Effect of Total-Body X Irradiation on Metabolism of the Rat. (19214)

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Increased metabolism following X irradiation has been claimed on the basis of indirect evidence from the dog(1,2) and of basal oxygen consumption data from the rat(3). The present report indicates that total-body exposure of the rat to X rays does not affect metabolism except under special conditions.

Experimental. Male, Sprague-Dawley rats were subjected to single, total-body X irradiation (200 kv, 15 ma, 0.5-mm Cu and 3.0-mm Bakelite filters, 72.5 cm target distance, 0.9-mm Cu half-value layer and 15 to 20 r per minute dose rate). Six to eight rats were irradiated simultaneously, equal numbers of each experimental group of a study being included in an exposure. The animals were individually caged beginning one week prior to initiating experimental procedures. Drinking water was available at all times. Body weight was measured between 8:30 A.M. and 10:00 A.M. daily in the following groups of 17 rats each in 2 experiments: (1) complete deprivation of food, (2) 800 r and complete deprivation of food, (3) free access to food, and (4) 800 r and free access to food. Measurements were also made of the water content of the gastrocnemius muscles and of the whole carcasses of starved and starved irradiated (800 r) rats. Water content was determined by drying to constant weight in an oven at 102°C. In 3 experiments oxygen consumption of irradiated and control rats was measured in a multiple Regnault-Reiset apparatus (4) allowing the simultaneous, individual study of 6 animals for a 3 hour period. Prior to irradiation the oxygen consumption of the rats was measured daily for about a week. In each study there were 6 control and 6 irradiated rats. Three rats from each group were studied in the morning and 3 in the afternoon, an individual rat being measured during the same session each day. During all pre-irradiation periods, the rats were allowed access to food only during the first hour following their stay in the metabolism ap-

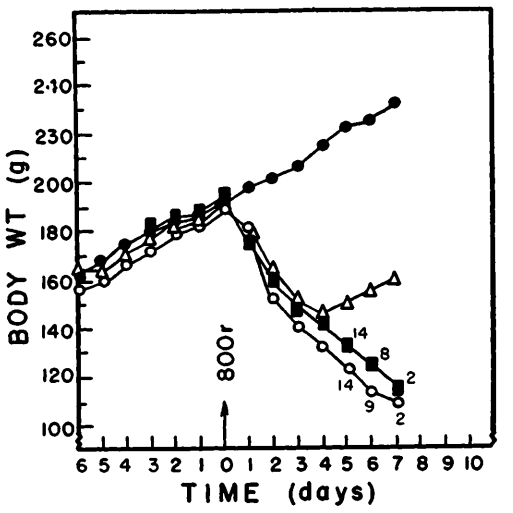

FIG. 1. Influence of total-body X irradiation upon body wt of starving rat. ● Fed, △ fed + 800 r, ■ starved, ○ starved + 800 r. Each point represents the mean of a group of 17 animals unless otherwise designated.

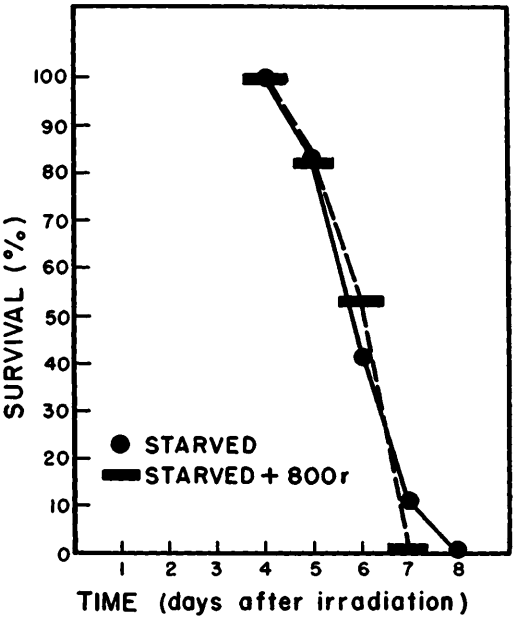

FIG. 2. Influence of total-body X irradiation upon survival time of starving rat. Each point represents the mean of a group of animals. Each group initially contained 17 rats.

