SOME ACUTE EFFECTS OF X-IRRADIATION (LD₁₀₀) ON
PLASMA AND ADRENAL TISSUE HISTAMINE IN RATS

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The effects of a lethal dose (1380 r) of X-irradiation on plasma and adrenal tissue histamine levels of rats were studied. Histamine levels (determined fluorometrically), adrenal weights, and blood counts were made at 1, 3, 5, 9, and 24 hours post-irradiation. X-irradiation was delivered from a G. E. beryllium window X-ray unit at 120 KVP, 5 ma with a $\frac{1}{4}$ mm Al filter at a target distance of 30 cm.

The plasma histamine response was triphasic (increase at 1-3 hours, decrease at 5 and 9 hours and return to control at 24 hours post-irradiation). The adrenal tissue histamine response was found to be biphasic (decrease at 1 to 9 hours and a return to control level at 24 hours post-irradiation).

A slight, but sustained, hypertrophy of the adrenal glands was noted. A sustained severe eosinopenia occurred while a slight leucocytosis was observed at the third hour post-irradiation. No change in erythrocytes was noted.

The data suggests that histamine may be involved in the action of the adrenal-pituitary axis following X-irradiation.
SOME ACUTE EFFECTS OF X-IRRADIATION \((LD_{100})\) ON PLASMA AND ADRENAL TISSUE HISTAMINE IN RATS

THESIS

Presented to the Graduate Council of the North Texas State University in Partial Fulfillment of the Requirements For the Degree of

MASTER OF SCIENCE

By

James L. Ferguson, B. S.
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INTRODUCTION

Ellinger (12, 13) proposes that radiation sickness can be classified as a typical case of the General Adaptation Syndrome of Selye. This syndrome as described by Selye (37) states that any severe change of an animal's environment may initiate compensatory actions by the animal. This compensatory action is the unspecific response of the body to stress, disregarding the causative agent. Such factors as cold, electrical stimulation, bacterial toxins, ionizing radiation, and other stressors have been employed experimentally to demonstrate the adaptive defense mechanisms of an animal. These defense mechanisms, according to the Selye concept, are triggered by the stimulation of the hypothalamic centers, which results in the stimulation of the anterior pituitary gland to effect the secretion of tropic hormones.

Bacq and Fischer (9) reported an increased output of ACTH in X-irradiated animals. Such increases in ACTH cause the cortex of the adrenal gland to discharge an increased quantity of adrenocorticosteroid hormones into the blood (the mineralocorticoids and the glucocorticoids). The mineralocorticoids act mainly on the equilibrium of sodium and potassium in the body, while the glucocorticoids act on the carbohydrate, protein, and lipid metabolism (8).
Schayer (36) implicated the adrenal cortex as a possible site for histamine regulation by showing that glucocorticoids have an inhibitory effect on the formation and binding of new histamine in rat skin tissues. Moreover, in the case of rat skin, he suggests that the action may be at any point in the complex chain of events which follows the entry of free histidine into the circulation of the skin and terminates with the decarboxylation and binding of the histidine. This decarboxylation and binding is thought to occur in mast cells. Telford and West (40, 41) state that after injections of corticoids into rats the histamine content of skin and small intestine was reduced while that of the stomach tissue was increased. The extent of change of histamine was found to be roughly proportional to the glucocorticoid activity.

When administered to animals, histamine provokes responses which are typical of the so-called "Alarm Reaction" of the stress concept proposed by Selye (37). This concept includes three pituitary-adrenal reactions: (a) alarm phase, during which the adrenal gland undergoes hypertrophy and increases secretion; (b) resistance phase, during which the animal increases resistance to the stress; (c) exhaustion phase, which terminates with the death of the animal if the stress is too great. The development and duration of each of the phases are dependent on the type and intensity of the stressor that is applied. The alarm phase is characterized by the inhibition of growth, adrenal hypertrophy, thymus-
lymphatic atrophy, eosinopenia, and a depletion of the ascorbic acid content of the adrenalglands, as reported by Fortier (17). Fortier also indicated that the discharge of ascorbic acid by the injection of histamine is indirect through stimulation of the pituitary gland, because hypophysectomy abolished such an effect. Gordon (19) suggests that the discharge of ascorbic acid from the adrenals initiated by a small dose of histamine was extremely rapid and was not prevented by a previous injection of cortico-adrenal extracts.

