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Effects of the Gamma Ray Irradiation on Histamine Metabolism in the Magnesium-Deficient Rats.

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Introduction

It has been well known that the hyperemia of the ears is the first visible symptom of the experimental magnesium (Mg)-deficiency in rats[6,8]. We found that the hyperemia was mediated by histamine, using the histamine-antagonists[13] and estimating the tissue histamine levels[9-11]. Recently we reported that the splenic histamine content in Mg-deficient rats increased markedly with the enlargement of spleen, in which there were an active hematopoiesis and an increase in the number of basophil cells[4,5].

In this study, examinations were carried out on to the effects of the gamma ray irradiation injurious to the hematopoietic organs, upon the appearance of the hyperemia and the histamine metabolism in the Mg-deficient rats.

Materials and Methods

Diets and animals: The composition of purified diet was the same as that reported previously[9]. Male Wistar rats, weighing about 40g body weight, were used. Before the beginning of experiments, rats were fed a control diet (Mg: 0.07%) for 3 days. Then the rats were divided into four groups. Two groups among these were given an irradiation, consisting of 600 rad, using Cs-137 (GC-40 type irradiator). After that, the irradiated rats were fed with a control diet (γ-ray irradiated control group), or with a Mg-deficient diet (Mg: 0.001%) (γ-ray irradiated Mg-deficient group) for 7 days. The other two groups were the non-irradiated control and Mg-deficient rats. The diet and distilled water for drinking were provided ad libitum. The rats were housed in stainless-steel or metabolic cages and kept at an ambient temperature 23-25°C with a controlled 12-hour light-dark cycle.

Preparation of samples and assay methods: Urine was collected daily from each rat for histamine estimation as previously reported[9]. On the 7th day of experiments, all rats were sacrificed by heart puncture under sodium pentobarbital anesthesia. Blood was collected into a heparinized syringe and the number of red blood cells (RBC) and that of white blood cells (WBC) were counted with colter counter (Model Dn, Colter Electronics Ltd. Luton, Beds, U. K.) and the hematocrit value was determined by micro capillary method. The skin of back and the spleen

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were each removed, weighed and immediately homogenized with Ultra-Turrax (Model TP 18–10, Janke Kunkel KG, Staufen i. Breisgau) in 9 ml of 0.4N perchloric acid for histamine assay[9]. Histamine in both the homogenate with 0.4N perchloric acid and the urine was extracted and determined spectrofluorometrically by the method of Shore *et al*[14,15]. The criteria of hyperemia of ears were used in scoring hyperemia in the same way as reported previously[13].

Statistical evaluation: The mean values of the results are shown in the figures and tables with standard error. Differences between the mean were tested for statistical significance by Student's *t*-test. The significance was established when the probability level was equal to, or less than, 5%.

Results

General observations

Fig. 1. shows the curves of the body-weight-gain in each group. The body-weight-gains of the gamma ray irradiated control and Mg-deficient groups were depressed for three days after the irradiation, and then, they increased gradually. The body-weight-gain of gamma ray irradiated Mg-deficient rats was significantly lower than that of gamma ray irradiated controls, on the 7th day of experiment. The body-weight-gain of Mg-deficient rats was significant lower than that of controls after 5 days of Mg-depletion. The food and water intake of each group is shown in Fig. 2. and 3. The food and water intake of the gamma ray irradiated control and Mg-deficient groups decreased significantly for 3 days after the irradiation, and then increased gradually to the non-irradiated levels.

