dase activity of the liver and its molybdenum concentration.

*Summary.* Vit. E-deficient rabbits with muscular dystrophy had elevated liver xanthine oxidase activity. This increase was observed in the presence or absence of added dietary molybdenum, and with 15 or 24% casein in the diet. Low intestinal xanthine oxidase levels were not increased during vit. E deficiency. Xanthine oxidase was not detected in rabbit heart, lung, kidney, spleen, brain, or skeletal muscle normally or during vit. E deficiency. The molybdenum content of various rabbit tissues was influenced by the Mo content of the diet, but was not altered by the presence or absence of vit. E.

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## Effect of Whole Body X-Irradiation on Ascorbic Acid of Rat Tissues.* (20681)

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Interest in the fate of ascorbic acid in irradiated tissues has been stimulated by several reports. It has been shown(1) that X-irradiation of ascorbic acid in aqueous solutions results in a loss of from 1.7 to 2.4 $\mu$ moles/1000 r. This is the order of magnitude of the effect of X-irradiation on a number of organic compounds in dilute solution(2). The destruction of ascorbic acid by X-irradiation, unlike thiamine and most organic compounds, is not inhibited by the presence of albumin and, indeed, proceeds to the same extent in blood plasma as in aqueous solution. However, only slight destruction of ascorbic acid occurred in minced rat muscle exposed to 22,000 r *in vitro* (1). Favorable clinical reports on the use of ascorbic acid in the treatment of X-ray sickness led to the study of the effect of X-ray therapy on the serum ascorbic acid of patients by Kretzschmar and Ellis(3). A reduction was noted during and after treatment. These workers also reported an immediate and sustained (12-30 days) reduction in plasma ascorbic acid of rabbits which had received 1500 r on one side of the body. Moreover, they found a reduction in muscle and kidney ascorbic acid in rats 1-48 hours after irradiation of these tissues. These reports indicate the possibility that cell ascorbic acid might be destroyed by the production of oxidizing radicals in cell water during X-irradiation(4). This type of immediate *in vivo* destruction has been reported by Skoog(5) to occur in the auxin of plants receiving from 600-2100 r. The prolonged effect on plasma ascorbic acid found by Kretzschmar and Ellis suggests that besides the possible primary oxidative destruction, there are also secondary factors which influence the *in vivo* ascorbic acid levels after

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X-irradiation. One of these factors might be reduction in food intake. In species not dependent upon dietary intake for ascorbic acid this factor should not be significant in relatively short term experiments. The report of Patt *et al.*(6) on adrenal weight and cholesterol content after whole body irradiation has established that irradiation activates the pituitary-adrenal system. Pituitary-adrenal activation might, therefore, be expected to be another factor in the alteration of *in vivo* ascorbic acid levels. Wexler *et al.*(7) have recently reported changes in adrenal ascorbic acid, after whole body irradiation, attributable to this factor.

Our investigation was undertaken to extend the earlier work to whole body irradiation and to other tissues. Data will be presented which indicate that some oxidation of ascorbic acid may occur in irradiated rats of the Long-Evans strain. In rats of the Wistar strain, on the other hand, the principal changes are the result of activation of the pituitary-adrenal system.

*Procedures.* Nineteen female Long-Evans (Rockland Farms) rats, body weight 138-208 g, and 54 Wistar (Carworth Farms) rats, body weight 94-162 g, were used. They were given tap water and Rockland Rat Diet (complete) fed *ad libitum.* This diet was supplemented with fresh leaf lettuce twice weekly. During the 72 hour post-irradiation period of study, very little food was eaten by the irradiated animals.

*Exp. 1.* Ascorbic acid was determined by the method of Roe and Kuether(8) in the adrenal gland, thymus, spleen, liver, kidney, thigh muscle, and whole blood. The animals were anesthetized with ether and were sacrificed by bleeding from the inferior vena cava. The blood was drawn into the required(9) amount of heparin (7.35 mg/ml) in saline solution. The tissues were then removed, dissected free of fat, weighed and placed into a pyrex homogenizer(10) with the required amount of 4% trichloroacetic acid (TCA), and carefully homogenized for one minute. Six liver homogenates made in this way contained an average of 15.8 mg ascorbic acid/100 g liver. When the sediment, from a centrifuged homogenate, was rehomogenized and the 2 supernatant layers combined, the average ascorbic acid value was 17. The difference is within experimental error, indicating that there was no additional ascorbic acid obtained by rehomogenization. Furthermore, hydrolysis of the homogenate by 1 N acetic acid (steam cone for 45 minutes, in an $H_2S$ atmosphere) yielded no increment. It was therefore concluded that a single extraction was sufficient for the complete removal of ascorbic acid.

