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Irradiation effects on the adrenal gland of rats undergoing inanition stress

S S HASAN and P K CHATURVEDI
Zoology Department, Garhwal University Campus, Pauri-Garhwal 246001, India

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Abstract. The effect of total body x-irradiation was studied on rats under inanition stress. In response to irradiation an increase in the activity of cortex and medulla was noted in inanition stress administered rats rather than in the normally fed animals. Similarly, rising levels of urinary catecholamines and 5-hydroxytryptamine were observed in the starved animals after irradiation.

Keywords. Inanition stress; irradiation; adrenal gland.

1. Introduction

Earlier studies suggest that ionizing radiation decreases the production of corticoids from the adrenal cortex (Nabors *et al* 1974; Nabors 1962; Berliner *et al* 1962; Stevens *et al* 1963). Similarly, earlier investigators (Nair 1965; Hasan *et al* 1977, 1978, 1979; Veninga and Brinkman 1962; Renson and Fischer 1959; Varagic *et al* 1967) have demonstrated that the radiation elicits release of 5-HT and catecholamine in rats. Further, the response of adrenal activity is mostly dose dependent to radiation (Dougherty and White 1946; French *et al* 1955). However, no result seems to have been reported on the effect of x-irradiation on the adrenal gland of rats undergoing inanition stress. In this investigation, urinary metabolites of catecholamine and 5-hydroxytryptamine *viz* VMA and 5-HIAA have been studied in relation to inanition stress after total body irradiation.

2. Material and methods

Male Holtzman strain rats (110–120 g) were used in all control and experimental groups. Water was allowed *ad libitum* to each group of rats.

Group I: rats were starved for 10 days.
Group II: rats were starved for 10 days and on the 6th day of starvation they were exposed to x-rays.
Group III: rats were fed on a standard commercial diet (Hindustan Levers Ltd., India) to serve as control for groups I and IV.
Group IV: rats were fed on a standard diet as mentioned in the group III and exposed to x-rays, on the day when rats of group II were irradiated.

Total body of animals of groups II and IV were exposed to x-rays, 1000 R (80 kV; 200 MAS; 1 sec; 80 cm distance).

The control and experimental rats were killed by decapitation at intervals of 24, 48 and 96 hr after irradiation/on the 6th day after starvation. Adrenals were dissected out and fixed in Bouin's fluid. Paraffin sections (5μ) were cut and stained with haematoxylin and eosin. Before the sacrifice, urine of each rat of the control and the experimental groups was collected in a specially devised box for 24 hr for the biochemical investigation of 5-HIAA (Subramaniyam and Narayanan 1973) and VMA (Armstrong *et al* 1957). Rate of mortality was also recorded during the stretch of experiments.

3. Results

The group undergoing inanition stress showed 29·4 % mortality from the 5th to the 10th day of starvation. On the other hand the group receiving a combined treatment of starvation and irradiation exhibited nearly 33·3 % mortality during this period which

Table 1. Chart of mortality.

Groups	Normal control *A*	Radiation *B*	Starvation *C*	Starvation + Radiation *D*
Total no. of rats taken	25	25	34	36
1st day of starvation	—	—	—	—
2nd day of starvation	—	—	—	—
3rd day of starvation	—	—	—	—
4th day of starvation	—	—	—	—
5th day of starvation	—	—	2	2
6th day of starvation	—	—	4	—
7th day of starvation/ 24 hr after irradiation	—	—	2	4
8th day of starvation/ 48 hr after irradiation	—	—	—	—
9th day of starvation/ 72 hr after irradiation	—	—	2	4
10th day of starvation/ 96 hr after irradiation	—	—	—	2
Percentage mortality	—	—	29·41 %	33·33 %

indicated an increase in the rate of mortality as compared to the non-irradiated starved rats (table 1).