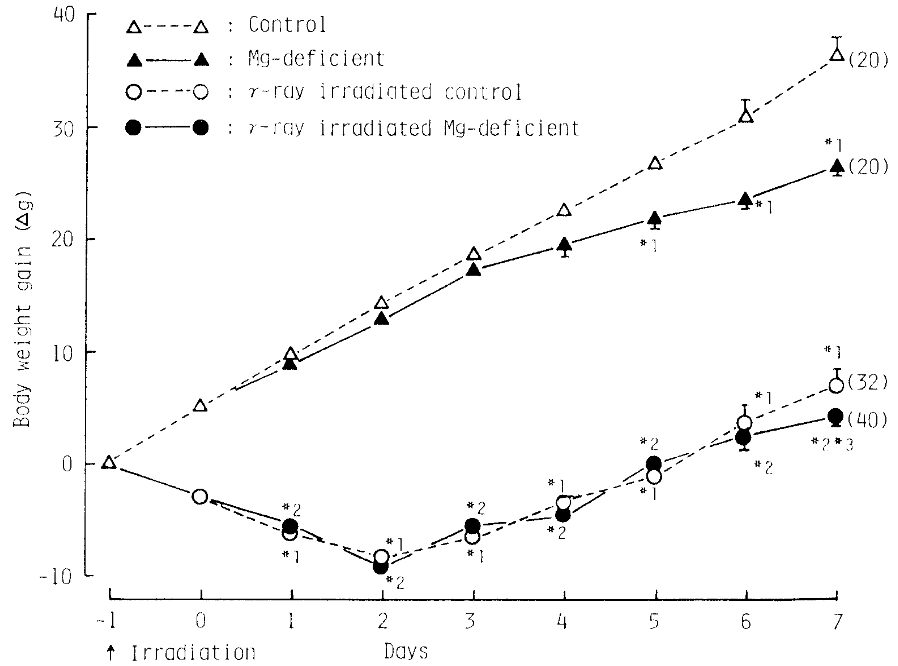

Fig. 1. Body weight gain in control, Mg-deficient, γ-ray irradiated control and γ-ray irradiated Mg-deficient rats. ( ): Number of rats. Vertical bars indicate standard error of the mean.
*1: Significantly different from control rats ($P<0.05$).
*2: Significantly different from Mg-deficient rats ($P<0.05$).
*3: Significantly different from γ-ray irradiated control rats ($P<0.05$).

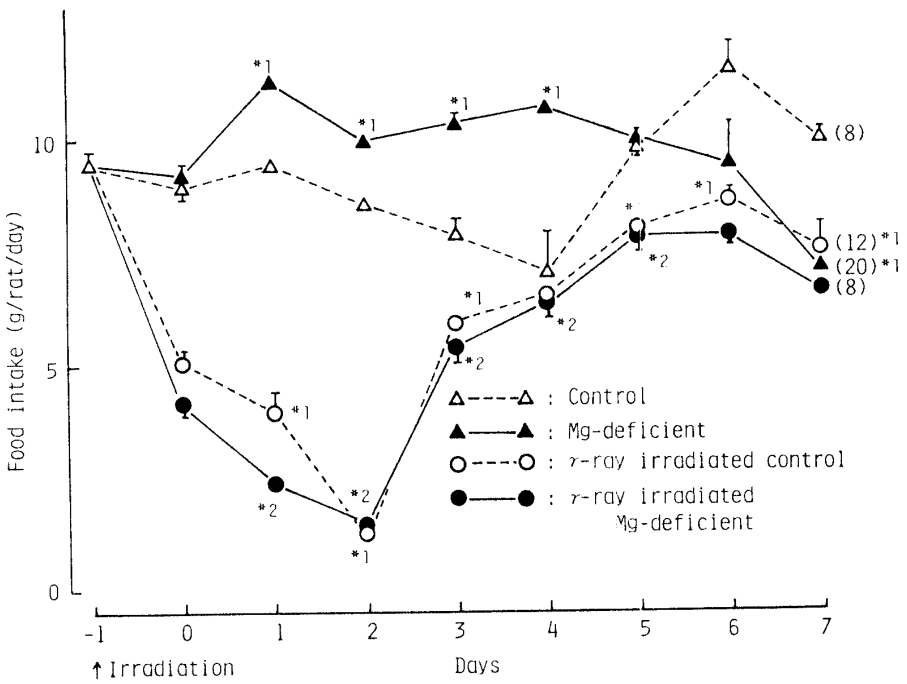

Fig. 2. Food intake in control, Mg-deficient, γ-ray irradiated control and γ-ray irradiated Mg-deficient rats. ( ): Number of rats. Vertical bars indicate standard error of the mean.
*1: Significantly different from control rats ($P<0.05$).
*2: Significantly different from Mg-deficient rats ($P<0.05$).