TABLE I. Tissue Ascorbic Acid Values for Control Animals of Wistar (W) and Long-Evans (L-E) Strains. All values are expressed as mg per 100 g (tissue) or ml (blood).

| Organ | Mean ascorbic acid value L-E | | Mean ascorbic acid value W | |
|---|---|---|---|---|
| Adrenal | 330 | ±30. * | 380 | ±20. * |
| Thymus | 39 | ± 3. | 33 | ± 1. |
| Spleen | 33 | ± 3. | 38 | ± 1. |
| Liver | 19 | ± 2. | 19 | ± 1. |
| Kidney | 8.9 | ± .4 | 8.2 | ± .5 |
| Muscle | 3.2 | ± .3 | 2.2 | ± .1 |
| Blood | .81 | ± .09 | .58 | ± .04 |

* Stand. error of mean.

TABLE II. Effect of Irradiation on Tissue Ascorbic Acid of Long-Evans Strain Rats. (All values expressed as mg per 100 g (tissue) or ml (blood)).

| Organ | Difference from control immediately after irradiation |
|---|---|
| Adrenal | —70 ±36 * |
| Thymus | — 4.0 ± 4.2 |
| Spleen | — 5.0 ± 3.6 |
| Liver | — 2.0 ± 2.8 |
| Kidney | + .4 ± .8 |
| Muscle | — .9 ± .4 |
| Blood | — .24 ± .11 |

* Stand. error of difference $= \sqrt{(\sigma m_1)^2 + (\sigma m_2)^2}$.

Ten unirradiated rats of the Long-Evans strain were sacrificed and the ascorbic acid of the tissues analyzed as indicated. These data constituted the *control values* (Table I). Subsequently 9 animals received 710 r whole body X-irradiation, and the same analyses were made immediately after irradiation. The irradiation factors were: 200 KV, 20-23 MA, ½ mm Cu + 1 mm Al filters, 19.3-20.0 r.p.m., and 86 cm t.o.d.

*Exp. 2.* A total of 54 female Wistar rats were used. The procedure was the same as in Exp. 1 except that a control animal was sacrificed with each irradiated animal. Ten irradiated animals, with controls, were sacrificed immediately after irradiation. Of these, 4 animals received 710 r, three 800 r, and three

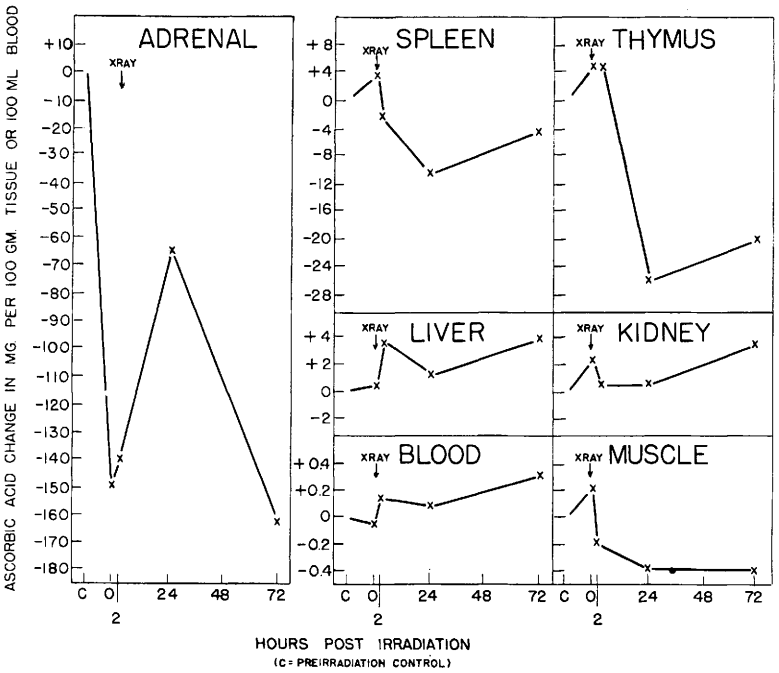


FIG. 1. Changes in tissue ascorbic acid of Wistar female rats after 1000 r whole body x-irradiation. Units of ordinates are chosen to represent one standard deviation of differences plotted, except in the case of the adrenal where each unit represents a quarter of a standard deviation.

