# In vivo Studies of the Relationship between the Activation of Lipid Metabolism, Postirradiation Bone Marrow Cell Proliferation and Radioresistance of Mice

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**Abstract.** The effect of adaptation to intermittent feeding on the in vivo biosynthesis of fatty acids and total lipids in the epididymal adipose tissue, the liver and the bone marrow was studied in adult male mice (CBA/JPh × C57BL/ /10 ScSnPh)F₁. At the same time the effects of the same experimental stimulus on the rate of regeneration (proliferation) of bone marrow cells after sublethal irradiation of animals and on the overall radioresistance of mice expressed as 30 days survival after whole-body gamma irradiation were determined. Intermittent feeding in mice has been shown to have a significant effect on the biosynthesis of fatty acids and total lipids in all the tissues studied, including bone marrow cells, the intensity of the effect being closely dependent on the duration of the experimental stimulus. Maximum stimulation of lipogenesis during realimentation was observed approximately within 1 week of adaptation, with a reduction of the metabolic responses thereafter. The intensity of bone marrow cell proliferation in mice irradiated in the realimentation phase was inversely proportional to the preirradiation degree of biosynthesis of fatty acids and total lipids: in a period of lower lipogenetic capacity of cells in the tissue studied (around the weeks 2—5 of adaptation) an increase in the regeneration potential of bone marrow cells was observed together with increased radioresistance of the mice. During the 1-week of adaptation the opposite proved to be the case. Attention is drawn to the possible participation of prostaglandins and lipid peroxides in the responses observed.

**Key words:** Fatty acids metabolism — Bone marrow — Cell proliferation — Radioresistance

## Introduction

A large body of radiobiological and other experimental evidence suggests a close dependence between quantitative (and qualitative) metabolic deviations and the cytokinetic parameters of cells of many tissue types. This is manifested in different resistance of cells (especially of renewal populations) and organisms to penetrating radiation (Pospíšil and Vácha 1983). In studying conditions of the regulation of cell proliferation, attention has recently been paid to substances relating to the lipid metabolism, in particular highly unsaturated fatty acids and their derivatives; most of the work in this respect has been done in vitro (Cornwell and Morisaki 1984). In order to extend our understanding of the problem to in vivo systems, it would appear useful to exploit results obtained from studies into the effects of intermittent food intake, a stimulus with a major effect on the metabolism of nutrients consumed (Fábry 1969). If animals under condition of intermittent feeding are realimented with a carbohydrate-rich diet, saccharides are used mainly to replace loss of energy sources resulting from starvation, i.e. glycogen and lipids. The nutritional regimen of regular alternation of fasting and subsequent realimentation results in a complex of adaptive changes in tissues, expressed inter alia in an enhancement of the processes involved in utilization of nutrients. In particular the biosynthesis of fatty acids and lipids gets on such a high level that it prompted the formulation of the concept of "adaptive hyperlipogenesis" (Tepperman and Tepperman 1964). Also, the intensity of the lipogenetic response has been found to be determined to a large extent by the duration of the experimental feeding schedule (Petrásek et al. 1970).

We could show previously that mice subjected to intermittent food intake exhibit different radioresistance in dependence on the length of the adaptation period; the highest resistance to whole-body gamma irradiation is accompanied by a favourable effect on haemopoiesis, including that of the stem cell populations (Kozubík and Pospíšil 1982, 1985; Kozubík et al. 1985). The aim of the present work was to investigate in more detail these effects, i.e. the relationship between the degree of the activation of lipid metabolism, and the proliferation of bone marrow cells or organisms surviving following radiation exposure.

## Materials and Methods

*Animals and the intermittent feeding schedule (IF)*

Male $(CBA/JPh \times C57BL/10 ScSnPh)F_1$ mice were kept under controlled light cycle (12 h light : 12 h darkness; $06:00 - 18:00 - 06:00$) at a temperature of $22 \pm 2\,°C$, 20 animals per cage.

At the age of 12 weeks the mice were subjected to alternating periods of 24 h fasting and subsequent 24 h feeding ad libitum (realimentation) for 1—6 weeks. The animals were given standard pelleted laboratory diet (DOS 2b/St VELAZ) with a carbohydrate content of about 50 cal%. Food was offered or removed two hours after the start of the "light" period. Water was supplied ad libitum. The intermittent feeding schedule was discontinued as soon as irradiation was started. After irradiation the experimental animals were given both food and water ad libitum. Both the total food consumption and body weight of animals on the intermittent feeding schedule did not significantly differ from those of animals fed ad libitum. The experimental animals were thus able to compensate for food deficiency during starvation by higher food intake on realimentation days. Control (C) animals had continual access to food ad libitum.

