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LATE EFFECTS IN MOUSE THYROID AND ADRENAL GLAND AFTER ^{60}Co γ-IRRADIATION OF THE BRAIN: AN ULTRASTRUCTURAL STUDY

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Abstract

A single dose of 8 or 20 Gy ^{60}Co γ rays was given to C3H male mice at 4 months of age. The ultrastructural changes in mouse pituitary, thyroid and the adrenal gland due to brain irradiation were observed at 4 days to 18 months after irradiation. The pituitary microvasculature showed degenerative lesions including swollen endothelial cells and cytoplasmic blebs in the capillary sinusoids of all irradiated animals at 4 days after irradiation and persisted at later times (12-18 months). Cell atrophy, an increase of lysosomal-like bodies in the endocrinal cells and interstitial fibrosis were found in the anterior pituitary at 12 months and became more severe at 18 months after irradiation. The posterior pituitary was more resistant to radiation as compared with anterior lobe. Late degenerative changes were noted in the thyroid gland of all animals after 20 Gy irradiation. These changes included interstitial fibrosis, follicular degeneration, and abnormal thickening of the basement membrane of follicular cells. Focal hyperplasia frequently appeared in the adrenal cortex at 12 and 18 months after irradiation. Many necrotic cells with cytoplasmic vacuolation and severe interstitial fibrosis were observed in the adrenal cortex of all irradiated animals. Cell vacuolation and degranulation were observed in the adrenal medulla at 18 months after irradiation.

Key Words: Brain irradiation, indirect effect, pituitary, long-term effect, endocrine organs, thyroid, adrenal, hyperplasia, mouse, ultrastructure.

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Introduction

It is known that radiation therapy within or around the cranium can lead to brain injury (Llena *et al*., 1976; Sheline *et al*., 1980). However, the consequences for other organs following brain injury from localized cerebral irradiation are poorly understood. Our studies on the indirect effect of brain irradiation on the cardiovasculature have demonstrated that degenerative changes occurred in the myocardium and smooth muscle cells of coronary arteries within 24 months after brain irradiation (Yang *et al*., 1990). Degeneration of smooth muscle cells of coronary arteries also occurred after the orbital region was exposed to iron ions (Yang, 1993). However, the severity of degenerative changes in the endocrine organs following brain irradiation was only judged by weight and histological structure. Description of the late effects at the ultrastructural level was lacking. It has been demonstrated that the endocrine changes in weight became increasingly prominent with increasing dosage and with time after deuteron irradiation to the rat pituitary (Van Dyke *et al*., 1959; Tobias, 1979; Woodruff *et al*., 1984). The low doses of deuteron irradiation affected growth and anabolism more than the sexual functions and the functions of the adrenal gland, whereas at higher doses (above 25 Gy) depressing effects on sex and adrenal functions became apparent (Van Dyke *et al*., 1959). Using the rate of growth retardation and the rate of shrinking of epiphyseal cartilage widths as parameters, Tobias found that the secretion rate of somatotropin and thyroid-stimulating hormone decreased exponentially with dose after deuteron irradiation to the rat pituitary (Tobias, 1979). Clinically, the neuroendocrine disturbance was found following radiation therapy when the hypothalamic pituitary axis was included in the radiation field (Huang *et al*., 1991; Sklar and Constine, 1995). The present study is an extension of our previous work to include the ultrastructural observations on the pituitary as well as the adrenals through 18 months after ^{60}Co γ-irradiation of the mouse brain. The specific objectives are as follows: to search for evidence of a differential effect of brain irradiation on pituitary cell type at the ultrastructural level and to determine the long-term effects on the endocrine organs of brain irradiation.

Materials and Methods

Male C3H mice, 4 months old, were divided into four groups: the first two groups received brain irradiation (8 and 20 Gy), the third was a sham-irradiated control group, and the fourth was an age-matched unirradiated control group. For brain-irradiated animals, the pituitary gland and the surrounding tissues in a field 0.6 cm in diameter were irradiated. The remainder of the body was protected by lead shielding, 9 cm thick. Mice in the sham-irradiated control group were completely shielded with 9 cm of lead (Yang *et al*., 1990). The lead mold for irradiating the animal consisted of the lower part and the upper part. The lower part was formed to hold a mouse in a prone position, while the upper part had a 0.6 cm diameter aperture located immediately above the pituitary gland. The radiation source was a Packer (USA) C9 ^{60}Co teletherapy unit (now discontinued), provided with a light localizer for centering the irradiated area. A dosemeter type 2570 was used to measure the dose rate in different field sizes. The source skin distance (SSD) was 60 cm, and the dose rate was 0.68 Gy/min (Yang *et al*., 1990). The heart dose under the shield was measured using a 30-329 PTW-Markus (Gottingen, Germany) chamber as the reference dose received by the non-irradiated areas. During each treatment, the incident-ray beam was aligned and centered to the 0.6 cm aperture. The beam was then directed vertically through the head of the mouse, which was anaesthetized with sodium pentobarbital (0.05 mg/g body weight) given intraperitoneally. Periodically, the general appearance, body weight, and tail length were recorded. Three to five animals from each group were sacrificed with ether at 4 days, and 3, 6, 12, and 18 months after irradiation. After opening the thorax, the animal was perfused with phosphate buffered saline (PBS) followed by a fixative containing 4% paraformaldehyde, 5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 for 5-8 minutes (Yang *et al*., 1978; Yang and Ainsworth, 1982). The pituitary, thyroid, adrenals and testis were dissected and weighed. The tissues were cut into 1 x 2 x 2 cm^3 tissue blocks, and washed in 0.1 M cacodylate buffer, post-fixed in 1% OsO_4 for 1 1/2 hours, dehydrated and embedded in epon LX112. One μm sections were stained with toluidine blue for light microscopy. Thin sections were stained with uranyl acetate and lead citrate and viewed under a Hitachi-600 electron microscope (Hitachi, Tokyo, Japan).

Results

The amount of scattered dose to the heart and the other organs under the shield was 11.2 cGy or less than 11.2 cGy in comparison to the 20 Gy pituitary dose. In order to obtain evidence of progressive change in the organs under brain irradiation, the status was evaluated by the weight and morphology at increasing times following irradiation. The body weights after the 20 Gy dose reduced at 18 months after irradiation (Yang *et al*., 1990). At the 8 Gy dose, the body weights were not significantly different from those of the control animals throughout the experimental period. After irradiation with 20 Gy, the pituitary weight was reduced to less than half normal by the end of the eighteenth month (Yang *et al*., 1990). The weight of the irradiated pituitary is not necessarily representative of its functional capacity, since different cell types may be damaged to different degrees. The thyroids were smaller at 12 and 18 months after 20 Gy of pituitary irradiation (Yang *et al*., 1990). The weights of the adrenals and the testis of both irradiated groups did not differ from the controls. However, late gross abnormalities such as focal hyperplasia and necrosis appeared in the adrenal cortex in all animals that received 8 Gy and 20 Gy to their brains (Table 1). These changes appeared in only one control pituitary at 18 months into the experiment.