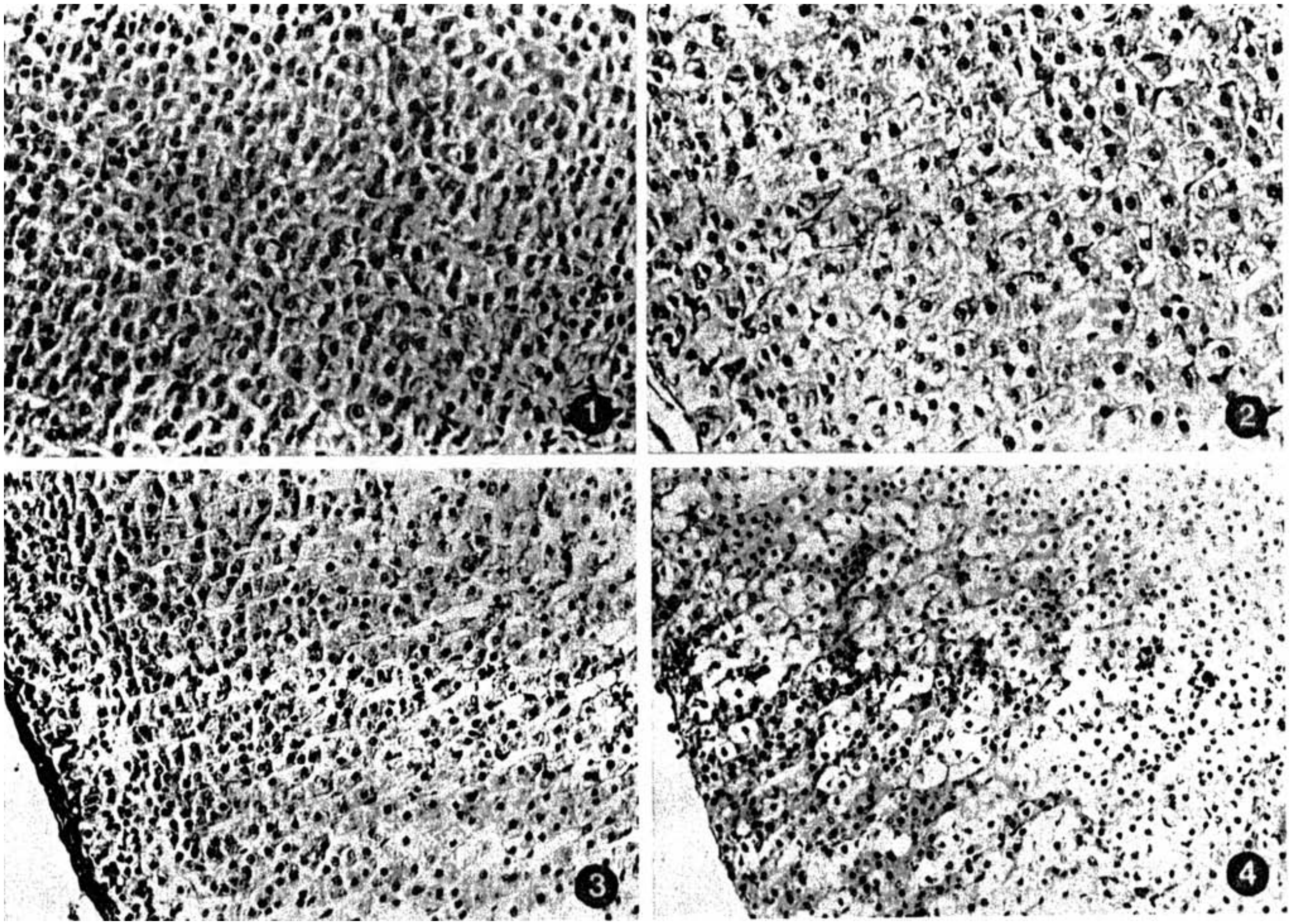
3.1a *Adrenal cortex*: Cells of the normal adrenal cortex were tightly packed and had a dense staining cytoplasm. Throughout the cortex, there was a rich vascular bed of sinusoids (figure 1). In rats undergoing inanition stress there was hypotrophy of cortical cells and most of the cells appeared vacuolated at the end of the 7th day of starvation. With increase in post-starvation period the columnar cells were widely separated by the appearance of large sinusoids which were conspicuously present in the region of zona reticularis (figure 3). Irradiation of the rats undergoing starvation brought about gradual hypertrophy of cells accompanied by degranulation of the cytoplasm; besides nuclei were markedly atrophied and had agranular nucleoplasm. By the end of 96 hr of post-irradiation most of the cells in the cortex appeared hypertrophied and showed increased depletion of granular contents of the cytoplasm indicating hyperactivity of cortical cells in response to irradiation (figure 4) as compared to the non-irradiated starved rats (figure 3). Contrary to this, normal diet fed irradiated rats showed gradual hypertrophy and vacuolization of cortical cells (figure 2) but the extent of hypertrophy and vacuolization was lesser than that observed in the starved irradiated rats (figure 4).