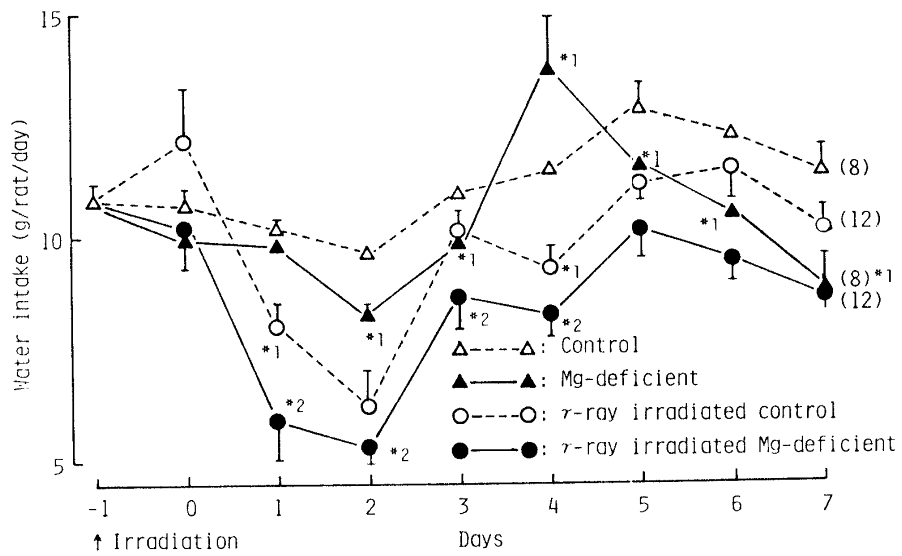

Fig. 3. Water intake in control, Mg-deficient, γ-ray irradiated control and γ-ray irradiated Mg-deficient rats. ( ): Number of rats. Vertical bars indicate standard error of the mean.
*1: Significantly different from control rats ($P<0.05$).
*2: Significantly different from Mg-deficient rats ($P<0.05$).

Hyperemia of ear

Fig. 4. shows the hyperemia of ears in each group. Hyperemia of ears began to be apparent in all Mg-deficient rats from the 3rd day and continued until the 7th day. However, no hyperemia was observed in the gamma ray irradiated Mg-deficient rats and in both the control groups.