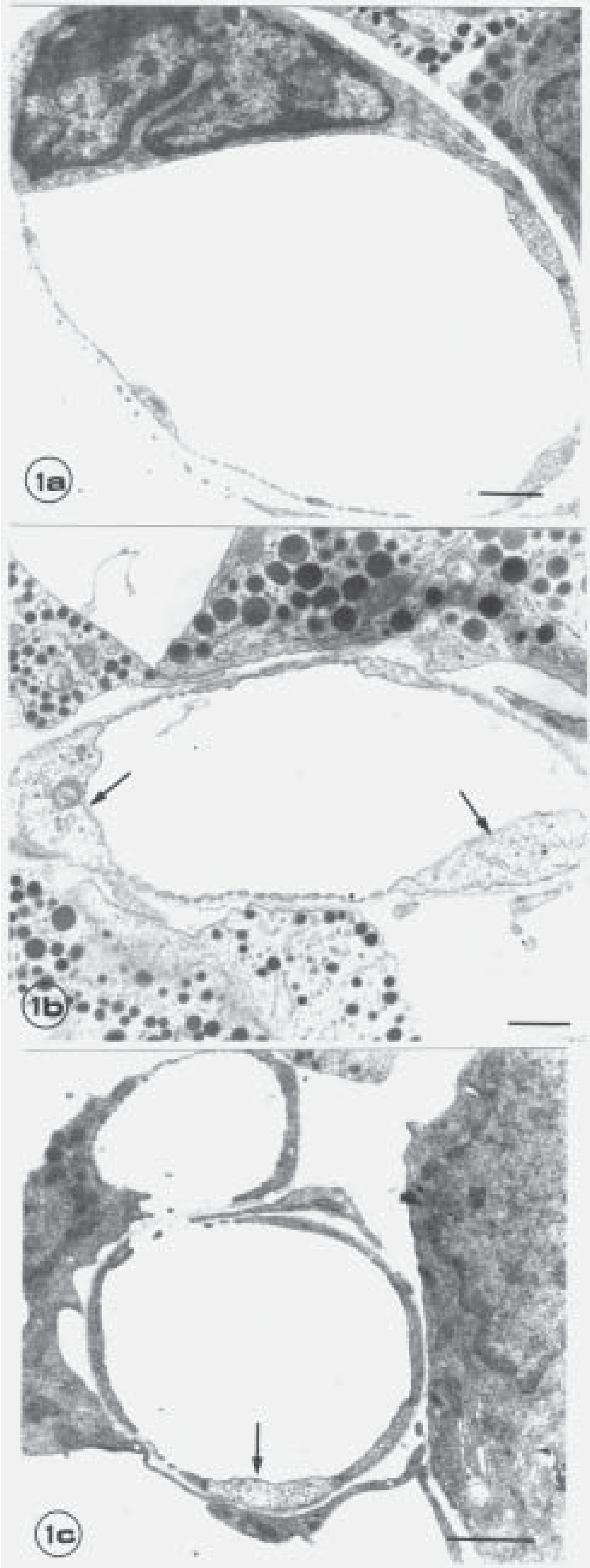
Table 1. Adrenal weights[a] and frequency of adrenal hyperplasia after brain exposures to ^{60}Co γ rays.

Dose in Gy	Time after irradiation (in months)		
	6	12	18
0	4.0 ± 0.5[#] (0%)[b]	4.3 ± 0.7 (0%)	4.3 ± 0.4 (33%)
8	4.5 ± 0.3 (0%)	5.5 ± 0.5 (100%)	5.5 ± 0.1 (66%)
20	4.1 ± 0.5 (0%)	4.1 ± 0.8 (66%)	4.1 ± 0.2 (100%)

[a]The number of adrenal glands per each dose group per time interval is 6.
[#]Mean ± standard error (SE);
[b]frequency of hyperplasia.

Pituitary gland

At 4 days after irradiation, the intercellular space of the anterior pituitary was slightly increased. The endothelial cells of capillary sinusoids in both anterior and posterior lobes were swollen with pale, electron-lucent matrix as compared with the control animals (Figs. 1a and 1b). The swollen endothelial cells persisted at 18 months after irradiation (Fig. 1c). The cells of pituitary parenchyma were differentiated in electron microscopy by the shape, size and electron density of their secreting granules and by characterization of their cytoplasmic organelles. The ultrastructural alterations in the parenchyma of the anterior lobe after 20 Gy were noticed at 6 months after irradiation and became progressively more severe at later times (12-18 months). The intercellular space was markedly increased and mild fibrosis and atrophy were observed. The somatotrophs of the unirradiated animal at 12 months after the experiment have numerous dense, spherical granules 350-450 nm in diameter (Fig. 2a). Cisternae of the rough endoplasmic reticulum are often parallel to the cell