**Irradiation.** The mice were whole-body gamma irradiated with a single dose from a $^{60}$Co source, during the morning hours (between 07:30 and 08:30); the experimental animals were irradiated following 24 h realimentation (after 1—6 weeks of intemittent feeding). Both sublethal (5 Gy) and lethal (9.5 Gy) doses were used. The dose rate was 0.5 Gy/min.

The cell proliferation assay (Hübner et al. 1981) is based on determination $^{125}$iododeoxyuridine ($^{125}$I-UdR) incorporation into bone marrow cells 10 days after sublethal irradiation. $^{125}$I-UdR, a thymidine analogue, is specifically incorporated into DNA and may be used as a label for proliferating cells. The mice were given $3.7 \times 10^4$ Bq $^{125}$I-UdR (Amersham International, Amersham, England) intraperitoneally in 0.4 ml saline. In order to inhibit endogenous thymidilate synthesis, $10^{-7}$ mol 5-fluoro-2′-deoxyuridine (SERVA, Feinbiochemica, Heidelberg, FRG) was injected intraperitoneally 30 min before the $^{125}$I-UdR injection (Hughes et al. 1964; Takada et al. 1971). Six hours after the injection of the isotope the mice were killed, their femora excised and placed for 48 h in 10% buffered formalin to remove radioactive iodine not incorporated into DNA (Bürki et al. 1971). The activity of the femora was measured using a Nuclear Chicago Automatic Gamma Well Counting System and expressed as per cent of the total activity administered.

**Lipid analysis.** Lipids were extracted from liver, epididymal adipose tissue and bone marrow samples according to Folch et al. (1957), incubated and analysed by a modification of the method of Hillyard and Entenman (1973). One hour before sacrificing animals by cervical dislocation, U-$^{14}$C-glucose (Institute of Nuclear Research, Prague; 7 400 kBq/1 kg body weight) was administered i.p. Bone marrow suspensions were obtained by quantitative flushing with 2 ml saline, and the organs were excised and weighed. Then all tissues were homogenised and extracted. Samples (2 ml) of the chloroform phase of the chloroform-methanol extracts of liver, adipose tissue and bone marrow were evaporated under nitrogen; the isotope incorporation into total lipids was measured. Another 2 ml aliquots of the chloroform phase were evaporated and hydrolysed in 30% alcoholic KOH for 5 h at 70—80 °C. The non-saponified portion of the lipids was extracted into petroleum ether. Fatty acids in the saponified part were extracted after acidification with 10 N HCl into petroleum ether and after evaporation their radioactivity was measured. Samples of total lipids and fatty acids were solubilised in 10 ml of the scintillation mixture STL 31 (Spolana Neratovice, Czechoslovakia). The radioactivity was measured in a liquid scintillation spectrometer (LKB-WALLAC 81 000, Broma, Sweden) according to the external standard method (results are expressed in terms of total radioactivity per gram wet tissue weight or per two femora). All the analyses were made in duplicate; in bone marrow suspensions determinations were made in pooled samples. The experimental animals were tested both after 24 h fasting and after 24 h realimentation (between 07:30 and 20:30).

**Statistics.** The values shown in the Figures are means ± S.E. Statistical significance of differences was evaluated using the $\chi^2$ test, the distribution test, and the Student's $t$-test. For biochemical and

cytokinetic studies at least eight animals per group were analysed; for experiments monitoring survival after lethal irradiation 20 to 40 animals per group were used.

