6. Jahan, I., Pitts, R. F., *Am. J. Physiol.*, 1948, v155, 42.

7. Talbot, N. B., Sobel, E. H., McArthur, J. W., Crawford, J. D., *Functional Endocrinology,* Harvard Univ. Press, 1952.

8. Talmage, Roy V., Kraintz, F. W., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 263.

9. Talmage, Roy V., Kraintz, F. W., Buchanan, G. D., *ibid.*, 1955, v88, 600.

10. Allport, N. L., Keyser, J. W., *Colorimetric Analysis,* v1, Chapman and Hall, 1957.

Received March 17, 1959. P.S.E.B.M., 1959, v101.

Effects of Extremely High X-Ray Intensities and Dosages on Mice. (24921)

W. S. MOOS, H. C. MASON,* M. COUNELIS AND R. A. HARVEY
(Introduced by J. P. Marbarger)
Dept. of Radiology, College of Medicine, University of Illinois, and Ill. State Psychopathic Inst., Chicago

Considerable information has been collected on effects of low and medium high dosages of ionizing radiation upon living organisms. Recently, interest has developed in delivering large amounts of such radiant energy to animals, due in part to ability to produce sufficiently high energy flux, and to increasing recognition that interactions between living organisms and ionizing radiation can now be investigated which might not be observable at lower radiation dosages and dose rates(1-6). However, data obtained with extremely high dosages and/or high intensity x-rays is rather scarce(7). Extremely high x-ray dosages and intensities referred to in this paper connote dosages of 10^5 r or more, and intensities of 10^5 r/minute or greater.

Methods. The x-ray tube was placed inside a lead lined cabinet with lead glass window. Temperature in cabinet stayed reasonably constant over considerable time. A strong fan served to remove ozone and stabilize temperature. High intensity radiation was produced by Machlett AEG 50 Beryllium window tube at 45 KVp and 40 mA. No filtration was added. Distance from target to animal's skin was 3.6 cm with dose rate of 2.1 x 10^5 r/minute. X-ray output of tube was repeatedly checked with chemical dosimeter (8). For this purpose ferrous sulfate reagent (0.5 ml, 5 mN) was placed in small glass cups fitting snugly into lucite holder and irradiated for various time intervals† (distance from target to level of liquid = 3.6 cm). Depth of fluid was 2-3 mm. Following irradiation, 0.3 ml of ferrous sulfate reagent was removed and diluted with 3 ml of 0.8 N sulfuric acid. Optical density after irradiation was compared to that of non-irradiated samples of solution in Beckman spectrophotometer at wave length setting of 3020 Å. The dose of 2.1 x 10^5 r/min thus obtained is representative of energy absorbed in first few mm of skin over area of 2.25 cm^2. This procedure compares quite well with mesh ionization chamber measurements performed earlier at same x-ray energies and similar exposure dose rates. X-ray absorption measurements were carried out with same type of dosimeter by placing shallow containers of liquid inside a lucite holder and covering holder with pieces of mylar or lucite varying in thickness 12 μ to 2.45 cm. In this particular instance, distance from tube window to liquid level was 5.5 cm and remained constant throughout. The resulting absorption curve (Fig. 1) shows that approximately 85% of x-rays are absorbed in the first cm of tissue. Output constancy measurements with a CdS crystal dosimeter were $\pm$ 5%; however, after tube had been in operation for about 150 to 200 hours a gradual reduction in output was observed to as low as 1.3 to 1.1 x 10^5 r/min. Some of our repeat

* Present address: State of Washington Health Dept., Seattle.

† Amount of x-rays delivered to ferrous sulfate solution did not exceed 5 x 10^4 r and therefore should be within useful range of this dosimetric method. This was confirmed by 2 measurements performed with Ceric-Cerous dosimetry system.

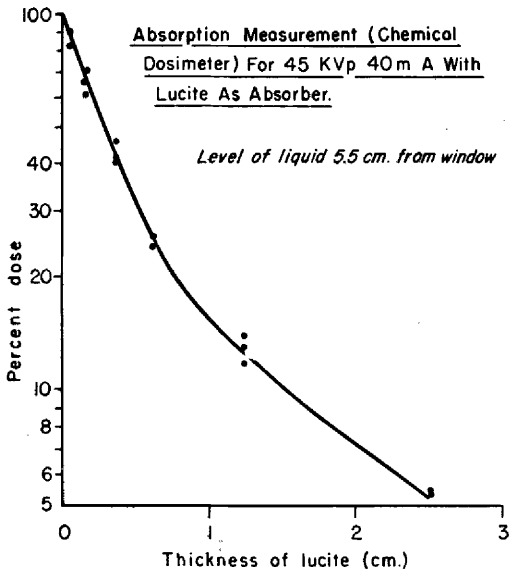


FIG. 1. Absorption of 45KV unfiltered x-rays. (Be-window only) in Lucite.

