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Effects of Total Body X-Irradiation on Some Constituents of Liver and Kidney of the Rat. (20225)

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To characterize certain metabolic alterations in the liver and kidney due to x-irradiation, phosphate distribution, glycogen, sulfhydryl, sodium, potassium, and water content as well as cytochrome oxidase activity of these organs were studied in rats subjected to lethal total body x-irradiation.

Inasmuch as the content of some of these constituents is known to be influenced by the secretion of the adrenals, some of these studies were done on adrenalectomized animals. Since decreased food intake is part of the post-irradiation syndrome, the effect of fasting alone on the above tissue constituents was also studied.

Materials and methods. Male Sprague-Dawley rats, weighing 235-280 g and fed Purina chow diet, were used throughout. The adrenalectomized rats were maintained on 0.95% NaCl drinking solution and used about 10 days after operation. Radiation dosage was 1000 r in the experiments on phosphate distribution in the liver and kidney, and on cytochrome oxidase activity in the liver; 880 r in the other experiments. Radiation factors were: 200 kv, 6 ma, ½ mm Cu 1 mm Al filter, target distance approximately 29 cm and 40 r/min dosage rate measured in air.* The majority of deaths occurred within 6 to 14 days after irradiation with 880 r and 4 to 6 days after irradiation with 1000r. A. *Phosphate Distribution.* Phosphate determinations on the tissue were made according to the method of Umbreit(1) except that the animals were killed by decapitation and the tissues were quickly removed, frozen in a dry ice-acetone bath, and lyophilized. Trichloroacetic acid extracts of the tissues were prepared and the barium-insoluble fraction was analyzed for inorganic phosphorus and easily hydrolyzable phosphorus(15 min., 100°C, 0.2 N HCl). All results are reported as mg P/100 g dry tissue. The irradiated animals, which had access to food and water, were sacrificed 1 to 6 days after irradiation. Food, but not water, was withheld overnight prior to sacrifice. B. *Liver Glycogen and Blood Glucose.* Food was withheld for periods indicated in Table II, while water or 0.95% NaCl solution (adrenalectomized animals) was allowed *ad libitum.* The animals were sacrificed by decapitation, and samples weighing approximately 1 gram were removed from the median lobe of the liver and immediately immersed in cold (3° to 5°C) saline. The samples were then cut into 6 nearly equal parts under cold saline, blotted on filter

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TABLE I. Phosphorus Distribution of Liver and Kidney of X-Irradiated Rats. Dosage 1000 r. Average values of dry tissue ± S.D.

	Liver			Kidney		
	No. of rats	Inorganic P, mg/100 g	Labile P, mg/100 g	No. of rats	Inorganic P, mg/100 g	Labile P, mg/100 g
A. Control	21	23.7 ± 4.3	79.8 ±10.3	25	32.5 ± 6.7	95.3 ± 9.6
B. Days after irradiation						
1	6	16.0 ± 3.8	60.0 ± 4.7	6	34.7 ± 7.3	106.6 ± 6.4
2	7	14.5 ± 1.8	55.2 ± 9.0	8	32.4 ± 8.4	99.8 ± 8.6
3	5	19.6 ± 4.4	54.2 ± 9.8	6	35.4 ± 5.9	97.2 ± 8.5
4	10	20.7 ± 6.3	64.2 ±17.2	10	36.7 ± 7.5	95.2 ± 6.8
5	8	24.0 ±10.5	80.2 ±12.1	8	29.3 ± 9.0	101.4 ± 8.4
6	5	19.6 ±11.2	84.4 ±17.3	6	24.3 ± 9.7	99.2 ± 5.6
C. No. of days without food						
3	3	21.5 ± 2.4	80.0 ± 7.2	3	28.7 ± 3.5	103.1 ± 4.1
4	3	16.9 ± 2.4	74.4 ± 4.6	3	28.7 ± 2.1	97.9 ± 4.5
5	3	19.7 ± 0.6	66.6 ± 5.8	3	34.2 ± 3.8	97.0 ± 3.7

TABLE II. Effect of Total Body X-Irradiation (880 r) on Liver Glycogen in Rats.

