The Effects of Total Body Proton Irradiation on the Peripheral Plasma Corticosterone Concentration and the Ultrastructure of the Zona Fasciculata in Mus musculus

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APPROVAL SHEET

This thesis is submitted in partial fulfillment

of the requirements for the degree of

Master of Arts

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ABSTRACT

Total body proton irradiation of mice produced two distinct elevations of the peripheral plasma corticosterone concentration; the first occurred within a few hours of irradiation, and the second occurred after several days. Specifically, when compared to the corresponding controls: animals treated with a dose of 850 rads showed a 37% elevation of plasma corticosterone at 1.5 h after irradiation with the second elevation of 30% at 288 h and 45% at 360 h; animals treated with a dose of 540 rads showed a 20% elevation at 3 h with the second elevation of 15% at 96 h. At all other sample times, there were no significant differences in the peripheral plasma corticosterone concentrations observed between the irradiated animals and their respective controls. The increment increase in peripheral plasma corticosterone in the irradiated animals was shown to be directly proportional to the irradiation dose. The time prior to the onset of the first increase in peripheral plasma corticosterone was inversely proportional to the irradiation dose whereas the time prior to the onset of the second increase in peripheral plasma corticosterone appeared directly proportional.

Adrenal zona fasciculata tissue from the experimental animals irradiated with a dose of 850 rads was examined with the electron microscope. The electron opaque matrix normally found within the lipoid inclusions had disappeared at 1.5 h and only structures which appeared to be coalesced membranes remained. The number of cytoplasmic vesicles increased. 24 h after irradiation, protuberances of the mitochondria were noted. The lipoid inclusions' matrices were still electron transparent. 72 h after irradiation, fragmentation and disruption of the inner mitochondrial membranes was observed and it could not be determined if some of the resulting structures were either altered mitochondria or lipoid inclusions. The outer mitochondrial membrane appeared to be transformed into cytoplasmic vesicles. From 96 h to 240 h the lipoid inclusions and mitochondria appeared increasingly similar to the corresponding organelles observed in the control zona fasciculata. At 288 h and 360 h the lipoid inclusions' matrices were again electron transparent. The fluctuation in electron density of the lipoid inclusions' matrices correlated with the observed changes in the peripheral plasma corticosterone concentrations.
THE EFFECTS OF TOTAL BODY PROTON IRRADIATION
ON THE PERIPHERAL PLASMA CORTICOSTERONE CONCENTRATION
AND THE ULTRASTRUCTURE
OF THE ZONA FASCICULATA IN MUS MUSCULUS
INTRODUCTION

Glucocorticoids are primarily secreted from the adrenocortical zona fasciculata and have an overall metabolic function of maintaining and promoting gluconeogenesis in addition to inducing eosinopenia, lymphopenia, and anti-inflammatory responses. Corticosterone, the major glucocorticoid synthesized within the mouse adrenal cortex (Turner, 1971), maintains plasma glucose homeostasis by coordinating hepatic and peripheral tissue metabolism. Corticosterone stimulates the production and release of peripheral gluconeogenic precursors. These precursors, fatty acids and glycerol from adipose tissue and amino acids and lactate from muscle tissue, are released into the circulatory system and taken up by the liver. Corticosterone stimulates the de novo biosynthesis of key gluconeogenic enzymes while an increased hepatic substrate pool insures sufficient amino acids from which to synthesize the enzymes. The free fatty acids absorbed by the liver inhibit glycolytic enzymes which oppose gluconeogenesis (Weber, 1968).

Total body irradiation, activating the pituitary-adrenal axis, elicits pituitary ACTH and subsequent glucocorticoid secretion (Binhammer & Crocker, 1963). Because of the diversified and powerful physiological effects of the
glucocorticoids, any fluctuation in the plasma concentration of these hormones would most probably influence the overall reaction of the organism to irradiation. Changes in adrenocortical cholesterol and ascorbic acid concentrations have been utilized as indices of the functional activity of the adrenal cortex. Cholesterol is the substrate for corticosterone synthesis whereas the ascorbic acid concentration is decreased during this process. Attempts to elucidate the effects of irradiation upon the adrenal cortex using these indirect methods have produced inconclusive results. Betz (1956) and Bacq and Fischer (1957) reported observing a biphasic decrease in both rat adrenal cholesterol and ascorbic acid concentrations following total body x-irradiation. However, North and Nims (1949), Hochman and Block-Frankenthal (1953) and Zhorno (1956) observed only an initial cholesterol and ascorbic acid decrease. Changes in the glucocorticoid function of the adrenals following the administration of exogenous ACTH or irradiation may be reliably evaluated only by determining the plasma glucocorticoid concentration (Ivanenko, 1965). Methods for the determination of plasma glucocorticoid concentration have been reviewed by Braunsberg and James (1961) and more recently by Van der Molen (1970). Fluorometric and spectrophotometric techniques frequently lack the sensitivity to measure steroids in physiological concentration. Gas-chromatographic procedures are very sensitive but require extensive and intricate manipulation. The isolation and characterization of corticosteroid-binding
globulin (CBG, transcortin) as an L-glycoprotein, containing hexose, hexosamine, fucose, and sialic acid (Bush, 1957; Daughaday, 1958a, b; Upton & Bondy, 1958; Slaunwhite & Sandberg, 1959) made feasible the development of an assay for corticoids (Murphy, 1967). CBG or transcortin is the most specific and prominent of the binding proteins. The test relies on the principle that a radioactive steroid competes with endogenous corticosterone for the binding sites of CBG. Dog plasma CBG, because of its high combining affinity and specificity for corticosterone, is used to bind tritiated corticosterone. When placed in solution, the unlabeled endogenous corticosterone in the sample extract quantitatively displaces the tritiated corticosterone. The competitive protein-binding assay is highly sensitive, especially in nanogram/ml ranges and is unaffected by many substances which interfere with the colorimetric and fluorometric methods.

