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Radiosensitivity of Mice in Relation to Heightened Metabolic Activity During and Immediately Prior to Exposure to 220 KV X-rays

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RADIOSENSITIVITY OF MICE IN RELATION TO HEIGHTENED METABOLIC ACTIVITY DURING AND IMMEDIATELY PRIOR TO EXPOSURE TO 220 KV X-RAYS

by

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INTRODUCTION

The manner in which certain factors influence the susceptibility of protoplasmic systems to ionizing radiations is not as yet well understood. Of prime importance among these factors is metabolic rate; it has been demonstrated that there exists a definite relationship between metabolic level and the lethal effects of such radiations. (1) At the outset it must be pointed out that there are two principal problems in this connection; that is to say, radiosensitivity can be studied in relation to metabolic rate prevailing (1) during or (2) subsequent to exposure. One of the most frequently used criterion of radiosusceptability is mortality rate.

There are various physical and chemical methods available for the experimental alteration of metabolic rate. Exercise is an efficient method of heightening the overall metabolic level; it has the particular advantage that it does not involve the artificial introduction of chemicals into the system. In general, there have been surprisingly few investigations into the question of radiosensitivity in relation to muscular activity (exercise involves principally this form of activity) during and subsequent to irradiation. Investigations regarding the relationship between exercise

subsequent to irradiation and mortality due to total-body irradiation have been notably lacking; conflicting results are on record. Kimeldorf and Jones (2) report that the lethality of total-body x-irradiation is increased significantly in rats with induced, repeated vigorous exercise (swimming) subsequent to exposure. However, in similar experiments Smith and Smith (3) report that x-ray lethality is not appreciably increased by post-irradiation exercise. It must be emphasized here that the few data revelant to the problem of radiosensitivity in relation to induced exercise prior to irradiation have been derived from experiments in which the exercise was induced a considerable time before irradiation; Kimeldorf and Jones (4) concluded from their investigations that exercise given daily for two weeks prior to irradiation results in no significant effect upon mortality.

As far as the writer has been able to ascertain, there have been no investigations directed specifically to the problem of radiosensitivity in relation to exercise induced during or immediately prior to exposure to lethal doses of ionizing radiations. It is to this issue that the present investigation is specifically addressed. As originally planned, the investigation was to be directed to the sole question of radiosensitivity in relation to induced, violent exercise during irradiation. The method of exercising employed, however,

proved unsatisfactory; hence, a revision of technique was indicated. The revised method involved induced, violent exercise immediately prior to exposure to x-rays; its advantages will be described. The preliminary results obtained with this second method were clear-cut and highly suggestive. It is with the data derived from this second method that the present report will be principally concerned.

The theoretical background of the experimental work reported here derives from the well recognized fact that a condition of anoxia is accompanied by increased radioresistance (1).

Briefly, this thesis is directed to a study of the radiosensitivity of mice, as measured by the ensuing mortality rate, in relation to heightened metabolic activity occasioned by induced, violent exercise during and immediately prior to exposure to a lethal dose of hard x-rays.

MATERIALS AND METHODS

The animals used in the present investigation were male Swiss albino mice, of the same age, secured from Carworth Farms. These animals were housed, fed, and watered according to accepted recommendations.

Two principal experiments were performed; these will be described in order.

Experiment I: The Effect of Exercise During Exposure on X-ray Lethality.

This experiment involved ninety-six mice; these were divided into four experimental and control groups as indicated below (Table I). The method of heightening the metabolic level during exposure was as follows: The animals were exercised in an electrically driven treadmill apparatus constructed of plastic (Fig. I). It revolved approximately twenty and two-thirds times per minute and measured five and one-half inches in diameter, large enough to contain four mice at one time. One side consisted of a removable wall to facilitate deposition and removal of the mice. Small wood splinters were glued to the floor to give the animal more traction.

