Adaptation to intermittent fasting as a factor modifying the radiation resistance of mice

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Summary. Mice adapted to intermittent food intake (24 h fasting:24 h feeding) for 2-3 weeks exhibit a higher resistance to whole-body gamma irradiation. This is manifested by increased survival of animals and a more effective recovery of blood leukocyte counts.

Due to a close relationship between the metabolic state of a biological system and manifestations of its radiosensitivity[1] attempts have been made to modify the radiation resistance of the mammalian organism by physiological means based on the control of food intake. A short-term reduction of the food intake before irradiation did not influence the $LD_{50/30}$-values in rats[2], but it did protect from acute gastrointestinal radiation deaths[3]. The experiments mentioned presumed a protective role of the reduction of metabolic processes at the time of irradiation and a possible response to these effects at the level of radiosensitive cell populations. Furthermore, a moderate increase in the radiation resistance of mice irradiated at the end of a 2-days re-alimentation period following a previous 2-days fast was reported, and the importance of stimulating the recovery of hemopoietic functions was discussed[4]. The results presented introduce novel aspects into the study of these problems and suggest the possibility of modifying the radiation resistance of the organism by the induction of adaptive metabolic changes which occur in the course of prolonged intermittent food intake.

Material and methods. Male mice of a non-inbred H strain, aged 12-14 weeks at the onset of the experiment, were used. The animals were caged in groups of 20 and kept under a controlled lighting regime (12 h light:12 h darkness), at a temperature of 22±1 °C. In contrast to the control animals given food ad libitum (standard laboratory diet with carbohydrate content of about 53 cal%), the feeding regime of the experimental groups was as follows: 24-h intervals of free access to food were alternated with 24-h intervals of fasting for a period of 1-6 weeks. Food was always given at the beginning of the light period. Access to tap water was not restricted. The animals were whole-body irradiated (^{60}Co gamma-ray source, dose rate 0.65 Gy/min) at the beginning of the light period, after a 24-h period of re-alimentation. From this time on, the mice were allowed free access to food. Blood samples were drawn from a fine incision in the tail vein. Repeated blood withdrawals were made and white blood cells counted by a Coulter Counter. Student's t-test and the χ^2-test were used for statistical evaluation of the results.

Results. The results evaluating the effect of the experiment on the survival of the animals are evident from figure 1. After irradiation with a dose of 8.70 Gy, a significantly increased survival was observed in mice adapted to intermittent fasting for 2-3 weeks (groups IF_2-IF_3). Only a delayed onset of dying is apparent in the other experimental groups. The period of the maximum effect (i.e., 2 weeks of adaptation) is accompanied by a better recovery of the leukocyte blood cell line following a sublethal dose of 5.26 Gy (fig. 2). This effect is highly significant from the 11th day after irradiation onwards.

Experiments evaluating the survival of intermittently fasted and irradiated animals were performed also on female mice. The results (data not given) indicated a similar radioprotective effect of the experimental regime on mice of both sexes.

Discussion. In the organisms of rats[5] and mice[6,7] adapted to intermittent food intake, there occur marked metabolic deviations characterized by the development of hyperphagia, hypertrophy of the digestive system, and further an increase in the activity of enzyme systems engaged in substrate metabolism, and the formation of larger, particularly fat, energy stores as well as their more effective mobilization. Assuming that these metabolic deviations participate in the changes of radioresistance of mice, the best protective effects of a 2-3 week period of intermittent fasting may be the result of optimal adaptation. The

