heart and liver extracts is similar in type and rapidity. The activity of these tissues appears to be due to some substance present in the tissues and not due to a pH effect. The presence of this substance in heart and liver tissues which splits digitoxin, and thus changes the solubility characteristics, may in some manner explain the action of digitoxin and its mode of excretion.

Summary. A study of the breakdown of digitoxin to form digitoxigenin by hydrochloric acid and extracts of dried heart, liver, kidney, and yeast has been carried out. The results of this study showed the hydrochloric acid and extracts of heart and liver have the ability to cause this breakdown, while extracts of kidney and yeast are inactive in this reaction. Reaction rate studies gave the following rate constants at 29.7°C: 0.85% hydrochloric acid 6.85×10^{-2}, heart extract (10 g dried, defatted powder per 100 ml phosphate buffer) 5.15×10^{-2}, liver extract 5.80×10^{-2}. Purification of the heart extract yielded a reddish brown solid which had a high enzymatic activity and gave typical protein reactions.

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Effect of X-Radiation upon Succinoxidase of Rat Kidney. (18772)

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Hammett(1), irradiating a solution of glutathione with radium, found a decrease in sulfhydryl groups and an increase in disulfide groups and suggested that radium irradiation in "non-tissue destroying amounts" had its main effect on cell growth by decreasing the concentration of sulfhydryl. Barron and coworkers(2-4) reported both *in vitro* and *in vivo* experiments showing inhibition of –SH enzymes following small amounts of X-irradiation. In the latter experiments, inhibition was relatively marked in kidney slices from X-irradiated animals with succinate as a substrate, presumably as a result of inhibition of the –SH enzyme, succinic dehydrogenase.

Since succinic dehydrogenase is a widely distributed enzyme whose activity as affected by irradiation could be easily measured, further study of the effect of irradiation on the succinoxidase system seemed indicated.

Methods. Litter-mate Sprague-Dawley rats weighing 150 to 200 g were divided into control and irradiated groups. The irradiated animals received 400 r to 800 r total-body X-radiation (200 kv, 15 ma, and 1.5 mm Cu and 3 mm Bakelite filters). Irradiation was given at approximately 17 r per min, and the animals were killed by a blow on the head within 2 hr following irradiation. Five additional animals were given 800 r (250 kv) at the faster rate of 180 r per min and killed within 7 min after irradiation. The kidneys were removed immediately and 5% homogenates were made in a glass homogenizer with cold distilled water. Kidney homogenates were assayed for succinoxidase, succinic dehydrogenase, and cytochrome oxidase activity by the method of Schneider and Potter(5) as applied by Case and Dickens(6). Kidney

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TABLE I. QO_2 of Kidney Homogenates from Control and Irradiated Animals.

	Succinic oxidase		Succinic dehydrogenase		Cytochrome oxidase	
	No. of animals	Avg $QO_2 \pm$ S.D.	No. of animals	Avg $QO_2 \pm$ S.D.	No. of animals	Avg $QO_2 \pm$ S.D.
Control	19	178.2 ± 18.9	12	68.8 ± 5.4	10	643.3 ± 22.4
After 400 r X-ray	10	191.5 ± 20.9	10	71.3 ± 8.4	7	708 ± 73.6
After 800 r X-ray	5	192.1 ± 12.9	10	69.3 ± 7.6	5	680 ± 7.4

TABLE II. QO_2 of Kidney Slices from Control and Irradiated Animals.

Substrate	None		Succinate		Pyruvate	
	No. of animals	Avg $QO_2 \pm$ S.D.	No. of animals	Avg $QO_2 \pm$ S.D.	No. of animals	Avg $QO_2 \pm$ S.D.
Control	9	21.9 ± 4.3	12	44.7 ± 8.6	10	34.7 ± 3.6
After 400 r X-ray	9	23.8 ± 4.5	11	47.8 ± 7.6	10	38 ± 5.9
After 800 r X-ray	4	22.6 ± 1.7	4	47.8 ± 4.4	3	38.1 ± 4.2

cortex slices were cut freehand. They varied considerably, but all were less than 0.18 mm in thickness. Individual slices were placed in flasks containing Krebs-Ringer phosphate buffer, and oxygen uptake was measured without added substrate and with the addition of 0.3 cc 0.1 M sodium succinate or 0.3 cc 0.1 M lithium pyruvate. Center wells contained 0.2 cc 6 N NaOH. The gas phase was oxygen. After the experiment the slices were removed and dried overnight in an oven at 100°C and weighed. The temperature for all experiments was 38°C and flasks were shaken at 120 oscillations per min. Lithium pyruvate was prepared by the method of Wendel(7).

