it would appear that the Embden-Meyerhof scheme represented the dominating event during this time interval. Also, since after the first 3 minutes, the keto acid levels increased as the hydroxy acid levels decreased, and since the concentrations of these metabolites then returned to normal pre-shock levels, it would appear that the aerobic phase predominated during this time interval. It is of considerable interest that the L/P ratio, at any particular time after shock, of all patients studied fell within a small range.

Further investigations are in progress to determine the relationship between the variations in the concentrations of lactic acid and pyruvic acid, or their ratios, as an index of the relative intensity of the convulsion in EST.

Preliminary data obtained on the blood levels of glucose, sodium, potassium, and calcium, indicated that the concentration of these metabolites varied considerably over a sixty minute period following EST, rising and falling several times during this time interval.

Summary. A simultaneous measurement has been made of lactic acid and pyruvic acid changes in the blood of neuropsychiatric patients before and after extreme muscular excitation induced by electroshock therapy (EST). The blood lactic acid level of all patients following EST rose sharply to a peak (six times pre-shock level) within 3 to 5 minutes and then returned to the normal level after 1 to 1½ hours. The blood pyruvic acid level of all patients rose immediately following EST for thirty seconds (4-6 mg %), then fell to a minimum (2-3 mg %) within 3 to 5 minutes and rose again to a peak three times the pre-shock level 20 min after EST.

We would like to thank Mr. Roscoe R. Schaffert and Miss Marion Amos for their kind assistance with the analyses and Dr. Seymore Bird for his kind cooperation in dealing with the patients. The calcium lactate was obtained through the courtesy of Dr. Merrill N. Camien, Department of Biochemistry, University of California at Los Angeles.

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Received August 25, 1953. P.S.E.B.M., 1953, v84.

Influence of Hibernation on Survival Time and Weight Loss of X-Irradiated Ground Squirrels.* (20647)

JOHN DOULL AND KENNETH P. DUBOIS.

From the U. S. Air Force Radiation Laboratory and the Department of Pharmacology, University of Chicago.

Numerous studies on the influence of temperature on the radio-sensitivity of various biological systems have been conducted and it has been concluded(1) that temperature changes within the range which can be tolerated by most living systems do not directly modify radiation reactions. However, the tissue injury resulting from the interaction of the products of ionizing radiations with cellular constituents is delayed when the temperature of the organism is maintained below normal following irradiation and survival is thereby prolonged. Although this effect has been demonstrated in several types of cells and tissues by a number of investigators, its occurrence in hibernating mammals(2-4) is

* This study was supported by funds provided under contract with the USAF School of Aviation Medicine, Randolph Field, Texas.

of particular interest because it provides another approach for studies on the mechanism responsible for the injurious action of ionizing radiations on mammalian tissues.

For the application of hibernating animals to research in radiobiology it is desirable to use a species which can be readily obtained, easily maintained under laboratory conditions and in which hibernation can be induced throughout the year. We have found that the hibernating ground squirrel (*Citellus tridecemlineatus*) meets these requirements and thus serves ideally for investigating the influence of toxicants on a hibernating species. The present communication describes experiments on the susceptibility of hibernating and non-hibernating ground squirrels to lethal doses of x-irradiation. The results of this investigation have shown that animals irradiated while in hibernation exhibit a prolonged survival time provided they are maintained in the poikilothermic state following irradiation.

Materials and methods. Male and female ground squirrels were trapped in the vicinity of Chicago during the summer months by placing a wire cage over the entrance to the burrow and flooding the burrow with water, forcing the animals into the cage. They were maintained in an air-conditioned laboratory at 68-75°F in individual cages and fed Rockland Rat Diet supplemented biweekly with carrots and cabbage. Hibernation was induced in adult animals of this species by placing the animals in a cold room at 5°C for 24 to 48 hours without food or water. Hibernation in this species is more profound than in other available hibernating animals and it is possible to expose the hibernating ground squirrels to light, noise, and even certain surgical procedures without arousing the animals. Furthermore, the hibernating ground squirrel does not return spontaneously to the homeothermic state as frequently as do most other hibernating species and in this respect offers a distinct advantage for studies involving hibernation. X-irradiation was administered in a single whole-body exposure by means of a Keleket Therapy X-ray Machine (250 KVP, 15 ma, 0.25 mm copper and 1 mm aluminum added filtration, 75 cm target distance, dose rate 35-36 r per minute). Groups of 12 animals were irradiated simultaneously in a rotating aluminum cage placed perpendicularly to the central ray so that each individually caged animal received an identical dose. When animals were irradiated while in the hibernating state, the entire cage was enclosed in a refrigerated box maintained at 5°C during the exposure.