experiments were made during this period of decrease and are marked accordingly. Other serious dose variations may occur due to slight changes in location of animal from proper target distance (3.6 cm TSD for all mice) due to inverse square effects. Despite proper marking devices, this can easily happen due to changes in breathing, convulsions, and struggling motions of animals during exposure. Furthermore, dose distribution over area covered by x-ray beam at the particular target distance is not uniform (Table I). Thus determination of absorbed energy is difficult and only approximate values can be obtained. Temperature measurements were carried out with a Thermister Tele-Thermometer (Yellow Springs Instrument Co.). The hypodermic needle type thermistor probe was always inserted into the thigh of hind leg of mouse, which was protected by quarter inch lead shield. This shield extended beyond the tip of the thermistor probe but did not touch the skin. This location of the probe was used in either abdominal or head irradiation. In some later experiments rectal temperature readings were also recorded. A total of 130 mature CFW mice have been studied, averaging about 25 g with extremes from 19-32 g. Food and water were not restricted until just before each experiment. Animals were secured, back down, onto small boards by adhesive tape over individual limbs. Mice were exposed to radiation of either the head or lower parts of body subsequently called abdominal irradiation (Fig. 1). The head of a 25 g mouse approximates about 1 to 1½ cm as measured from the mandibular bone to up-

TABLE I. Dose Distribution.

Distance from axis of beam, cm	% dose parallel to tube axis	% dose right angle to tube axis
2.5		.3
2.0	.4	2.3
1.5	.6	18.
1.0	30.	36.
.5	80.	80.
.0	100.	100.
.5	80.	80.
1.0	50.	40.
1.5	27.	25.
2.0	16.	2.7
2.5	1.2	.3
3.0	.3	

Field size 4 cm diameter.
CdS detector target distance = 3.8 cm.

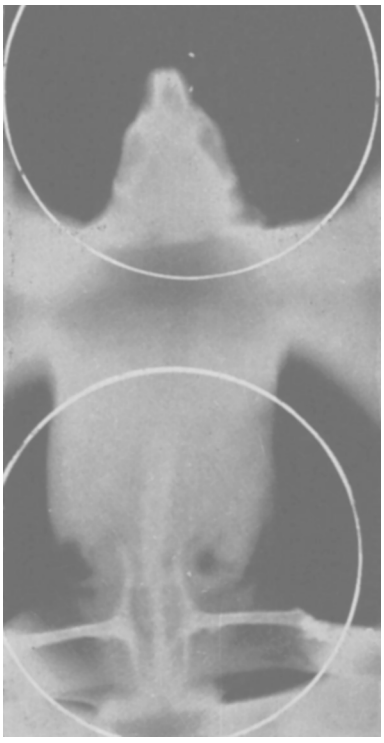


FIG. 2. Radiograph taken with 25 kv x-rays shows areas of mouse body exposed to x-rays. In this figure head and abdominal treatment fields are shown on one animal.

per parietal bone of skull. Body depth through the abdomen to dorsal side is somewhat greater. Mice that were difficult to handle were injected with small amounts of Nembutal. Additional radiation experiments revealed no variation in survival time of these from that of non-anesthetized animals. To obtain reliable temperature measurements, all animals were placed under the tube for 3 to 10 minutes prior to x-ray treatment. Normally, little change in temperature was observed. The temperature of target cooling water was regulated to vary within 1 degree (C°) of irradiation cabinet temperature, before and during operation of tube. This cooling water also kept entire headpiece of x-ray tube at constant temperature by circulating the cooling water through water jacket surrounding tube head, with exception of the window. The following studies were carried out during and after exposure of mice: A. Determination of survival time following: 1. Head exposure only at normal body temperature. 2. Abdominal exposure at normal body temperature. 3 and 4. Repetition of 1 and 2, but at artificially lowered body temperature. B. Recording changes of body temperature.

Results. Mean survival time of mice after a surface‡ dose of 2 x 10^6 r (2.1 x 10^5 r/min) to head or abdomen was determined. Mean survival time of head irradiated animals was 11.8 ± 1.4 minutes at normal body temperature. A survival time of 14.9 ± 2 minutes followed irradiation of abdomen. This somewhat prolonged survival in contrast to head irradiation was rather persistent throughout. Above values demonstrate mean survival times without regard to body volume bathed by x-ray beam. If we examine survival times in respect to total absorbed energy, assuming that the abdominal area exposed to radiation is about 3.6 times as large(9) as that of head, much greater sensitivity of brain to irradiation under these circumstances becomes clearly evident (Table II). Equal amounts of absorbed energy by either of the 2 body portions results in much shorter survival time for head irradiation than for abdominal treatment. This greater response of brain has also been observed with a dose of 10^5 r by Rajewsky(7), who recorded a survival time of approximately 9 hours following irradiation of mouse rump in contrast to 90 minutes after head irradiation.