TABLE I. Influence of Total-Body X Irradiation Upon Water Content of the Rat.

Treatment group	Time after irradiation (days)	No. animals	Carcass water content					Gastrocnemius water content				
			Mean wet wt	Stand. error	Mean dry wt	Stand. error	Mean % water	Mean wet wt	Stand. error	Mean dry wt	Stand. error	Mean % water
Starved	1	7	191.9	±5	56.8	±1.9	70.4	1.478	±.090	.380	±.028	74.4
" + 800 r		6	205.9	±3.5	60.7	±1	70.5	1.560	±.092	.394	±.025	74.8
"	2	8	184.6	±4.8	53.8	±1.8	70.6	1.235	±.035	.304	±.010	75.5
" + 800 r		9	187.9	±5.2	55.4	±1.4	70.3	1.342	±.054	.329	±.010	75.4
"	5	15	147.3	±3.6	42.3	±1	70.8	.977	±.040	.736	±.009	75.4
" + 800 r		10	133.3	±4	41.9	±1.6	68.3	.821	±.034	.701	±.008	75.5

paratus. Subsequently the experiments were as follows: (1) Exp. No. 1, 800 r and complete deprivation of food; (2) Exp. No. 2, 1000 r and complete deprivation of food, and (3) Exp. No 3, 800 r with access to food according to pre-irradiation pattern. Following irradiation, measurements of oxygen consumption were taken daily until death.

Results and discussion. The weight loss of starved rats was similar to that of irradiated, starved rats. Exposure to X radiation did not accelerate the rate of weight loss or hasten the death of rats that were completely deprived of food (Fig. 1 and 2). Neither did X irradiation influence the water content of the gastrocnemius muscle or of the entire carcass of the starved rat (Table I) indicating that water retention was not responsible for the failure to find an increased rate of weight loss in the starved-irradiated rat. France(5), found slight but statistically significant increases in the muscle water of the fed rat and in the total-body water of the fed mouse after 700 r. These increases might result from small differences in the nutritional status of his irradiated and control animals. Fig. 3 and an analysis of variance(6) show that: (1) irradiation did not increase the oxygen consumption of either the fed or the starved rats to values above the pre-irradiation control levels; (2) irradiated, starved animals had an overall trend toward a higher oxygen consumption than their non-irradiated starved controls; (3) rats with free access to food following exposure showed levels of oxygen consumption similar to those of the non-irradiated, fed controls, and (4) in agreement with previous studies(7,8) starvation was followed by a decrease in oxygen consumption.

The findings that the rate of weight loss of starving, irradiated rats and of starving controls is similar and that the basal oxygen consumption of fed, irradiated rats and of fed controls is similar strongly suggest that total-body X irradiation does not affect metabolism. Like oxygen consumption data from the fed, irradiated mouse(9), the observations(10,11) that tissue slices taken from irradiated animals show either no change or a decrease in oxygen consumption, and the report(9) that

FIG. 3. Influence of total-body X-irradiation upon basal oxygen consumption of the rat. Each point represents the mean of a group of 6 rats unless otherwise designated.

irradiated mice are less sensitive to progressive hypoxia lead to the same conclusion.

In the previously reported experiments which claimed an increased metabolism it was found that (1) dogs exposed to single, total-body X irradiation in the lethal dose range lost weight at greater rates than did pair-fed controls, and (2) following single, total-body X irradiation over the dosage range of 54 r to 972 r rats showed post-absorptive oxygen consumptions significantly in excess of their pre-irradiation control levels. The differences between these data, and our own seem more apparent than real. Thus the small number of dogs (3 control and 3 experimental) could readily have given misleading results, since there was considerable variability in the rates at which individual dogs lost weight when either partially or totally starved. The differences in oxygen consumption data can be explained by the possibility that in the study of Kirschner *et al.*(3), the post-irradiation measurements might have increased during the same period of time in the absence of irradiation. In our experiments the chances for such errors were obviated by the simultaneous study of non-irradiated, but otherwise similarly treated rats. Moreover, in the present experiments as well as in the recent report on the mouse(9) significant day to day variations in the controls were observed.