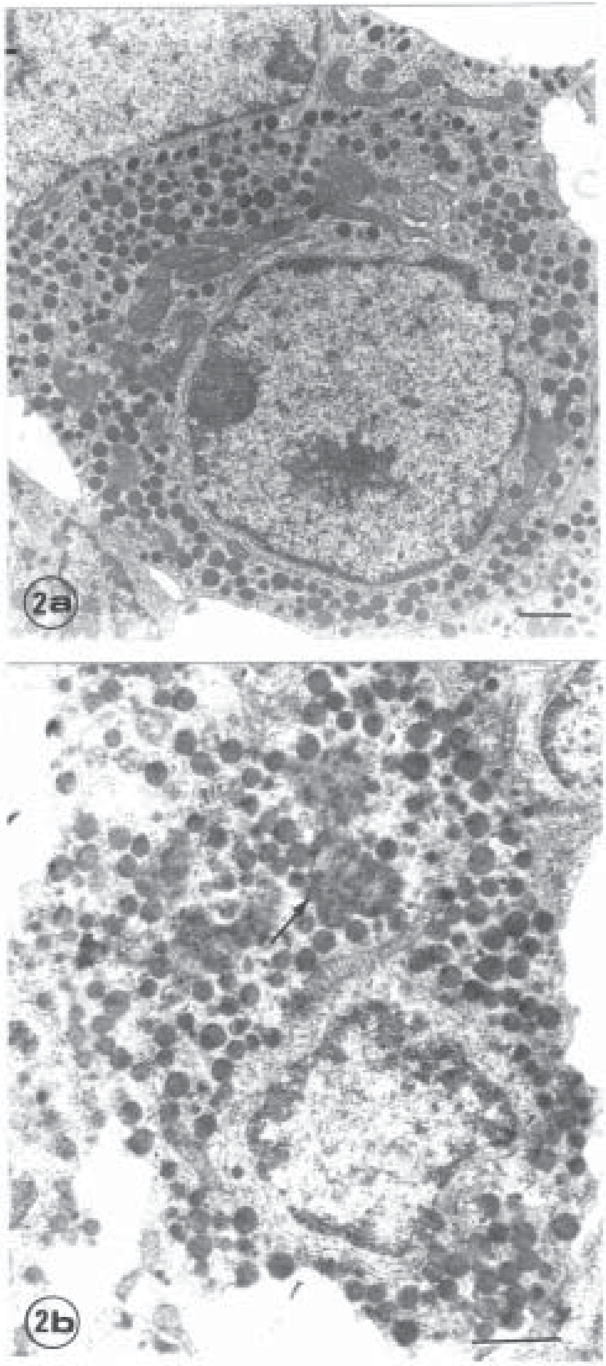
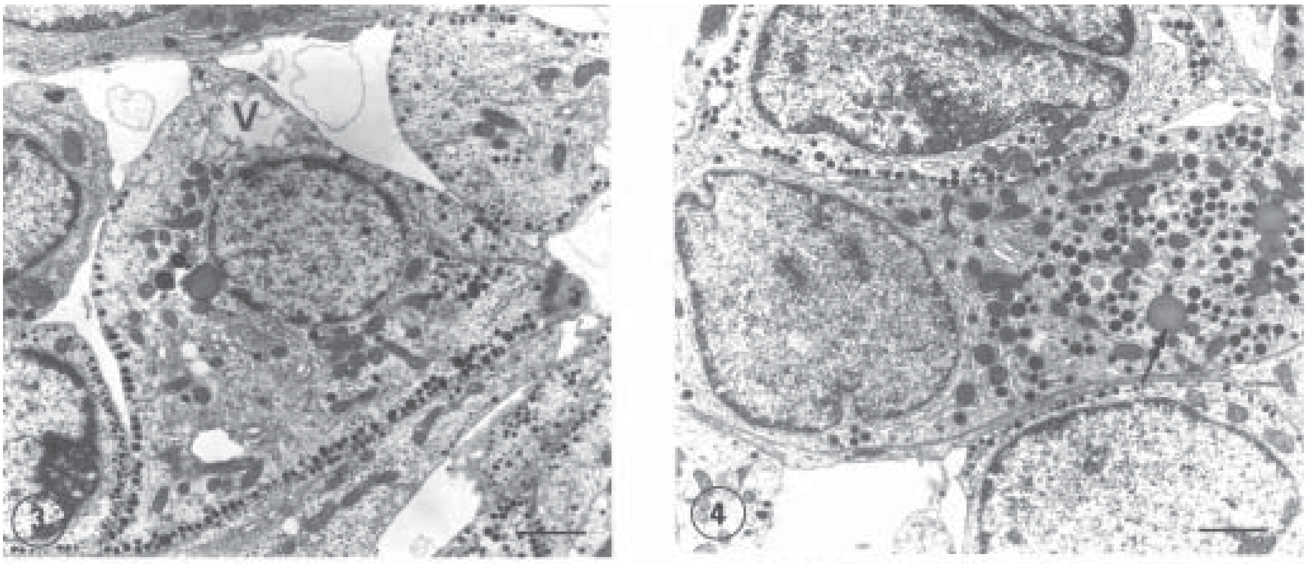
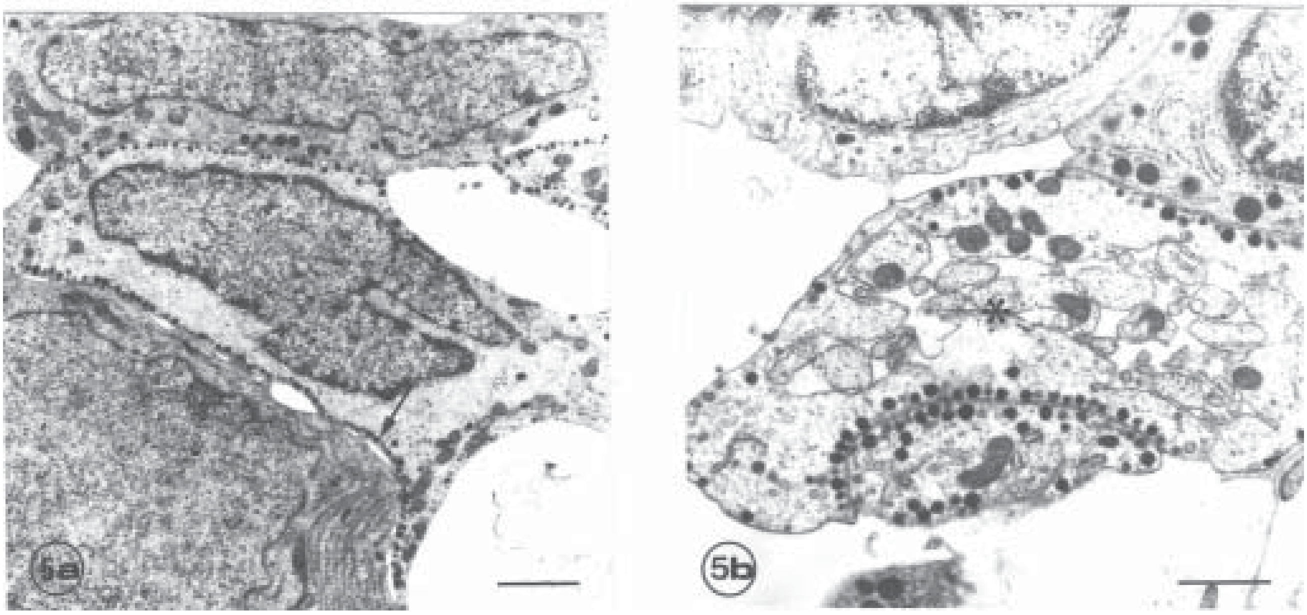


Figure 1. (**a**) Pituitary capillary sinusoid of a control mouse at 4 days into the experiment. (**b**) Swollen endothelial cells (arrows) were found at 4 days and (**c**) persisted through 18 months after 20 Gy irradiation.

Figure 2. Pituitary somatotrophs. (**a**) Many secretory granules in the cytoplasm of control mouse, 12 months into the experiment. (**b**) The lysosomal-like bodies (arrow) increased 12 months after 20 Gy irradiation.
Bars = 1 µm (Figs. 1 and 2).





Figures 3 and 4. Pituitary thyrotrophs (**Fig. 3**) and gonadotrophs (**Fig. 4**) at 18 months after 20 Gy irradiation showing vacuolation (V; Fig. 3) and the lysosomal-like bodies (arrow; Fig. 4) in the cytoplasm. Bars = 1 µm.

Figure 5. Pituitary corticotrophs. (**a**) A control mouse 12 months into the experiment. Secretory granules (arrow) arranged around the edge of cytoplasm. (**b**) Degeneration appeared in the cytoplasm (*) at 18 months after 20 Gy irradiation. Bars = 1 µm.

surface, but occasionally they form the concentric systems. At 18 months after 20 Gy irradiation, the ultrastructure of somatotrophs observed was relatively normal, except the lysosomal-like bodies in the cytoplasm was increased (Fig. 2b). The thyrotrophs are polygonal in shape and contain small granules, 140-180 nm in diameter. At 18 months after 20 Gy irradiation, the lysosomal-like bodies and vacuolation appeared in the cytoplasm of most thyrotrophs observed (Fig. 3). The gonadotrophs are not of a single cell type and can be characterized by the coexistence of small and large secretary granules. The gonadotrophs were more resistant to radiation as compared with other types of parenchymal elements (Fig. 4). The corticotrophs in control pituitary are irregular stellate in shape (Fig. 5a). Cytoplasm is of low density and has a

