further studies should definitely prove progesterone to be locally negative in the feather papilla, this would show its systemic action to be upon some intermediate link. Since the bird's own thyroid is apparently unaffected by progesterone in circumstances where the substance is adequate for growth induction in the papilla, that gland is probably not the participant despite the very evident experimental effectiveness. In these aspects, the newly discovered role of progesterone casts some doubt on the concept that surges of thyroidal activity initiate the normal events of molt and suggests a revised approach to an important problem.

Summary. The respective local actions upon the feather papilla of thyroxine and of progesterone were examined. Application was made by intradermal injection to the immediate vicinity of the resting, quiescent and plucking-stimulated papillae. Thyroxine was effective in causing proliferation in all 3 circumstances; progesterone proved negative with this technic in the resting and quiescent states; it may have exerted a slight augmentation in the third.

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Fate of Ascorbic Acid in Early Radiation Damage.* (21983)

PETER D. KLEIN, DOROTHY T. HANDA, AND ROBERT W. SWICK.
(Introduced by F. Schlenk.)
From the Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill.

There is a paucity of information regarding the role of ascorbic acid in radiation damage. In light of the reported involvement of ascorbic acid in the conversion of folic acid to citrovorum factor(1) and, thereby, in nucleic acid synthesis, its sensitivity *in vivo* to irradiation is of interest. Thus far, the study of ascorbic acid has been concerned chiefly with its changes in the adrenal gland(2). We have investigated a variety of rat tissues from the standpoint of the concentration and total organ content of ascorbic acid as well as organ weight after whole-body X-irradiation. These organs included cervical lymph nodes, thymus, liver, spleen, kidney, intestinal mucosa, testis, brown adipose tissue, muscle, adrenals, and brain. The results indicate a highly selective action of irradiation on tissue ascorbic acid.

Methods. Male Sprague-Dawley rats, weighing 125-200 g, receiving a stock Purina Chow diet *ad lib.*, were irradiated at a rate of 23 r/min with a 250-kvp X-ray machine at 15 ma with a 0.5 mm Cu plus a 1.0 mm Al filter and the HVL equal to 2.0 mm of Cu. The target distance was 34 inches from the table supporting the animals. At 4, 28, and 72 hours after irradiation, groups of 4 to 6 animals were sacrificed. The organs were dissected out, blotted, and weighed on a torsion balance. A 9-inch strip of small intestine was slit, washed, and scraped to provide a sample of intestinal mucosa. The tissues were homogenized in 10% trichloroacetic acid; all of the ascorbic acid was converted to the oxidized (dehydro) form and determined as the osazone by the method of Roe and Kuether (3). No differentiation of oxidized and reduced ascorbic acid was carried out. Typical control values and their variation are shown in Table I. The values for concentration, total ascorbic acid and organ weight for the experimental groups are reported as a percent of the control values.

* Work performed under the auspices of the U.S. Atomic Energy Commission.

TABLE I. Ascorbic Acid Concentration and Organ Weights of Normal and Chloretone-Treated Rats.*

Organ	Normal		Chloretone-treated†	
	Ascorbic acid, mg %	Organ wt, mg/g	Ascorbic acid, mg %	Organ wt, mg/g
Adrenals	311.6 ± 28.8	.21 ± .01	391.4 ± 16.9	.20 ± .00
Thymus	66.8 ± 3.2	4.02 ± .57	64.4 ± 2.3	2.92 ± .29
Spleen	49.7 ± 2.9	4.44 ± .16	51.5 ± 2.2	3.56 ± .39
Cervical lymph nodes	46.5 ± 2.0	.81 ± .06	60.5 ± 3.0	.93 ± .17
Brain	41.7 ± .2	9.18 ± .32	38.6 ± .9	8.13 ± .04
Intestinal mucosa, 9 in.	33.0 ± 2.7	2.03 ± .04	38.0 ± 3.1	1.84 ± .12
Left testis	29.9 ± .9	5.70 ± .16	28.5 ± 1.3	5.72 ± .63
Liver	27.2 ± .9	54.5 ± 4.0	36.7 ± 1.3	61.3 ± .2
Left kidney	16.0 ± .1	4.87 ± .17	19.3 ± 1.4	5.34 ± .24
Pancreas	6.5 ± .5	3.62 ± .46	6.7 ± .5	2.50 ± .04
Skeletal muscle	5.0 ± .3	—	4.6 ± .3	—
Brown adipose tissue	4.5 ± .4	1.68 ± .12	4.7 ± .5	1.18 ± .12