Hameed and Haley (22) have reported a biphasic increase of plasma and adrenal corticosterone in the rat at 2.5 and 48 hours post-X-irradiation with 650 r. Comparable data of biphasic adrenal responses after whole-body X-irradiation have been reported by a number of workers; however, the second response is more variable with respect to onset and magnitude (16, 26). The synthesis and secretion of the adrenocorticoids is thought to be dependent upon a neuro-endocrine mechanism. This neuro-endocrine mechanism has been called by Bacq and Alexander (8) the adrenal-pituitary axis. This adrenal-pituitary axis regulates the production of ACTH, which in turn controls the release of the adrenocorticoids. Neither ACTH nor the adrenocorticoids have been shown to bring about the liberation of histamine or affect its action after it is released. However, Carryer and Code (10) indicate that both the pituitary hormone (ACTH) and the glucocorticoids of the adrenal glands may inhibit the biogenesis of histamine.
Although much has been written to indicate that there is a close correlation between the conditions of shock and the liberation of histamine from damaged tissue, Prosser et al. (32) were the first to show an increase in histamine release from tissues of animals exposed to mid-lethal to lethal doses of X-irradiation in the first few hours after exposure. Haeger et al. (21) have shown a leucocytosis in the rat to occur two to twenty-four hours after X-irradiation. However, with this leucocytosis a drastic reduction in the absolute eosinophil count has been observed by Lott and Gaugl (26, 27) following X-irradiation. Archer (5) has explained the eosinopenia that is observed in rats under stress conditions on the basis of changes in free histamine found in the tissues. Patt and his associates (30) studied adrenal weight and histamine tolerance in the rat and observed changes that seemed to be dependent upon radiation dose and time after irradiation. However, Wilson (43) noted that the histamine blood level in adrenalectomized animals is lowered and resistance to injected histamine is raised by adrenocortical extracts.

Although the role of histamine in the inflammatory process is now relatively well known, its role in the radiation syndrome has not been fully elucidated. Since, as seen in the foregoing review, metabolism of histamine appears to be closely associated with the adrenal gland, it would seem feasible that an attempt to correlate histamine changes in irradiated animals with certain changes in the adrenal-pituitary axis ought to
be made. Such was the general aim of the present study.

The specific aims of the present study were to determine the effect of lethal dosages of X-irradiation upon levels of histamine in the blood plasma and adrenal tissues and see if these histamine changes could be used to explain the initial shock reaction (within 24 hours) associated with the radiation syndrome. The parameters measured were erythrocyte count, leucocyte count, absolute eosinophil count, adrenal weights, blood plasma histamine, and adrenal tissue histamine.
MATERIALS AND METHODS

Male albino Sprague-Dawley rats of an average weight of 204 ± 22.6 gm were used in this study. The stock animals were supplied by the Ferguson Laboratory Animal Supply, Inc., Lewisville, Texas. Animals were kept in a community cage in a temperature-controlled animal house which was continually lighted. The lights were left on constantly to try to negate any diurnal histamine level changes.

The rats were fed Wayne Dog Chow and tap water ad libitum. Following X-irradiation, the rats were transferred to the laboratory and maintained there until the designated time of sacrifice. In all of the test experiments, irradiation and testing were performed in groups of three to five animals.

Filtered X-irradiation (\( \frac{1}{2} \) mm aluminum filter) was delivered from a General Electric beryllium window unit at 120 KVP and 5 ma. All test rats in this study received a lethal dose of 1380 r (138 r/min). The target distance was 30 cm, with an exposure time of 10 minutes. To insure uniform exposure, all X-irradiated rats were placed in a plexiglass tube-motor apparatus and rotated at the rate of one rpm. The sham-irradiated rats were treated identically to the test rats.