Two distinct maximal elevations of corticosterone estimated by the fluorometric method, the first occurring within a few hours of irradiation and the second occurring after several days, were reported for rats and mice following x-irradiation (Van Cauwenberge et al., 1957), β-irradiation (Moroz and Dubrovin, 1968), and γ-irradiation (Moroz and Pinelis, 1968). However, there has been no report concerning the effects of proton irradiation upon the peripheral plasma corticosterone concentrations. Particle irradiation with its increased LET would most probably affect a greater degree of irradiation induced damage to critical organs.
Fine structural alterations in adrenocortical mitochondria and lipoid inclusions have been observed following the administration of exogenous ACTH and hydrocortisone and irradiation. Lever (1956 a) noted that the outer mitochondrial membranes in the rat appeared polylaminar following exogenous ACTH administration. Lever (1956 b) observed openings through the adrenocortical outer mitochondrial membranes in hypophysectomized rats given exogenous ACTH. Sabatini, De Robertis, and Bleichmar (1962) noted protrusions from the limiting mitochondrial membranes of hypophysectomized rats which had also been administered ACTH and speculated that the precursor of the glucocorticoid or the hormones themselves were first synthesized in the mitochondria and then released into the cytoplasm. Mitochondria are considered the probable sites of corticosteroidogenesis because four key steroidogenic enzymes essential for the conversion of cholesterol to corticosterone (20α hydroxylase, 22R hydroxylase, desmolase, and 11β hydroxylase) have been isolated from zona fasciculata mitochondrial fractions (Roberts and Creange, 1968). Hydrocortisone treated mice demonstrated an increase of depleted lipoid inclusion vacuoles in the zona fasciculata and zona reticularis (Zelander, 1959) and ACTH treated human adrenal glands demonstrated a decrease in the zona fasciculata lipoid material (Belt, 1960). Vacuolar formations developed in the zona fasciculata lipoid inclusions of rats administered x-irradiation (Klug, 1967). Therefore,
mitochondria and lipoid inclusions are considered to be involved in steroidogenesis.

These experiments were designed to determine the formerly unstudied effects of total body proton-irradiation upon the adrenal cortex at both the sub-lethal and lethal doses of 540 and 850 rads. The adrenocortical synthetic activity following these doses will be determined by the fluctuations in the peripheral plasma corticosterone concentrations estimated by the competitive protein binding radio assay of Murphy (1967). If increases in the peripheral plasma corticosterone concentration are observed following irradiation, they will afford the opportunity to examine with the electron microscope the fine structural alterations that concomitantly occur. Special attention will be given to the mitochondria, lipoid material, smooth endoplasmic reticulum and cytoplasmic vesicles.
MATERIALS AND METHODS

ANIMALS

340 male CDI Charles River Breeding Laboratory mice, age 70 days and weighing 34-40g, were used. The animals were housed individually in 11x7x5 inch polypropylene cages on a bedding of San-i-Cell corncob (Chesapeake Feed Co.) within a temperature (74±3°C) and light (photoperiod of 12h light, 6AM-6PM, and 12h darkness, 6PM-6AM) controlled room. All animals were maintained on a diet of Purina 50-10-C rat food and water provided ad libitum. The animals were acclimated to the conditions of the room for a period of not less than 9 days prior to the day of irradiation. To minimize "stress" responses due to variables other than irradiation, noise was kept to a minimum.

ANIMAL GROUPS

Irradiated

140 animals were administered total body 260 Mev proton irradiation at a mid line tissue dose (MTD) of 850±30 rads. Six animals were decapitated at each of the following time periods after irradiation: 1.5, 3, 5, 8, 24, 48, 72, 96, 120, 144, 192, 240, 288, and 360 h.
100 animals were administered total body 260 Mev proton irradiation at a MLD of 540±30 rads. Six animals were decapitated at each of the following time periods after irradiation: 1.5, 3, 5, 24, 72, 96, 120, 192, 240, 288, 360, and 408 h.

**Sham Irradiated**

30 mice were rotated in the holder at 12 rpm for the same period of time as those animals which received an irradiation dose of 850±30 rads. Six of the animals were decapitated at each of the following time periods after sham irradiation: 1.5, 3, 5, 8, and 24 h.

**Control**

156 mice were used as controls. 6 of the animals were decapitated at each of the sacrifice times for both doses of irradiation and for the sham irradiation.

**IRRADIATION PROCEDURES**

Total body 260 Mev proton irradiation was delivered at a rate of 96±5 rads/min to two different groups of experimental animals. The first group of animals received a dose of 850±30 rads and the second group received a dose of 540±30 rads. Dose measurements were performed using standard ionization chambers positioned along the central axis of the proton beam. Calibration of the ion chambers was according to the standard procedures utilizing a Faraday cup as the primary dose standard (Raju *et al.*, 1968). Ion chamber results were confirmed by LiF thermoluminescent dosimeters.
A specially designed revolving lucite animal holder allowed the precise positioning in the proton beam of 6 animals simultaneously. The holder was revolved at 12 rpm to insure a uniform distribution of the proton dose to each animal.

ESTIMATION OF THE PERIPHERAL PLASMA CORTICOSTERONE CONCENTRATION

Preparation of CBG solution

Heparinized dog plasma was obtained daily from a 63 kg male Irish Wolfhound and was centrifuged for 20 minutes at 2,500 rpm. A 1.25 ml aliquot of the resulting plasma was diluted to 50 ml with distilled water. This solution was dialyzed for 6 hours in seamless cellulose tubing, molecular weight retention of 8-12,000, (Union Carbide) immersed in 250 ml of Ringers solution maintained at 40°C. The dialyzate was the 2.5% CBG solution.

Preparation of the standards

The working standard solutions of non-radioactive corticosterone at concentrations of 0.01ug/ml and 0.10ug/ml were maintained in ethanol at -23°C. These were made monthly from a fresh stock standard of 10ug/ml. For each set of determinations, six standards and up to 70 samples were run in duplicate.

Extraction of samples

Following decapitation the peripheral blood was collected from each animal in a heparinized centrifuge tube. The samples were immediately centrifuged at 10°C for 20
minutes at 2,000 rpm. A 0.1 ml aliquot of the plasma in
duplicate was immediately extracted with 2.5 ml methylene
chloride (Fisher, spectroanalyzed) and frozen at -23°C. The
samples were warmed to room temperature on the day of analysis.
A 1.5 ml aliquot of distilled water was added to each vial and
the vials were gently shaken and placed in a freezer at -23°C
to freeze the water phase to insure complete separation of the
organic phase. 1 ml of the methylene chloride extract was
placed into a 75x12 mm test tube and evaporated in a 40°C dry
bath under a stream of air. All of the experimental and con­
trol samples for each treatment time were analyzed concurrently.

**Binding procedure**

1.0 ml of a 81uc/100 ml ethanol solution of 1,2-³H
corticosterone (New England Nuclear, S.A. = 1.75ug/0.25 mc)
was added to 50 ml of the 2.5% CBG solution. A 1.0 ml
aliquot of the H³-corticosterone-CBG solution was dispensed
into each sample or standard tube containing 1.0 ml of the
methylene chloride extract. The tubes were shaken on a
vortex mixer for 2 minutes and placed in a 45°C water bath
for 10 minutes to facilitate an equilibrium of the steroid
with the plasma. The tubes were removed from the waterbath,
and shaken again on a vortex mixer for 2 minutes and then
placed in an ice water bath for 10 minutes. 40 mg of
Florisil (Floridin Co., 60/100 mesh), which had been previously
washed 12 times in distilled water and then dried at 90°C and
stored at 60°C, was added to each tube and the contents shaken
for 2 minutes on a vortex mixer. The tubes were returned to
a water bath at 45°C for an additional 10 minutes to allow the Florisil to settle. A 0.5 ml aliquot of the supernatant was removed and placed in a plastic scintillation vial with 10 ml Bray's solution (Bray, 1960). The samples were counted for tritium for 10 minutes in a Nuclear Chicago Series 724 liquid scintillation counter. A standard curve was constructed by plotting the counts per minute versus the nanograms of known corticosterone standards. The experimental values were interpolated from the standard curve.