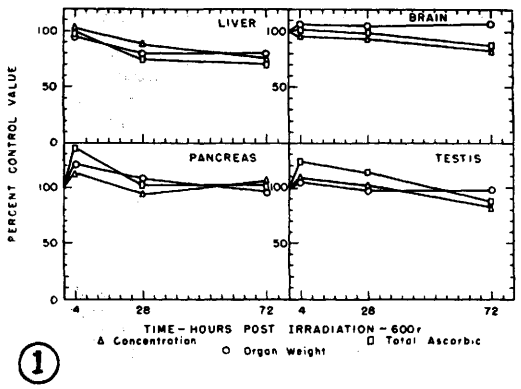
* Avg of 3 animals and its stand. dev.
† 1 mg of chloretone/ml drinking water for 6 days.

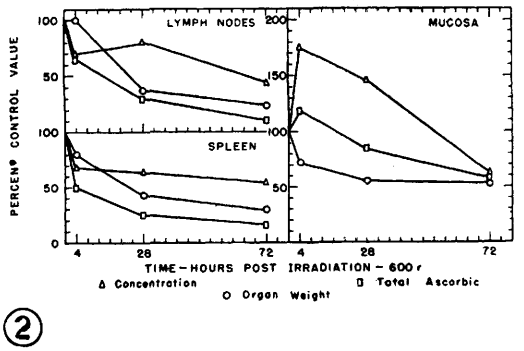
Results. After exposure to 600 r there were no gross changes in concentration or organ content of ascorbic acid or in organ weight in liver, brain, pancreas, testis (Fig. 1), muscle, kidney and brown adipose tissue. The adrenal glands showed an immediate drop in concentration at 4 hours, as has been noted by other workers(2); however at 28 hours the level had returned to normal and remained unchanged thereafter. The remaining 4 tissues: thymus, lymph nodes, spleen, and intestinal mucosa, underwent the rapid changes shown in Fig. 2. The total ascorbic acid content of lymph nodes, thymus, and spleen decreased to 30, 25, and 10%, respectively, of the original values. The concentration of ascorbic acid in these organs dropped abruptly within 4 hours to 65-70% and changed slowly thereafter. After 300 r, the changes in these organs were similar to those observed at the higher dose level while after 100 r the losses of ascorbic acid were small from the thymus and spleen and negligible from the lymph nodes.

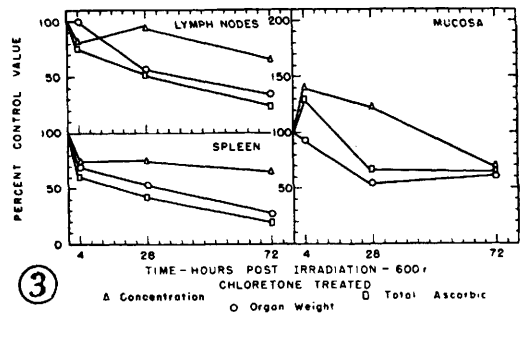
The changes observed in the intestinal mucosa 4 hours after X-irradiation were unique. Despite a loss of tissue weight, the total ascorbic acid content of this tissue had increased because of the concentration which had risen to 170% of the control value. This response likewise diminished slightly with lower doses of irradiation, but, even at 100 r, the concentration of ascorbic acid was 135% of the control value 4 hours after irradiation.

We investigated the water content of these four tissues after irradiation to determine, in the thymus, lymph nodes, and spleen, if there is an edema present which masks a loss of cells containing high concentrations of ascorbic acid, or in the mucosa, if there has been a dehydration of the tissue which caused an apparent increase in concentration. From Table II (which shows percent water content of organs of rats after exposure to 600 r), it can be seen that changes in water content of tissues could not account for the observed changes in ascorbic acid concentrations. In the lymph nodes there was even some dehydration.