This study was divided into three series: (a) pre-tests, (b) sham-irradiated series, and (c) X-irradiated series. A
minimum of six rats was used for each sampling time of the sham-irradiated series. Eighteen rats were used for the resting controls. The sham-irradiated and test animals were sacrificed at predetermined intervals of 1, 3, 5, 9, and 24 hours after X-irradiation or sham-irradiation. To check the effects of rotation, another group of rats, the pre-test series, was taken directly from the animal quarters and sacrificed after being rotated, but not sham-irradiated or X-irradiated. Six physiological parameters were monitored during this study: (a) red blood cell count, (b) white blood cell count, (c) the absolute eosinophil count, (d) the weights of decapsulated adrenal glands, (e) the plasma histamine, and (f) the adrenal tissue histamine. The red and white cell counts were made from tail samples obtained after the rats had been anesthetized with intraperitoneal injections of Nembutal (33 mg/gm body weight). To aid in the blood sampling, each rat's tail was immersed in warm water. Following blood sampling from the tail, the animals were immediately guillotined and more blood samples taken for the plasma histamine determinations. The adrenal glands were then removed and decapsulated, weighed (mg/pair/100 gm weight) and stored for tissue histamine analysis.

The red and white blood cell counts were made using the direct-chamber counting method. Absolute eosinophil counts were made following the direct-chamber counting method of Pilot (31).
Anton and Sayre's (1) modification of the Shore et al. (38) method was employed to determine the adrenal tissue histamine in this study. The procedure is outlined as follows:

The paired adrenals were homogenized in 2.0 ml of 0.4 N HClO₄ in a glass homogenizer submerged in ice, and the homogenate was transferred to a 12 ml pyrex centrifuge tube. One-half ml of H₂O was added with thorough mixing and centrifuged at 10,000 rpm in a refrigerated centrifuge for 15 minutes. Two ml of the supernatant were transferred to another centrifuge tube containing 2.0 ml of H₂O and mixed. The dilute supernatant fraction was then poured into a 50 ml pyrex centrifuge tube containing 3.5 gm of K₂PO₄ and mixed. Twenty ml of isoamyl alcohol were added, the mixture was mechanically shaken, and centrifuged at 3,000 rpm for 5 minutes to separate the phases. Twenty ml of the organic layer were pipetted into another 50 ml pyrex centrifuge tube containing 4.0 ml of 0.01 N HCl plus 15 ml of heptane. The mixture was mechanically shaken for 6 minutes and then centrifuged for 5 minutes at 3,000 rpm after the organic phase was aspirated and discarded. Three and one-half ml of the acid extract were pipetted into another centrifuge tube containing 0.5 ml of 10 N NaOH and mixed. To this mixture 2.5 gm of NaCl and 20 ml of CHCl₃ were added and mechanically shaken for 3 minutes, followed by centrifugation at 3,000 rpm for 3 minutes to separate the phases. The washed extract (top layer, 4.0 ml) was then pipetted into another centrifuge tube to which 1.5 gm of NaCl were added and thoroughly
mixed. To this mixture were added 20 ml of isoamyl alcohol, and the resultant mixture was mechanically shaken for 6 minutes and centrifuged at 3,000 rpm for 5 minutes. Twenty ml of the organic phase were pipetted into another pyrex centrifuge tube containing 2.0 ml of 0.1 N HCl plus 15 ml of heptane and this mixture was shaken for 6 minutes, centrifuged at 3,000 rpm for 5 minutes, and the organic layer aspirated and discarded. The aqueous layer was saved for analysis.

Fluorophor formation was accomplished by adding the following reagents: 0.25 ml of sample, 0.25 ml of 0.1 N HCl, 1.10 ml of 1.0 N NaOH, 0.225 ml of OPT reagent, wait 4 minutes, and add 0.05 ml of 2.0 M citric acid. The fluorophor was then measured in a Turner fluorometer, model 110. The fluorometer was equipped with a No. 7-60 primary filter and a No. 2A secondary filter. The data are expressed in mg of histamine per gm of adrenal tissue.