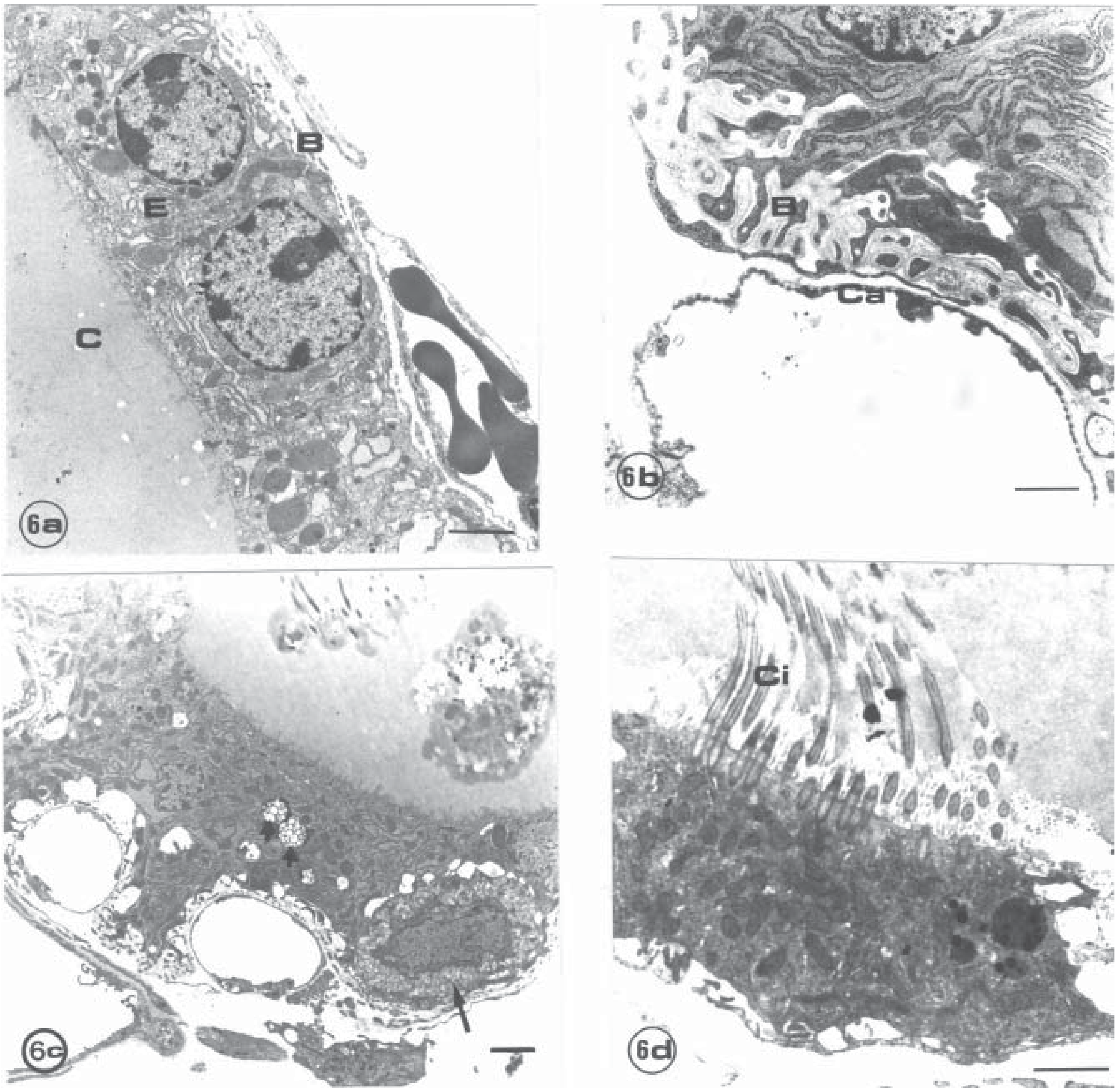
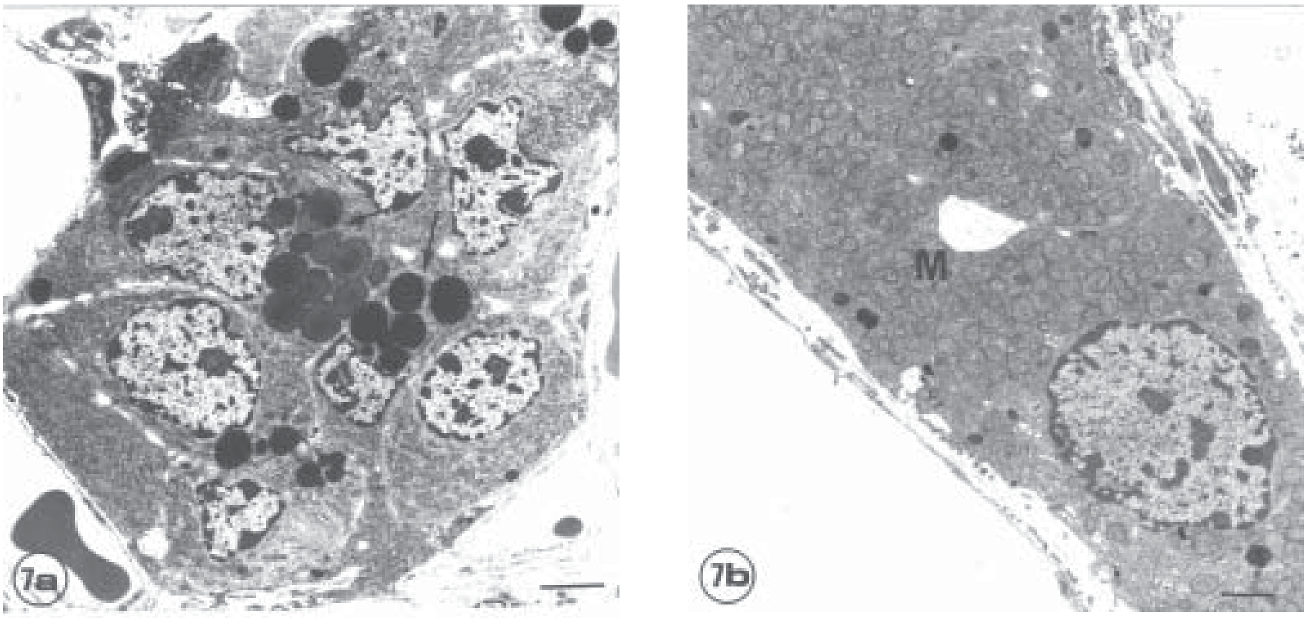


Figure 6. (**a**) Thyroid gland of a control mouse showed well arranged follicular epithelia (E). Colloid (C). Basement membrane (B). At 18 months after 20 Gy brain irradiation, the thyroid gland showed (**b**) thickening of basement membrane (B) with normal capillaries (Ca). (**c**) Degeneration of follicular cells (arrow), lysosomal-like bodies (arrowheads) were revealed. (**d**) Extended cilia (Ci) in the lumen. Bars = 1 μm (a, b, and d) and 2 μm (c).

sparse endoplasmic reticulum. Relatively few granules accumulated in these cells; granules that did were 200-250 nm in diameter and located for the most part immediately beneath the cell membrane. At 18 months after 20 Gy irradiation, many corticotrophs showed vacuolation (Fig. 5b). After irradiation of the pituitary with 8 Gy, parenchymal elements in all pituitary lobes showed the same type of alterations as previously observed but the changes appeared less prominently. The cells in the posterior lobe were less damaged.



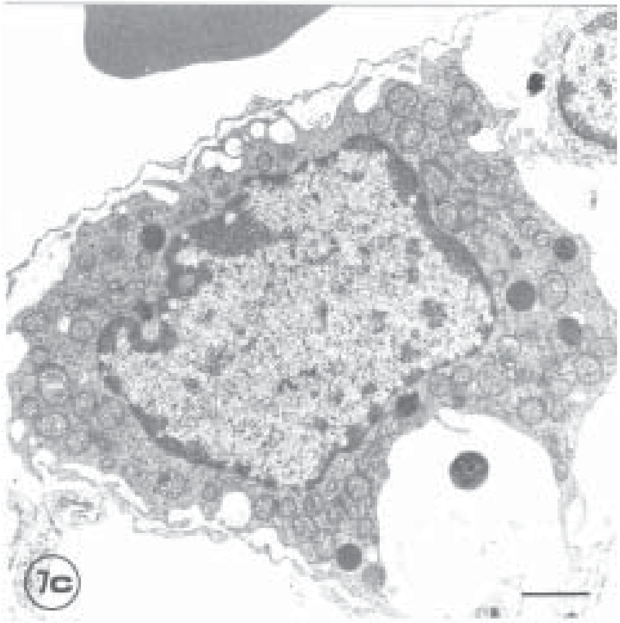


Figure 7. Adrenal cortex of control mouse consisted of 3 layers of tissues. (**a**) Cell in zona glomerulosa was spherical. Lipid droplets (arrows). (**b**) Cell in zona fasciculata arranged in bundles. Mitochondria (M). (**c**) Cell in zona reticularis was polygonal. Bars = 2 μm (a) and 1 μm (b and c).