‡ Surface dose in this case means dose integrated over first 2-3 mm depth of ferrous sulfate solution.

TABLE II. Mean Survival Times of Mice. 2 × 10^6 r (2.1 × 10^5 r/min.)

Irradiated body parts	Temp. of body	Wt of body part(9), g	Mean survival time (min.)
Head	Normal	4- 6	11.8 ± 1.4*
Abdomen	"	15-16	14.9 ± 2.0
Head	Lowered	4- 6	104.0 ± 17.2
Abdomen	"	15-16	116.0 ± 17.0

* Stand. error of mean.

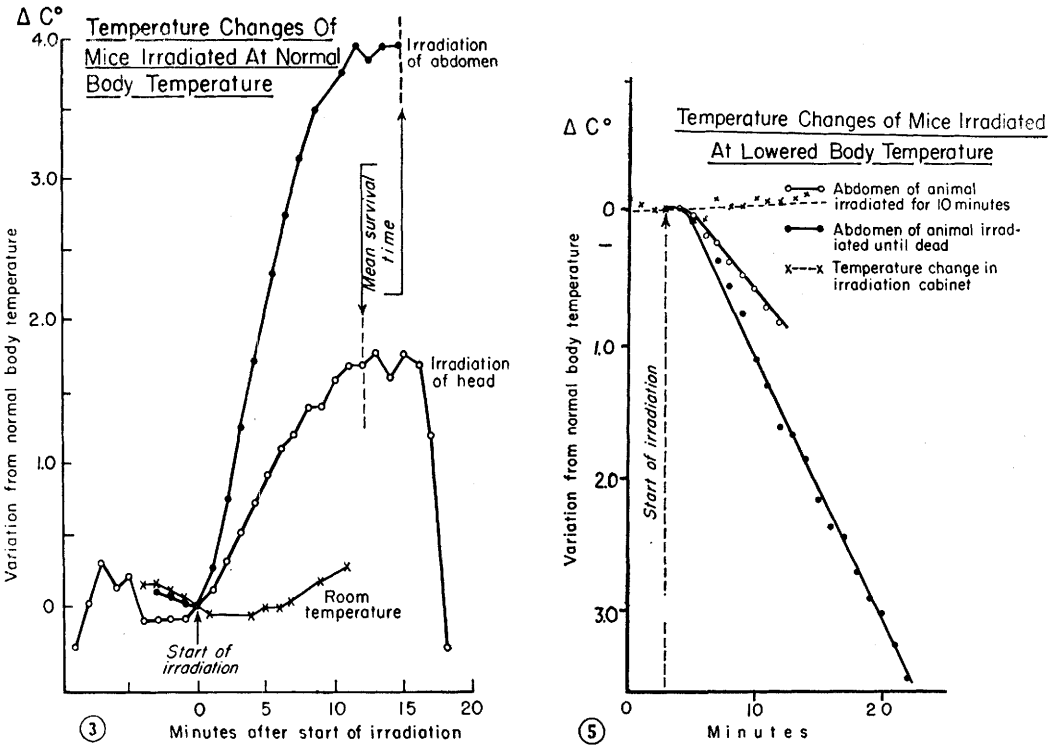
We were also concerned with the toxic amounts of ozone produced by highly intense x-ray beam. To eliminate any possibility of accelerated death from this factor, survival times of animals exposed to the beam without any ventilation in the x-ray tube cabinet, were compared with mice irradiated under identical conditions, but with constant use of forced air stream between tube window and head (in addition to cabinet ventilation). The data so obtained exhibit no discrepancy in survival time between the 2 groups; this is in accord with findings of Brace and Andrews(5). However, one can be reasonably certain that the level of ozone produced by such highly intense ionizing radiation would have a profound effect on animals if they lived longer(10). In our experiments, radiation damage was so severe as to overshadow any other effects.