Results. To ascertain the susceptibility of ground squirrels to x-irradiation, 6 groups each containing 12 non-hibernating animals were exposed to doses of x-ray ranging from 400 r to 1200 r. Observations on the mortality and changes in body weight were made daily for 30 days following irradiation. The results of these measurements on 72 animals indicated that the approximate 30-day LD_{50} of x-ray for ground squirrels is 700 r. Non-hibernating ground squirrels exposed to 800 r of x-ray and kept at room temperature following irradiation exhibited 80% mortality within 10 days and 100% mortality within 30 days after irradiation. In a group of 24 ground squirrels, which were irradiated while in the hibernating state and maintained at 5°C thereafter, no mortality was observed within 30 days after the administration of 800 r. However, when 6 of these animals were returned to room temperature either at 2 weeks or at 4 weeks after irradiation, they developed the typical radiation syndrome and died within 10 days. Thus the ultimate mortality was the same as if the animals had been irradiated on the day that they were returned to room temperature and the homeothermic state. Similar results were obtained using doses of 1000 r and 1200 r of x-ray. In 2 groups of 12 non-hibernating ground squirrels given 1200 r, death of all of the animals occurred within 6 days following irradiation, whereas in the animals given this dose while in hibernation and maintained in hibernation following irradiation, no deaths occurred within 30 days after irradiation. However, when 6 of the hibernating, irradiated ground squirrels were returned to room temperature all of these animals died within 6 days.

When 12 ground squirrels were irradiated while in the non-hibernating or homeothermic state and then maintained at 5°C, none of the animals went into hibernation and these animals exhibited only a slight prolongation of survival time as compared with non-hibernating animals maintained at room temperature following irradiation. It is thus clear that maintaining the animals at a low environmental temperature following x-irradiation does not prolong the survival time in this species. In order to obtain the increased survival time following x-irradiation it is necessary that the animals be in hibernation during the irradiation and remain in hibernation following the exposure to x-ray. In the few instances in which the hibernating, irradiated ground squirrels spontaneously came out of hibernation after irradiation the animals did not return to the poikilothermic state and died within a few days even though they were maintained at 5°C. The prolonged survival time observed in the hibernating, irradiated ground squirrels did not alter the ultimate mortality of these animals, however, since even those animals in which hibernation was not spontaneously nor artificially terminated eventually died. Deaths began in these animals on the 54th day after 800 r, on the 42nd day after 1000 r, and on the 38th day after 1200 r of x-ray. It, therefore, appears that although the development of sufficient radiation injury to cause death of the animals is markedly delayed by hibernation, it is not prevented.

Measurements of the body weights of irradiated ground squirrels were used as an indication of the response of the hibernating and non-hibernating animals to various doses of x-ray. The results of body weight measurements following 800 r of x-ray are shown in Fig. 1, in which each value on the curves represents the average for at least 12 animals. From these data it may be seen that ground squirrels which were irradiated while in hibernation and maintained at 5°C thereafter lost about the same amount of weight as did non-irradiated hibernating controls. When these irradiated animals were returned to the homeothermic state by placing them at room temperature a rapid decrease in weight occurred terminating with death of the animals. Ground squirrels given 800 r while in the homeothermic state and maintained at room