STATISTICAL METHODS

Student's t test was employed to determine significant differences between the peripheral plasma corticosterone levels of the irradiated animals and their corresponding controls. Significance was defined as the 99% level ($p < .01$) whereas, $p < .001$ was defined as highly significant.

ELECTRON MICROSCOPY

Fixation

Immediately following decapitation, both adrenal glands from each animal were excised and the surrounding fat and connective tissue removed without injury to the capsule. A center section approximately 1 mm in diameter was cut with a razor blade perpendicular to the long axis of the gland so that the medulla would be located in the center of the section and the cortex on the periphery. The sections were fixed at room temperature in a 3-4% glutaraldehyde solution buffered with 0.1M phosphate at pH 7.4. The 0.1M phosphate buffer at
pH 7.4 was used as the buffering solution for both rinsing and post-fixation. Following fixation the tissues were rinsed briefly three times then stored up to three months at 4°C in buffer. Post-fixation was accomplished in 2 hours at room temperature in a buffered 1% OsO₄ solution.

**Dehydration**

The tissues were dehydrated by rinsing briefly in 50% acetone followed by storage for 24 hours at 4°C in a 70% acetone-2% uranyl acetate solution. Complete dehydration was insured by rapidly rinsing at room temperature in graded acetone solutions.

**Infiltration and embedding**

Infiltration was accomplished in 4 days with Epon 812 epoxy resin according to the method of Luft (1961). Just prior to embedding, the tissues were cut with a razor blade into wedge-shaped sections. These sections were oriented and flat embedded in Beem capsules. The epon was polymerized by heating at 60°C for 3 days.

**Sectioning and staining**

Thin sections were obtained with a Dupont diamond knife on an LKB Ultratome III ultramicrotome. The sections were picked up by means of a non-coated 1 hole copper grid, stained 1-2 minutes with lead citrate and then transferred to a formvar coated 1 hole copper grid in the manner described by Galey and Nilsson (1966).
Observations and photography

The thin sections were examined in a Zeiss EM 9S-2 electron microscope and photographed on Agfa-Gevaert Scientia 7x7 cm film. The negatives were developed in Methanol-U developer and printed on Agfa-Gevaert Fotorite contrast paper, grades 2-4.
RESULTS

ESTIMATION OF PERIPHERAL PLASMA CORTICOSTERONE CONCENTRATIONS

Sham irradiation (Table 3)

The sham irradiated animals did not demonstrate significant increases in the peripheral plasma corticosterone concentration when compared to their corresponding controls. Therefore, all increases in the peripheral plasma corticosterone concentrations observed in the irradiated animals were attributed to the effects of proton irradiation.

Irradiation at a dose of 850±30 rads (Table 2 and Fig. 1,3)

At 1.5 h after irradiation the peripheral plasma corticosterone of the irradiated animals demonstrated a highly significant (p < .001) 37% increase in concentration when compared to the corresponding control animals. By 24 h after irradiation the peripheral plasma corticosterone concentration of the irradiated animals appeared to decrease to a value slightly below that of the control animals and at 48 h the concentration appeared to increase and remain slightly elevated until 288 h. However, none of these fluctuations were significant. A significant (p < .01) 30% increase of the peripheral plasma corticosterone of the irradiated animals was observed at 288 h. A highly significant (p < .001)
increase of 45% in the peripheral plasma corticosterone was noted at 360 h. By 288 h the animals were extremely debilitated and the majority of the animals sacrificed at this time were moribund. At 360 h all animals sacrificed appeared moribund.

Irradiation at a dose of 540±30 rads (Table 1 and Fig. 2,3)

A significant (p < .01) 20% increase of the peripheral plasma corticosterone concentration in the irradiated animals was demonstrated at 3 h when compared to the corresponding control animals. Corticosterone concentrations slightly below the control values were observed at 24 h after irradiation; however, at 96 h they were observed to increase to a highly significant (p < .001) 20% when compared to the corresponding controls. No significant differences in the peripheral plasma corticosterone concentration were observed between the irradiated animals and their respective controls from 120 h until the termination of the experiment at 408 h.

ULTRASTRUCTURAL ALTERATIONS IN THE ADRENAL ZONA FASCICULATA FOLLOWING IRRADIATION AT A DOSE OF 850±30 RADS

Ultrastructural observations of control animals (Fig. 4,5,6,7)

The observed fine structure of the non-irradiated mouse zona fasciculata was in general similar to that described by previous workers (Lever, 1955; Ashworth, Race, and Mollenhauer, 1959; Zelander, 1959; Sato, 1967). The capillaries of sinusoids which were located throughout the zona fasciculata appeared to be collapsed. The capillaries were bordered by endothelial cells which contained oblong nuclei
oriented in a direction parallel to the long axis of the capillary, oval mitochondria, free ribosomes, and secretory vesicles. A continuous periendothelial space separated the vascular endothelium from the epithelial cells. Two basement membranes were observed within this space, one bordering the epithelial plasma membrane and the other bordering the vascular endothelial plasma membrane. Cells described either as fibroblasts by Zelander (1959) and Sheridan and Belt (1964) or as macrophages by Brenner (1966) and Sato (1967) were located in the periendothelial space in addition to numerous microvilli and collagen fibrils.

Nuclei, irregular in shape and with occasional, well-marked indentations, were observed to be generally located in the center of the epithelial cell. The nuclear envelope, consisting of a double-membrane, was not continuous due to the presence of sparsely scattered nuclear pores. Peripherally located chromatin material and a single nucleolus, both highly distinguishable, were found within each epithelial cell.

Agranular or smooth endoplasmic reticulum was diffusely dispersed throughout the cytoplasm. The agranular endoplasmic reticulum was either in multi-lamellar profiles or was found singly. Occasionally, it was in juxtaposition with the mitochondria, nuclei, or lipoid inclusions. Granular or rough endoplasmic reticulum was not seen. The ribosomes were usually in the form of polysomes; however, individually
occuring ribosomes were occasionally encountered in the cytoplasm.

Numberous cytoplasmic vacuoles bound by a single membrane were observed to be diffusely situated throughout the cytoplasm. Frequently they were found in close proximity to the mitochondria and lipoid inclusions. These cytoplasmic vacuoles appeared similar to the vacuoles observed by Lever (1955) and Sato (1967), who suggested that they might possibly be a component of the Golgi apparatus. They were composed of a system of smooth membranes, small vesicles, and large vacuoles.