Noah and Brand's (29) procedure was used to determine plasma histamine levels of the animals in this study. This technique, also a modification of the Shore et al. (38) method, employs glass elution columns which have two constrictions with the following dimensions: total length 160 mm, 22 mm opening, 3.5 mm internal diameter at the first constriction, which is 50 mm below the opening, 1 mm internal diameter at the second constriction, which is 120 mm below the opening (See Fig. 1). The columns were packed with 50 mg of cellulose acid succinate (CAS).
The CAS was prepared by mixing 1.25 gm anhydrous sodium acetate, 10 gm of succinic anhydride, 75 ml of glacial acetic acid, and 2.5 gm of cellulose all in a heavy-walled pyrex bottle which was stoppered with a drying tube. The bottle was then placed in an oven at 100°C. for 48 hours and removed occasionally for gentle shaking.

The CAS was filtered on filter paper with gentle suction. Then the CAS was washed with 500 ml of water, 500 ml of 1% HCl, and again with 1,000 ml of water. The washed CAS was resuspended in water, filtered, and washed with water. Then the CAS was dried with 95% ethanol, absolute ethanol, and then in a vacuum oven at 50°C. for 3 hours. The CAS was then ready for packing the columns. After the columns were packed, the packing was used for ten extractions before being replaced with new CAS. The columns were cleaned by washing them with 95% ethanol and absolute ethanol between extractions. After fluorophor formation from the extracts from the columns the plasma histamine concentration was stated in terms of µg/L.
RESULTS

In the graphs each circle represents the mean value of at least six animals. The letter "C" represents unrotated animals that were brought directly from the animal quarters and sacrificed. The pre-test animals were sham-irradiated animals that were brought from the animal quarters and sacrificed after they had been rotated ten minutes in the plexiglass chamber.

Figure 2 depicts the effects 1380 r X-irradiation on the total erythrocyte (A) and leucocyte (B) count in rats. It is clear that such dosage did not alter the red blood counts during the 24 hours post-irradiation. On the other hand a significant increase in leucocyte count was observed at the 3rd hour post-irradiation. Following a return to control levels at the 5th and 9th hours post-irradiation a significant decrease in leucocytes was noted at the 24th hour post-irradiation.

As shown in Figure 3, the eosinophil response to a lethal dose (1380 r) of whole-body X-irradiation was striking. It was noted that there was an immediate and sustained eosinopenia in the irradiated animals. The sham-irradiated animals did not vary markedly from the resting controls or pre-test animals in their absolute eosinophil counts.

Figure 4 and Table I show the effects of a lethal dose (1380 r) of X-irradiation on the paired wet adrenal weights.
Fig. 2 -- The effect of 1380 r whole-body X-irradiation on erythrocyte and leucocyte counts in rats. (Each circle represents a mean of at least six rats.)
Fig. 3 -- The effect of 1380 r whole-body X-irradiation on the eosinophil count in rats. (Each circle represents a mean of at least six rats.)
Fig. 4 -- The effect of 1380 r whole-body X-irradiation on mean adrenal weights, per pair. (Each circle represents a mean of at least six rats.)
TABLE I

A SUMMARY OF THE EFFECT OF 1380 R WHOLE-BODY X-IRRADIATION ON ADRENAL WEIGHTS*

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Hours Post-Irradiation</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control Pre-test</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Control (Unrotated)</td>
<td>15.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+4.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-irradiated</td>
<td></td>
<td>+1.5</td>
<td>+1.2</td>
<td>+1.5</td>
<td>+1.3</td>
</tr>
<tr>
<td>1380 r Whole-body</td>
<td>19.4**</td>
<td>19.7**</td>
<td>20.2**</td>
<td>20.1**</td>
<td>21.6**</td>
</tr>
<tr>
<td></td>
<td>+1.5</td>
<td>+1.6</td>
<td>+1.9</td>
<td>+1.6</td>
<td>+1.7</td>
</tr>
</tbody>
</table>

* Adrenal Weights in mgs (per pair) per 100 gms Rat Weight (+ Standard Deviation).
**Changes from Pre-test Sham-irradiated rats are statistically significant (Student's t; P < .05).
From the data shown here it is evident that there was a sustained hypertrophy of the adrenal glands after whole-body exposure to X-irradiation. The data, summarized in Table I, were found to be statistically significant regarding the increase in adrenal wet weight at all sampling times post-irradiation. It was also evident that the observed hypertrophy appeared to become larger with time. The sham-irradiated groups showed no significant change from the pre-test series.