Body temperature exhibits considerable change during either type of (head or abdomen) irradiation; therefore, in additional experiments mouse body temperature was reduced 4-8°C during exposure. This was achieved by placing blocks of ice around the animal, whose entire back soon was bathed in ice water. This caused no interference with x-ray beam since the mice were tied down with backs resting on restricting board. Survival time obtained in this manner was much prolonged, 7-8 times for head as well as for abdominal irradiation (Table II). Whether this phenomenon is in direct relationship with keeping body temperature at levels more com-

patible for animal organism, or whether some indirect kind of protection for body chemistry is afforded by cooling, remains to be answered. Mice irradiated at lowered body temperature provided opportunity to study over a longer period of time the effects of radiation, whereas the others had usually expired by time of removal from the animal holder. Mice (cooled) abdominally irradiated showed no extraordinary symptoms that had not been observed in animals exposed to smaller dosages of radiation. The animals were hunched, huddling together, as if seeking warmth. Periods of lethargy intermingled with short intervals of great excitability, the latter becoming less and less frequent. Water and food were consistently ignored. Respiration became increasingly difficult toward the end, but was continued often over long periods of time (1-3 hours) though the animal was on its side or back, and apparently unconscious. In some instances, the hind extremities appeared to be paralyzed, but this could not be confirmed. Pain perception in the tail was maintained. Head irradiated (cooled animals) demonstrated identical symptoms to those discussed above. However, paralysis of one or other of the extremities occurred more often. Periods of excitement were more frequent and longer, and the animals demonstrated complete loss of orientation. Circular, screw or eel type convulsive motions at surprising speeds were noted in most instances(11). The mice maintained only very labile balance when able to stand at rest, or remained as placed when not in a stage of excitement. Many of these sequelae seem to be the same or similar to those observed by Gerstner *et al.*(12) at lower x-ray dosages (12,500 r). Cooling protracts the above mentioned disturbances and symptoms in head irradiated animals. This became evident in 2 instances when such mice were freed from the animal holder before death occurred and similar screw type motions were observed.

Changes in body temperature. One can observe a reduction of body temperature as a function of dose and time after exposure to medium and high dosages of x-rays, Langham *et al.*(2). The interest in these particular experiments was to determine variations of temperature during irradiation. The average increase of body temperature after 10 minutes of head irradiation (2×10^6 r) was 1.7°C whereas it amounted to 3.9°C for the same irradiation time of the abdominal area (Fig. 3). This rise in temperature could perhaps in part be attributed to the absorbed energy of the high intensity beam and heat from the tube window. To compare these temperature changes with those from other heat producing sources, the following experiments were carried out. Temperature increase of the x-ray tube window during operation was determined with a copper-constantan thermocouple. Then a metal disc of equal diameter as the tube window was heated to about the same temperature (measured with the same thermocouple) as the window. This was achieved by a 15 watt electric bulb housed in a plastic container to which the disc was attached. The abdomen of the mouse was then placed first under the lamp heated disc (1.6 cm from disc) and temperature changes recorded. After temperature returned to normal the same mouse was moved under the x-ray tube and irradiated about the same amount of time at 1.6 cm from the window (3.6 cm from target) and again temperature measurements were made.

At the time these particular experiments were performed, we had to take into consideration that the tube output was reduced to about 1.2×10^5 r/min. The results obtained indicate a greater body temperature increase with x-irradiation than with the simulated treatment using the lamp: however, temperature increase is smaller than observed previously as one would expect with reduction of x-ray tube output (Fig. 4). These observations could be interpreted to mean that part of the temperature rise in the animal is due to heat from the window, whereas the remaining part must be due to the absorbed radiant energy (ionizing) and/or the reaction of some biological process in the animal. This latter assumption seems to be supported not only by the fact that body temperature drops considerably despite continuation of treatment after the animal has expired, but also by phantom measurements. Such a phantom consisted of a mouse shaped thin plastic bag filled with 10 or 20 ml of water. This bag was irradiated



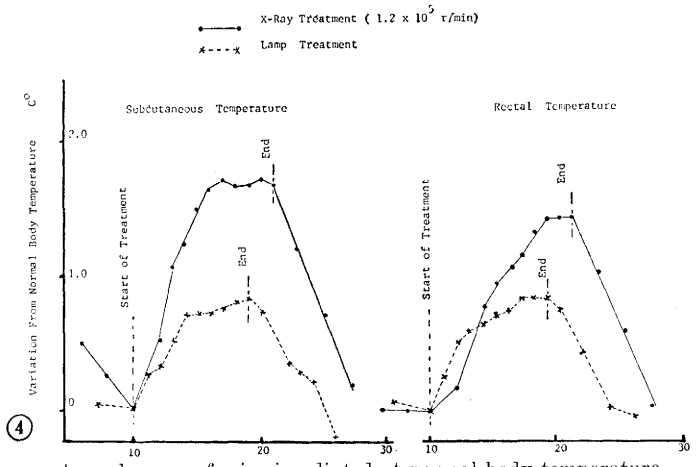


FIG. 3. Temperature changes of mice irradiated at normal body temperature.