Abundant mitochondria demarcated by a double-membrane envelope were oval shaped and of a variety of sizes. The mitochondrial internal structures were observed to be either the rectimembraneous or tubular form or the cyclomembranous form as described in detail by Zelander (1959). Amorphous, osmiophilic intramitochondrial inclusions which appeared to be lipoid in composition were frequently visible.

Double membrane bound lipoid inclusions appeared numerous and of a variety of sizes. Irregularly layered membranous structures were observed occasionally in the periphery of the inclusions. The lipoid inclusions were often observed to be in close proximity with mitochondria or nuclei.

Bodies which corresponded to pigment bodies as described by Zelander (1959) were observed to be composed of intensely dense regions, very light areas, small dense granules, and laminated rings.
Ultrastructural observations of irradiated animals

The nuclei and its contents in both the epithelial and endothelial cells, capillaries with the associated endothelial cells, and the periendothelial space were not observed to be morphologically altered following irradiation. The number of polysomes did not appear to have fluctuated; however, quantitative studies were not performed.

1.5 h (Fig. 8,9)--The morphology of the lipoid inclusions was greatly altered as no typical lipoid inclusions with osmiophilic matrices were observed. Structures which appeared to be either altered mitochondria or lipoid inclusions were observed in great quantity. These structures, which were termed vacuoles, consisted of electron transparent spaces which contained an osmiophilic substance usually located adjacent to the surrounding membrane. The major portion of this osmiophilic substance appeared to be membranous in nature whereas the remainder appeared as a homogeneous matrix. The size of the vacuoles was not uniform but tended to be slightly larger than that observed for control lipoid inclusions. A few vacuoles appeared to consist of two components. These components may have been either the product of fusion or might possibly represent vacuoles which were becoming subdivided. The number of mitochondria was greater than that observed for control zona fasciculata. Agranular endoplasmic reticulum, both the amount found singly and that associated with mitochondria and lipoid inclusions
or vacuoles, was increased. Cytoplasmic vesicles were observed in greater quantity.

24 h (Fig. 10,11,12)--The electron opaque matrix of the vacuoles was decreased in volume more than that observed at 1.5 h. The membranous structures were still evident; however, they were not in the quantity observed previously. A separation of the outer mitochondrial membrane away from the inner mitochondrial membrane produced intramitochondrial sinus-like spaces or vacuoles which gave the mitochondria a swollen appearance. Concomitantly, within the mitochondria the osmiophilic ground substance or matrix increased in volume and density. The outer mitochondrial membrane completely disappeared from a few mitochondria and the intramitochondrial structures appeared to be dispersed in the cytoplasm; this created the erroneous impression of increased cytoplasmic vesicles. The amount of agranular endoplasmic reticulum was less than the amount apparent at 1.5 h. Photography of the irradiated cells was hindered by the poor resolution and contrast of all structures.

48 h (Fig. 13,14)--Typical lipoid inclusions still were not observed. An extremely electron opaque band was observed on the rim of the vacuoles and the encompassed membranous structures became more osmiophilic and appeared to have coalesced. Many vacuoles had small protuberances. These protuberances appeared to have been either cytoplasmic vesicles which had fused with the vacuole or future cytoplasmic vesicles which had not completely budded from the
vacuole. Many of the vacuoles were abutted and appeared to have fused. The internal mitochondrial membranes were disrupted and produced intramitochondrial vacuoles of varied sizes. The amount of osmiophilic mitochondrial matrix decreased from that observed previously. The amount of both cytoplasmic vesicles and endoplasmic reticulum decreased. The resolution of all structures remained poor.

72 h (Fig. 15, 16)—The outer mitochondrial membrane was disrupted and appeared to have been directly transformed into cytoplasmic vesicles. The extensive inner mitochondrial membrane disruption produced intramitochondrial vacuoles. The amount of both electron opaque matrix and membranous substance observed within the vacuoles increased. At 1.5 h and 24 h it was assumed that the vacuoles were most probably altered lipid inclusions and not altered mitochondria. This assumption was based on the observation that both normal mitochondria were still present while typical lipid inclusions were not observed. However, because the mitochondria were so altered at 72 h it was impossible to determine if the structures termed vacuoles were truly altered mitochondria or lipid inclusions. No lipid inclusions characteristic of the control tissue were observed. The amount of endoplasmic reticulum and cytoplasmic vesicles was greatly decreased. Resolution was better than that obtained for the 48 h tissue; however, it was poorer than that obtained from control tissue.

96 h (Fig. 17)—The matrices of the lipid inclusions, excluding small vacuolar areas, were electron opaque. The
integrity of both the inner and outer mitochondrial membrane was observed; however, electron transparent sinus-like spaces were observed within the mitochondria. The intramitochondrial matrix was less electron opaque than that observed within the mitochondria of the control zona fasciculata. The matrices of the vacuoles with the exception of small circular areas were electron opaque. Membranous structures were still observed within a few vacuoles. Typical osmiophilic lipid inclusions were observed in a small quantity. Resolution was almost equal to that observed from the control tissue.

120 h (Fig. 18)—The integrity of both the interior and exterior mitochondrial membranes was observed. The matrices of the vacuoles were generally electron opaque; however, small electron transparent areas still existed. The amount of both agranular endoplasmic reticulum and cytoplasmic vesicles was approximately equal to that observed in control tissue. Resolution was comparable to that obtained from control tissue.

144, 192, & 240 h (Fig. 19, 20)—These three time periods were grouped together because no essential differences either in the fine structure of the cytoplasm or in the organelles were observed in the respective tissues. The organelles, other components of the cytoplasm, and their respective resolution were similar to those found in the control zona fasciculata. The only distinctive difference was the lipid inclusions. One end of a few of the inclusions
was vacular and membrane bound vesicles appeared within these vacuoles; however, typical osmiophilic lipoid inclusions were also observed.

288 h (Fig. 21,22)—Contrary to the previous three observation periods, typical osmiophilic lipoid inclusions were not observed. Vacuoles similar to those observed at 48 h appeared. The integrity of both the inner and outer mitochondrial membranes was preserved; however, there was an increase in the density of the osmiophilic intramitochondrial matrix. Endoplasmic reticulum and cytoplasmic vesicular structures increased in number. Resolution of all structures was poor.