The effects of a lethal dose (1380 r) of whole-body X-irradiation on plasma histamine may be seen in Figure 5 and are summarized in Table II. As seen in Figure 5, the plasma histamine concentration showed a significant rise from the initial concentration at the third hour post-irradiation. However, the histamine level dropped to sub-control levels at the fifth and ninth hour post-irradiation. Recovery in the histamine level occurred at the twenty-fourth hour post-irradiation. The sham-irradiated animals were at no time significantly different in plasma histamine levels from the pre-test series.

As shown in Table II, the differences in the means of the histamine levels observed in all of the X-irradiated animals were statistically significant from the means of the pre-test series.

The effects of 1380 r X-irradiation on adrenal tissue histamine are depicted in Figure 6 and summarized in Table III. In Figure 6 it can be seen that there was a decrement
Fig. 5 -- The effect of 1380 r whole-body X-irradiation on the plasma histamine level in rats. (Each circle represents a mean of at least six rats.)
### TABLE II

**A SUMMARY OF THE EFFECT OF 1380 R WHOLE-BODY X-IRRADIATION ON PLASMA HISTAMINE LEVELS**

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Control Pre-test</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>9</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Unrotated)</td>
<td>3.96 ± .55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-irradiated</td>
<td>4.05 ± .28</td>
<td>3.65</td>
<td>3.75</td>
<td>4.09</td>
<td>4.22</td>
<td>3.94</td>
</tr>
<tr>
<td>1380 r Whole-body</td>
<td>4.38 ± .45</td>
<td>4.87**</td>
<td>2.23**</td>
<td>2.76**</td>
<td>3.49**</td>
<td>3.49**</td>
</tr>
</tbody>
</table>

* Plasma Histamine in ug/L (± Standard Deviation).
**Changes from Pre-test Sham-irradiated rats are statistically significant (Student's t; p < .05).
Fig. 6 -- The effect of 1300 r whole-body X-irradiation on the histamine content of adrenal glands in rats. (Each circle represents a mean of at least six rats.)
### TABLE III

A SUMMARY OF THE EFFECT OF 1380 R WHOLE-BODY X-IRRADIATION ON ADRENAL HISTAMINE LEVELS*

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Control Pre-test</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>9</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Unrotated)</td>
<td>26.2 ±4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-irradiated</td>
<td>28.6 ±5.7</td>
<td>27.1</td>
<td>29.2</td>
<td>27.9</td>
<td>29.2</td>
<td>27.9</td>
</tr>
<tr>
<td>1380 r Whole-body</td>
<td>21.4** ±4.1</td>
<td>21.2**</td>
<td>20.8**</td>
<td>23.9</td>
<td>27.0</td>
<td></td>
</tr>
</tbody>
</table>

* Adrenal Tissue Histamine in ug/gm of tissue (+ Standard Deviation).
**Changes from Pre-test Sham-irradiated rats are statistically significant (Student's t; p < .05).
in histamine level in the adrenal tissues at 1, 3, 5, and 9 hours post-irradiation. The decrease, however, as indicated in Table III, was significant from the means of the pre-test series only at 1, 3, and 5 hours post-irradiation. At 9 and 24 hours post-irradiation, the differences observed between the test series and the sham-irradiated controls were not significantly different.

If one compares the data in Figures 5 and 6, it may be noted that there was little correlation between the changes in tissue histamine and circulating histamine. X-irradiation brought about an immediate and relatively sustained decrease in adrenal histamine. On the other hand, the same dosage of X-irradiation brought about a biphasic response in circulating histamine levels: id est, an initial enhancement followed by a decrease in histamine. Interestingly, recovery to control levels were noted in both parameters at the same time: id est, 24 hours post-irradiation.
DISCUSSION

The experimental procedures used in this study for determining plasma (Noah and Brand, 29) and adrenal tissue (Anton and Sayre, 1) histamine levels were both modifications of the method of Shore et al. (38). Multiple assays were carried out in an effort to give statistical validity to the use of these newer extraction and analysis procedures for histamine. Preliminary trials using these modified methods resulted in recoveries of eighty-nine percent for Noah and Brand's method for plasma histamine and eighty-six percent for Anton and Sayre's method for tissue histamine. It was concluded that the newer methods yielded more valid histamine determinations than Shore's procedure.

