15. Spellberg, M. A., Keeton, R. W., and Ginsberg, R., *Arch. Path.*, 1942, v33, 204.

16. Blumberg, H., MacKenzie, C. G., and Seligson, D., *Fed. Proc.*, 1942, v1, 187.

17. Schenck, J. R., Simmonds, S., Cohn, M., Stevens, C., and du Vigneaud, V., *J. Biol. Chem.*, 1943, v149, 355.

18. Simmonds, S., and du Vigneaud, V., *ibid.*, 1942, v146, 685.

19. Kensler, C. J., Rudden, M., Shapiro, E., and Langemann, H., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 39.

Received January 26, 1954. P.S.E.B.M., 1954, v85.

Mouse-Strain Differences in Response to Radiation. (20883)

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In the course of an experiment(1) on the protective effect of hormones on the mortality of irradiated mice, there was observed a considerable difference in the radiation doses delivered to the heads of mice of 2 different strains necessary to elicit the same degree of mortality. The doses differed by more than a factor of 2 in the dba and Marsh-albino mice.

The difference in acute radiation mortality has now been examined in 2 additional mouse strains, making a total of 4. This is a report on the results to date, which were obtained with each strain represented by several hundred mice of either sex in the age range from 8 to 15 weeks. The entire experiment is being repeated with larger numbers of mice as well as with additional mouse strains.

Procedure. In order to establish the acute mortality curves, mice of the dba, Marsh, C57 black, and C3H strains were irradiated as follows: (a) whole body exposed, (b) only the head irradiated, and (c) the head shielded and the remainder of the body irradiated. All mice were irradiated at a distance of 30 cm to the midsection with an x-ray beam having a H.V.L. of 0.9-mm copper and a dose rate of 150 r per minute in air. The x-ray generator was monitored continuously for constancy of output. Doses reported herein are air doses. For whole-body radiation 10 mice were placed in a 6"-diameter Petri dish and covered with a perforated aluminum disc ½-mm thick. Since the space was limited, it was not necessary to immobilize the animals by anesthesia. Measurements of dosage when the dish was filled with purple-heart wood to simulate the setup when filled with mice showed that the radiation dose intensity decreased from 100% at the center to 91.8% at the outer periphery of the dish. Thus, the doses to the mice varied approximately 4% above and below the average dose in the dish. For irradiation of the head only the mice were held in position by a half cylinder of 1/16" lead which functioned both as an immobilizing device and as a radiation shield. Only the head was exposed. The mice and shield were fastened to a wooden board in 2 rows of 3 mice each, nose to nose, so that the heads of 6 mice could be irradiated simultaneously. Over the bodies of the mice covered with 1/16" lead 2 additional layers of 1/8" lead rubber were laid. Transmission of the x-rays through one layer of 1/8" lead rubber is 0.06%. The boundary between the irradiated head and shielded body was defined by the edge of the lead-rubber shield, which ran across the cervical vertebrae. Measurements of radiation dose in this setup were made with the mice simulated by purple-heart wood mock-ups. At the center of the mouse body under the shield the dose due to scattered x-rays was 4% of the dose delivered to the exposed head. For body irradiation without head the above procedure was modified by using a semicylinder of ½-mm-thick aluminum instead of lead to restrain the mice. The heads were shielded by 1/8" lead. The edge of the lead defined the boundary between head and body and ran across the cervical vertebrae. Dosage measurements with the purple-heart wood mock-ups showed that the

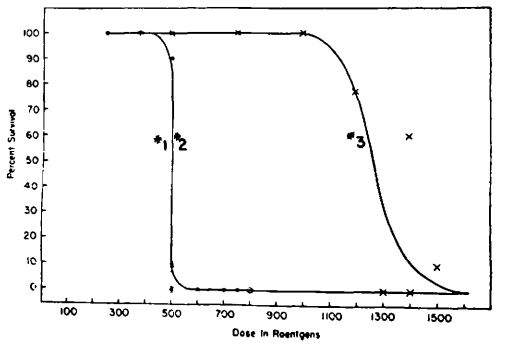

FIG. 1. Survival curves for the DBA strain. ● #1 whole body exposure; ○ #2 head exposure; × #3 body exposure, head protected.

dose in the center of the shielded head due to scattered x-rays was 13% of the dose delivered to the exposed body.

Results. The survival rates plotted against radiation doses for 4 strains of mice under the 3 conditions stated above are shown in Fig. 1, 2, 3, and 4. These curves show there is a marked strain difference in the response of these mice to the 3 different conditions of radiation. When the whole body is irradiated, the resulting survival curves for the 4 strains are practically identical, having a median lethal dose (M.L.D.) at about 528 r. The survival was 50% at 12 days. Thus, 528 r is the L.D.-50% at 12 days.