360 h (Fig. 23,24)—The extensive disruption of the internal mitochondrial membrane produced numerous intra-mitochondrial vacuoles. The disruption of the outer mitochondrial membrane produced numerous cytoplasmic vesicles. As evidenced at 1.5 h the number of mitochondria increased. No typical osmiophilic lipoid inclusions were observed. Vacuoles, which no longer contained the thick osmiophilic rim, were observed in great quantity. Some osmiophilic membranous structures were encompassed by the vacuoles. The amount of both endoplasmic reticulum and cytoplasmic vesicles was greatly increased. The resolution of all structures was poor.
Legend for Table 1

Changes in the peripheral plasma corticosterone concentration following total body proton irradiation at a dose of 540 rads. The plasma corticosterone concentration of the irradiated animals and corresponding control animals at each time are expressed as mean values ± S.E. The plasma mean corticosterone concentrations of the irradiated animals are expressed as a percentage of the mean plasma corticosterone concentration of the corresponding control animals.
TABLE 1

Peripheral plasma corticosterone concentrations
Mean ug/100ml plasma

<table>
<thead>
<tr>
<th>Time following Irradiation</th>
<th>Control</th>
<th>Irradiated</th>
<th>Percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>30.8 ± 1.3</td>
<td>32.1 ± 1.3</td>
<td>5</td>
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<td>(12)</td>
<td>(12)</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>29.7 ± 0.9</td>
<td>36.9 ± 1.8</td>
<td>20*</td>
</tr>
<tr>
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<td>(12)</td>
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<tr>
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<td>30.8 ± 1.3</td>
<td>4</td>
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<tr>
<td>(12)</td>
<td>(12)</td>
<td></td>
<td></td>
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<tr>
<td>24</td>
<td>32.5 ± 1.1</td>
<td>32.0 ± 1.0</td>
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<tr>
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</tr>
<tr>
<td>72</td>
<td>30.4 ± 1.0</td>
<td>31.8 ± 0.9</td>
<td>4</td>
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<tr>
<td>(12)</td>
<td>(12)</td>
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<tr>
<td>96</td>
<td>31.2 ± 0.7</td>
<td>36.5 ± 1.2</td>
<td>15**</td>
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<tr>
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<td>(12)</td>
<td></td>
<td></td>
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<tr>
<td>120</td>
<td>30.4 ± 1.2</td>
<td>32.8 ± 1.1</td>
<td>8</td>
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<tr>
<td>(12)</td>
<td>(12)</td>
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<tr>
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<tr>
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<tr>
<td>288</td>
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<tr>
<td>408</td>
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<td>29.3 ± 1.0</td>
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<td>(12)</td>
<td>(12)</td>
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</tbody>
</table>

Number in parentheses refers to the number of determinations for the group directly above

**p < .001 and *p < .01 with respect to the corresponding control values
Legend for Table 2

Changes in the peripheral plasma corticosterone concentration following total body proton irradiation at a dose of 850 rads. The plasma corticosterone concentration of the irradiated and corresponding control animals at each time are expressed as mean values ± S.E. The irradiated animals' mean plasma corticosterone concentration at each time is expressed as a percentage of the mean plasma corticosterone of the corresponding control animals.
TABLE 2

Peripheral plasma corticosterone concentrations
Mean ug/100ml plasma

<table>
<thead>
<tr>
<th>Time following Irradiation</th>
<th>Control</th>
<th>Irradiated</th>
<th>Percent change</th>
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<tbody>
<tr>
<td>1.5</td>
<td>30.5 ± 1.5</td>
<td>48.4 ± 2.2</td>
<td>37**</td>
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<td>3</td>
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<td>5</td>
<td>32.3 ± 1.8</td>
<td>33.0 ± 2.5</td>
<td>3</td>
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<td>(12)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>32.5 ± 1.6</td>
<td>35.4 ± 1.7</td>
<td>9</td>
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<td>(12)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>29.6 ± 1.6</td>
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<td>-5</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>28.7 ± 1.6</td>
<td>31.7 ± 1.9</td>
<td>10</td>
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<td>(12)</td>
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<tr>
<td>72</td>
<td>29.1 ± 1.6</td>
<td>32.1 ± 1.7</td>
<td>10</td>
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<td>(12)</td>
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</tr>
<tr>
<td>96</td>
<td>25.8 ± 1.7</td>
<td>29.2 ± 1.6</td>
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<tr>
<td>120</td>
<td>28.2 ± 1.7</td>
<td>32.9 ± 1.5</td>
<td>15</td>
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<td>(12)</td>
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<tr>
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<td>(12)</td>
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</tr>
<tr>
<td>192</td>
<td>29.9 ± 1.3</td>
<td>34.4 ± 1.6</td>
<td>14</td>
</tr>
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<td></td>
<td>(12)</td>
<td>(12)</td>
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<tr>
<td>240</td>
<td>29.4 ± 1.5</td>
<td>34.3 ± 1.9</td>
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<td></td>
<td>(12)</td>
<td>(12)</td>
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<tr>
<td>288</td>
<td>32.0 ± 1.8</td>
<td>45.7 ± 3.7</td>
<td>30*</td>
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<td></td>
<td>(12)</td>
<td>(12)</td>
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<tr>
<td>360</td>
<td>28.4 ± 1.0</td>
<td>50.8 ± 2.4</td>
<td>45**</td>
</tr>
<tr>
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<td>(12)</td>
<td>(12)</td>
<td></td>
</tr>
</tbody>
</table>

Number in parentheses refers to the number of determinations for the group directly above.

**p < .001 and *p < .01 with respect to the corresponding control values.
Legend for Table 3

Changes in the peripheral plasma corticosterone concentration following sham-irradiation. The plasma corticosterone concentration of the sham-irradiated animals and corresponding control animals at each time are expressed as mean values ± S.E.
TABLE 3

Peripheral plasma corticosterone concentrations
Mean ug/100ml plasma

<table>
<thead>
<tr>
<th>Time following Irradiation</th>
<th>Control</th>
<th>Sham Irradiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 (12)</td>
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<td>28.7 ± 1.5 (12)</td>
</tr>
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<td>3 (10)</td>
<td>29.9 ± 2.0</td>
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</tr>
<tr>
<td>8 (12)</td>
<td>32.5 ± 1.6</td>
<td>32.8 ± 1.3 (12)</td>
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<tr>
<td>24 (12)</td>
<td>29.6 ± 1.6</td>
<td>28.7 ± 1.3 (12)</td>
</tr>
</tbody>
</table>

Number in parentheses refers to the number of determinations for the group directly above.
Legend for Figure 1

Changes in the peripheral plasma corticosterone concentration following total body proton irradiation at a dose of 850±30 rads. The plasma corticosterone concentrations are expressed as mean values ± S.E. Control animals are represented by the broken line.
Legend for Figure 2

Changes in the peripheral plasma corticosterone concentration following total body proton irradiation at a dose of 540±30 rads. The plasma corticosterone concentrations are expressed as mean values ± S.E. Control animals are represented by the broken line.
Legend for Figure 3

Changes in the peripheral plasma corticosterone concentration following total body proton irradiation at doses of 850 and 540±30 rads. The mean plasma corticosterone concentrations of the irradiated animals at each time are expressed as a percentage of the mean plasma corticosterone concentration of the corresponding control animals. The animals irradiated with a dose of 540 rads are represented by the broken line.
### KEY TO STRUCTURES CITED