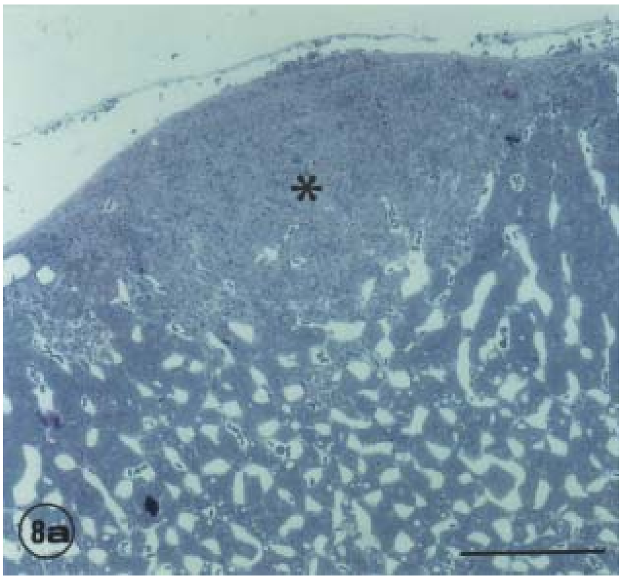
Thyroid

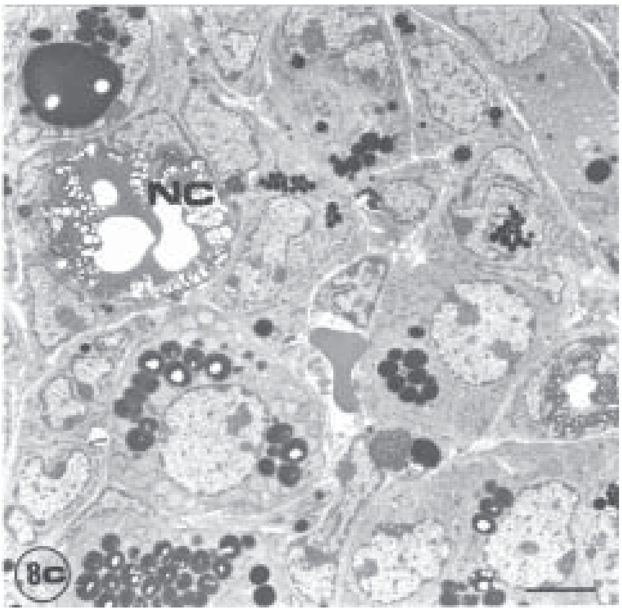
The ultrastructure of thyroid follicular epithelium of the control group is shown in Figure 6a. Degenerative changes were noticed at 12 months and became more severe at 18 months in all animals after irradiation. These changes included interstitial fibrosis, abnormal thickening of basal lamina (Fig. 6b), degeneration of follicular cells (Fig. 6c). The cilia structure was extended into the lumen of some follicular cells at 18 months after 20 Gy irradiation (Fig. 6d). There were no apparent damages in the blood capillaries.

Adrenals

The adrenal gland has two distinct parts: the adrenal cortex and the adrenal medulla. The adrenal cortex is divided into three layers: zona glomerulosa, zona fasciculata, and zona reticularis. Cells in zona glomerulosa were spherical, contained lipid droplets in the cytoplasm (Fig. 7a). Cells in zona fasciculata were arranged in bundles. Numerous mitochondria in spherical shape and few lipid droplets were filled in the cytoplasm (Fig. 7b). Cells in zona reticulata were polygonal (Fig. 7c). Fewer mitochondria and lipid droplets were observed in the cytoplasm than those in the cells of the other two regions.

Localized foci of cellular hyperplasia were present in the adrenal cortex at 12 and 18 months after 8 and 20 Gy irradiation. In contrary, hyperplasia were present in controls with lower incidence (Table 1). Focal hyperplasia were rare in the adrenal medulla after irradiation. Some of the secretory cells in the zona glomerulosa layer were degranulated or were atypical in size. Little connective tissues existed (Figs. 8a and 8b). Many necrotic cells existing in the interstitium showed cytoplasmic vacuolation (Fig. 8c). Some cells in the zona glomerulosa arranged irregularly and protruded into the zona fasciculata layer (Fig. 9a). Numerous lipid drops existed inside the cells (Fig. 9b). The necrotic cells and severe interstitial fibrosis were found in this area (Figs. 9c and 9d). Other abnormal cells, of a different type, were arranged irregularly





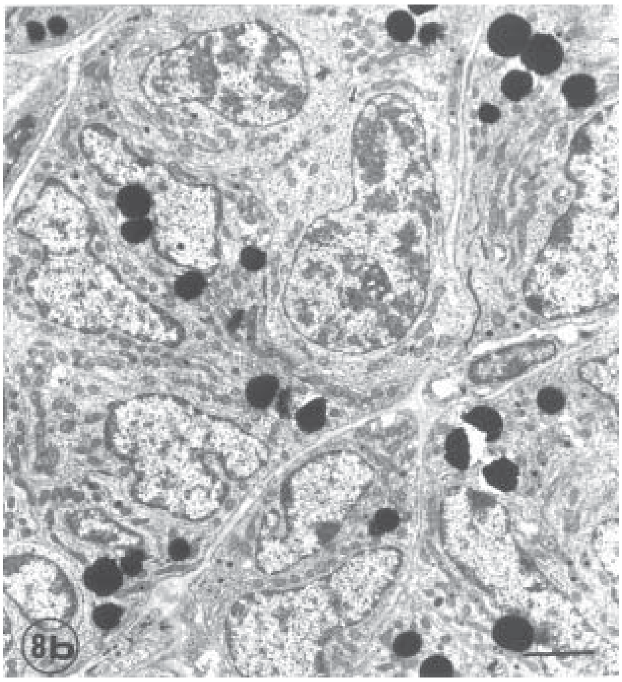


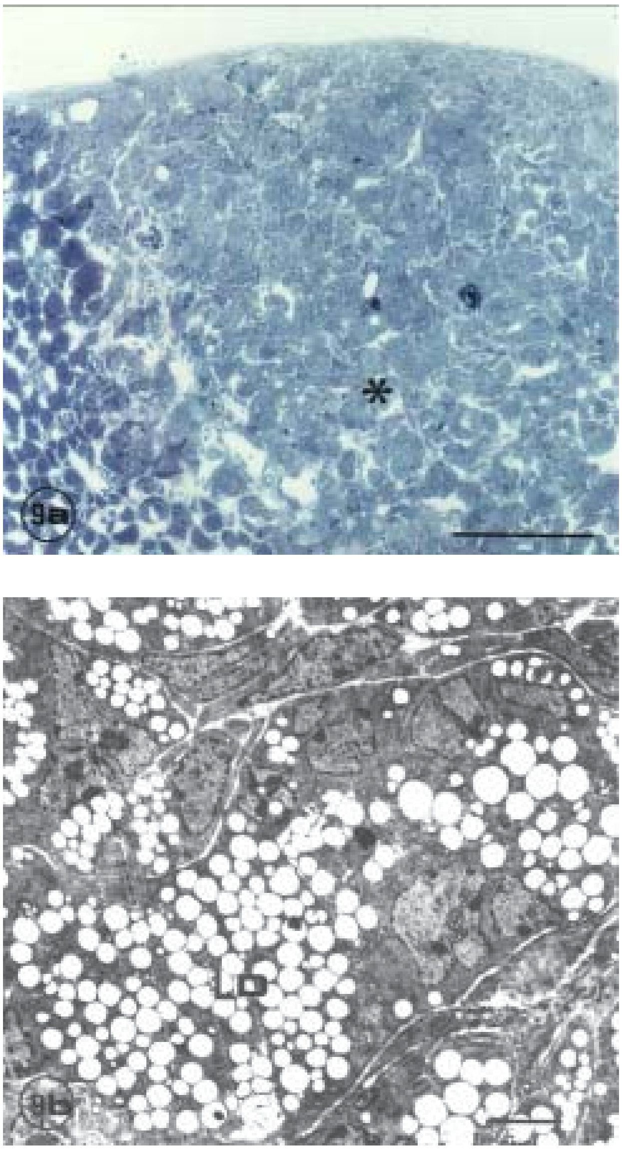
Figure 8. At 12 months after 8 Gy brain irradiation, hyperplasia appeared in cortical region. (**a**) Light micrograph showed hyperplasia (*). (**b**) At an ultrastructural level, cells were arranged tightly without intercellular space. (**c**) Necrotic cells (NC) appeared. Bars = 0.1 mm (a), 2 µm (b), and 4 µm (c).