One group of thirty-two mice was exercised for ten minutes prior to irradiation, and then for an additional twenty minutes during the administration of the radiation; a second group, comprising thirty-two mice, was irradiated during a period of twenty minutes while the animals were at rest in the immobilized treadmill, without previous exercise; a third group of twenty control animals was exercised for thirty minutes in the treadmill; a fourth group of twelve control animals was subjected neither to exercise nor irradiation.

The radiation conditions for Experiment I were as follows: Type of machine, G.E. Maxiscope; 220 kv; 7 ma;

Fig. 1. The electrically driven treadmill, constructed of plastic, was employed in simultaneously exercising four mice during exposure to x-radiation.

FIGURE 1

target-to-animal distance, 50 cm; 40r/min; added filter of 2 mm Al(equivalent to .07 mm Cu).

Experiment II: Effect of Exercise Immediately Prior to Exposure on X-ray Lethality.

In this experiment a total of eighty-seven mice were involved. These were divided into experimental and control groups as will be described below (Table II). The method of exercise employed in this second experiment consisted in suspending each mouse by its tail with strips of adhesive tape to a horizontal bar clamped above a pan about one foot long containing water. To avoid having its nose submerged the mouse arches its back and paddles violently with its front feet; the hind feet paddle in air with equal activity (Fig. 2). The animals were irradiated in a plastic container covered by a wire screen which prevented escape of the animals. Four animals were placed in the container which was partitioned with pieces of lead. The center of the container was walled-off to insure more uniform irradiation of the animals.

One experimental group of thirty-eight mice was exercised violently for ten minutes prior to irradiation, and then, immediately irradiated for three and one-half minutes in a plastic dish; a second experimental group of thirty-nine mice was irradiated for a period consisting of three and one-half minutes in the plastic dish; the third group of ten animals was exercised by the swimming method for ten minutes.

Fig. 2. The following is the second method for inducing exercise: The pan, filled with water, is brought up to the head of the mouse which has been securely fastened by his tail with strips of adhesive tape to the horizontal bar. To avoid having its nose submerged the mouse arches its back and paddles violently with its front feet; the hind feet paddle in air with equal activity.

Fig. 3. The plastic container, partitioned into four compartments by lead plating strips, used to expose the mice to the x-rays immediately after the exercise. Four animals at a time were exposed; the screen prevented the escape of the mice. The center of the container was walled off to insure more uniform irradiation of the animals.