3.1b *Adrenal medulla*: Chromaffin cells of normal control animals contained secretory granules and the nuclei were large and prominently stained and had granular nucleoplasm (figure 5). In response to inanition stress the medullary cells got hypotrophied and the cells appeared vacuolated. With increase in post-inanition period the cells further became hypotrophied, the cytoplasm appeared almost degranulated, sinusoids were found filled with erythrocytes (figure 7). Total body x-irradiation of starved rats hastened the process of vacuolization of the chromaffin cells with nuclear atrophy and dilation of blood vessels (figure 8). In addition, sinusoids were found filled with erythrocytes (figure 8). Normal diet fed irradiated rats showed degranulation of the cytoplasm of chromaffin cells and copious secretions were observed around the nuclei indicating the secretory activity of the medullary cells (figure 6) in response to whole body irradiation.

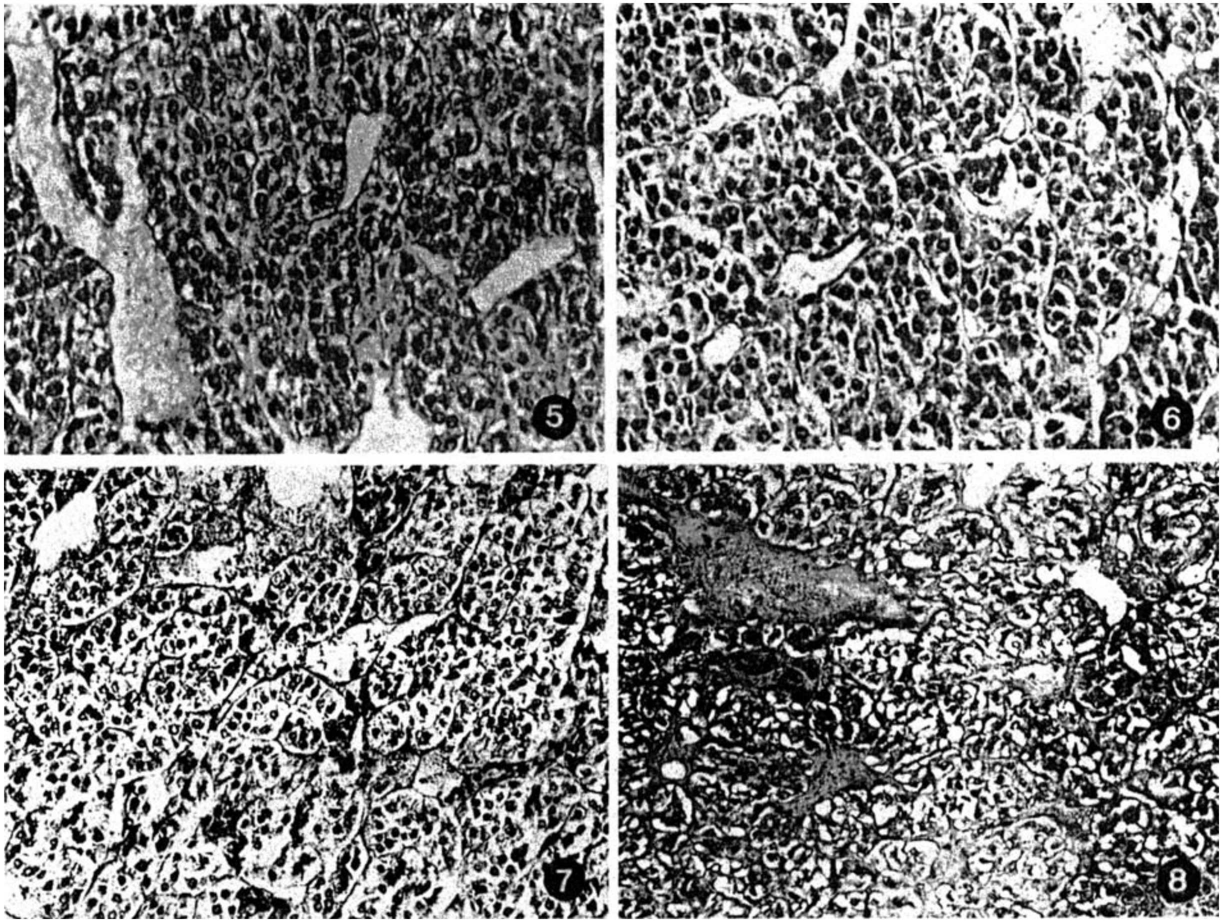
3.2 *Biochemical studies*

3.2a *5-HIAA* (*table* 2): In all the three experimental groups, *i.e.*, the normal diet fed group, the inanition stress administered rats and the irradiated starved rats there was rising concentration of 5-HIAA in their urine as compared to the normal control. But the level of urinary excretion of 5-HIAA was greater in the irradiated rats undergoing inanition stress than in the normal diet fed irradiated group and the non-irradiated inanition administered group.

3.2b *VMA* (*table* 3): As compared to the normal control the level of urinary concentration of VMA was higher in the normal diet fed irradiated control rats and the starved irradiated rats. However, there was an increase in the concentration of VMA in the urine of rats receiving combined treatment of starvation plus irradiation compared with the levels of the normal diet fed irradiated control and the non-irradiated inanition administered rats.