## Results

Figure 1 summarises the results of measurements of incorporation of $U^{14}C$--glucose into total lipids and fatty acids in fat tissue, liver and bone marrow of control and experimental (i.e. fed intermittently for 1—5 weeks) animals. The intensity of incorporation of the labelled precursor into the tissues suggests a considerable depression of biosynthesis of fatty acids in the adipose tissue and the liver of all experimental animals fasted for 24 h; in the bone marrow it was impossible to determine fatty acids in fasted mice due to technical reasons. At the same time a suppression of the synthesis of total lipids in the adipose tissue and the bone marrow was recorded in the fasted animals compared with the fed ones, i.e. those realimented for 24 h (in the liver higher level of biosynthesis of total lipids was recorded in the fasted mice compared with the fed ones). In the
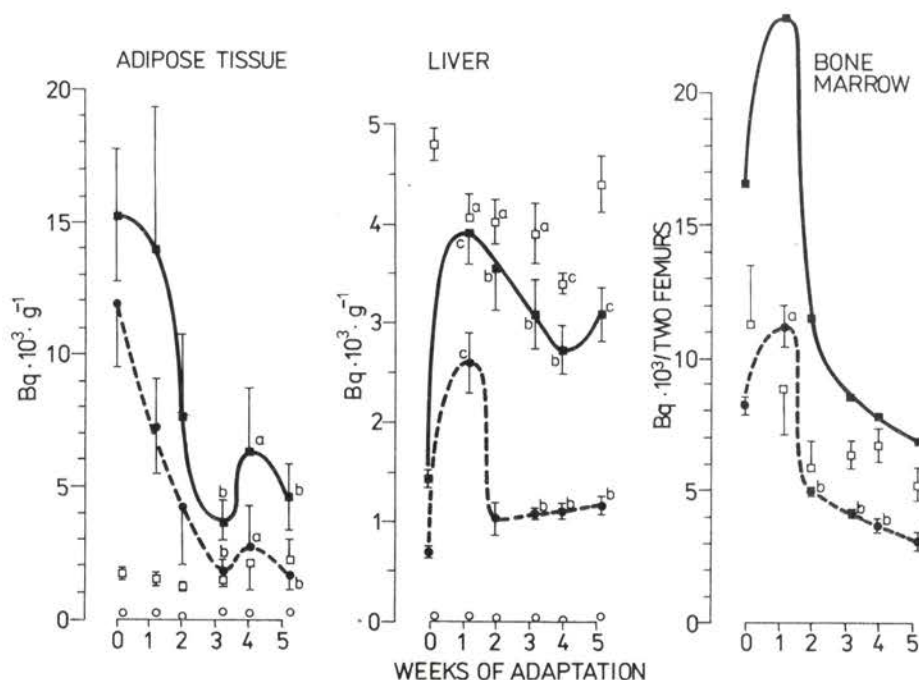


**Fig. 1.** Incorporation of $U^{14}C$-glucose into total lipids (■) and total fatty acids (●) in realimented and fasted mice (open symbols) in the adipose tissue, liver and bone marrow of control (interval "0") and 1—5 weeks adapted animals. *a*: $p < 0.05$, *b*: $p < 0.01$, *c*: $p < 0.001$ as compared with control values.

realimented animals the intensity of the lipogenetic reactions exhibited considerable fluctuations in dependence on the duration of experimental conditions; in all tissues monitored a clear drop of U-$^{14}$C-glucose incorporation into both fatty acids and total lipids was observed following the first week of adaptation. This drop was preceded by a phase of stimulation, with maximum intensity of lipogenesis around the first experimental week as clearly reflected in the liver and the bone marrow.

Figure 2 shows changes in the intensity of $^{125}$I-UdR incorporation into the DNA-synthesising bone marrow cells in control and in animals adapted for 1—6 weeks, monitored on day 10 after whole-body gamma irradiation with 5 Gy in two independent experiments (experiment I was performed during November and December, experiment II during January and February). It is clear that intermittent food intake during the preceding week depresses or does not affect the postirradiation recovery of cell proliferation, while two to four-week experimental feeding conditions prior to irradiation stimulate postirradiation recovery; after intermittent feeding over five to six weeks the stimulatory effect disappeared almost entirely.
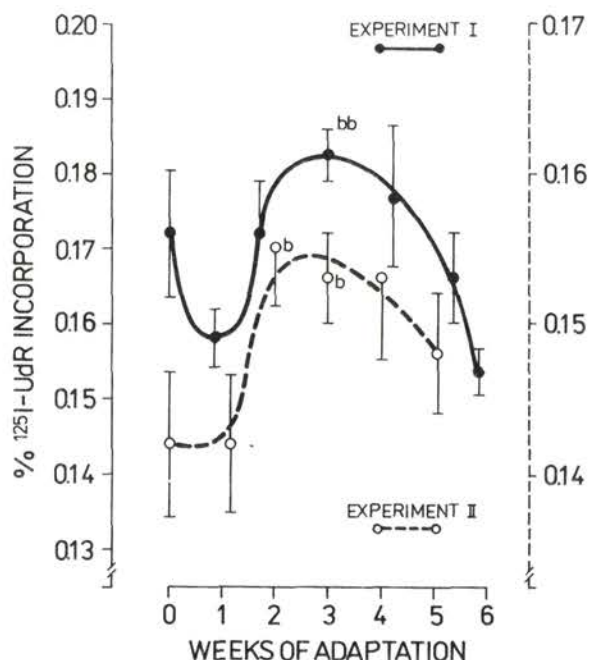


**Fig. 2.** Incorporation of $^{125}$I-UdR into DNA synthesising bone marrow cells of control (interval "0") and 1—6 weeks adapted mice measured on day 10 after sublethal (5 Gy) whole-body irradiation; two independent experiments. b: $p < 0.01$ vs. control animals (experiment II); bb: $p < 0.01$ vs. animals adapted for 1 week to intermittent feeding (experiment I).