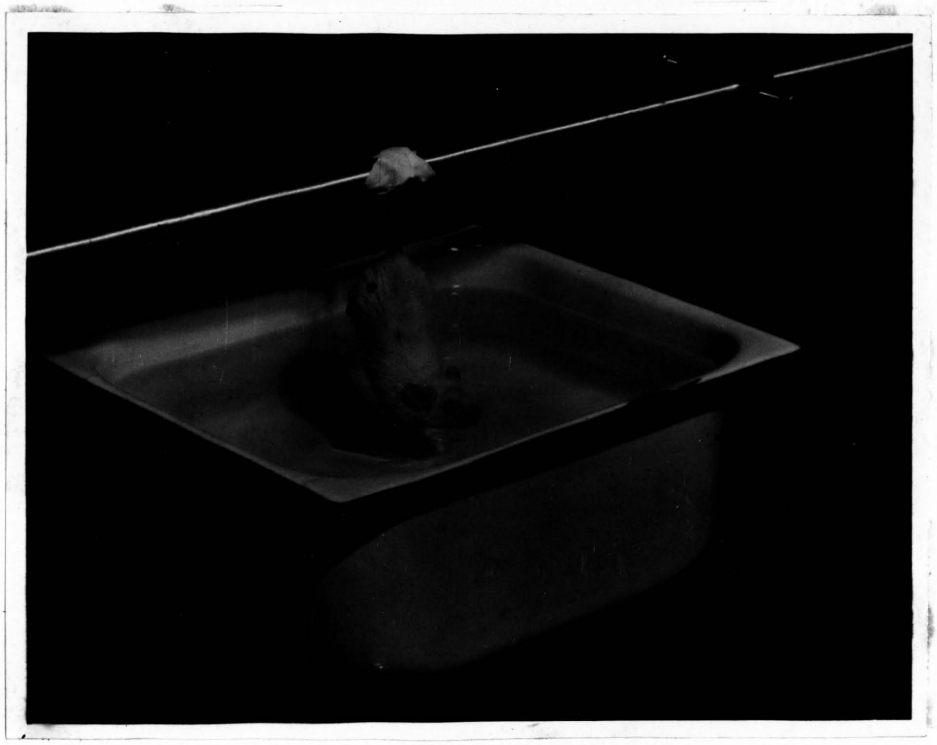


FIGURE 2

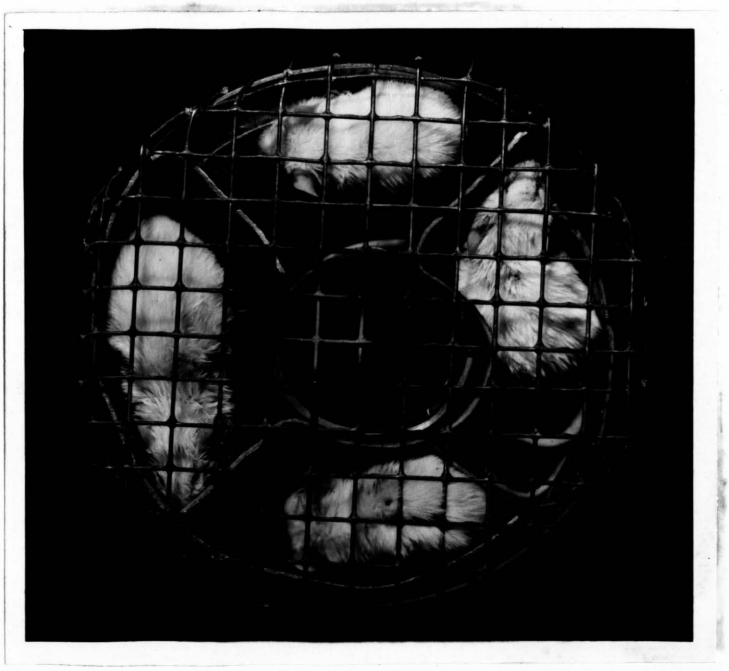


FIGURE 3

The radiation conditions accompanying Experiment II were as follows: Type of machine, G.E. Maxiscope; 220 kv; 10 ma; target-to-animal distance, 25 cm; 228 r/min; added filter of 2 mm Al(equivalent to .07 mm Cu).