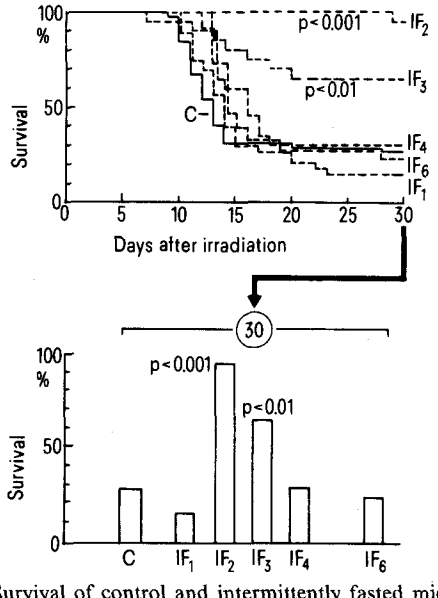

Figure 1. Survival of control and intermittently fasted mice up to the 30th day after irradiation with 8.7 Gy. —— C, controls; ---- IF_1-IF_6, animals adapted 1-6 weeks to intermittent fasting. Minimum number of animals per group, 20.

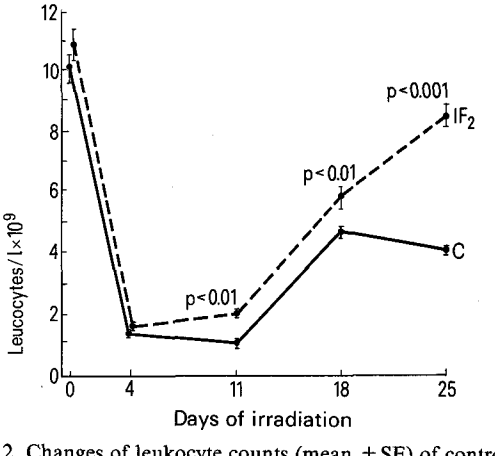

Figure 2. Changes of leukocyte counts (mean ± SE) of control and intermittently fasted mice irradiated with 5.26 Gy. —— C, controls; ---- IF_2, animals adapted 2 weeks to intermittent fasting. 20 animals per group were used.

optimal situation conditioning the highest radioresistance can be understood as a preponderance of the favorable effects of intermittent fasting over the simultaneously acting unfavorable influences, e.g. stress due to a reduced caloric intake after a long period of fasting.

The type of metabolism in which the energy potential of a system is restored, in particular on account of the free energy of lipids, generally characterizes processes of long-term adaptation to stress effects[8]. The results presented may therefore be discussed especially from the point of view of the role of lipid metabolism. This consideration is supported by some earlier experimental results. Increased radiation resistance of mice exhibiting RQ-values greater than 1.0 before irradiation was demonstrated, and the importance of higher fat energy stores and of the subsequent sparing of proteins during the postirradiation catabolic reaction was considered[9]. Analogous mechanisms may participate in the radioprotective action of long-term acclimatization of animals to cold[10]. A greater body mass due to higher storage of fat was observed in mice selected for higher radioresistance[11]. The possible active role of some components of lipid metabolism in the regulation of cell populations, and/or of their radiosensitivity cannot be neglected. It was shown that increasing the content of fat reserve substances in yeast cells decreased their radiosensitivity[12]. Further, lipogenesis as well as lipid composition of the bone marrow are altered in medullary hemopoiesis changes, and causal relationships between these functions are suggested[13,14]. It is of course possible that some other metabolic or enzyme pathways, independent of fat metabolism, which facilitate the repair or recovery processes in the irradiated organism, may be involved in the radioprotective effects of the intermittent food intake.