1000 r. Since there was no marked difference attributable to the change in dose, these animals are considered together and all subsequent groups received 1000 r. Two groups, each of 5 irradiated animals with 5 controls, were sacrificed 2 and 24 hours after irradiation, and 7 irradiated animals with 7 controls were sacrificed 72 hours after irradiation.

The data of Exp. 1 are presented in Table II, and of Exp. 2 in Fig. 1. In each case the difference between irradiated and control animals is presented. (K. Mather, quoted in (3)).

*Discussion.* From Table II it can be seen that in Long-Evans rats irradiated at 710 r (approximately a mid-lethal dose) a statistically significant decrease in the ascorbic acid content of the adrenals, muscle, and blood occurs immediately after irradiation. Less marked decreases occurred in the thymus, spleen, and liver. Our data for the Long-Evans rat, therefore, confirm the findings of Kretzschmar and Ellis(3), that the ascorbic acid of muscle and plasma is decreased immediately after irradiation, and show a marked decrease in adrenal ascorbic acid as well. That this immediate decrease in muscle and blood ascorbic acid is dependent upon the strain of the animal studied is suggested by the results of Exp. 2. The strain dependence of response to irradiation has been repeatedly emphasized.

The data of Exp. 2 for the animals sacrificed immediately after irradiation are plotted (Fig. 1) as zero hour points although it required about 50 minutes to deliver the dose. The earliest changes, in tissues other than the adrenal, as brought out in Fig. 1, are not individually statistically significant. However, consideration of the 4 tissues—thymus, spleen, liver, and kidney—together suggests a tendency for the earliest values in the irradiated animals to be higher than the controls. It is possible that this represents a synthesis of vitamin by injured cells as reported by Loofbourow and his associates for various living tissues (rat, mouse, and chicken embryos and adult newt), subjected to lethal ultraviolet radiation, X-irradiation, mechanical and chemical injury(11-13). Of interest also, is the report of Richmond, Altman and Salomon

(14), that bone marrow and spleen homogenates from animals sacrificed immediately after irradiation exhibit an increased capacity for the synthesis of hemin and globin.

The changes presented in Fig. 1, are probably due to activation of the pituitary-adrenal system as a result of the "non-specific stress effect" of X-irradiation(6,7,15). It is particularly to be noted that the changes in the liver and blood are the opposite of the change noted in the adrenals. Sayers *et al.*(16) have reported a rise in liver and plasma ascorbic acid with reduction of adrenal ascorbic acid caused by loss of 1.1 ml blood per 100 $cm^2$ of body surface. The finding of an adrenal ascorbic acid level below normal at 24 hours and a secondary more severe reduction at 72 hours, we attribute to the severity of the irradiation which is at least 100 r above the dose required for about 90% mortality. A biphasic change of this type in the adrenal cholesterol has been reported(6) in rats receiving 900 r whole body irradiation.

The decrease in ascorbic acid of the muscle, thymus and spleen in Exp. 2, since it occurs after an initial increase, is probably not due to direct oxidation of the vitamin. The data indicate that the changes in ascorbic acid which occur in the tissues of rats of the Wistar strain are due to secondary physiologic factors and not to primary oxidation.

*Summary.* Evidence obtained from an experiment with rats of the Long-Evans strain suggests that mid-lethal whole body X-irradiation (710 r) may directly reduce tissue ascorbic acid. In rats of the Wistar strain, however, except for the adrenal glands, a higher dose (1000 r) did not result in an immediate reduction in tissue ascorbic acid but rather a tendency to increase was noted. In both strains, the effect of X-irradiation on the adrenal ascorbic acid could be ascribed to activation of the pituitary. In the Wistar strain animals, the changes in the ascorbic acid of the liver and blood could also be explained by this mechanism.

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