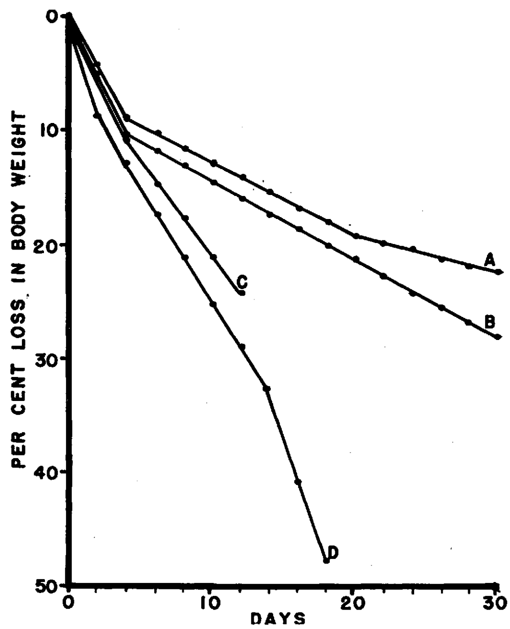

FIG. 1. Effects of hibernation and x-irradiation (800 r) on body wt of ground squirrels *(Citellus tridecemlineatus)*. A. Unirradiated, hibernating. B. Irradiated during hibernation and maintained thereafter at 5°C. C. Maintained in homeothermic state during and following irradiation. D. Irradiated while in the homeothermic state at room temperature and maintained at 5°C after irradiation.

temperature following the irradiation exhibited a rapid decline in body weight. Thus the changes in body weight supported the mortality data and indicated that radiation damage is markedly retarded in irradiated, hibernating animals.

Discussion. The initial chemical events such as the production of free radicals or the direct alteration of biological compounds by ionizing radiations are probably not influenced by temperature differences of the magnitude seen in hibernating and non-hibernating ground squirrels. Evidence in support of this statement was obtained in this investigation by the observation that although hibernation markedly prolonged the survival time of x-irradiated ground squirrels it did not influence the ultimate mortality of these animals. This finding was interpreted as meaning that the same initial events occurred in both the hibernating and non-hibernating animals on exposure to x-irradiation, but that in the hibernating animals the repercussions of this initial damage are not manifested to the same degree as in the normal animal. The marked lowering of the metabolic rate during hibernation is apparently responsible for the prolongation of the survival time in the irradiated, hibernating animals either through postponing the tissue damage or by markedly slowing the rate of disruption of the cellular metabolic processes by x-ray. Studies by Smith and Grenan(3) have indicated that at least some of the blood changes characteristically seen after x-ray, do not occur while irradiated woodchucks and marmots are in the hibernating state. Petersen and DuBois (6) found that lethal doses of x-ray produce an increase in the activity of alkaline phosphatases of the spleens of ground squirrels. The amount of increase in enzyme activity was less in hibernating, irradiated animals than in non-hibernating animals receiving the same dose of x-ray.

The results of this investigation showed that ground squirrels irradiated while in the homeothermic state and maintained thereafter at 5°C failed to exhibit any significant prolongation of survival time. It seems likely that the inability of irradiated ground squirrels to undergo hibernation with a consequent decrease in metabolic rate accounts for this lack of protection. It has been demonstrated that amphibians(5) kept at low temperatures after x-irradiation exhibit a prolonged survival time. Thus if it were possible to induce hibernation and the coincident lowering of the metabolic rate immediately following irradiation, a protective effect might be noted in ground squirrels irradiated while in the non-hibernating state. Extension of the results of this investigation may provide further insight into the mechanism of radiation lethality.

Summary. The LD_{50} of x-ray for non-hibernating ground squirrels (*Citellus tridecemlineatus*) is approximately 700 r. Non-hibernating animals given 800 r of x-ray exhibited over 80% mortality within 10 days. Ground squirrels irradiated while in hibernation and maintained in the hibernating state following irradiation exhibited no mortality within 30 days following exposure to x-ray, but when these animals were returned to the homeothermic state either at 2 weeks or at 4 weeks after irradiation, death ensued within 10 days. Animals which were maintained in hibernation following lethal doses of x-ray eventually succumbed, although survival was greatly prolonged. Thus, while hibernation markedly increased the survival time in this species following lethal doses of x-ray, it did not alter the ultimate mortality.

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Received August 28, 1953. P.S.E.B.M., 1953, v84.

A Growth Factor Required by *Donovania granulomatis*.*† (20648)

W. KNOWLTON HALL, ROBERT B. DIENST, AND CALVIN H. CHEN.

From the Departments of Biochemistry, Microbiology and Public Health, Medical College of Georgia, Augusta, Ga.

Little is known concerning the exact requirement for growth of the Donovan body (*Donovania granulomatis*). Anderson(1) reported the cultivation of the Donovan body

* This investigation was supported by grants from Lederle Laboratories Division of American Cyanamid Corp. and the Division of Research Grants of the National Institutes of Health, Public Health Service.

† A preliminary report was presented at the meeting of Fed. Am. Soc. for Exp. Biol., New York City, April 14-18, 1952.