Results of thirty-day survival studies of animals subjected to lethal doses of gamma irradiation following metabolic modifications as described above, are shown in Fig. 3. As compared with controls, a non-significant decrease of the degree of 30 days survival after irradiation can be observed in animals adapted for one week and, a clear improvement of the survival in experimental weeks 3 and 4 (with a gradual drop during the subsequent weeks of adaptation).
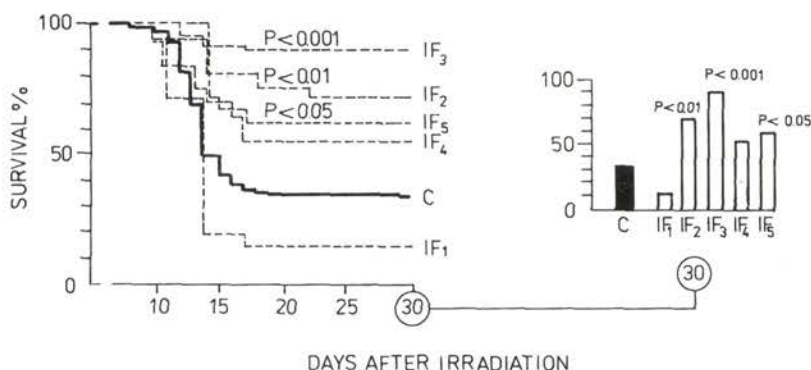


**Fig. 3.** Effect of the duration of the intermittent feeding scheme on survival of mice irradiated after 24 h of realimentation. C = controls, $IF_1 - IF_5$ = animals adapted to intermittent feeding for 1—5 weeks. Statistical significance of differences between control and experimental animals is shown.
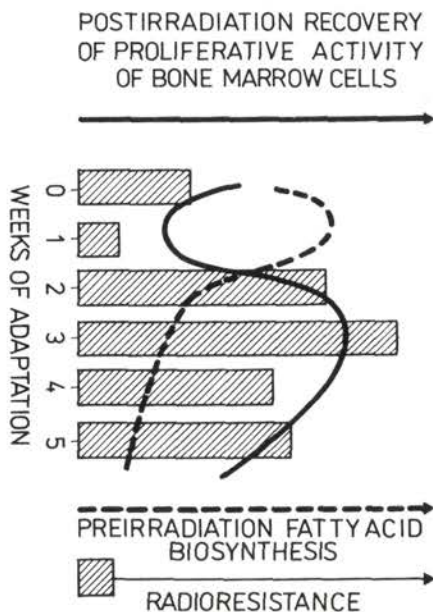


**Fig. 4.** Schematic representation of the relationships between the preirradiation degree of biosynthesis of fatty acids (lipogenesis) and the postirradiation intensity of the recovery of bone marrow cell proliferation (measured on day 10 after irradiation) and the radioresistance of mice in terms of survival during 5 weeks of adaptation to intermittent feeding.

## Discussion

The amount of available nutrients has marked effects on both quantitative and qualitative deviations in the metabolism from the first days of adaptation to the experimental nutrition schedule. Even fasting for one day reduces the glucose turnover in rats to half, with other glucogenic substrates being exploited preferentially (Rémésy and Demigné 1983). Realimentation with a high-carbohydrate diet activates the respective enzymes and restores energy reserves: glycogen, and especially lipids (Bouillon and Berdanier 1981; Williams and Berdanier 1982; Careche et al. 1985). Our results of monitoring of fasted mice also suggest that due to drops in both glucose supply and turnover the biosynthesis of fatty acids and lipids is largely depressed. Following feeding lipogenesis is enhanced, and even 24 h after starting realimentation the levels measured in the tissues monitored are well above those for fasted animals (with the exception of the biosynthesis of total lipids in the liver, where there are no major differences between fasting and realimentation during adaptation). From the viewpoint of the dynamics of these changes in realimented animals it is significant that maximum lipogenetic effect in the tissues monitored (except for adipose tissue) was observed after about the first week of adaptation, with a gradual decrease of lipogenesis stimulated in this way during the subsequent period. Also it follows from these results that in mice, the development of intensity changes in the metabolic responses over time are very similar to that reported for rats on similar feeding scheme (see Petrásek et al. 1970). The response of the adapting organism seems to be of a generally applicable nature. A further important finding is that the metabolic responses studied are also manifested in the bone marrow. This means that an association may be established between metabolic influencing of an organism and its radioresistance from the viewpoint of the role of the critical organ in the radiation damage, the bone marrow.