The effect of stimulating synthesis of ascorbic acid on subsequent response to irradiation was next investigated. Administration of chloretone at a level of 20 mg/day for 7 days has been shown to result in urinary excretion of as much as 20-30 mg of ascorbic acid per day, in contrast to normal excretion of 0.5-2.0 mg/day(4). It does not necessarily follow, however, that concentration of ascorbic acid in tissues is correspondingly increased, since a low kidney threshold has been shown to exist in other species(5). That this appears to be the case may be seen by comparing the values for ascorbic acid concentration of tissues of rats given chloretone (1 mg/ml of drinking water) with those of untreated animals (Table I). Tissues of rats treated with chloretone exhibited, for example, the following increases in ascorbic acid concentration: liver, 35%;







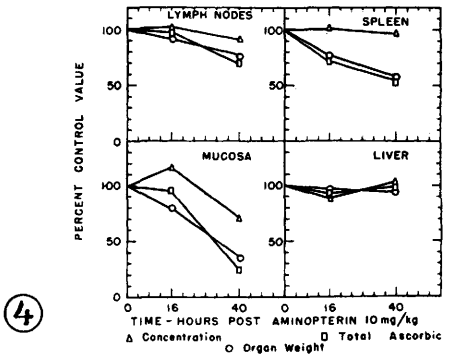


FIG. 1. Ascorbic acid changes in normal rat liver, brain, pancreas, and testis after 600 r.

FIG. 2. Changes in ascorbic acid in normal rat lymph nodes, spleen, and intestinal mucosa after 600 r.

FIG. 3. Ascorbic acid changes in chloretone-treated rat lymph nodes, spleen, and intestinal mucosa after 600 r.

FIG. 4. Changes in ascorbic acid in rat lymph nodes, spleen, intestinal mucosa, and liver after aminopterin treatment.

lymph nodes, 30%; intestinal mucosa, 15%; and spleen, 4%. These animals served as chloretone-treated, non-irradiated controls for the following irradiated animals. The remaining 24 rats were irradiated with 600 r and chloretone administration continued after irradiation. Their tissues displayed changes in concentration of ascorbic acid, total content and organ weight (Fig. 3) similar to those seen in previous experiments. The effect of chloretone was apparent in higher final levels of ascorbic acid in tissues of treated animals.

TABLE II. Organ Water Content.

Hr post-irradiation	% organ wt		
	Lymph	Spleen	Mucosa
0	81.8	78.1	81.7
4	76.9	76.0	80.7
28	79.5	77.3	82.0
72	77.8	75.5	82.8

and in a slight reduction of the rise in mucosal concentration. Administration of chloretone did not, however, abolish the decrease in concentration in lymphoid organs or stimulate recovery within 72 hours.

Alternatively, the effect of blocking the conversion of folic acid to citrovorum factor on the levels of ascorbic acid was studied. When aminopterin was administered intraperitoneally in doses of 10 mg/kg of body weight to a group of rats, the concentration and total content of ascorbic acid in lymph nodes, spleen, mucosa, and liver underwent the changes shown in Fig. 4. Aminopterin caused an involution of lymph nodes and spleen, but the loss of total ascorbic acid paralleled the loss in tissue weight, leaving the concentration unchanged. There was a small but significant rise in mucosal concentration after 16

hours, and no changes were observed in the liver.

Discussion. After irradiation with 600 r the majority of organs analysed presented no striking changes in ascorbic acid within 72 hours. However, organs of a lymphoid nature quickly dropped in both concentration and total content of ascorbic acid, while the mucosa increased in concentration during the same interval. Certain possibilities appear evident as explanations of the phenomena. For example, it is tempting to explain the rise in mucosal concentration and decreases in lymphoid tissue by means of a migration of lymphocytes to the intestinal epithelium. The rise in mucosal concentration after aminopterin treatment lends credence to this possibility, since such a migration has been observed after both aminopterin treatment and X-irradiation(6,7) and a histological examination of the small intestine confirms the presence of increased number of lymphocytes.†

The influx of lymphocytes cannot be wholly responsible for the findings because the increase in lymphocytes appeared to be insufficient to account for the total rise and the accumulation of such cells was not, apparently, matched by a corresponding loss of material from the lymphoid organs themselves.

The stability of ascorbic acid concentration in these organs after treatment with aminopterin would suggest that blocking conversion of folic acid to citrovorum factor does not in itself affect concentration of ascorbic acid. These data are, themselves, at variance with those of Williams(8) and Schwartz and Williams(9) who found a decrease in liver ascorbic acid concentration 13 days after feeding of aminopterin at a level of 4 mg/kilo of diet. This discrepancy may be due either to route of administration or to high dosage (LD_{100}—72 hours) employed in the present study which was, in our consideration, necessary to duplicate the acute aspect of irradiation. Even at this level, however, the concentration of ascorbic acid in tissues did not decrease within 40 hours.

