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STIMULATORY EFFECT OF INTERMITTENT FEEDING ON HEMOPOIETIC RECOVERY IN SUBLETHALLY GAMMA-IRRADIATED MICE

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Abstract

The effect of three-week adaptation to intermittent feeding on the recovery of the hemopoietic functions of mice after sublethal gamma irradiation was investigated. Measurement of oxygen consumption, carbon dioxide output and the respiratory quotient demonstrated an increased metabolic rate in the intermittently fed animals and an accentuation of lipogenic processes. This metabolic state persisted even after irradiation. An improvement in the recovery of hemopoietic functions after irradiation was demonstrated in adapted animals, which was reflected by the increased proliferative activity of the hemopoietic cell populations (more intensive incorporation of ^{125}I-UdR into the DNA of cells of the spleen, thymus and femoral bone marrow), by more rapid renewal of spleen weight, more rapid recovery of the femoral bone marrow cellularity and increased levels of granulocytes in peripheral blood.

We have previously pointed out the possibility of increasing the radiation resistance of mice by their prolonged adaptation to intermittent food intake. The favourable effect of two to six weeks' regular alternation of twenty-four hour periods of fasting and realimentation on the survival of animals after whole-body gamma irradiation with mid-lethal doses has been demonstrated. The optimum effect was achieved after two to three weeks' adaptation to intermittent feeding, where the animals were irradiated in the well-fed condition, i.e. after twenty-four hours' realimentation. After the renewal of the normal feeding regimen a fall of thus induced radiation resistance was recorded (10, 11).

The cause of the increase in radiation resistance under such conditions may be supposed to be adaptive changes in the intermediary metabolism, both quantitative and qualitative. Intermittently fed animals gradually develop metabolic reactions marked by a faster processing and better utilization of nutrients, increased formation of energy reserves and stimulation of mechanisms allowing a faster mobilization of energy resources (6, 15, 16). The aim of the present investigation was to show the effect of these metabolic conditions on the restoration of hemopoietic functions in sublethally gamma-irradiated animals.

Material and Methods

The mice used in the experiments were F_1 male hybrids (CBA/JPh×C57BL/10ScSnPh). The animals were adapted to conditions in the experimental room for 2 to 3 weeks. Mice were kept under controlled lighting (12 h light : 12 h darkness; 6 a.m.–6 p.m.–6 a.m.) at a temperature of 22±2°C, 20 animals per cage (unless otherwise stated).

The animals were subjected to intermittent feeding from the age of 12 weeks. Unlike the controls, which were fed (standard laboratory diet DOS 2b/St VELAZ; carbohydrates content of about 50 cal %) continuously (ad libitum), the experimental animals were fed as follows: 24-hour intervals of fasting alternated with 24-hour intervals of free access to

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food (realimentation) for a period of three weeks. The food was always offered and removed two hours after the start of the 'light' period. Water was supplied ad libitum. The regimen of intermittent feeding was finished at the time of irradiation. After irradiation the experimental animals were given both food and water ad libitum.

The mice were subjected to whole-body irradiation, a single dose from a ^{60}Co source, always in the morning (between 7.30 a.m. and 8.30 a.m.), i.e. in the case of the experimental animals following 24 hours' realimentation. In all experiments the dose used was sublethal, 5.26 Gy. The dose rate was 0.5 Gy/min.

Measurement of the total oxygen consumption was made and the carbon dioxide output was determined by means of an automatic gas analyzer employing the open-circuit system. From the start of experiment the mice were kept individually in glass chambers of 0.5 litre in volume, placed in the experimental room. The control animals were kept under the same conditions except for the feeding regimen. After three weeks' experimental feeding the actual measurements began. In the case of experimental animals measurements were taken after 24 hours' realimentation, i.e. around 8 a.m. The irradiated animals and the controls were measured at the same time of day. The chambers containing the animals were connected to the measuring instrument (which was installed in the adjoining room to minimize stress) 45 min before the start of measuring, by which time the animals had calmed down entirely and exhibited a minimum of physical activity. The oxygen consumption and carbon dioxide output values given in the results are the means from the interval between the 45th and the 60th min from the attachment of the chamber to the analyzer and are expressed in ml/g body weight/h. These data were used to calculate a respiratory quotient (RQ).