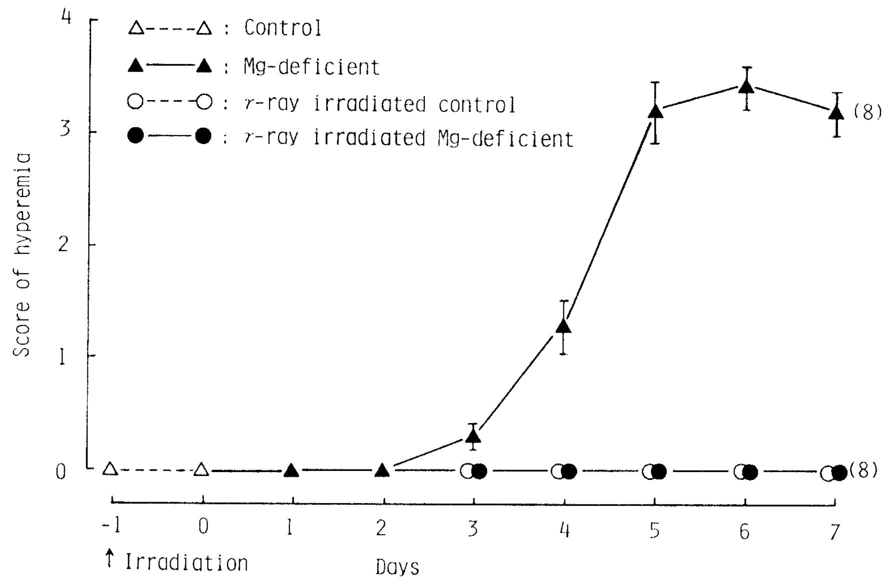

Fig. 4. Hyperemia of ears of Mg-deficient and γ-ray irradiated Mg-deficient rats. ( ): Number of rats. The following criteria were used for the scoring of hyperemia: 1: Hyperemic at the base of the ear, 2: Hyperemic over half of the ear, 3: Hyperemic over three quarters of the ear, 4: Hyperemic over the entire ear.

Urinary histamine

Fig. 5. shows the urinary histamine excretion in each group. Urinary histamine levels increased rapidly from the 5th day in Mg-deficiency, but in control did not significantly change during the experimental period. These results were similar to the previous reports[9,13]. The gamma ray irradiated Mg-deficient group showed lower urinary histamine levels than those of the non-irradiated Mg-deficient group.

Spleen weight

Table 1. shows the spleen weight in each group on the 7th day of experiments. Spleen weight in the Mg-deficient rats increased about 2-fold, compared to that in the control rats. But spleen weights in the gamma ray irradiated control and Mg-deficient groups were about 25% of those in controls.

Hematological data

Table 2. shows the hematological data in each group on the 7th day of experiments. The hematocrit value and the number of RBC were not significantly different between control and Mg-deficient rats, but in the gamma ray irradiated control and Mg-deficient groups were about 70% of those in control rats. The number of WBC in the Mg-deficient rats increased to about 4-fold, compared to that in control rats, but that in the gamma ray irradiated control and Mg-deficient groups decreased to about 25% of those in controls.