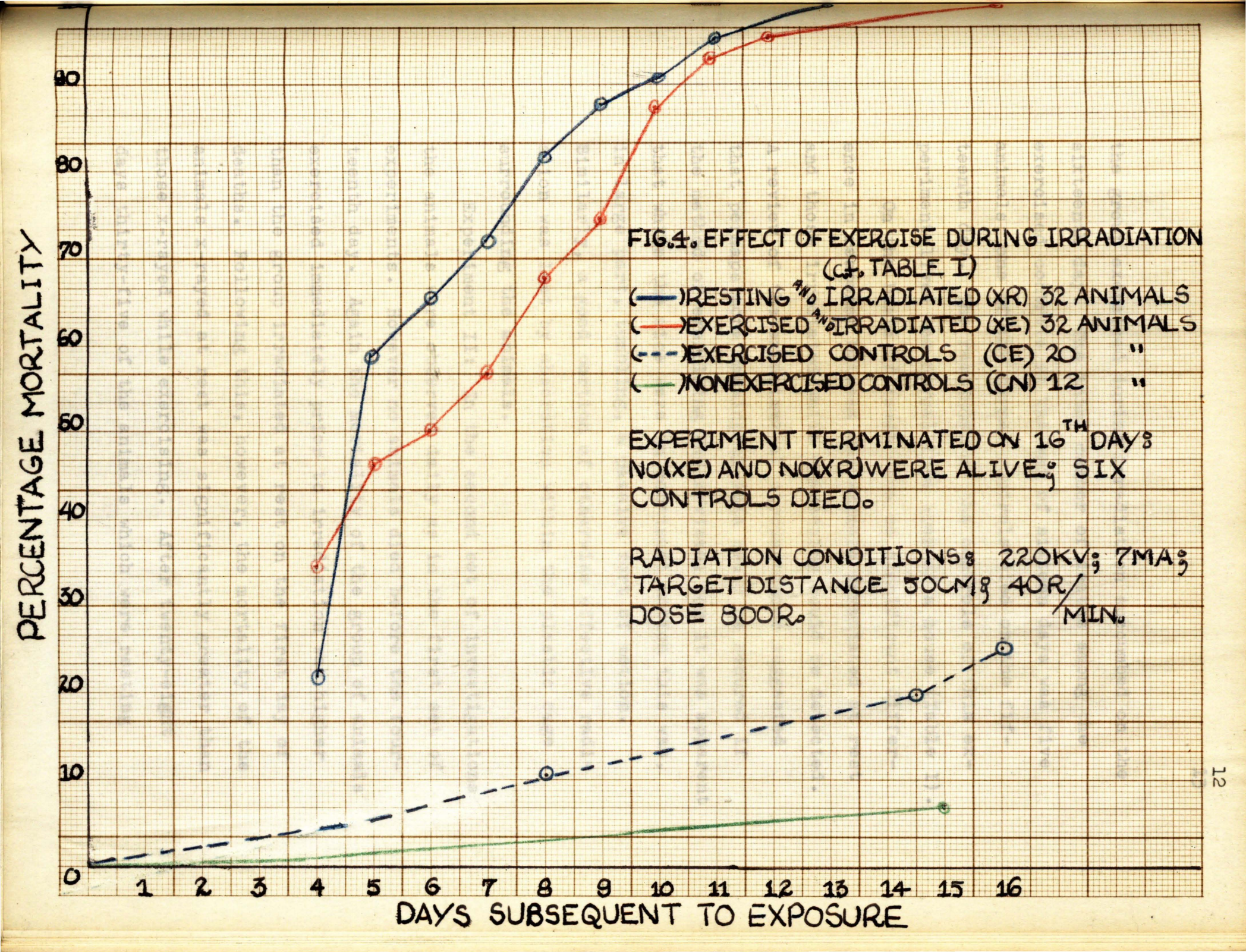
OBSERVATIONS

The response of experimental and control groups, in terms of mortality rate, in both experiments were regularly observed following each experiment. The data gathered are described below.

Experiment I: The data summarized in Table I reveals, upon inspection, that at the end of a period of sixteen days subsequent to irradiation all the experimental animals were dead. That is, those irradiated during exercise and those irradiated resting. Deaths in these groups began on the fourth day subsequent to irradiation and continued in almost daily occurence throughout the following twelve days for both the animals x-rayed while exercising and those x-rayed at rest; the difference in death rate was not significant. However, following the fourth day the numerical mortality among the animals x-rayed while exercising remained generally below that of the animals x-rayed while resting. On the eleventh day subsequent to irradiation they again coincided as to the number of dead animals. The last animal of the group irradiated while resting died on the thirteenth day, while the last animal of

TABLE I EFFECT OF EXERCISE DURING IRRADIATION

GROUP DESIGNATION	NUMBER OF ANIMALS	DEATHS OCCURRING ON SUCCESSIVE DAYS SUBSEQUENT TO EXPOSURE															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
EXERCISED AND IRRADIATED (XE)	32	0	0	0	7	12	2	2	3	2	1	2	0	1	0	0	0
RESTING AND IRRADIATED (XR)	32	0	0	0	11	4	1	2	4	2	4	2	1	0	0	0	1
EXERCISED ONLY (CE)	20	0	0	0	0	0	0	0	3	0	0	0	0	0	1	0	1
RESTING ONLY (CN)	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0