and loosely in the zona glomerulosa (Fig. 10a). These cells exhibited pale cytoplasm in comparing to the surrounding normal tissues. Many of these cells showed pyknotic nuclei (Fig. 10b). Some cells in the hyperplasia region were smaller in size as compared with the surrounding normal cells (Fig. 11a). Cytoplasmic vacuolation and interstitial fibrosis were observed (Figs. 11b and 11c). No morphological changes in the hyperplasia free region of anterior lobe of the adrenal gland were observed at 12 and 18 months after irradiation. The vacuolation and degranulation in the cytoplasm were frequently observed in the adrenal medulla in all 20-Gy irradiated animals (Figs. 12a and 12b).

Discussion

There has been little information on whether irradiation of the brain will result in the degeneration of peripheral organs. This concern is especially important in Taiwan, as the incidence of nasopharyngeal cancer in this area is particularly high, and local irradiation is a common treatment for this disease. Although the radiation dose which the peripheral tissues receive is low, and causes no immediate effects, there is concern that certain late effects may affect the peripheral organs of the body. Our previous studies demonstrated that there were structural changes in the heart after brain exposures to ^{60}Co γ-rays and ^{56}Fe heavy charged particles (Yang *et al*., 1990; Yang, 1993). From the present study, degenerative changes at the ultrastructural level were observed in the thyroid and the adrenal gland after brain irradiation. These changes occurred at 12 months and became more severe at 18 months after irradiation.

The present and the previous results indicated that the weight of the pituitary as well as the thyroid were reduced since 12 months after irradiation. It has been reported that the weights of pituitary and thyroid were significantly decreased in young (30-40 days old) rats at 3 months after X-rays or



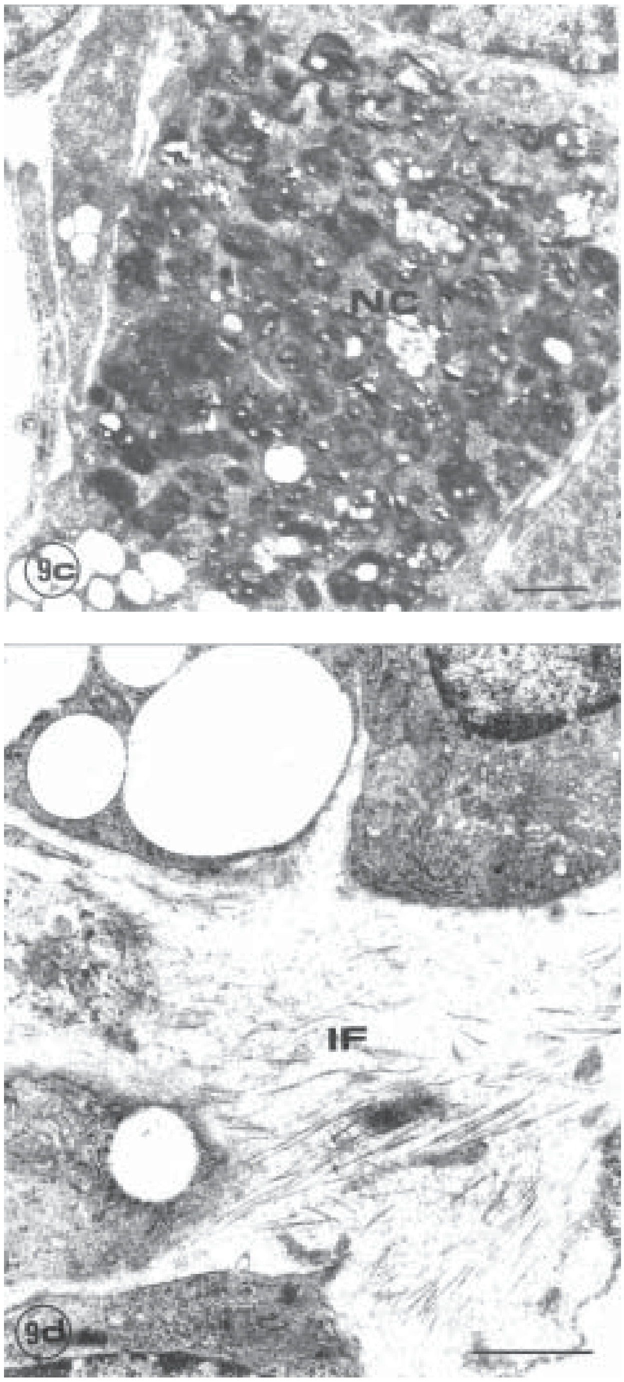
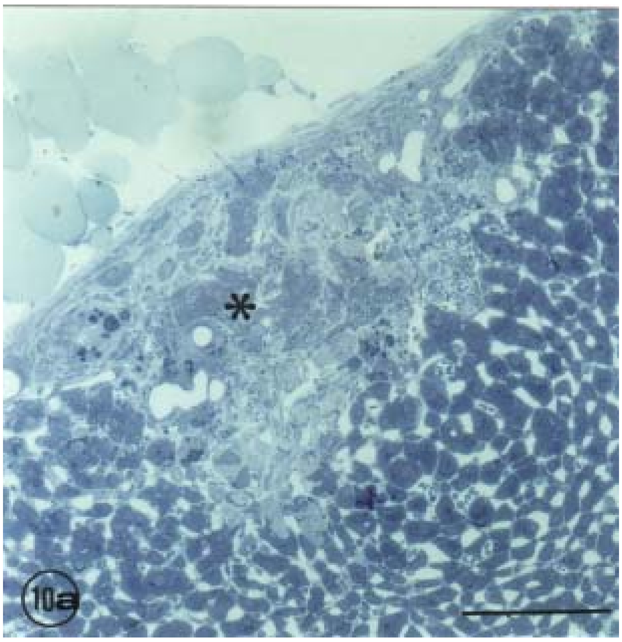


Figure 9. (**a**) At 12 months after 8 Gy irradiation, hyperplasia (*) intruded into zona fasciculata. (**b**) At an ultrastructural level, numerous lipid droplets (LD) were revealed in the cytoplasm. (**c**) Necrotic cell (NC) and (**d**) interstitial fibrosis (IF) were also observed. Bars = 0.1 mm (a), 4 µm (b), and 1 µm (c and d).

deuteron irradiation to the pituitary (Van Dyke *et al*., 1959; Woodruff *et al*., 1984).

In our experiments, the weight changes of these organs appeared until 12 months after irradiation. This discrepancy might be due to the difference in radiation quality, radiation dose, and the animal age when the irradiation initiated. The dosages used in this experiment apparently did not affect the weight changes in the testis. The weights of the adrenals were not different from the control group. However, late developing adrenal hyperplasia appeared in all animals that received 8 Gy and 20 Gy to their brains. It has been reported that the weight changes in thyroid at lower dose is more sensitive than the changes occurred in adrenal and testis. However, the adrenals and testis became more sensitive when the dosage was higher than 25 Gy (Van Dyke *et al*., 1959).