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Chromatin Material</td>
</tr>
<tr>
<td>CF</td>
<td>Collagen Fibrils</td>
</tr>
<tr>
<td>CV</td>
<td>Cytoplasmic Vesicles</td>
</tr>
<tr>
<td>enB</td>
<td>Endothelial Basement Membrane</td>
</tr>
<tr>
<td>epB</td>
<td>Epithelial Basement Membrane</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic Reticulum - Agranular</td>
</tr>
<tr>
<td>G</td>
<td>Golgi</td>
</tr>
<tr>
<td>L</td>
<td>Lipoid Inclusion</td>
</tr>
<tr>
<td>M</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>mv</td>
<td>Microvilli</td>
</tr>
<tr>
<td>N</td>
<td>Nucleus</td>
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<tr>
<td>NP</td>
<td>Nuclear Pore</td>
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<td>Nu</td>
<td>Nucleolus</td>
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<tr>
<td>P</td>
<td>Polysome</td>
</tr>
<tr>
<td>PES</td>
<td>Periendothelial Space</td>
</tr>
<tr>
<td>R</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>SV</td>
<td>Secretory Vesicles</td>
</tr>
<tr>
<td>V</td>
<td>Vacuole</td>
</tr>
</tbody>
</table>
Legend for Figure 4

Control zona fasciculata. A nucleus with nuclear pores, chromatin material, and nucleolus can be seen. Golgi is also located in the cytoplasm. X 14500.

Legend for Figure 5

Control zona fasciculata. The epithelial and endothelial basement membranes can be seen in the peri-endothelial space. Two red blood cells are located in the capillary. X 13500.
Legend for Figure 6

Control zona fasciculata. Collagen fibers and microvilli can be seen. Note the lipid inclusions with the internal membranous structures. X 14500.

Legend for Figure 7

Control zona fasciculata. Numerous rectimembranous mitochondria and lipid inclusions with osmiophilic matrices are observed. Cytoplasmic vesicles and polyribosomes are also noticeable. X 28500.
Legend for Figure 8

Zona fasciculata 1.5 h after 850±30 rad proton irradiation. Vacuoles with electron transparent spaces and osmiophilic membranous structures are shown. Agranular endoplasmic reticulum is associated with vacuoles. X 14500.

Legend for Figure 9

Zona fasciculata 1.5 h after 850±30 rad proton irradiation. Mitochondria with decreased osmiophilic ground matrix and intact outer and inner membranes can be seen. X 36000.
Legend for Figure 10

Zona fasciculata 24 h after 850±30 rad proton irradiation. Matrices of the numerous vacuoles are more electron transparent than at 1.5 h. X 5000.

Legend for Figure 11

Zona fasciculata 24 h after 350±30 rad proton irradiation. Note the protuberance of the outer mitochondrial membrane. The mitochondrial matrix has an increased osmiophilic ground matrix. X 19000.

Legend for Figure 12

Zona fasciculata 24 h after 850±30 rad proton irradiation. Note the swollen appearance of the mitochondria. X 23750.
Legend for Figure 13

Zona fasciculata 48 h after 850±30 rad proton irradiation. Vacuoles have an extremely electron opaque band on their periphery. X 28500.

Legend for Figure 14

Zona fasciculata 48 h after 850±30 rad proton irradiation. Vacuoles as described in Fig. 13 are seen. Note the cytoplasmic vesicle attached to the vacuole. X 24000.
Legend for Figure 15

Zona fasciculata 72 h after 850±30 rad proton irradiation. The outer and inner mitochondrial membranes are disrupted. Outer mitochondrial membranes appear to be transformed into cytoplasmic vesicles. X 24000.

Legend for Figure 16

Zona fasciculata 72 h after 850±30 rad proton irradiation. Vacuoles which may be either altered mitochondria or lipid inclusions are seen. X 24500.
Legend for Figure 17

Zona fasciculata 96 h after 850±30 rad proton irradiation. Lipoid inclusions' matrices are electron opaque. Membranes of the mitochondria are intact but intra-mitochondria sinus-like spaces are seen. X 11500.

Legend for Figure 18

Zona fasciculata 120 h after 850±30 rad proton irradiation. Lipoid inclusions have electron opaque matrices. X 24000.
Legend for Figure 19

Zona fasciculata 144 h after 850±30 rad proton irradiation. Mitochondria and lipid inclusions are similar to those observed in control tissue. X 18500.

Legend for Figure 20

Zona fasciculata 192 h after 850±30 rad proton irradiation. Lipoid inclusions have membranes within a vacuolar space. X 13500.
Legend for Figure 21

Zona fasciculata 288 h after 850±30 rad proton irradiation. Vacuoles similar to 48 h can be seen. The mitochondrial membranes are intact. Note the overall poor resolution of the cells. X 14500.

Legend for Figure 22

Zona fasciculata 288 h after 850±30 rad proton irradiation. Vacuoles with osmiophilic rims can be observed. Mitochondria have increased osmiophilic ground matrix. X 31550.
Legend for Figure 23

Zona fasciculata 360 h after 850±30 rad proton irradiation. Vacuoles have lost osmiophilic matrices and the mitochondrial ground matrix is more electron opaque. X 5220.

Legend for Figure 24

Zona fasciculata 360 h after 850±30 rad proton irradiation. Note mitochondrial membrane disruption and numerous vacuoles. X 25500.
DISCUSSION

Total body proton irradiation elicited adrenocortical responses which consisted of two distinct elevations of the peripheral plasma corticosterone concentration. The first elevation occurred within a few hours of irradiation and the second elevation occurred after several days. The time of onset of the first elevation appeared inversely proportional to the irradiation dose whereas the time prior to the second response appeared directly proportional to the irradiation dose. The magnitude of both the first and second adrenocortical responses was directly proportional to the irradiation dose.

Patt et al. (1948) and Hameed and Haley (1964) demonstrated that hypophysectomy prevented the initial adrenocortical response after irradiation and speculated that the initial response was a hypothalamo-hypophysial-adrenal response. The hypothesis that irradiation activated the hypothalamo-hypophysial-adrenal axis is supported by Bacq and Fischer (1957) who prevented the initial adrenocortical response by using a hypothalamic inhibitor of nembutal and morphine. The participation of the hypothalamus and/or pituitary in mediating the adrenal response was corroborated
by the demonstration that irradiation caused a release of ACTH from the adenohypophysis (Binhammer and Crocker, 1963). However, the mechanism by which the hypothalamo-hypophysial-adrenal axis is activated has not been elucidated. The compensatory adrenal hypertrophy usually observed following unilateral adrenalectomy was inhibited by localized head irradiation and Mirand and Hoffman (1956) speculated that the irradiation directly affected either the pituitary or the hypothalamus. Mateyko and Edelmann (1954) locally irradiated the pituitary of rats and observed a subsequent increase in hypophysial ACTH concentration. These workers believed that these results indicated that the adrenocortical response was the result of a direct and specific effect of irradiation on the pituitary. Contrary to these hypotheses, Bacq et al. (1960) observed that the adrenocortical response following irradiation was prevented by hypothalmic lesions and speculated that the hypothalamus was involved in the adrenal response. However, a non-specific nature of the stimulation mechanism was demonstrated by Langendorff and Lorenz (1950) who showed that adrenocortical responses following body irradiation with the head shielded were comparable to the responses following total body irradiation.