PERCENTAGE MORTALITY
0
10
20
30
40
50
60
70
80
90
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
DAYS SUBSEQUENT TO EXPOSURE
FIG.4. EFFECT OF EXERCISE DURING IRRADIATION
(cf. TABLE I)
(——) RESTING AND IRRADIATED (XR) 32 ANIMALS
(——) EXERCISED AND IRRADIATED (XE) 32 ANIMALS
(- - -) EXERCISED CONTROLS (CE) 20 "
(——) NONEXERCISED CONTROLS (CN) 12 "
EXPERIMENT TERMINATED ON 16TH DAY; NO (XE) AND NO (XR) WERE ALIVE; SIX CONTROLS DIED.
RADIATION CONDITIONS: 220KV; 7MA; TARGET DISTANCE 50CM; 40R/MIN. DOSE 800R.

the group exercised during irradiation succumbed on the sixteenth day. The total number of deaths among the exercised controls at the end of sixteen days was five animals; one of the normal controls died on the fifteenth day. The remainder of the controls of this experiment lived the normal life span of a mouse (Table I).

On the basis of these data no significant difference in response between the animals irradiated at rest and those irradiated while exercising could be detected. A review of the experimental circumstances suggested that perhaps more meaningful data could be secured if the method of exercising was revised; for it was apparent that while the mice were in continual motion this was, in large part, tumbling, a passive sort of motion. Similarly, a good portion of otherwise effective radiation was lost by absorption within the plastic cage surrounding the animals.

Experiment II: In the second set of investigations the animals were observed daily as in the first set of experiments. However no animals died before the fourteenth day. Again the mortality of the group of animals exercised immediately prior to irradiation was higher than the group irradiated at rest on the first day of deaths. Following this, however, the mortality of the animals x-rayed at rest was significantly greater than those x-rayed while exercising. After twenty-eight days thirty-five of the animals which were resting

TABLE II EFFECT OF EXERCISE IMMEDIATELY PRIOR IRRADIATION

GROUP DESIGNATION	NUMBER OF ANIMALS	DEATHS OCCURING ON SUCCESSIVE DAYS SUBSEQUENT TO EXPOSURE
		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 31 32 33
EXERCISED AND IRRADIATED (XE)	38	0 0 0 0 0 0 0 0 0 0 0 0 0 0 3 1 0 2 2 2 0 1 1 2 1 1 0 0 1 0 0 0 0
RESTING AND IRRADIATED (XR)	39	0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 4 5 6 6 2 4 0 1 2 1 0 2 0 1 0 0 0 0 0
EXERCISED ONLY (CE)*	10	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
AS ABOVE		34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66
(XE)		0 0 0 1 1 2 1 1 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0
(XR)		0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
(CE)		0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