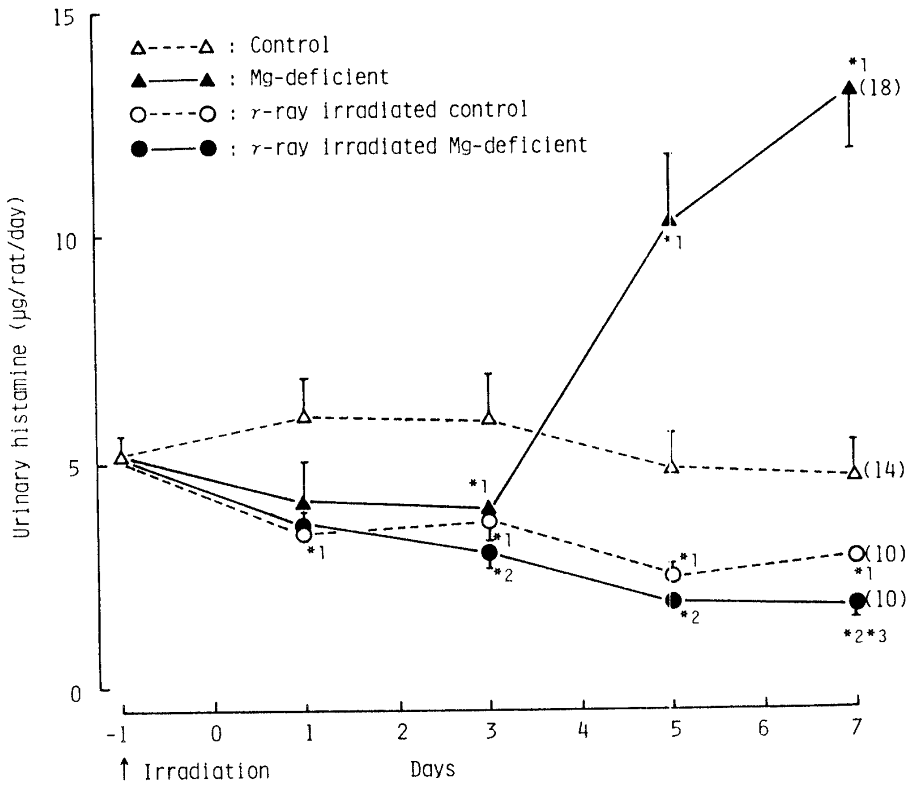

Fig. 5. Urinary histamine level in control, Mg-deficient, γ-ray irradiated control and γ-ray irradiated Mg-deficient rats. ( ): Number of rats. Vertical bars indicate standard error of the mean.
*1: Significantly different from control rats ($P<0.05$).
*2: Significantly different from Mg-deficient rats ($P<0.05$).
*3: Significantly different from γ-ray irradiated control rats ($P<0.05$).

Table 1. Spleen weight in control, Mg-deficient, γ-ray irradiated control and γ-ray irradiated Mg-deficient rats.

		Spleen weight	
		(mg)	(mg/100g. body wt.)
Contorl	(19)	328.6±13.0	361.2±15.8
Mg-deficient	(18)	637.2±33.3*1	788.1±42.4*1
γ-ray irradiated control	(23)	85.1± 5.6*1	136.6± 5.2*1
γ-ray irradiated Mg-deficient	(32)	90.0± 4.3*2	154.9± 5.7*2,*3

Each value indicates mean±S.E. ( ): Number of rats.
*1: Significantly different from control rats ($P<0.001$).
*2: Significantly different from Mg-deficient rats ($P<0.001$).
*3: Significantly different from γ-ray irrdiated control rats ($P<0.05$).

Tissue histamine

Table 3. shows the histamine contents of the spleen and skin on the 7th day of experiments. Histamine contents of the spleen in Mg-deficient rats increased about 6-fold, compared to those in controls. However, in the gamma ray irradiated control and Mg-deficient groups, the histamine

Table 2. Hematologic data in control, Mg-deficient, γ-ray irradiated control and γ-ray irradiated Mg-deficient rats.

	Hematocrit (%)	RBC ($\times 10^4$ cells/mm^3)	WBC (cells/mm^3)
Control	35.6±0.6(18)	478.3±15.2(16)	6715.1±1218.7(15)
Mg-deficient	36.1±0.6(18)	489.1±32.0(15)	29093.6±6592.8(12)*1
γ-ray irradiated control	25.5±0.8(27)*1	337.7±14.2(14)*1	1372.8± 216.9(13)*1
γ-ray irradiated Mg-deficient	23.3±0.6(36)*2	392.1±13.9(15)*2, *3	1767.1± 234.7(16)*2

Each value indicates mean±S.E. ( ): Number of rats.
*1: Significantly different from control rats ($P<0.05$).
*2: Significantly different from Mg-deficient rats ($P<0.05$).
*3: Significantly different from γ-ray irradiated control rats ($P<0.05$).

Table 3. Histamine contents of the spleen and skin in control, Mg-deficient, γ-ray irradiated control and γ-ray irradiated Mg-deficient rats.

		Histamine content (μg/g)	
		Spleen	Skin
Control	(8)	3.1±0.4	32.0±3.5
Mg-deficient	(6)	17.7±2.4*2	33.0±1.5
γ-ray irradiated control	(4)	3.8±0.7	19.2±3.3*1
γ-ray irradiated Mg-deficient	(4)	3.9±0.4*2	19.2±5.6

Each value indicates mean±S.E. ( ): Number of rats.
*1: Significantly different from control rats ($P<0.05$).
*2: Significantly different from Mg-deficient rats ($P<0.01$).

content of the spleen was of no significant difference from that of controls. No significant difference was observed between the control and the Mg-deficient rats in the dermal histamine content. However, in the gamma ray irradiated control and Mg-deficient groups, dermal histamine contents decreased significantly to about 60% of those in the non-irradiated control and Mg-deficient rats.