When monitoring the state of leukopoiesis the endotoxin mobilization of granulocytes was used. White blood cell counts were measured eight hours after the intraperitoneal injection of 20 μg endotoxin (Lipopolysaccharide B, S. typhosa, Difco, USA) in 0.2 ml physiologic solution per mouse (18). Blood was withdrawn from a light incision in the caudal vein. The numbers of leukocytes in the blood were determined on a Coulter Counter, model ZF (Coulter Electronics, Ltd., England). Numbers of granulocytes and lymphocytes were calculated after differentiation of blood smears. The number of nucleated cells of the bone marrow of the femur was also determined on the Coulter Counter in a suspension obtained by perfusion of the femurs following the removal of their epiphyses.

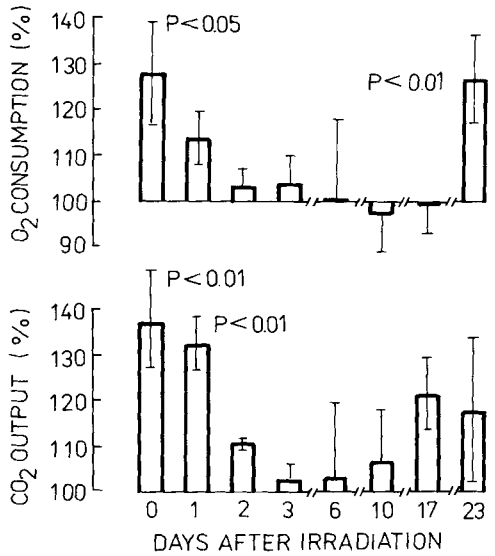

Fig. 1. Consumption of O_2 and CO_2 output in mice adapted to intermittent feeding and measured before (0) and at various intervals after irradiation. The results are expressed as percentages of the control group.

Table 1

Values of the respiratory quotient of mice from the period before and after irradiation. All data were obtained from fed animals, in the case of those subjected to intermittent feeding after 24 h realimentation

	Controls	Intermittent feeding
Before irradiation	0.92±0.02	1.06±0.10
After irradiation (days)		
1	0.88±0.04	1.02±0.04*, ***
2	0.98±0.05	1.05±0.04**
3	0.98±0.01	0.94±0.08
6	0.87±0.10	0.91±0.07
10	0.94±0.06	1.03±0.09
17	0.95±0.08	1.16±0.11*
23	1.01±0.10	0.95±0.10

* $p<0.05$.
** $p<0.01$, compared with unirradiated controls.
*** $p<0.05$, compared with irradiated controls.

The intensity of cell proliferation in the spleen, thymus and bone marrow was monitored using the incorporation of ^{125}I-iododeoxyuridine by DNA-synthesizing cells (^{125}I-UdR) (5, 8, 17). At various time intervals (before and after irradiation) animals were injected intraperitoneally with 3.7×10^4 Bq ^{125}I-UdR (Amersham, England; spec. act.

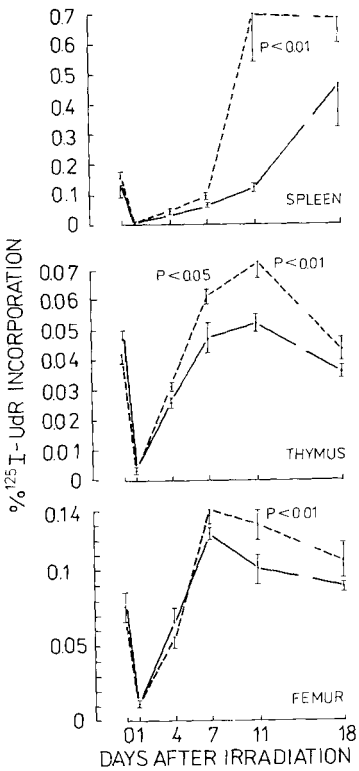

Fig. 2. Changes in the intensity of ^{125}I-UdR incorporation into the cells of the spleen, thymus and femoral bone marrow of mice adapted to intermittent feeding and monitored before (0) and at various intervals after irradiation. The results are expressed as percentages of applied activity. Control animals (—), animals adapted to intermittent feeding (– – –).