Regarding the erythrocyte data, Bacq and Alexander (8) stated that following LD_{100} radiation anemia will develop if the animal lives long enough. However, the data here indicate that this anemia did not develop within the first twenty-four hours post-irradiation. This finding was not unexpected since Supplee et al. (39) have shown that, due to the 80-120 day turnover time of erythrocytes in the rat, anemia would not likely appear until late in the radiation syndrome. Although the erythrocyte count may not be directly related to histamine concentration of the blood or plasma, Fulton et al. (18) have indicated that after whole-body irradiation there is an
increased extracellular fluid and plasma volume without a concomitant decrease in the total red blood cell count.

Archer, in his monograph (6) on the role of eosinophils in the body, has presented evidence that almost all of the blood histamine is contained in the white blood cells and particularly in the eosinophils. Indeed, he stated that the eosinophil was the main carrier of blood histamine. Code (11), however, believes that the basophil leucocyte, like the tissue basophil mast cell, is an important storage place for histamine. Samson and Archer (35) presented data to show that the basophil contains significant amounts of histamine while other white cells do not. However, they admit that the histamine content of human basophils was much lower than the histamine content of rat mast cells.

The role of certain leucocytes as histamine liberators has been reported by Lutz et al. (28). They noted that two basic peptides in lysosomes of normal bovine polymorphonuclear leucocytes were potent histamine liberators. These histamine-liberating peptides were found to contain high amounts of arginine and demonstrated more effective histamine liberating properties when there was an alternative sequence of arginine and glycine. They further suggested that these arginyl peptides increased vascular permeability through mast cell disruption.

Riley and West (33) have presented evidence that tissue mast cells are the likely site for the intracellular location of much of the histamine found in rats. According to Archer
(2,3) and Archer and Jackas (4), the disruption of eosinophils causes the release of eosinophil peroxidase. This peroxidase initiates the lysis of the basophil granule. Both the disruption of eosinophils and lysis of basophils could result in an increased free histamine. Lott (25) presented data showing that there was a sharp and sustained eosinopenia after exposure to sublethal and lethal dosages of X-irradiation. If this drop in eosinophils in fact was due to lysis of these cells, then they would release the peroxidase which in turn would result in lysis of mast cells, initiating a release of histamine into the plasma or tissues. Such action might account for the initial increase in the plasma histamine noted in this study. However, it does not explain the secondary decrease in plasma histamine and then the return to control levels that followed. One might conjecture that there would have to be another mechanism besides the eosinophil lysis (decreased binding?) to account for the return of histamine to control levels. The hypothesis of Archer (5) that eosinophils inactivate histamine cannot be disregarded on the basis of the present data; however, his hypothesis would be strengthened if the eosinophil response had followed the plasma histamine response after X-irradiation.

When administered to animals, histamine elicits responses which are typical of the so-called "Alarm Reaction" of Selye (37). After administration of histamine, Fortier (17) has shown that there is an inhibition of growth, adrenal hypertrophy, thymus-lymphatic atrophy, eosinopenia, and a depletion
of the ascorbic acid content of the adrenal glands. Indeed, adrenal hypertrophy was one of the earliest used indices of adrenal activity during stressful conditions. Patt et al. (30) have shown unilateral hypertrophy to follow six hours after exposure to X-irradiation. The slight adrenal hypertrophy that was observed in the present study might be explained on the basis of Selye's (37) General Adaptation Syndrome. In the alarm phase an animal that has been exposed to a stress agent demonstrates eosinopenia, a hypertrophy of the adrenal gland, and an increased secretion, supposedly due to the influence of ACTH. Indeed eosinopenia has been used as a bioassay tool for ACTH.