The pituitary gland is located near the geometric center



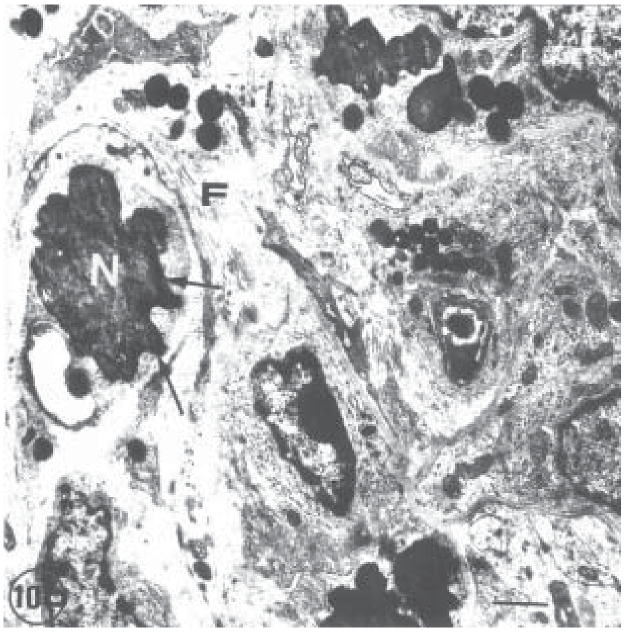
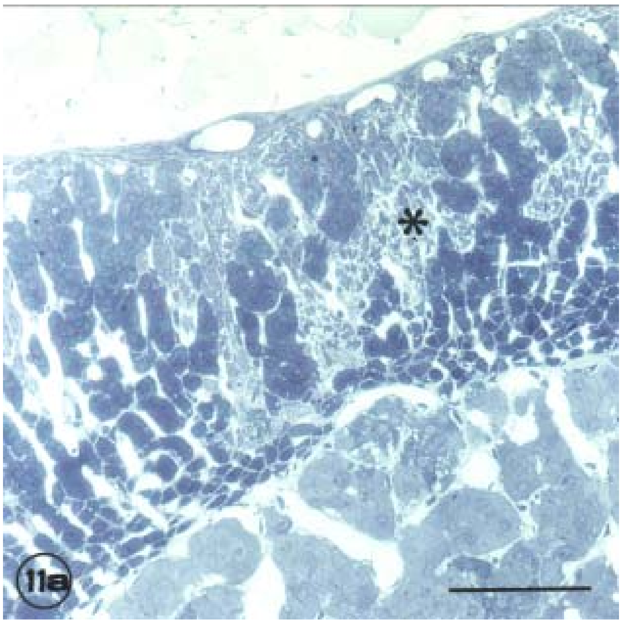


Figure 10. (**a**) Hyperplasia (*) in the adrenal zona glomerulosa at 12 months after 8 Gy brain irradiation. At higher magnification (**b**), these cells exhibited pale cytoplasm with pyknotic nuclei (N, arrow). Fibrosis (F). Bars = 0.1 mm (a) and 1 μm (b).

of the head and is protected by the sella turcica. In recent years, new technologies have been developed in order to reduce the damages to the peripheral tissues during radiation therapy (Tobias, 1979, 1985). The fine structures of pituitary from normal rats have been well studied (Yoshimura and Nogami, 1981; Yoshimura *et al*., 1982). However, the degenerative changes in the ultrastructural level after irradiation have not been investigated. Our results showed radiation-induced fibrosis and various degenerative changes following irradiation to pituitary which progressed over time after irradiation. We also demonstrated that damages in capillaries appeared at 4 days after irradiation and persisted through the entire experiment. The progressive central fibrosis probably reflects patterns of vascular flow after radiation-induced vascular insufficiency (White, 1976; Yang *et al*., 1978). The ultrastructural data revealed that the corticotrophs were more sensitive to radiation than the other types of endocrinal cells. The degenerative changes in thyrotrophs and somatotrophs were not as severe as the corticotrophs. The posterior pituitary contains the non-myelinated axons of neurosecretory cells, and the results showed it was more resistant to radiation than the anterior pituitary. These data correlated with the clinical observations and other animal studies (Woodruff *et al*., 1984). Late developing pituitary tumors were not detected in the control and irradiated animals. In an independent study, Tobias (1979) found a number of pituitary tumors among late-surviving rats that received neutron radiation. On the other hand, rodents exposed to whole body or whole head irradiation of γ-rays or X-rays usually develop relatively few pituitary tumors.

The endocrine organs under pituitary control showed varying degrees of degenerative changes at the ultrastructural level. The degenerative changes of thyroid appeared at 18 months after irradiation. Thyroid atrophy and a decrease in size of follicular cells in 20-day old rats after X irradiation of pituitary have been reported (Lawrence *et al*., 1937). Thyroid malfunction was also observed in human and in animal studies after pituitary irradiation (Jenkins *et al*., 1972; Slater *et al*., 1988). From the present study, one of the main ultrastructural features of lesions, the hyline material, is due to the overproduction of a basement-like material by follicular cells. The increasing thickness of basal lamina could have hindered the release of thyroid hormone into the blood capillaries and therefore caused the malfunction of thyroid. Long microvilli and cytoplasmic projections extended into the lumen containing fine granular material of moderate electron density. The capillaries and the small venules showed normal appearance throughout the experimental period, which indicated that ultrastructural changes in the thyroid are secondary to pituitary and are not a direct effect of irradiation (Alcaraz *et al*., 1990).

Focal hyperplasia were first observed in the adrenal cortical tissues at 12 months after 8 Gy and 20 Gy brain irradiations. It has been reported that the incidence of tumors



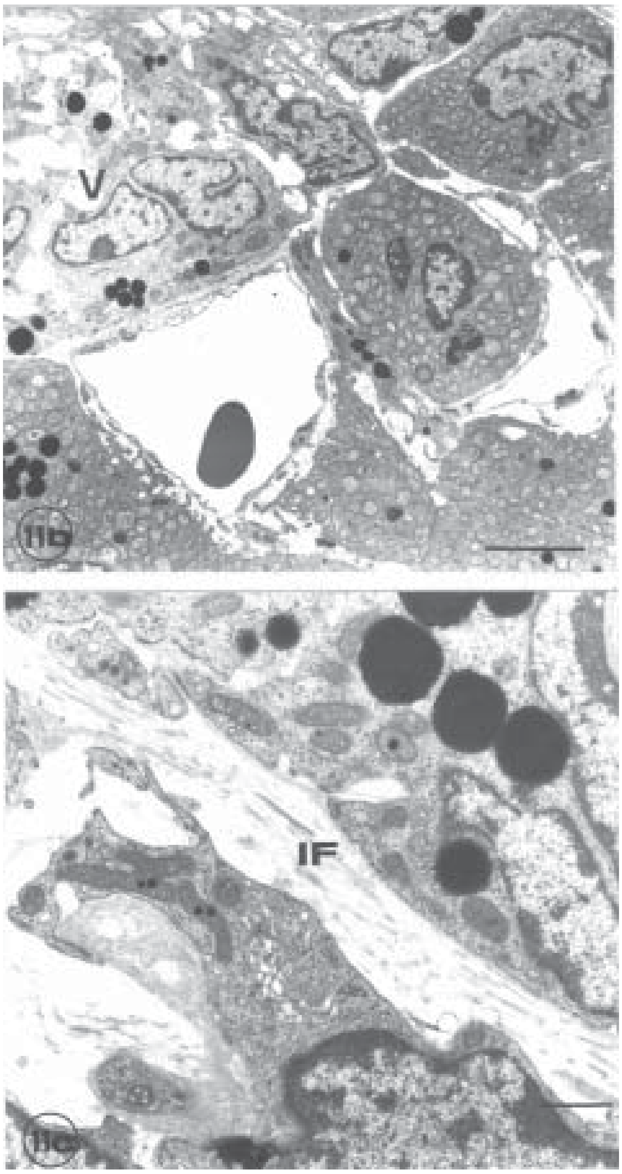


Figure 11. (**a**) Cells in the hyperplasia region (*) of adrenal cortex were smaller in size as compared with the surrounding normal cells 12 months after brain irradiation. (**b**) At ultrastructural level, cell vacuolation (V) was observed in the cytoplasm. (**c**) Interstitial fibrosis (IF). Bars = 0.1 mm (a), 4 µm (b), and 1 µm (c).

in adrenals was greater in groups which had received the low doses of pituitary radiation than in controls, whereas at higher doses of radiation (higher than 25 Gy), the appearance of tumors was virtually suppressed (Woodruff *et al*., 1984). This might be due to the destructive action of deuteron irradiation of the pituitary. Hyperplasia were also observed in the control animals at 18 months into the experiment. But, the incidence is low (33%) compared with those in the irradiated groups (66%-100%). Focal hyperplasia in the irradiated pituitaries were frequently multiple, more so than in controls.