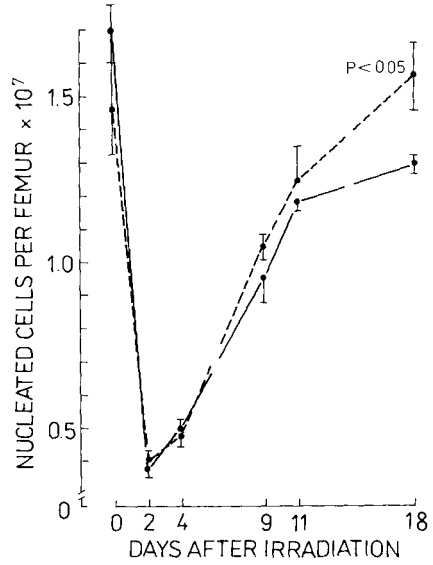

Fig. 3. Changes in the number of nucleated cells of the femur in control (—) and experimental animals (– – –), the latter fed intermittently before irradiation, measured before (0) and at various intervals after irradiation.

1.5×10^{12} Bq/mmol) in 0.4 ml physiologic solution. In order to inhibit the synthesis of thymidilate animals were injected with 5-fluoro-2′-deoxyuridine (SERVA, G.F.R.) at 10^{-7} mol in 0.2 ml distilled water (8, 19) 30 min before ^{125}I-UdR administration. Six hours after the administration of ^{125}I-UdR the animals were killed by decapitation, the spleen, thymus and left femur were excised and placed in 10% buffered formalin for 48 h to remove radioactive iodine not incorporated into DNA (4). The radioactivity of the organs was measured using the Nuclear Chicago Automatic Gamma Well Counting System and expressed as a percentage of the total activity administered.

The data presented are the means ($\bar{x}$) ±SE. At least ten animals were evaluated in each group. The statistical significance of the results was assessed using Student's t-test.

Results

The results of the monitoring of the total metabolic intensity of mice by means of the respiratory exchange of O_2 and CO_2 are given in Fig. 1. Compared with controls fed ad libitum, the animals adapted to intermittent feeding for three weeks exhibited an increased intensity of metabolic processes. The state of increased respiratory exchange persists for several days after irradiation in the intermittently fed mice. After a certain transitional period in which the intensity of metabolism does not differ greatly between the two groups, the adapted animals display a renewed increase in respiratory exchange towards the end of the postirradiation monitoring period. The RQ values of experimental animals were around 1.0 even after irradiation, i.e. in the period of free access to food. The controls exhibited a similar metabolic state on the 2nd, 3rd and 23rd days after irradiation (Table 1).

Fig. 2 shows changes in ^{125}I-UdR incorporation into cells of the spleen, thymus and the bone marrow of the femur. In experimental animals a marked increase in incorporation of labelled precursor into the DNA of the cells of all the hemopoietic organs studied was noted in the interval between the 7th and 18th days after irradiation. These results demonstrate a greater proliferation of the cells of regenerating hemopoietic tissues in the intermittently fed mice.

In accord with these results, higher numbers of nucleated cells of the femur (Fig. 3) and greater spleen weights (Fig. 4) were recorded in the experi-

Table 2

Numbers of lymphocytes and granulocytes in one μl peripheral blood 8 h after the injection of endotoxin on the 15th and 17th days after irradiation in control and experimental animals, the latter fed intermittently before irradiation

Days after irradiation	Controls		Intermittent feeding	
	Lymphocytes	Granulocytes	Lymphocytes	Granulocytes
15	381±102	9 299±2 232	378±78	13 000±2 233
17	436±85	10 949±1 085	358±60	17 561±1 789*

* $p<0.01$, compared with controls.

mental animals at the stated interval after irradiation. After three weeks' adaptation to intermittent feeding a reduction in the weight of the thymus (Fig. 4) was observed. After irradiation (except for the 7th day) the pattern of thymus regeneration was similar in both control and experimental animals.

In order to monitor the effects of intermittent feeding on the state of leukopoiesis after irradiation endotoxin-mobilized numbers of granulocytes were used (Table 2). Differences were observed in the recovery phase, during the third week after irradiation. Higher blood granulocyte counts were found in the experimental animals. Numbers of lymphocytes in the peripheral blood were not affected.