		No. of animals	Treatment	Liver glycogen, %
Normal				
Control	Food & water *ad lib.*	5	0	5.33 ± .15*
	Fasted 24 hr	23	0	.10 ± .05
Irradiated	" 24 " after	12	0	2.08 ± .74
	" 24 " before & after	7	0	2.12 ± .50
Adrenalectomized				
Control	Fasted 24 hr	2	0	.09 ± .01
Irradiated	" 24 " after	4	0	.39 ± .17
Control	" 48 "	2	0	.01 ± .00
Irradiated	" 48 " after	4	0	.39 ± .44
Control	" 24 "	4	ACE†	.51 ± .22
Irradiated	" 24 " after	3	ACE†	4.44 ±3.37

* Mean ± S.D.

† Upjohn Adrenal Cortex Extract inj. in 5 doses of 1 ml each for 24 hr, last injection 1 hr prior to irradiation or beginning of fast.

paper, and placed in 30% KOH. Glycogen was extracted according to the method of Good, Kramer, and Somogyi(2), as modified by Sjogren, *et al.*(3), and determined by the anthrone method of Morris(4). Blood samples were collected in oxalated beakers from the point of decapitation and analyzed for glucose according to the method of Somogyi(5). C. *Sulfhydryl Content.* The sulfhydryl content of the liver and kidney was determined by a modification of the amperometric method of Benesch and Benesch (6). A rotating platinum electrode was employed as described by Herbert and Denson (7). Since certain difficulties were encountered in the titration with the use of sulfosalicylic acid (5 ml of a 22% solution for 0.5 g tissue sample), the protein precipitant was changed to 0.1 ml of a 10% solution of phosphomolybdic acid. No difference was found in the results employing either protein precipitant. For 16 hours before and throughout the experiment, the animals were fasted but were allowed water or 0.95% NaCl (adrenalectomized animals); they were sacrificed by decapitation. D. *Na, K and H_2O Content.* Tissue water content was determined by drying the samples to constant weight at 100°C. The Na and K contents were determined according to the procedure of Robertson and Peyser(8) using a Beckman flame photometer. The data presented are not corrected for the blood content of the tissue. For 16 hours before and throughout the experi-

ment, the animals were fasted but were allowed water or 0.95% NaCl (adrenalectomized animals); they were sacrificed by the administration of ether. E. *Cytochrome Oxidase.* Cytochrome oxidase activity was determined according to the method of Schneider and Potter(9). The irradiated animals, which had access to food and water, were sacrificed by decapitation 1 to 6 days after irradiation. Food but not water was witheld overnight prior to sacrifice.

Results and discussion. A. *Phosphate distribution.* The procedure employed here for the determination of labile phosphate (ATP) seemed to give higher values for the labile organo-phosphates than those previously reported. As early as 1 day after irradiation both inorganic and labile phosphate (ATP) in the liver were subnormal (Table I). The liver inorganic phosphate remained at this low level through the third day after irradiation and the ATP-phosphorus continued to decline slowly. On the fourth day, both inorganic and ATP-phosphorus in the liver rose, the inorganic phosphorus approaching the control level where it remained until death. The ATP-phosphorus, however, reached the control range on about the fifth day after irradiation and apparently continued to rise slowly. Fasting of 3 days duration did not produce any pronounced changes in the inorganic and labile phosphate of the liver (Table I). However, 4 and 5 days fasting caused a decrease of inorganic and labile phosphorus. It seems, therefore, that the radiation was responsible for the decrease in inorganic and labile phosphorus observed in the liver during the first 3 days after irradiation and for the increase in labile phosphate during the terminal stage. The fall in the ATP content following irradiation may be linked with the presence of increased glycogen. The increase of ATP content of the liver during the terminal stage may indicate a reduced glycogenetic capacity of the liver of the irradiated animals. In the kidney, the changes which occurred in the inorganic and labile phosphate content were not significant (Table I). Fasting of 3 to 5 days duration also did not produce any significant changes in the inorganic and labile phosphorus content of the kidney (Table I).