A non-specific neuroendocrine reaction to irradiation was first described by Selye (1950) and termed the "stress adaptation" syndrome. This concept asserts that any stress-ful agent such as hemorrhage, trauma, infectious disease, surgical operation, cold, ionizing irradiation, and ultra-
violet radiation will activate the hypothalamo-hypophysial-adrenal axis either by direct stimulation of the hypothalamic nerve center or through an epinephrine mediated response (Casarett, 1968). In the present studies, the first increase in the peripheral plasma corticosterone concentration following irradiation at both doses was probably the result of a general stress adaptation syndrome. The resultant augmented secretion of ACTH from the adenohypophysis elicited the increased secretion of corticosterone from the adrenal cortex. This hypothesis is consistent with the observation that the peripheral plasma corticosterone concentration returned to control values by 24 h following irradiation at both doses. Because the proton irradiation was not a continuous stress, the high titers of circulating corticosterone probably inhibited continued secretion of ACTH via a hypophysial mediated simple negative feedback mechanism.

The onset and magnitude of the second and terminal adrenocortical response was variable following irradiation at a dose of 850 rads. The variation of onset gave the erroneous impression that two distinct second adrenocortical responses occurred, one at 288 h and the other at 360 h. All animals which demonstrated a high peripheral plasma corticosterone concentration at these two times appeared moribund just prior to being sacrificed.

The second increase in the peripheral plasma corticosterone concentrations following proton irradiation at a dose of 850 rads most probably was a compensatory response.
The hypophysial-adrenal axis was probably activated by proton induced biochemical and physiological alterations characteristic of the gastrointestinal (GI) and hematopoietic syndromes. These syndromes observed after irradiation at lethal doses are the result of the destruction of the reproductive capacity of the progenitor cells for the functional hematopoietic tissue and gastrointestinal mucosa (Patt and Quastler, 1963). The functional villus mucosa cells are produced in the crypts and migrate by way of the crypt mouth and villus epithelium to the villus tip with the eventual extrusion of the mucosa cells into the intestinal lumen (Lesher, Fry and Cohn, 1961). The destruction of the mitotic ability of the intestinal crypt cells by irradiation would result eventually in the thinning of the functional mucosa. If the irradiation dose was sufficient to produce edema of the connective tissue, the resultant restriction of the vascular supply might produce denudation of the existing functional mucosa. Both the thinning or denuding of the intestinal mucosa impede the glucose, thiamine, pyridoxine (Detrick et al., 1962) and intrinsic factor-bound B-12 (Okuda and Fujii, 1968) intestinal uptake. Additionally, the denudation inhibits the intestinal reabsorption of water which diffuses into the lumen from the capillaries and the resultant dehydration and electrolyte imbalance contribute to vascular collapse and hemoconcentration. The decrease in the absorption of nutrients and fluids alters the blood glucose levels, electrolyte balance, and fluid volume. All of these alterations are probably detected by the hypothalamus and
thereby promote appropriate compensatory adrenal adjustment via adenohypophysial ACTH.

Lesher and Sallese (1966) reported that conventional irradiation at doses up to 1000 rads was not sufficient to suppress the repair-recovery ability of the crypt cells and induce denudation of the villi. Subsequently, only a slight decrease in glucose absorption and total water balance are observed (Bromfield and Dykes, 1964). These observations for conventional irradiation do not preclude the possibility that proton irradiation at doses of 850 rads might arrest the cryptic mitotic activity. Denudation of the villi and the resultant hemoconcentration and decreased plasma glucose are feasible at these doses because proton irradiation has been demonstrated to be more effective in producing the GI syndrome than conventional irradiation (Ashikawa et al., 1965; Sondhaus et al., 1965).

The primary causes of death in mammals exposed to ionizing irradiation at doses between 200 and 1000 r are bacterial septicemia and hemorrhage. These conditions are the result of bone marrow hyperplasia and the resultant neutropenia and thrombopenia, respectively. The vascular structure of the marrow is disrupted soon after x-irradiation at a dose of 1000 r and the marrow becomes aplasic with severe cellular depletion several days after irradiation (Bond et al., 1964). Hemorrhage due to increased vascular fragility and decreased circulating platelets occurs in nearly all mammals irradiated at a dose of 450 r (Bond,
Fliedner, and Archambeau, 1965). Compared with conventional irradiation, proton irradiation produces hemorrhage earlier and more extensively (Dalrymple, 1966) and induces a greater leukocyte depression (Taketa, 1964). Therefore, resultant hemoconcentration, plasma fluid volume decrease, and bacterial septicemia are more likely to occur following proton irradiation. Subsequently, compensatory activation of the hypothalamo-hypophysial-adrenal axis is more likely to occur. Most probably, the GI and hematopoietic syndromes produced by proton irradiation at a dose of 850 rads synergistically activate the hypothalamo-hypophysial-adrenal axis. This hypothesis is consistent with the observation of other workers who also reported a correlation of a second increase of the peripheral plasma corticosterone concentration with acute hematopoietic symptoms (Betz, 1956; Hameed and Haley, 1964; Fedotov, Moroz, and Pantyushina, 1967).

It is most likely that morphological alterations of the central nervous system (CNS) were not induced by the proton irradiation because the doses used in these studies were greatly below the 5000 r threshold reported necessary to induce CNS damage (Stahl, 1962). Additionally, the immediate death characteristic of the CNS syndrome was not observed. The involvement, however, of the CNS in mediating the effects of the irradiation must be recognized. Calvo (1967) has speculated that the nervous system is responsible for the sinusoidal distension in bone marrow which is followed by diapedesis of red cells and granulocytosis in bone marrow
parenchyma observed after irradiation. Additionally, bio-
chemical alterations of the CNS might have been produced
which contributed to the observed alterations.