Figures 1–4. TS of adrenal cortex of normal and experimental rats, HE (×160) **1.** normal control showing the presence of stainable granular contents in the cortical cells; **2.** 96 hr after x-irradiation showing appearance of vacuoles in the cells indicating liberation of cortical hormones from the cortical cells; **3.** 10 days after starvation showing nuclear atrophy, nuclei with agranular nucleoplasm and cortical cells being interlaced by the rich bed of sinusoids; **4.** 10 days after starvation/96 hr of post-irradiation showing increased hypertrophy of adrenocortical cells, almost complete depletion of granular material of the cytoplasm and a marked nuclear atrophy.



Figures 5–8. TS of adrenal medulla of normal control and experimental rats, HE, (×160). **5.** Normal control showing medullary cells filled with stainable granular contents and cells with large vesicular nuclei; **6.** 96 hr after irradiation showing nuclear atrophy, depletion of cytoplasmic granular contents and sinusoids with almost negligible amount of erythrocytes; **7.** 10 days after starvation showing hypertrophy of medullary cells, depletion of cytoplasmic granular contents and sinusoids with visible erythrocytes; **8.** 10 days after starvation/96 hr of post-irradiation showing increased depletion of stainable granular contents of the cytoplasm with marked nuclear atrophy and dilated sinusoids being filled by erythrocytes.

Table 2. Urine- 5-HIAA (mg/24 hr) (mean ± SD).

Time of collection of urine (hr)	Normal control *A*	Radiation *B*	Starvation *C*	Radiation + Starvation *D*
24	0·916 ±0·125	1·644 ±0·106 $A:B$ $P<0·01$	1·282 ±0·051 $A:C$ $P<0·01$ $B:C$ $P<0·01$	1·894 ±0·245 $A:D$ $P<0·01$ $B:D$ $P<0·02$ $C:D$ $P<0·01$
48	0·888 ±0·082	1·094 ±0·068 $A:B$ $P<0·01$	1·674 ±0·086 $A:C$ $P<0·01$ $B:C$ $P<0·01$	2·325 ±0·165 $A:D$ $P<0·01$ $B:D$ $P<0·01$ $C:D$ $P<0·01$
96	0·799 ±0·077	1·087 ±0·211 $A:B$ $P>0·01$	1·826 ±0·263 $A:C$ $P<0·01$ $B:C$ $P<0·01$	3·539 ±0·122 $A:D$ $P<0·01$ $B:D$ $P<0·01$ $C:D$ $P<0·01$

Table 3. Urine- 3-methoxy, 4-hydroxy mandelic acid (mg/24 hr) (mean ± SD).