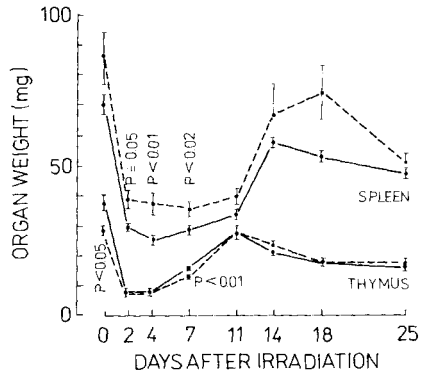

Fig. 4. The weight of the spleens and thymuses of control (—) and experimental animals (– – –), the latter fed intermittently before irradiation. The values were measured before (0) and at various intervals after irradiation.

Discussion

The results of measurement of the incorporation of ^{125}I-UdR into the DNA of spleen, thymus and bone marrow cells, the monitoring of the cellularity of bone marrow, endotoxin-mobilized peripheral granulocyte levels and spleen weight show that prolonged intermittent feeding favourably affects the recovery of the hemopoiesis after irradiation. The metabolic influences thus exerted are an effective stimulus, reinforcing the regeneration processes in hemopoietic organs of irradiated animals. It is important that this stimulatory effect influences also granulopoiesis, which is the decisive function in overcoming the effects of bone marrow syndrome. At the same time, however, one must expect the stimulus used to cause a certain stress. This is shown by the results of the monitoring of thymus weights. The lower weight of the thymus in the period before irradiation may be due to the lympholytic effect of increased levels of glucocorticoids.

It should be stressed that the effects observed are the result of a long-term adaptation to the altered nutritional regimen; realimentation after a single 24-hour period of fasting does not modify radiation resistance (11).

As has been demonstrated, the adaptation of mice to intermittent feeding is accompanied by a significant activation of the overall metabolism. This state persists for a certain time after irradiation. Changes in the respiratory quotients show that the adapted animals, in contrast to the controls, exhibit activation of those metabolic pathways which lead to an increased transformation of the carbohydrates into fat (activated lipogenesis). Experiments on rats lead one to suppose that animals adapted to a sudden food supply are capable of increased transformation of all types of nutrients (2, 6). There is an increase not only in the formation of energy reserves, due to the activation of the lipogenic, glycogenosynthetic and gluconeogenic systems (16, 20, 21, 24), but the functionability of the systems capable of mobilizing these energy sources is reinforced (6, 16). This results in an increased rate of metabolism of nutrients received and their better utilization (1, 2, 6). The increased metabolic rate and the accelerated recov-

ery of the hemopoietic functions after irradiation in intermittently fed animals are apparently interrelated processes. Other radiobiologic experiments have shown that active energy metabolism is a condition of effective postirradiation repair and regeneration. It has, for instance, been demonstrated that animal species with a higher intensity of metabolism also have a higher level of repair and more rapid recovery of hemopoietic tissue, after irradiation, which correlates with the increased radiation resistance of these animals (13, 23).

The question of the direct or indirect mediation of the metabolic effects induced by intermittent food intake on the hemopoiesis of irradiated (and also unirradiated) organisms remains open, as does that of the level at which these effects occur. Our preliminary results indicate that under the experimental conditions used there is a major effect on the kinetics of the stem cell populations (CFU-S), consisting in an expansion of their pool at the time of irradiation and their more intensive proliferation after irradiation. It may be that both a more favourable energy situation and certain specific mechanisms may contribute to these effects. It is known that intermittent feeding induces in rats a proliferation of the cells of adipose tissue, these being smaller and more numerous than the adipocytes of controls (3). Assuming a similar situation in bone marrow, one may consider the possibility of the regulation of the proliferation of hemopoietic cell populations by means of factors produced by the adipocytes of the marrow. A preadipocytic cell line has been isolated from the bone marrow of mice, which has the ability of promoting proliferation of CFU-S through a short range cell-to-cell interaction (9, 12).

The manner of increasing the postirradiation recovery of hemopoiesis as described here can be added to those experimental means of adaptive increase in radiation resistance already known, such as exposure of animals to prolonged action of cold (7), hypoxic hypoxia (22) and long-term thyroid hormone administration (14). However, the physiologic character of the adaptive changes induced by intermittent feeding has to be emphasized.

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