* RESTING CONTROLS OMITTED DUE TO LACK OF ANIMALS

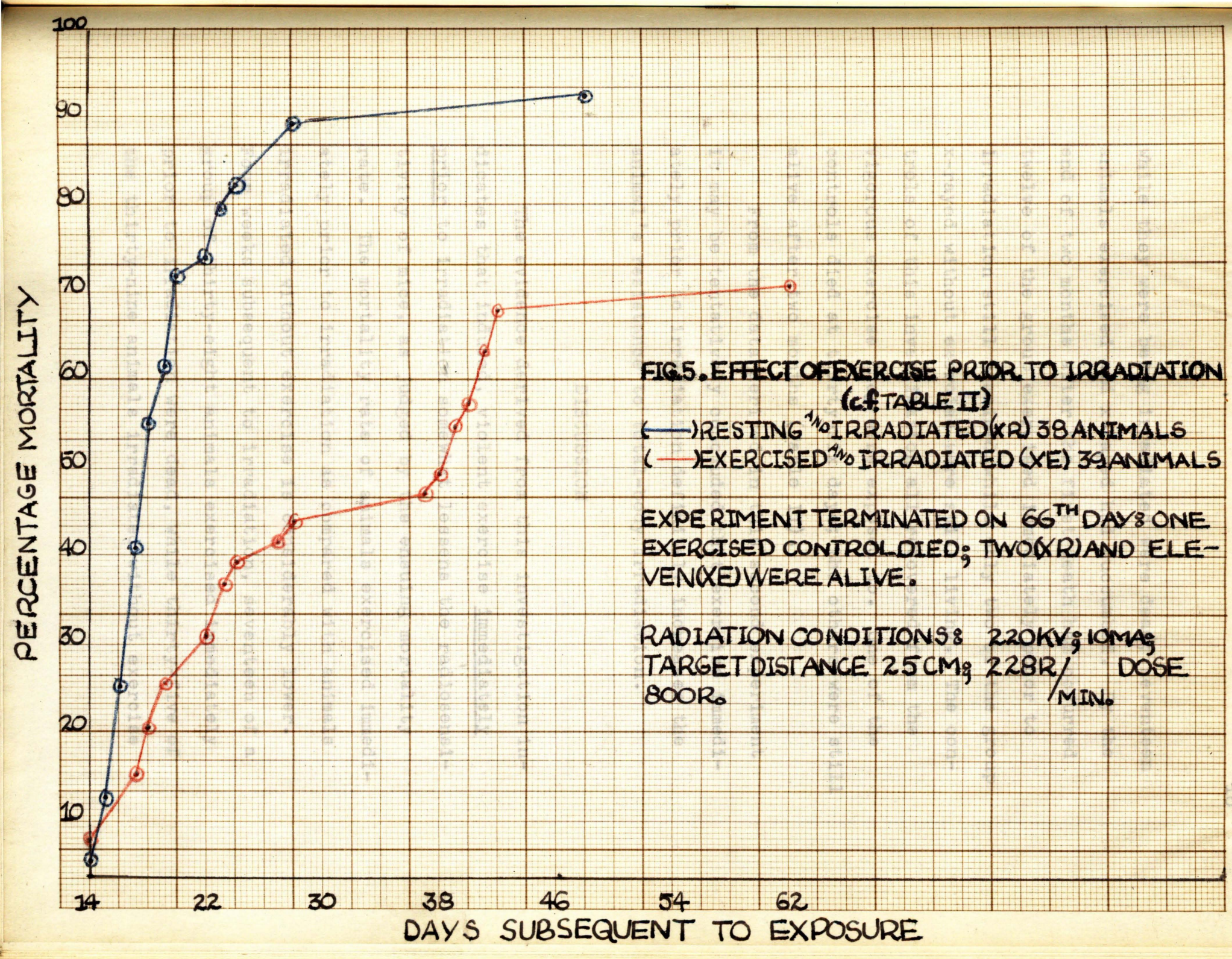


FIG.5. EFFECT OF EXERCISE PRIOR TO IRRADIATION (c.f. TABLE II)

(——) RESTING AND IRRADIATED (XR) 38 ANIMALS

(——) EXERCISED AND IRRADIATED (XE) 39 ANIMALS

EXPERIMENT TERMINATED ON 66TH DAY: ONE EXERCISED CONTROL DIED; TWO (XR) AND ELEVEN (XE) WERE ALIVE.

RADIATION CONDITIONS: 220 KV; 10 MA; TARGET DISTANCE 25 CM; 228 R/MIN. DOSE 800 R.

while they were being irradiated were dead; seventeen animals exercised and x-rayed had succumbed. By the end of two months after the first death had occurred twelve of the group exercised immediately prior to irradiation still remained while only two of the group x-rayed without exercise were still living. The controls of this investigation all recovered from the vigorous exercise they were exposed to. One of the controls died at thirty-six days; the others were still alive after two months (Table II).