FIG. 4. Variation of body temperature of mice exposed to x-ray treatment or heat treatment. (Abscissa: time in min.)

FIG. 5. Two examples of temperature changes of mice irradiated at subnormal body temperature.

with x-rays or the lamp in the same manner as the mice discussed earlier. The temperature changes recorded under these conditions were about equal for both sources. However, one of the problems has not been solved, namely determination of the true x-ray window temperature. Though both thermocouple measurements (x-ray window and metal disc on lamp) were adjusted to show equal temperature readings, it was impossible with this arrangement to find out how much of the observed temperature increase is caused by the

heat of the x-ray window alone and to what extent absorbed x-ray energy in the thermocouple itself contributes to the temperature change. It could be very well possible that the actual window temperature is lower than the thermocouple indicates.

Fig. 5 shows changes of body temperature during x-ray exposure of animals cooled by ice water. In all instances temperature was reduced below normal, and life was prolonged regardless of treatment area. At the very least a doubling of dose was required to kill the animal within 25 minutes.

Additional information has been sought by investigating changes in breathing pattern, electrocardiogram tracings, as well as blood changes and histological picture, since the above findings present a variety of possible reasons for super-acute radiation death of the animals. Results obtained in these studies are being analyzed.

Summary. The effects of extremely high x-ray dose rates and dosages on mice irradiated at normal and subnormal body temperatures were studied. Considerable lengthening of survival time is obtained when body temperature is lowered during irradiation. A slight elevation of body temperature during irradiation was noticed.

We wish to thank Drs. F. Heinmets and A. Kroeger for assistance in chemical dosimetry, and Drs. L. Skaggs and R. K. Clark for evaluation of manuscript. Helpful discussions with Dr. J. Ovadia were greatly appreciated.

1. Brooks, P. M., *Radiation Research,* 1956, v4, 206.
2. Langham, W., Woodward, K. T., Rothermel, S. M., Harris, P. S., Lushbaugh, C. C., Storer, J. B., *Radiation Research,* 1956, v5, 404.
3. Rothermel, S. M., Woodward, K. T., Storer, J. B., *Radiation Research,* 1956, v5, 433.
4. Kent, S. P., Swanson, A. A., *Radiation Research,* 1957, v6, 111.
5. Brace, K. C., Andrews, H. L., *Peaceful Uses of Atomic Energy,* 1955, XI, 115.
6. Spalding, J. F., Wellnitz, J. M., Schweitzer, W. H., *Radiation Research,* 1957 v7, 65.
7. Rajewsky, B., Aurand, K., Heuse, O., *Zeitschrift fur Naturforschung,* 1953, v8, 524.
8. McDonnell, W. R., Hart, E. J., *J. Am. Chem. Soc.,* 1954, v76, 2121.
9. Mason, H. C. Mason, B. T., Moos, W. S., *Brit. J. Radiology,* 1955, v28, 495.
10. Claman, H. G., *Toxicity of Ozone, Science,* 1956, v124, 213.
11. Hornberger, W., *Strahlentherapie,* 1951, v85, 459.
12. Gerstner, H. B., Pickering, J. E., Dugi, A. J., *Radiation Research,* 1955, v2, 219.

Received March 19, 1959. P.S.E.B.M., 1959, v101.

Carbohydrates and Gastrointestinal Absorption of Radiostrontium and Radiocalcium in the Rat.* (24922)

R. H. WASSERMAN AND C. L. COMAR
Lab. of Radiation Biology, N. Y. State Veterinary College, Cornell University, Ithaca, N. Y.

Ability of lactose to increase gastrointestinal absorption and retention of calcium has been known for many years and has been adequately confirmed under various conditions and in different species. The effects of lactose on calcium metabolism and other physiological functions have been reviewed recently by Duncan(1) and Atkinson *et al.*(2). Although the action of lactose has been widely studied, little attention, with one exception, has been given to the influence of other sugars and sugar derivatives on calcium. Fournier and his colleagues(3-5) determined the net retention of calcium in rats as influenced by ingestion of sugars by use of conventional calcium balance technic. Certain sugars, in addition to lactose, were capable of enhancing calcium retention in the growing and lactating rat; *e.g.*, xylose, mannose, raffinose, mannitol and sorbitol. It was not feasible with calcium balance method to determine extent

* Supported by Armed Forces Special Weapons Project. The authors gratefully acknowledge technical assistance of L. A. Cape and S. N. Duberman.