	6:15
	2:40	2:45	4:18	3:41	5:34	4:40
	2:55	2:40	5:05	5:03	8:17	19:27
	3:10	3:05	12:04	2:55	6:48	5:20
	3:05	2:55	2:53	4:50	4:02	6:55
Median	3:05[a]	2:45	3:30	3:41	7:15	6:09

Swimming time shown in minutes-seconds.

[a]Significantly different from controls ($P < .10$).

TABLE 2. EFFECT OF INTRAVENOUS INJECTION OF DIETHYL-HYDANTOIN UPON SURVIVAL TIME OF RATS EXPOSED TO A TOTAL BAROMETRIC PRESSURE OF 160 MM HG (37,500-FT EQUIVALENT)

50 mg/kg	saline	150 mg/kg	saline	50 mg/kg[a]	saline
4:00	3:00	3:00	3:00	5:15	5:15
5:00	4:00	4:00	4:00	5:40	5:24
8:00	7:00	10:30	4:30	9:45	5:54
14:00	8:00	11:00	5:00	11:45	10:12
15:00	11:00	22:00	6:00	12:30	13:48
8:00	5:00	6:10	2:30	3:30	2:48
12:00	6:00	11:05	6:30	5:00	4:10
13:00	13:00	14:58	8:00	8:10	4:54
14:00	16:00	19:21	15:00	13:00	6:12
26:00	23:00	25:32	2:30	20:00	8:42
2:10	1:30	6:20	6:30	12:45	3:50
5:25	4:30	6:20	7:00	12:50	4:10
7:10	5:00	9:45	8:30	14:55	5:15
9:32	5:30	16:50	9:30	18:30	7:30
22:25	11:30	18:00	11:00	24:30	12:06
Median 9:32	6:00	11:00[b]	6:30	12:30[c]	5:24

Survival time shown in minutes-seconds.

[a]Repeat run conducted with rats previously used in swimming experiment.

[b]Significantly different from controls ($P < .05$).

[c]Significantly different from controls ($P < .02$).