Total body proton irradiation at a dose of 850 rads
colosed ultrastructural alterations in the mouse adrenocortical
zona fasciculata. The sequential ultrastructural alterations
in the lipoid inclusions and the mitochondria and the fluctu-
atations in the amount of endoplasmic reticulum and cytoplasmic
vesicles were observed to occur concomitantly with both
periods of increased corticosterone secretion. The most
prominent ultrastructural alteration observed at both 1.5 h
and 288 h was the total absence of lipoid inclusions with
osmiophilic matrices. The exact role of lipoids in the pro-
cess of corticosteroidogenesis has not been elucidated.
However, lipoid inclusions have been observed extensively in
steroid producing cells. The lipoid content of the zona
fasciculata has been observed to increase during periods of
relative inactivity (Selye and Stone, 1950; Bachmann, 1954;
Borowiez, 1965). Vacuoles which appeared to be altered
lipoid inclusions were observed in great quantity and con-
sisted of membrane-bound electron transparent spaces with
peripherally located electron opaque material. If the
vacuoles were truly altered lipoid inclusions, then the
majority of the osmiophilic lipoid matrix had been depleted.
Concomitantly with this apparent lipoid depletion, an increase
in the number of mitochondria, an increase in the amount of
agranular endoplasmic reticulum associated with mitochondria, and a decrease in the electron opaqueness of the intramitochondria matrices were observed. These ultrastructural alterations suggest a mechanism by which the process of increased synthesis of corticosterone occurs. Lipoid soluble precursors of corticosterone are most likely stored within the lipoid inclusions and the observed decrease in electron opaqueness of the lipoid inclusions' matrices might represent the transfer of these precursors from the inclusions to the mitochondria. Most probably, this precursor transfer was accomplished via the agranular endoplasmic reticulum. This hypothesis is consistent with the recent discoveries that the mitochondria and the endoplasmic reticulum are both probable sites of steroidogenesis (Roberts and Creange, 1968).

It has been repeatedly demonstrated that the number of mitochondria observed within the zona fasciculata was proportional to the level of cellular activity (Miller, 1953; Lever, 1956; Borowiez, 1965). It has also been shown that the mitochondria were the sites of some of the enzymatic reactions of corticosteroidogenesis because the conversion of cholesterol from the major corticosteroid precursor is performed by a series of enzymatic systems located in the mitochondria. The enzymes 3β-hydroxysteroid dehydrogenase and 21-hydroxylase which convert pregnenolone to progesterone and 11-deoxycorticosterone, respectively, have been isolated from microsomal fractions, demonstrating the role of the endoplasmic reticulum in steroidogenesis (Roberts and Creange, 1968).
The mechanism by which the completely synthesized hormone is secreted from the epithelial cell in control tissue has not been elucidated. The decrease in the osmiophilic intramitochondrial ground substance with the simultaneous increase in the number of cytoplasmic vesicles suggests that in the irradiated tissue, corticosterone was transported by cytoplasmic vesicles from the mitochondria to the cellular membrane and eventually to the capillaries or sinusoids. The movement of the cytoplasmic vesicles towards the cell membrane and their fusion with the membrane were observed in both irradiated and non-irradiated zona fasciculata. The formation of protuberances and associated intramitochondrial vacuolation and the disruption of the outer mitochondrial membrane gave the appearance that the outer mitochondrial membrane was being transformed directly into cytoplasmic vesicles. Therefore, the completely synthesized corticosterone is most probably transported through the cytoplasm in vesicles which are derived directly from the mitochondria. It cannot be ascertained from these experiments whether or not the observed ultrastructural processes which accompanied the high secretion of corticosterone are peculiar to irradiated zona fasciculata tissue. The ultrastructural occurrences up to 24 h are very consistent with the ultrastructural alterations following the administration of ACTH. Sabatini, DeRobertis, and Bleichmar (1962) reported that the zona fasciculata mitochondria appeared to "open up" so that the intramitochondrial vesicles were expelled into the cyto-
plasm in hypophysectomized rats which had received restitu-
tional ACTH. The similarity between the ultrastructural
observations of zona fasciculata from animals that have been
irradiated and those that have been administered ACTH suggests
that the first increase in concentration of peripheral plasma
corticosterone was due to the increased secretion of adeno-
hypophysial ACTH. These findings are consistent with the
hypothesis that the first increase in peripheral plasma
corticosterone was a stress response.

The mitochondrial damage observed at 72 h was not
permanent because normal mitochondria were observed at 120 h,
144 h, 192 h, and 240 h. The extensive intramitochondrial
vacuolation apparent at 72 h rendered electron transparent
structures which resembled vacuoles. It appears, therefore,
that mitochondria were transformed into vacuoles and perhaps
eventually into lipoid inclusions. This interpretation would
offer an explanation for the presence of the osmiophilic
membranous structures that were observed in the periphery of
the vacuoles. A mitochondria-lipoid inclusion interrelation-
ship has been observed by other workers. Yamori, Matsuura,
and Sakamoto (1961) speculated that different types of lipoid
inclusions or granules existed and some of the inclusions were
produced by the accumulation of lipoid material within the
mitochondria and their subsequent release into the cytoplasm.
Ashworth, Race, and Mollenhauer (1959) also reported a mito-
chondria-lipoid inclusion interrelationship. They observed
a rapid increase of zona fasciculata which was correlated
with a decrease of lipoid inclusions. These observations, plus the appearance of double membranes and vesicles at the periphery of the lipoid inclusions caused these workers to speculate that the lipoid inclusions were transformed into mitochondria. This hypothesis is not consistent with the more recent observations that mitochondria with their distinctive DNA and RNA are autonomous (Novikoff and Holtzman, 1970).

The degree to which the inner and outer mitochondrial membranes were disrupted at 72 h has not been previously reported. The extensive morphological alterations appear to be a direct result of the proton irradiation and were not manifested until this later time. These alterations, however, were not accompanied by increased concentrations of peripheral plasma corticosterone. No typical lipoid inclusions with osmiophilic matrices were observed at this time. If the osmiophilic lipoid inclusions' matrices represent stored essential corticosterone precursors, then their depletion, might prevent the adrenal from secreting additional amounts of corticosterone. During the interim between the appearance of electron transparent lipoid inclusions' matrices and the reappearance of electron opaque matrices, the rate of corticosterone secretion probably was limited by the rate of synthesis of the corticosterone precursors. Apparently, the synthesis rate of these precursors was sufficient to enable the adrenal to maintain peripheral plasma concentrations of corticosterone equal to that observed in control animals.
Therefore, the lack of adrenal response concomitant with the mitochondrial alterations at 72 h was due to the high amount of corticosterone secretion demonstrated at 1.5 h at which time the stored corticosterone precursors were exhausted.

Most likely, slight morphological alterations appeared at 96 h following irradiation at a dose of 540 rads and these alterations were responsible for the second increase in the peripheral plasma corticosterone concentrations. The animals irradiated at this lower dose did not demonstrate as high an initial adrenocortical response as did the animals irradiated at a dose of 850 rads. Consequently, the stored corticosterone precursors were not depleted and the organ responded to the morphological alterations.
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