The overall trend toward a small but significant difference in oxygen consumption between irradiated, starved rats and non-irradiated, starved controls indicates that the metabolism of irradiated, starved animals is higher than that of controls. It is interesting to note that the greatest differences occurred during the time when widespread tissue regeneration becomes apparent in the fed rat (12). Should similar regeneration occur in the starved animal, it is conceivable that an increased oxygen consumption could be related to the processes involved in regeneration. The absence of significant differences in oxygen consumption during the first 2 or 3 days following irradiation in the fed groups and the small differences in the starved groups strongly suggest that cellular destruction is accompanied by little or no increase in metabolism. Failure to find indications of increased metabolism from the data on rates of weight loss in similarly treated rats may be explained on the basis that the differences in metabolism were too small to be reflected in body weight. Moreover, the data of Kirschner *et al.*(3) show a decided lack of parallelism between oxygen consumption and body weight changes.

Summary and conclusions. The resting oxygen consumptions of irradiated and control, fed animals are similar. The rate of weight loss of starving, irradiated rats is similar to that of starving controls. The water content of the whole carcass and of the gastrocnemius muscle of the starving rat is unchanged by irradiation. Oxygen consumption falls during starvation. The oxygen consumption of the starving, irradiated animal is higher than that of its simultaneously starved control. Apparently the metabolism of rats having free access to food is not affected by irradiation and the metabolism of the irradi-

ated, starved animals is higher than that of their non-irradiated controls.

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Nutrition of *Leptospira canicola*. II. A Chemically Defined Basal Medium Containing Purified Rabbit Serum Albumin.* (19215)

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It was shown by Greene *et al.*(1) that *Leptospira canicola* could be grown in a chemically-defined medium of amino acids, minerals, purines, pyrimidines and vitamins supplemented with dialyzed rabbit serum. Attempts to fractionate the dialysed serum by ammonium sulfate precipitation resulted in about 80% loss of the activity and the remaining activity was not concentrated in any fraction. In the present investigation an active fraction obtained by a modified procedure has been found to replace dialysed rabbit serum.

Experimental. The chemically-defined basal medium was prepared by introducing 0.5 ml each of solution A (36 mg % of each of the 19 amino acids employed previously)(1), solution B (vitamins, purines and pyrimidines in the concentrations given previously)(1) and solution S (autoclaved and filtered aqueous solution of the following salts at mg per cent concentrations given in the parentheses: Na_2HPO_4 (312), KH_2PO_4 (420), NaCl (1280), Na_2CO_3 (32), KCl (32), $CaCl_2$ (32)) into each 15 x 127 mm test tube. The tubes were autoclaved for 30 minutes at 15 lb pressure, 0.5 ml of solution G (36 mg % filter-sterilized aqueous solution of L-glutamine) and a selected volume of test material were added to each tube, each tube was inoculated with a culture of the test organism and each solution was diluted to 6 ml. The pH of the medium was 7.2-7.4. The transfer medium was prepared by adding 0.8 ml of filter-sterilized, dialysed rabbit serum‡ to each tube containing an autoclaved mixture of 0.5 ml of solution S, 3.96 mg of Witte's peptone and 4.3 ml of water. *Leptospira canicola*§ was transferred weekly by inoculating this medium with 0.4 ml of a 7-day culture of the spirochete and incubating the mixture for 7 days at 32°. The inoculum was prepared by centrifuging (2000 RPM for ½ hr) the 7-day growth, suspending the cells in a quantity of solution S sufficient to produce a turbidity of 30-40 de-

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