1 Z.M. Bacq and P. Alexander, Fundamentals of radiobiology. Pergamon Press, Oxford 1961.
2 M. Dragic, V. Ninkov, S. Hajdukovic and J. Karanovic, Strahlentherapie *136*, 617 (1968).
3 S.T. Taketa, in: Biological aspects of radiation protection, p.219. Eds T. Sugahara and O. Hug. Igaku Shoin Ltd, Tokyo 1971.
4 T. Sugahara, T. Tanaka and A. Vacek, J. Radiat. Res. *8*, 152 (1967).
5 P. Fábry, Feeding pattern and nutritional adaptation. Academia, Prague 1969.
6 V. Šimek, H.M. Tuyet and R. Petrásek, Acta soc. zool. bohemoslov. *3*, 212 (1973).
7 A. Kozubík, PhD thesis, Faculty of Sciences, Purkyně University, Brno 1979.
8 L.E. Panin, Energeticheskiye aspekty adaptatsii. Medicina, Leningrad 1978.
9 M. Pospíšil and L. Novák, Nature *184*, 1814 (1959).
10 R. Ghys, Nature *198*, 603 (1963).
11 T.H. Roderick, Genetics *48*, 205 (1963).
12 V.M. Alekseyeva, Biofizika (USSR) *111*, 101 (1958).
13 G.M. Levis, A.A. Efstratiadis, J.D. Mantzos and C.J. Miras, Radiat. Res. *61*, 342 (1975).
14 M. Tavassoli, D.N. Houchin and P. Jacobs, Scand. J. Haemat. *18*, 47 (1977).

Clara cell surface of the rat: scanning and transmission electron microscopic study[1]

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Summary. In normal young rats, groups of Clara cells in the bronchioles showed the formation of many cytoplasmic blebs on their cytoplasmic domes. Detached blebs rested on the bronchiolar epithelial cells. The scanning (SEM) and transmission electron microscope (TEM) studies suggest localized changes of Clara cell surface activities by increased formation of cytoplasmic blebs which may represent the apocrine type of secretion.

Clara cells are unique non-ciliated epithelial cells located in the bronchioles of many mammals. Their histochemical and ultrastructural characteristics indicate that these are secretory cells. Investigators were divided concerning their mode of secretion. Clara[2] who first described these cells in detail favored the mechanism of apocrine secretion, or decapitation of the apical cytoplasm. This mechanism was supported by more recent papers dealing with normal animals by Etherton et al.[3,4], and experimental animals exposed to hypoxia by Smith et al.[5] and Heath et al.[6,7], or treated with naphthalene by Mahvi et al.[8]. On the other hand, based on TEM and freeze fracture studies, Wang et al.[9], Kuhn et al.[10], Yoneda[11] and Yoneda and Birk[12] suggested the process of exocytosis or merocrine type of secretion, and Niden[13], Stinson and Loosli[14] and Pack et al.[15] illustrated that both apocrine and merocrine types probably occurred. The present report will show that in limited areas of the normal rat bronchioles, hyperactive Clara cells appear to undergo apocrine secretion or decapitation.

45 Sprague-Dawley rats at various ages from neonates to adults were used. Each animal was anesthetized with ether, the trachea was exposed, and the chest was opened to allow the lungs to collapse. 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2, 480 mosm) was then injected slowly into the lungs through the trachea until the lungs filled the chest cavity. The entire lung was removed and placed in glutaraldehyde solution for at least 1 h. Each lobe of the lung was then cut along the long axis of the major airways to expose bronchiolar surfaces. The lung slices were then critical point dried and gold coated and observed in a JEOL JSM-35 SEM. Selected SEM specimens were reprocessed according to the methods of Hung et al.[16] for TEM observations. The specimens were placed in absolute alcohol, processed for embedding in araldite[17], thin sectioned, stained and observed in a JEOL 100S TEM. Some of the glutaraldehyde fixed lungs were processed for paraffin embedding and the sections stained with hematoxylin and eosin for light microscopy.

The light and electron microscope observations showed that the bronchioles were covered by the ciliated and Clara cells (figs 1 and 6). All Clara cells had characteristic cytoplasmic domes which sometimes showed surface convolution and microvilli (fig. 2). Occasionally, small cytoplasmic blebs projected from these cells (fig. 2). In 2 31-day-old rats, among the regular epithelium were small loci of apparently hyperactive Clara cells (fig. 1), which were covered by various amount of cytoplasmic blebs on their protruded domes (figs 1 and 2). The blebs were spherical and of random sizes (fig. 3) and were attached to the cell through a narrow cytoplasmic neck (fig. 4). In these specific loci, many detached blebs were seen to rest on the Clara or