Arora and Lahiri (7) have presented data to show that the metabolism of histamine is intimately associated with the adrenal glands. Rose and Brown (34) have demonstrated that adrenalectomized animals become very sensitive to the toxic effects of histamine. After adrenalectomy the histamine level rises in the blood as well as in several other tissues; for example, the gut lining. Moreover, Houssay (23) has demonstrated that hypophysectomy also sensitized animals to the toxic action of histamine, although in a lesser degree than adrenalectomy. Guillemin (20) and Fleming and Geierhass (15) have shown that an increased sensitivity to histamine is not only an indication of an increase in histamine, but may also be due to a lack of corticoids and a deficiency in body catecholamines. In this respect, whole-body irradiation has been
shown by a number of workers (14, 16, 22, 26) to stimulate a biphasic plasma corticoid response in rats. The first elevation in the glucocorticoids observed in this study at 5 hours post-irradiation might be due to the depressing action of the glucocorticoids on histamine levels. Thus, when the circulating corticosterone is at a high level, the histamine level decreased in the plasma.

The initial increase in plasma histamine that was noted at 1 and 3 hours post-irradiation fits very well with Weber and Steggerda's (42) data on blood pressure drops in rats at the second and third hours post-irradiation. The very effective vasodilator, histamine, has been shown to cause a pronounced drop in blood pressure when injected into the blood stream. The blood pressure in X-irradiated rats has also been shown to return to normal after the first reaction. This would seem to follow very closely the plasma histamine response to whole-body X-irradiation noted in this investigation.

This study has shown that the irradiated animals demonstrated a significant and sustained decrease in adrenal histamine at 1, 3, and 5 hours post-irradiation. This decrease occurred during the same period at which the plasma histamine was at its highest level after irradiation. One must remember, however, that the relative concentration of the tissue histamine is drastically higher than that found in the plasma. This is probably due to the greater number of mast cells in the tissues as compared with the blood, or it may be due to
changes in the release and/or binding of histamine in the blood and tissues. The diminution of histamine of the adrenal glands might be explained by the observations of Leinweber and Braun (24). These workers found that there is a suppression of histidine decarboxylase activity of rat tissues under moderate to severe stress. With this suppression there would be a decreased production (release?) of histamine from histidine in the tissues. On the other hand, the initial rise in plasma histamine may be due to the fact that X-irradiation brings about a faster release and/or production of histamine than corticoid production; therefore, the depressant action of the corticoids could not be manifested until they were in sufficient quantities by the 3-5 hours post-irradiation.

The early symptoms in the radiation syndrome are similar to that of the histamine response in some animals. One might, therefore, conjecture that when the histamine levels were decreased in both the blood and adrenal tissues post-radiation and during an eosinopenic period, one would expect to see a pronounced histamine response by the animal in toto, at least clinically.

The question then arises as to whether one might use plasma histamine analysis as a radio-biological indicator tool and/or a prognostic tool in dealing with animals, including humans, exposed to ionizing radiation.
SUMMARY

This study concerned the effects of a lethal dose (1380 r) of X-irradiation on the histamine response in rats. Parameters measured were (1) plasma and adrenal tissue histamine determined fluorometrically; (2) adrenal weights; (3) circulating eosinophils using Pilot's method; and (4) total red and white blood cell counts. Blood samples were taken prior to and at various intervals post-irradiation.

It was found that

(1) the plasma histamine response was triphasic, id est an increase at one and three hours post-radiation, a decrease at five and nine hours post-radiation, and a return to control levels by the twenty-fourth hour post-radiation;

(2) the adrenal tissue histamine response, on the other hand, was biphasic, id est a decrease at the one, three, five, and nine hours post-radiation and a return to control levels at the twenty-fourth hour;

(3) a slight, but sustained, hypertrophy of the adrenal glands occurred;

(4) an immediate, pronounced, and sustained eosinopenia developed;

(5) no change in erythrocyte count was evident; and
(6) a slight leucocytosis was observed at the third hour post-radiation.

The rise in plasma histamine was explained on the basis of a possible delayed corticoid production. The observed decrease in adrenal histamine was explained on the basis of a possible depression of histidine carboxylase activity in various tissues and cells including eosinophils and mast cells.

The data in this study indicate (1) a definite histamine response to X-irradiation that may be used as a radio-biological indicator tool; (2) that the initial symptoms in the radiation syndrome may be histaminic in nature; and (3) that histamine may play an important role in the action of the adrenal-pituitary axis following X-irradiation.
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