Results. The determinations of all 3 components of the succinoxidase system in both normal and irradiated animals agree with the figures of Schneider and Potter for rat kidney homogenates. These results are given in Table I. No inhibition of the succinoxidase system occurred even with exposure to 800 r. Five additional animals were exposed to 800 r at the faster rate of 180 r per min and killed within 7 min thereafter. Assay of succinic dehydrogenase, the –SH moiety of succinoxidase, in these animals also showed the same values as the control animals.

The respiratory inhibition in irradiated animals reported by Barron(4) was determined in slices, and not in cell-free homogenates. Similar studies were therefore made on the respiration of kidney slices as shown in Table II. No statistically significant difference could be found between the activities of tissue slices taken from irradiated and from control animals, whether without substrate, or with pyruvate or succinate.

Discussion. The experiments given here were carried out in a manner similar to that employed by Barron *et al.* and as nearly as possible under the conditions which gave these workers their maximum effects. The degree of inhibition of tissue respiration reported by these workers varied considerably, and was not regularly related to the amount of irradiation given or to the time after irradiation at which the tissues were examined. Their animals, the same strain as used in the present study, received radiation ranging between 100 r and 800 r. The tissue slices were examined at times varying, generally, between 4 and 120 hr following irradiation. With the range of doses and within the time intervals just stated, the reported inhibition of succinate oxidation by kidney slices was 36.5% to 79%. The inhibition of the respiration of slices from other organs, using succinate and other substrates, was less marked and less consistent, and this determined the choice of kidney for the present studies. Preliminary experiments with animals killed at 4 and at 24 hr following irradiation also showed no difference in the respiration of the kidney homogenates; therefore, the tissues were examined as soon as possible following irradiation to minimize any possible metabolic reversal of the expected inhibition, and only the higher doses of radiation were given.

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It would appear likely that any additional minor modifications of the conditions used herein would also not cause inhibition of these enzyme reactions. Du Bois and co-workers at the University of Chicago(8) observed no inhibition of succinic dehydrogenase in kidney slices from mice exposed to 2000 r γ rays. Neither did they find, in tissues similarly exposed, any inhibition of the –SH enzyme, adenosinetriphosphatase, which was readily inhibited by irradiation *in vitro*(2). In possible contrast, Dinning and his associates(9) have reported reduction of the endogenous respiration and of the choline and succinate oxidizing activity of bone marrow in 5 mature hens exposed to 300 r of X-rays. Approximately similar decreases were found in all the birds, regardless of the interval, from 1 to 17 days, after irradiation. No claim was made, however, that these reductions represented direct inhibitions of the enzymes by the irradiation. In view of the very rapid changes which occur in bone marrow cells after even moderate irradiation(10), the metabolic changes observed can most probably be ascribed to the altered cellular composition of the tissue.

In contrast to these *in vivo* results, moderate amounts of irradiation have been shown to inhibit –SH enzymes and other enzymes, *in vitro*(2,3,11). But since the addition of proteins and other substances to solutions of enzymes minimizes the inhibition produced by irradiation(11,12), an enzyme can be expected to be more sensitive to irradiation when in a dilute and pure solution than when within a living cell. Forssberg(13), for example, found a 25% inhibition of catalase when it was irradiated with 800 r as a saline solution, but no inhibition of the catalase activity in the rat tissues examined immediately after administration of the same dose to the whole animal.

The emphasis in these studies is on the effects of only moderate doses of irradiation, because of their striking physiological consequences and clinical usefulness, and because of the interest in their possible mode of action. In the rat, hematological changes are caused by much less than 100 r total-body irradiation, and 800 r causes death in 80% or more of the animals. In the present experiments, sulfhydryl enzyme inhibition, not found with doses of 800 r, cannot therefore explain the physiological effects of sub-lethal amounts of irradiation. These results also seem to question any selective *in vivo* effect of moderate doses of irradiation on –SH enzymes. It is probable that following moderate amounts of irradiation the pattern of enzyme reactions in a given tissue may sometimes be changed, but secondarily and as part of the general response, like changes in bone marrow. There appears to be no evidence that the still smaller but still biologically effective doses act *in vivo* directly on any enzyme systems yet known.

Summary. 1. The succinoxidase system, as well as its component enzymes, the –SH enzyme succinic dehydrogenase, and cytochrome oxidase, were determined in rat kidney homogenates within two hours after rats were X-irradiated with 400 r or 800 r. No inhibition of any of the enzymes was found. 2. The metabolism of kidney slices from rats given the above amounts of X-radiation was studied manometrically with sodium succinate and lithium pyruvate substrates. No statistically significant differences were obtained between the oxidative activities of the kidney slices from the control and irradiated animals. 3. The lack of correlation between certain of the *in vivo* and *in vitro* effects of irradiation upon enzyme systems is discussed.

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