In evaluating the role of metabolic deviations associated with the radiation damage to the organism two parameters were chosen: the incorporation of ^{125}I-UdR into DNA-synthesising cells on day 10 after whole-body sublethal irradiation, and the survival of animals exposed to lethal doses of gamma irradiation. On day 10 after the irradiation the intensity of the labelled precursor incorporation into bone marrow cells peaks, and it can be understood as a measure of the initial degree of damage to the hemopoietic stem cells, as well as of that of postirradiation repair and regeneration (Kozubík et al. 1985). The two indices of the radiation damage vary in parallel with the different influence on the metabolism: a postirradiation lethality increase is observed at a low degree of postirradiation bone marrow cell proliferation in sublethally irradiated animals, and a reduced postirradiation lethality upon a high degree of bone marrow cell regeneration. The relationship between metabolic conditions of the

organism and its postirradiation response can thus be considered from the viewpoint of the role of radioresistance of bone marrow cells and manifestations of the bone marrow syndrome. The question arises how the activation of biosynthesis of fats and fatty acids is associated with the postirradiation response of the organism. This association may be very complex. It has been shown (Kozubik et al. 1985) that intermittent feeding for three weeks improves the proliferation potential of the stem cells of an irradiated organism, and that contributions are made to this effect not only by an increased cell proliferation potential but also by the stimulatory role of the haemopoietic induction microenvironment. Hence the role of mechanisms affecting postirradiation regeneration of surviving haemopoietic populations should be considered. However, the possibility cannot be ruled out that radiosensitivity of haemopoietic stem cells is affected in a way that increases or decreases their postirradiation survival. The involvement of different activation rate of lipid metabolism in these mechanisms which are decisive for the haemopoietic recovery of an irradiated organism may hypothetically be determined in particular by the relationship of fatty acids to the formation of their products, i.e. lipid peroxides, possibly prostaglandins. This hypothesis assumes that in parallel with a higher endogenous biosynthesis of the fatty acids studied in our experiments there is an improved exploitation and incorporation of fatty acids from exogenous sources, i.e. arachidonic acid as a precursor of prostaglandins. Prostaglandins, products of the cyclooxygenase pathway of the transformation of polyunsaturated fatty acids, can affect postirradiation recovery of haemopoiesis. Kurland et al. (1978) have postulated the role of type E prostaglandins as being humoral factors depressing proliferation of myeloid stem cells. Our recent work showed that postirradiation recovery of haemopoiesis can be enhanced by administering indomethacine, a substance suppressing prostaglandin formation (Pospíšil et al. 1986). Many other reports (for a review see Cornwell and Morisaki 1984) have suggested that prostaglandins are effective regulators of cell proliferation, and emphasised the concentration-dependence of the effects. Highly unsaturated fatty acids — prostaglandin precursors — have been found to act on proliferation in a stimulatory manner up to certain concentrations, while at higher concentrations they have inhibit any effects. Fatty acids at the same time, produce free radicals and lipid peroxides. Their quantities also depend on the concentration of their precursors, and they have a radiosensitising effect (Goncharenko 1973), inhibit cell growth at high concentrations or even act cytostatically (Cornwell and Morisaki 1984). The degree of postirradiation recovery is then determined by the sum of the effects of both prostaglandins and lipid peroxides. The deterioration of marrow haemopoiesis regeneration and the decreased radioresistance observed in our experiments in the first week of adaptation to intermittent feeding may have been associated with deleterious

effects of excess activation of the lipid metabolism during this interval, i.e. with increased formation of lipid peroxides or prostaglandins. During the later phases (between weeks 2 and 5 of the adaptation period) characterised by a drop of the total lipid metabolism in the bone marrow to below control values, less lipid peroxides and prostaglandins were likely formed, and their expected radiosensitising effect and depressing effect on cell proliferation was weaker. Figure 4 schematically illustrates the possible associations existing between response of an organism to irradiation and the initial state of the biosynthesis of fatty acids. Further speculations are premature; above all it should be established whether in vivo changes in the degree of activation of bone marrow lipid metabolism are accompanied by changes in the biosynthesis of substances of the prostaglandin group.

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