From the curves for the dba strain (Fig. 1) it is seen that the survival curve with head radiation is practically identical with that for whole-body radiation whereas, when the head was protected, over double the dosage was required to produce the same effect. This would seem to indicate that in this dba strain a radiosensitive center is located in the head region.

On the other hand, the curves for the Marsh strain (Fig. 2) show that the survival curve for the head radiation lies in a higher dosage range, at about double the dosage for whole-body exposure, whereas the curve for whole-body-with-head-shielded radiation has moved to a lower dosage range than the corresponding curve for the dba strain.

The survival curves for the C57 black mice (Fig. 3) present still another picture in that there is a more marked separation resulting from the 3 methods of radiation. In this strain the survival curve following head radiation falls at a higher dosage level, and the curve for the whole body with head shielded falls at a lower level than the corresponding curves for the Marsh and dba strains. These

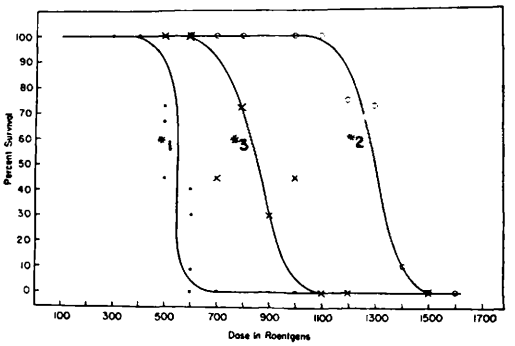

FIG. 3. Survival curves for the C57 Black strain. ● #1 whole body exposure; ○ #2 head exposure; × #3 body exposure, head protected.

findings could be explained if the head region were less sensitive to radiation than the remainder of the body. The curves for the C3H strain show this difference to a more marked degree in that the head-radiation curve is at a still higher dosage level, whereas the curve plotted for the mice radiated whole body with head shielded is at a lower level, suggesting that the head region in this strain is appreciably less sensitive to radiation than that in any of the other 3 strains.

With the families of curves arranged in the order shown, it should be noted that, as the head-radiation curves move progressively toward the higher dosage levels, the curves for the whole-body-except-head radiation move progressively toward the lower dosage levels.

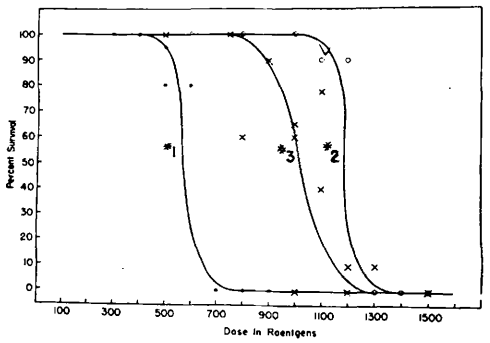

FIG. 2. Survival curves for the Marsh strain. ● #1 whole body exposure; ○ #2 head exposure; × #3 body exposure, head protected.

TABLE I. M.L.D. Values for the Three Conditions of Irradiation.

Mouse strain	Whole body, r	Head, r	Body except head, r
Dba	500	500	1265
Marsh	570	1185	1018
C57 black	550	1300	858
C3H	492	1443	735
Avg	528		

This is shown more clearly in Table I where the M.L.D. is given for comparison. From Table I it may be inferred that, as the head region becomes less sensitive to radiation, the remainder of the body becomes more radiosensitive.

The mortality curves shown in Fig. 1, 2, 3, and 4 refer to an acute mortality. Thus, the survival times for 50% of the mice given whole-body irradiation were in the interval of 10 to 12 days. When the head alone was irradiated, 50% survived for periods of from 12 to 20 days; and in the case of body-with-head-protected irradiation the survival was 50% for times ranging from 15 to 25 days.

The animals were examined daily for body changes. The most obvious symptoms of injury were an arched back, bristling fur, reduction of activity, and loss of weight. No evidence of infection was found. In those animals that survived for a sufficiently long time, hair and eye changes were seen. The C57 black and C3H mice which survived 1400 r to the head showed epilation of head hair from the 19th to the 21st days. About 2 weeks later the epilation was replaced by a growth of gray hair. This gray hair showed the sharply defined boundary between head and body across the cervical vertebra. At the lower dose of 1100 r partial epilation was seen. The hair did not fall off spontaneously, but could be rubbed off easily.

Eye changes were readily discernible with doses of 1400 and 1500 r delivered to the head. Inflammation of the epithelia surrounding the orbit preceded the appearance of visible cataracts at 3 weeks after radiation.