Time of collection of urine(hr)	Normal control *A*	Radiation *B*	Starvation *C*	Starvation + Radiation *D*
24	0·965 ±0·195	1·257 ±0·089 $A:B$ $P<0·01$	1·231 ±0·134 $A:C$ $P>0·01$ $B:C$ $P>0·01$	1·535 ±0·143 $A:D$ $P<0·01$ $B:D$ $P<0·01$ $C:D$ $P<0·01$
48	0·826 ±0·072	1·242 ±0·078 $A:B$ $P<0·01$	1·475 ±0·127 $A:C$ $P<0·01$ $B:C$ $P<0·01$	1·700 ±0·056 $A:D$ $P<0·01$ $B:D$ $P<0·01$ $C:D$ $P<0·01$
96	0·832 ±0·072	1·098 ±0·081 $A:B$ $P<0·01$	1·769 ±0·080 $A:C$ $P<0·01$ $B:C$ $P<0·01$	1·956 ±0·101 $A:D$ $P<0·01$ $B:D$ $P<0·01$ $C:D$ $P<0·01$

4. Discussion

From the results it appears that administration of inanition stress leads to atrophy and vacuolization of cortical cells; besides there occurs dilation of vascular beds of sinusoids in the adrenal cortex. This indicates that the adrenal cortex continues to secrete a minimum amount of cortical hormone in order to meet the salt, water and glucose metabolism of the body during the course of inanition stress. The rats undergoing inanition stress when exposed to total body irradiation showed increased hypertrophy of the cortical cells accompanied by almost complete depletion of the cytoplasm indicating enhanced hyperactivity of the adrenal cortex following total body x-irradiation during the inanition stress. In response to irradiation the non-starved rats also showed hypertrophy and vacuolization of cells in the cortex but the extent of changes were much more striking than those observed in the starved rats. Similar changes in the adrenal cortex of rats following irradiation have been reported in our earlier studies (Hasan *et al* 1977, 1978, 1979). This investigation attributes to the fact that x-irradiation induces hyperfunctioning and forced elimination of cortical hormone from the adrenal cortex in the starved animals rather than in the normally fed animals.

It is observed that under the influence of inanition stress there occurs regression in the size of medullary cells with consequential nuclear atrophy. In addition, chromaffin cells showed vacuolization and sinusoids appeared dilated suggesting increased liberation of hormones from the chromaffin tissues in the circulation. Starved rats when exposed to total body x-irradiation show an enhanced depletion of content of chromaffin cells and dilation of blood vessels with increasing number of erythrocytes in the sinusoids. Hyperactivity of the adrenal medulla is also demonstrated after ^{60}Co irradiation (Hasan *et al* 1977, 1978, 1979). These histological changes indicate increased hyperactivity of the medullary cells in the starved rats compared with the normally fed rats after total body irradiation.

Increased excretion of urinary catecholamine and 5-HIAA was noted after total body x-irradiation. The rise in concentration of 5-HIAA and VMA may be associated with the hyperactivity of 5-hydroxytryptamine and catecholamine in the body as the former happens to be the metabolite of the latter. Similarly, increase in the contents of 5-HT and catecholamine was reported by earlier workers after irradiation (Nair 1965; Hasan *et al* 1977, 1978; Veninga and Brinkman 1962; Renson and Fischer 1959; Varagic *et al* 1967). Rats undergoing inanition stress showed an increase in the excretion of 5-HIAA and VMA in the urine. Thus, it seems that during the period of inanition stress activity of 5-HT and nor-adrenalin gets augmented and probably this increase leads to rising levels of 5-HIAA and VMA in urine of starved rats. Further, the starved rats when exposed to x-rays showed an increase in the excretion of 5-HIAA and VMA and the concentration of these urinary metabolites were greater than in those of the non-irradiated starved rats and the normally fed irradiated rats. Further rise in the levels of 5-HIAA and VMA may be attributed to a stimulatory action of x-irradiation which possibly seems to have accelerated the already enhanced activity of 5-HT and nor-adrenalin owing to inanition stress. Thus, the biochemical studies in the present investigation further corroborate our histological observations on the adrenal gland.

The fact that x-irradiation induces more severe changes in the adrenal gland and brings about an increased excretion of urinary catecholamine and 5-HT in the starved rats rather than in the normally fed rats, favours our data recorded on the rate of mortality.

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