Furthermore, the effect of stimulating the synthesis of ascorbic acid on changes in concentration or organ content of ascorbic acid after irradiation is negligible, suggesting that an impairment of the synthetic process does not seem to be responsible. Nor does the stimulation of ascorbic acid synthesis by chloretone seem to be abolished by irradiation.

This then focuses attention on the relationship of ascorbic acid to the particular tissue in which its concentration is so readily changed by X-ray. The observed rapid rise of ascorbic acid and the well known inhibition of mitosis in the intestinal mucosa after irradiation suggest that the accumulation of ascorbic acid is a result of arrested mitosis and that ascorbic acid is requisite to and/or consumed by mitotic activity. A similar suggestion has been made by Stern and Timonen (10) working with lily anthers. Failure to observe similar changes in the other tissues may be due to their relatively much lower mitotic activity. Coupled with this, the loss of ascorbic acid from lymphoid tissues may be explained by the known radiation-induced fragility of several cell types in relatively high concentration in these organs.

Summary. 1. There were no gross changes in concentration or total content of ascorbic acid within 72 hours after 600 r of X-ray in rat liver, brain, pancreas, testis, muscle, kidney, or brown adipose tissue. Thymus, lymph nodes, and spleen showed a drop in ascorbic acid concentration within four hours, and the content of ascorbic acid continued to drop throughout the period studied. Intestinal mucosa showed a sharp increase in ascorbic acid concentration during the first 4 hours followed by a gradual decline. 2. Stimulation of ascorbic acid synthesis by chloretone administration does not modify the effects of irradiation, nor does the hydration of these tissues change in a fashion which might account for the observed phenomena. 3. The observed changes in concentration could not be duplicated by the administration of aminopterin at a level

† We wish to thank Dr. F. Wassermann for preparation of the material and Dr. A. M. Brues and Mrs. Agnes Stroud for assistance in interpretation of the specimens.

of 10 mg/kg body weight.

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Relationship of Prolonged Drainage of Bile through Pancreatic Duct System to Pancreatitis.* (21984)

FREDERICK S. CROSS, FRANCISCO L. RAFFUCCI, EDWIN L. BRACKNEY, AND OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota Medical School, Minneapolis.

The role of reflux of bile into the pancreatic duct system in the etiology of acute hemorrhagic pancreatic necrosis has been a subject of intensive investigation for many years. It is well known that when sterile bile is injected into the pancreatic duct of experimental animals, under certain conditions, a fulminating hemorrhagic pancreatic necrosis results quite regularly. However, there is a great deal of evidence that under other more physiologic conditions the presence of bile in the pancreatic duct is harmless to the pancreas. Mann and Giordano(1) found that, if the pressure exerted during retrograde injection of bile into the pancreatic duct were maintained within physiologic limits, no pancreatitis developed. It was shown in this laboratory(2) in cats that when the common bile duct was occluded distal to the entrance of the pancreatic duct so that bile could flow into the pancreatic duct under secretory pressure of the liver or pressure of the contracting gall bladder, pancreatitis developed in only a small proportion of animals, unless the cats were fed fatty meals supplemented with bile salts. Leven(3) and Colp and Doubilet(4) showed that reflux of iodized oil into the pancreatic duct could be demonstrated in cholangiograms in about 20% of patients with common bile duct drainage. This reflux was attributed in most cases to spasm of the sphincter of Oddi. Hicken and McAllister(5) using a water soluble contrast agent and very low injection pressures found that even in patients with no intraductal pathology, reflux of contrast material into the pancreatic duct could be demonstrated on routine cholangiograms in a high percentage of cases. Fisher *et al.*(6) drained the bile through the pancreatic duct by connecting a catheter in the common bile duct with a catheter in the pancreatic duct. In their 4 experiments the longest follow-up was 7 days. None of these animals developed any pancreatitis.

The experiments reported here, from August 1950 to July 1951, were designed to show the effect upon the pancreas of draining all of the bile secreted by the dog's liver through the pancreatic duct system over long periods of time.

Methods. The entire biliary flow in a series of dogs was caused to pass through the pancreatic duct into the duodenum by one of 3 operations. Type I: the duct of Santorini

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