The endocrine changes became increasingly more prominent with increasing dosage and with time. The lower the dose, the longer the period which elapsed before the maximum effect resulted. It has been reported that the order of decreasing sensitivity of the pituitary target organs to pituitary damage was adrenals, thyroid, then testis (Van Dyke *et al*., 1959). Our ultrastructural results indicated that the sensitivity to radiation of the pituitary parenchymal cells correlated well with the weight changes and with the alterations in the endocrine organs after brain irradiation. The relationships between pituitary abnormalities and target organ hyperplasia are of great interests. From the present study, it is premature to speculate that a pituitary cell secreting one hormone is more sensitive to radiation than another, but this possibility deserves considerations in future studies.

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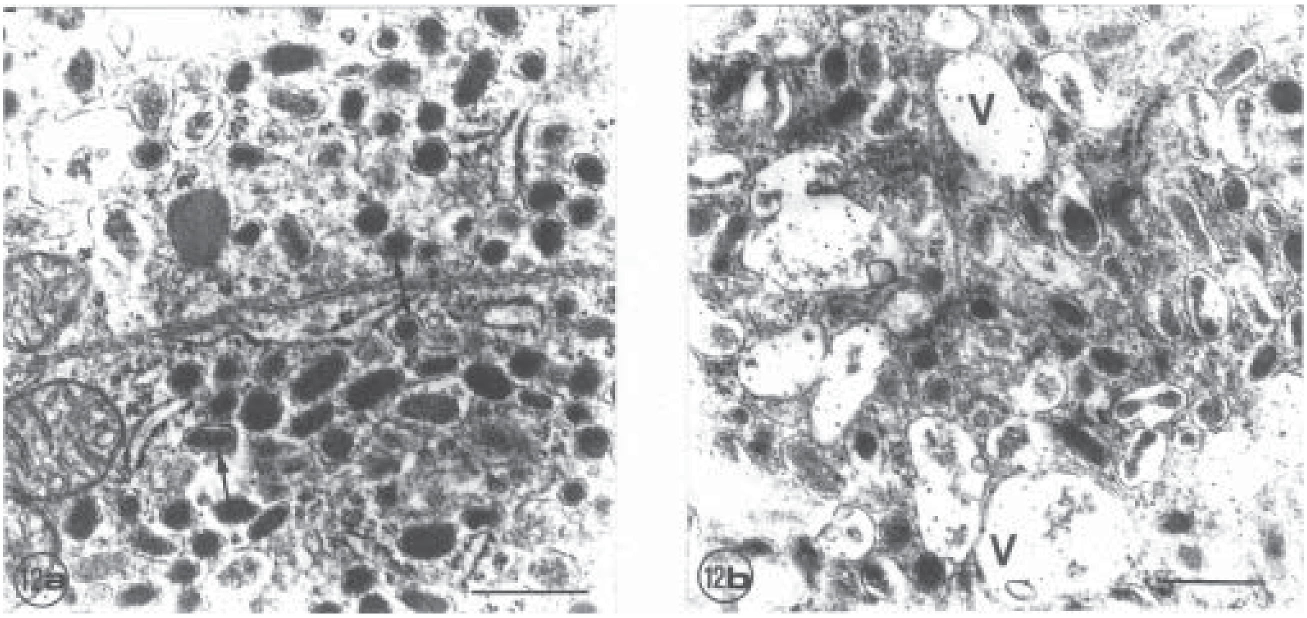

Figure 12. Adrenal medulla. (**a**) A control mouse 18 months into the experiment. Abundant granules (arrows) were in the cytoplasm of medulla. (**b**) The contents in the granules disappeared at 12 months after 8 Gy irradiation. Vacuoles (V) appeared in the cytoplasm. Bars = 1 μm.

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Discussion with Reviewers

T. Seed: Would the authors like to briefly discuss/speculate as to the mechanism(s) by which damage to the pituitary results in the noted arising pathology to non-irradiated thyroid and adrenal glands?
Authors: A variety of endocrine disturbances were observed following treatment with external radiation therapy when the pituitary is included in the treatment field. The secretion rate of somatotropin and thyroid-stimulating hormone decreased after rat pituitary exposure to deuteron beams. We suspect that the damages of pituitary due to brain irradiation would affect the hormonal secretion which result in the degenerative changes in the related endocrinal organs such as thyroid and adrenals. In addition, the humoral factors, such as "circulating toxins" could be set free as a consequence of brain irradiation and could cause the degenerative changes in the thyroid glands and adrenals.

T. Seed: If the irradiated mice were supplemented with pituitary hormone, would you still expect to see the pathologies in the adrenals and thyroid glands?
Authors: Hormone replacement could restore the physiological functions of endocrine organs. This has been employed in the patients received cranial irradiation. Changes would be back to normal after supplement with pituitary hormone.

M. Alcaraz: The authors state that there is little information on the effect of brain irradiation on the peripheral organs, and this is especially important in the treatment of certain types of cancer (nasopharyngeal cancer). Why did they choose such apparently low doses of radiation to study these effects? Once the results of their studies are known, what would be overall evaluation be of these effects on the peripheral organs that could have repercussions in oncological treatment?
Authors: To the best of our knowledge there has been little information on whether irradiation of the brain will result in the degeneration of the peripheral organs. This concern is especially important in Taiwan, as the incidence of nasopharyngeal cancer in this area is particularly high, and local irradiation is a common treatment for this disease. Although the radiation dose which the peripheral tissues receive in total is less than 50 Gy and causes no immediate effects, there is concern that certain late effects may affect other organs of the body. The follow-up study after cranial irradiation was only emphasized on the hormonal secretion of pituitary gland. However, there exist only few reports of ultrastructural changes of the pituitary and other endocrine organs after brain irradiation. The purpose of the present study, using mouse as a model system, is to investigate the long term effects after single doses of ^{60}Co γ-irradiation. The radiation doses used in this study are below the acutely lethal range to mice. In our opinion, a fair amount of further research would definitely be needed in order to extend this line of experiment to oncological treatment.

Z. Somosy: Can you confirm your data about different radio-sensitivity of unlike parts (somatotrophs, thyrotrophs, etc.) of pituitary gland by immunohistochemistry and/or morphometry?
Authors: Because the dose-response curves have not been implemented, it is not appropriate to confirm our data about radiosensitivity of different parts of parenchymal cells of pituitary gland either by immunohistochemistry or by quantitative morphometry.

Z. Somosy: Did you find any morphological changes in tumor free region of adrenal gland after 12 months?
Authors: We did not find any morphological changes in the tumor or hyperplasia free region of adrenal gland at 12 months after irradiation.