Discussion. The progressive changes in the values of M.L.D. with decreasing sensitivity of the head region of the mouse are greater than the data shown in Table I would indicate. The dosage measurements on mock-up mice indicated that in the whole-body-without-head exposures there was a scattered dose given to the shielded head, which was 13% of the body dose. Therefore, the M.L.D. values under the column for whole-body-except-head irradiation are probably lower than they would be if it were possible to irradiate the mouse body without scattering dosage into the head region.

The probable contribution of the scattered dose into the head is illustrated in the dba mice where 13% of the 1265 r (M.L.D. for whole body without head) is 164 r which, in turn, is 33% of the M.L.D. of 500 r for head only. In the C3H mice 13% of 735 r (M.L.D. for whole body without head) is 96 r. This, however, is only about 7% of the M.L.D. of 1443 r for head only. Thus, the scatter of radiation has the effect of decreasing the observable strain differences in mortality. In the C3H mice the scattered dose is probably negligible, whereas in the dba mice it assumes a significant role in the acute mortality observed.

The wide range of sensitivity of the head region shown by the M.L.D. values of Table I are not easily accounted for by the assumption of a single radiosensitive center in the head, namely the pituitary. The ratio of M.L.D. for irradiation of head only to that for whole-body-without-head irradiation is 0.40 for dba, 1.1 for Marsh, 1.5 for C57 black, and 1.96 for C3H mice. Measured by this ratio, there is more than a 4-fold change of sensitivity of the head compared with the remainder of the body. This estimate does not take

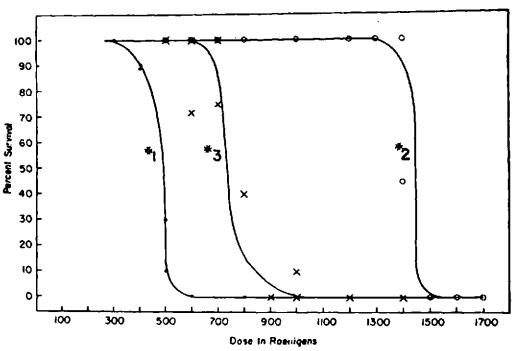

FIG. 4. Survival curves for C3H strain.
● #1 whole body exposure; ○ #2 head exposure;
× #3 body exposure, head protected.

into account the scattered dose to the head which was discussed above.

Two instances of different degrees of radiosensitivity of the head have been reported in other animals. Allen(2) reported that a lead shield over the heads of mongrel dogs, permitting not more than a 50-r dose to the head, enabled 90% of the dogs to survive a 450-r whole-body dose. This dose, given without head shield, caused 100% mortality. On the other hand, Lamerton *et al.*(3) found that doses of 600 and 1500 r to the head of the Wistar-albino rat produced little effect on the growth curve of young rats. They concluded that in these rats pituitary damage was not an important factor in subsequent rat growth. They report, however, in a comparison between rats given 450-r whole-body-with-head exposure and others given 500-r without-head exposure that the head-shielded rats showed the protective effect against radiation.

The dba mice have the same high degree of radiosensitivity in the head region as that reported by Allen for mongrel dogs. The other mouse strains show less sensitivity. The C3H mice appear to have the low head sensitivity of the Wistar rats, as reported by Lamerton.

That the sensitive center in the head might be the pituitary was indicated by the protective action of the adrenosteroid hormones against radiation mortality, as reported by Mirand *et al.*(1). Both cortisone and desoxycorticosterone acetate were found to reduce radiation mortality by their effects on the pituitary-adrenal axis. The assumption was made that the extent of pituitary damage as a result of ionizing radiation determines the degree of adrenal insufficiency which might be the large factor contributing to the death of the animals. The fact that both DCA and cortisone increased the survival time in the dba mice suggests that a state of adrenal insufficiency was avoided either by rendering the pituitary less sensitive to ionizing radiation or by preventing an early marked manifestation in adrenocortical insufficiency after radiation.

Summary. 1. The acute radiation mortality of mice exposed to x-rays shows that there are radiosensitive regions in the mouse head. 2. The L.D.-50% at 12 days for acute mortality due to whole-body exposure to x-rays is essentially the same for 4 mouse strains and is about 528 r. 3. The sensitivity of the head region varies with mouse strain. In the most sensitive strain, the dba, the M.L.D. for head exposure is the same as for whole-body exposure. Up to more than a 4-fold decrease in head sensitivity in the strains is found in the following order: dba, Marsh, C57 black, and C3H mice.

1. Mirand, E. A., Reinhard, M. C., and Goltz, H. L., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 397.

2. Allen, J. Garrott, in Army Med. Service Res. Project Rep., Ann. Rep., June, 1952, Office of the Surgeon General, U. S. Army, pp. 549-552.

3. Lamerton, L. F., Elson, L. A., and Harriss, E. B., *Br. J. Radiol.*, 1953, v26, 568.

Received December 22, 1953. P.S.E.B.M., 1954, v85.