Discussion

The gamma ray irradiated control and Mg-deficient groups showed diarrhea. So their body-weight-gains were depressed remarkably during 3 days after irradiation (Fig. 1). These phenomena suggest that the gamma ray irradiation is injurous to the stomach and intestine, in addition to the hematopoietic organs (Table 2).

In the Mg-deficient group, an appearance of pinnal hyperemia was noted on the 3rd day of Mg-depletion, growing most remarkable on the 6th day (Fig. 4). These data were similar to the previous reports[12,13]. However, in the gamma ray irradiated Mg-deficient rats, no appearance of pinnal hyperemia was noted (Fig. 4).

Urinary histamine levels in Mg-deficient group began to increase from the 5th day of Mg-depletion, but in gamma ray irradiated Mg-deficient rats did not (Fig. 5). We reported previously that the degree of the hyperemia was correlated to the increase of urinary histamine level during Mg-deficiency[12]. Bēlanger *et al.*[1], Bois *et al.*[2] and Kreaeuter and Schwartz[7] suggested a histamine liberation from the mast cells in Mg-deficient rats. We confirmed the degranulation of dermal mast cell in the early phase of Mg-deficiency[3], and found the increase of basophil cells in the spleen[4]. Hence the assumption that the increased histamine level in plasma and urine during Mg-deficiency might be due not only to the histamine liberation from mast cells, but also from basophil cells. Present results suggest that mast cells and basophil cells, both of which contain histamine, were injured by gamma ray irradiation, and histamine liberation from these cells were inhibited.

We observed the hematopoiesis of the spleen in Mg-deficient rats[4]. The splenic weight in Mg-deficient rats increased about 2-fold than that of control, but that of gamma ray irradiated rats was lighter than controls (Table 1). And also, the number of WBC increased remarkably in Mg-deficient rats, however, in the gamma ray irradiated rats the numbers of WBC and RBC decreased, and likewise in case of the hematocrit value. These data suggest that in the gamma ray irradiated Mg-deficient rats, hematopoietic organs, including bone marrow and spleen, were injured by gamma ray irradiation, and therefore, the hyperemia of the ears might not appear.

Summary

The effects of gamma ray irradiation on histamine metabolism in magnesium deficient rats were studied. Young Wistar male rats were irradiated with gamma ray from Cs–137. Thereafter, the rats were fed a control or a magnesium deficient purified diet ad libitum for 7 days. A significant depression of body-weight-gain was noted in the gamma ray irradiated groups, for 3 days after irradiation, then increased gradually. The number of white blood cells in magnesium-deficient rats was significantly increased on the 7th day of magnesium-deficiency. In the gamma irradiated rats, hematocrit value, numbers of red blood cells and white blood cells were significantly decreased. In the magnesium-deficient rats, an appearance of the pinnal hyperemia was noted on the 3rd day of depletion, growing most remarkable on the 6th day. In the gamma ray irradiated magnesium-deficient rats, no hyperemia was noted during the experiment. In the magnesium-deficient rats, an increasing urinary histamine level was noted on the 5th day of depletion, but in the gammer ray irradiated magnesium-deficient ones urinary histamine level was unchanged. The dermal histamine contents showed no significant difference between the control and magnesium-deficient rats. However, in the gammer ray irradiated rats, was noted a significant depression of the dermal histamine content. In the magnesium-deficient rats, the splenic histamine contents increased significantly on the 7th day of deficiency. However, in the gammer ray irradiated rats, no increment was observed. An increase in spleen weight was observed in the magnesium-deficient rats, but not in the gamma ray irradiated Mg-deficient rats. These results suggest that the hyperemia of ears, the increase of the urinary and splenic histamine levels in the magnesium-deficient rats may chiefly be related to the basophil cells from hematopoietic organs.

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