From the data derived in this second experiment it may be tentatively concluded that exercise immediately prior to irradiation definitely increases the animal's resistance to total-body irradiation.

DISCUSSION

The evidence derived from this investigation indicates that induced, violent exercise immediately prior to irradiation somewhat lessens the radiosensitivity of mice, as judged by the ensuing mortality rate. The mortality rate of animals exercised immediately prior to irradiation as compared with animals irradiated without exercise is considerably lower. Four weeks subsequent to irradiation, seventeen of a group of thirty-eight animals exercised immediately prior to irradiation were dead, while thirty-five of the thirty-nine animals irradiated without exercise

had succumbed. This means that 44.7% of the mice exercised immediately prior to irradiation had died, while 89.7% of the non-exercised irradiated mice were dead. At the termination of the experiment (sixty-six days) eleven animals exposed to x-rays immediately following violent exercise were still alive; however, only two of the animals exposed to the x-rays without exercise still survived.

As indicated, the results in the first experiment, (involving the treadmill) were not very informative (Fig. 4). Any difference between the mice exercised immediately prior to and during irradiation and those irradiated without exercise was not apparent. This suggested a revision of the method. The disadvantages were that the induced exercise was not entirely active; it consisted largely of passive tumbling. Furthermore, each of the four mice irradiated at one time received varying doses of radiation, for there was little uniformity in their respective positions in reference to the center of the x-ray beam. Another factor to be considered in the irradiation of this group of animals is that the plastic walls of the treadmill absorbed a good amount of what otherwise would have been effective radiation. Thus the necessity of devising the swimming technique described for the second experiment. This proved much more satisfactory since it involved violent, active exercise.

From the second experiment the following tentative conclusion was drawn: There is a definite relationship existing between the physiologic condition occasioned by violent exercise and the deleterious effects of ionizing radiations. It is a well-known fact that violent exercise produces in the organism an oxygen debt, a condition of anoxia. The animals cannot accumulate a sufficient quantity of molecular oxygen, and the energy required for the exercise is derived mostly from anaerobic oxidations.

LaCassagne and LaTarjet (1) subjected newborn mice to a state of asphyxiation, by nitrogen or carbon dioxide, occasioning a severe anoxic condition, and then exposed these mice to x-radiation. They found that animals irradiated in the anoxic condition survived, and subsequently grew at the same rate as the non-irradiated controls; however, control mice irradiated under normal conditions of oxygenation died within twelve days.

It would appear that the results of the experiment reported here may be reasonably interpreted in terms of the marked lowering of the concentration of molecular oxygen in the mice subjected to enforced exercise immediately prior to irradiation. In view of the fact that the mice were exposed immediately subsequent to the cessation of exercise renders it more than likely that they had not repaid any appreciable amount of the

oxygen debt incurred. The total exposure period was three and one-half minutes.

It has long been known that radiosensitivity is directly related in many instances to the concentration of molecular oxygen. As previously stated there seems to have been very little, if any, experimental work directed, in this radiological problem, to altering the molecular oxygen concentration by means of exercise. The clear-cut results derived in this experiment, although admittedly preliminary in character, indicate strongly the value of this approach and the need for its further exploitation.

SUMMARY

A method originally employed for studying the effect on x-ray mortality in mice of induced exercise during exposure has been appraised as inadequate; the data derived with this method were judged unreliable in reference to the question posed.

An alternative method of inducing heightened metabolic activity immediately prior to irradiation is described. The results indicate that a condition of heightened metabolic activity, occasioned by exercise immediately prior to exposure decreases the radiosusceptability of mice when exposed to a lethal dose of x-rays; appropriate controls were employed.

Some theoretical implications of the data derived from this work are discussed.

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