B. *Liver Glycogen and Blood Glucose.* The blood sugar in the irradiated animals fasted 24 hours after irradiation was found to be definitely elevated, 91 mg %, compared with 70.5 mg % for the control fasted animals. A similar observation has been made by Kohn (10).

As can be seen from Table II, the amount of hepatic glycogen was higher in the irradiated than in the control non-irradiated animals. After 24 hours of fasting, liver glycogen values were 2% as compared with 0.1% in the non-irradiated control group. These observations are in agreement with similar findings of other investigators(11-13). In adrenalectomized animals qualitatively similar results were obtained (Table II). To test the ability of the x-irradiated rats to form liver glycogen, animals were fasted 24 hours prior to irradiation and the fasting continued for an additional 24 hours. In striking contrast to the non-irradiated control fasting animals, the x-irradiated animals had a liver glycogen content up to 2% (Table II). It is likely that this production of glycogen is the result of an increased gluconeogenesis stimulated by the adrenals. It has been found by Patt and his associates(14) that total body x-irradiation (900 r) of rats resulted in a considerably increased functional activity of the adrenal cortex. However, the increased cortical activity alone cannot account for the observed liver glycogen accumulation since adrenalectomized animals also show some liver glycogen accumulation under these conditions (Table II). In this connection reference may be made to the observation that normal fasting rats, subjected to low pressures, will show an increase in the glycogen content of the liver(15). Apparently, total body x-irradiation impairs the mechanism for the physiological mobilization of liver glycogen. Further studies will be necessary to elucidate the cause of the impairment of glycogenolysis in the irradiated animals.

C. *Sulfhydryl Content.* The normal control value for liver, average 175 mg GSH/100 g tissue (Table III), agrees well with that reported by Peterson, Beatty and West(16) and by Ingbar, Otto, and Kass(17). Apparently, reduced glutathione is the principal acid-

TABLE III. Effect of Total Body X-Irradiation (880 r) on Sulfhydryl Content of the Liver in the Rat.

Control animals			X-irradiated animals starved 16 hr		
Sacrificed after hr starvation	No. of animals	Non-protein SH, mg GSH/100 g	Sacrificed hr after irradiation	No. of animals	Non-protein SH, mg GSH/100 g
16—SSA*	8	177 ± 14†	2	6	185 ± 18
16—PMoA‡	4	171 ± 22	4	4	208 ± 9
40	7	177 ± 17	12	9	219 ± 21
88	7	215 ± 12	24	10	239 ± 33
			72	8	263 ± 23
40—A§	4	137 ± 9	24—A	5	87 ± 13

* SSA—Sulfosalicylic acid (used as protein precipitant). † Mean ± S.D. Test for significance: In normal rats 1 day post-radiation "p" is less than 0.1, in adrenalectomized rats 1 day post-radiation "p" is less than 0.2. ‡ PMoA—Phosphomolybdic acid (used as protein precipitant). § A—Adrenalectomy.

soluble non-protein SH compound in the liver and kidney extract, since the values obtained by the amperometric method, employed in these studies, agree well with those reported by Woodward who employed a manometric method specific for reduced glutathione(18). Table III shows an increase in the sulfhydryl content of the liver following x-irradiation. Peterson, Beatty and West(16) found no change in the glutathione level of the liver in rats before the sixth day following 500 r total body x-irradiation, and a lowering between 6 to 19 days post-irradiation. The discrepancy in the results is probably due to the different x-ray dosage employed. An explaantion for the rise in the hepatic non-protein SH, observed after irradiation, can only be speculative at this time. An increased breakdown of glutathione-containing proteins, due to increased functional activity of the adrenal cortex observed after total body x-irradiation (14), may occur under these conditions. In addition, denaturation of cell proteins caused by the radiation, may increase the rate of proteolysis. In this connection reference may be made to the findings of Barron and his associates(19) that sulfhydryl-containing enzymes are readily oxidized by radiation and that it is possible to reactivate such enzymes after moderate radiation doses (100-200 r) on addition of a reducing agent. However, larger doses of x-irradiation were found to produce irreversible inactivation, which was attributed to protein denaturation. In contrast to normal animals, a reduction in the sulfhydryl content of the liver was observed in adrenalectomized animals after total body x-irradiation (Table III). The reasons for this difference in response are, at present, obscure. The absence of the adrenals may inhibit the rate of protein breakdown sufficiently to account for the observed effects. In contrast to the changes in GSH values found in the liver, no significant change was found in the sulfhydryl content of kidney after radiation; the control value (7 animals fasted overnight) was 144 ± 26 mg GSH/100 g tissue as compared with 158 ± 23 mg GSH/100 g tissue found 24 hours after irradiation (8 animals).

D. *Na, K and H_2O Content.*† The finding that there was no significant effect of radiation upon the sodium, potassium or the water content of the liver and kidney up to 48 hours after irradiation with perhaps the exception of a slight over-all increase in sodium in the kidney, agrees with similar observations of Bowers and Scott(20,21).

E. *Cytochrome Oxidase.*† Only negligible changes were found in the activity of cytochrome oxidase in the liver after lethal total body x-irradiation. This finding agrees with similar observations of other investigators who have studied the effect of total body x-irradiation on various other enzymes(22-24).

It is probable that several mechanisms are responsible for the changes in certain constituents of the liver and kidney observed after lethal total body x-irradiation. The nature

† Presentation of the experimental data is omitted since they only confirm the findings of other investigators. For details, see: Army Medical Research Laboratory Report No. 104, 20 Nov. 1952.

of these mechanisms is, at present, still rather obscure, and further studies are necessary for its elucidation.

Summary. 1. Lethal total body x-irradiation (1000 r and 880 r) of rats produced the following changes in certain constituents of the liver or kidney. 2. As early as 1 day after irradiation, both inorganic and labile phosphate (ATP) in the liver were definitely subnormal. The liver inorganic phosphorus remained at this low level through the third day after irradiation while the labile phosphate continued to fall slowly. On the fourth day, both inorganic and labile phosphate in the liver rose; the inorganic phosphorus approached the control level where it remained until death. The labile phosphate, however, reached the control range on about the fifth day after irradiation and apparently continued to rise slowly until death. In the kidney, the changes which occurred in phosphate distribution were not pronounced. 3. The fasting of rats immediately following total body x-irradiation resulted in what appeared to be a retarded rate of glycogenolysis. After 24 hours fasting, following radiation, liver glycogen values were 2% as compared with 0.1% in the fasted non-irradiated group. In adrenalectomized animals the liver glycogen content was also found to be higher after irradiation and fasting than the liver glycogen content found after fasting only. 4. A definite increase in the sulfhydryl content was found in the liver of fasted x-irradiated animals as compared with non-irradiated fasted animals. Removal of the adrenals produced a decrease in the sulfhydryl content of the liver of fasted animals. It was further reduced by irradiation. No significant changes were observed in the sulfhydryl content of the kidney of normal rats after irradiation. 5. There was no significant effect of x-irradiation upon the sodium, potassium or the water content of the liver and kidney up to 48 hours after irradiation with perhaps the exception of a slight increase in the sodium in the kidney. 6. Liver cytochrome oxidase activity showed only slight variations from the time of radiation exposure to subsequent death. 7. The possible influence of fasting on these liver and kidney constituents has been considered.

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