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Thymus Lipids in Continuously Irradiated Rats

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Summary

Male Wistar rats were irradiated continuously with a daily dose of 0.19 Gy (120 days), 0.57 Gy (90 days) and 0.96 Gy (35 days) of gamma rays. An other group of rats was irradiated continuously with graded doses of gamma rays, up to total exposures ranging from 3.83–19.15 Gy. Depending on both the daily dose and total exposure, there was a decrease in phospholipid content in the thymus which correlated well with thymus weight changes. The decrease in triacylglycerol content was a less reliable sign of radiation damage. The phospholipid content reflecting the patterns of organ cellularity is a valuable indicator of the extent as well as recovery from radiation – induced injury to the thymus.

Key words

Rats – Continuous irradiation – Thymus lipids

Introduction

Thymus is one of the central organs of immune responses in the organism. Exposure to ionizing radiation causes a rapid loss of thymus mass as the cells are destroyed. After single lethal whole-body irradiation (14.35 Gy) of rats with X-rays the concentration of triacylglycerols (TG) gradually increased while that of phospholipids (PL) decreased only nonsignificantly (Ahlers *et al.* 1973). Similar changes were also seen after exposure to a nonlethal dose of X rays (2.39 Gy) but with a later onset and earlier recovery – they recovered as early as 28 days after exposure (Ahlers *et al.* 1981). Considering the sharply decreasing thymus weight, lipid concentration changes do not provide a reliable picture as the changes in lipid content (Ahlers *et al.* 1981). Analysis immediately after cessation of continuous irradiation of rats with a daily exposure rate of 0.57 Gy of gamma rays for 25 days showed an increased TG concentration and decreased PL concentration (Ahlers *et al.* 1967).

In the present experiment, male rats were continuously irradiated with graded daily exposure rates of gamma rays and both major lipid fractions were determined in thymus.

Material and Methods

Male SPF Wistar rats (VELAZ, Prague,) weighing about 160–180 g, were continously irradiated with gamma rays from a ^{60}Co source in an open experimental gamma field. Animals were placed in plastic cages in groups of four-five, fed LD chow (VELAZ, Prague) and tap water *ad libitum*. Some animals were irradiated with daily exposure rates of 0.19 Gy for 120 days (non-lethal exposure), 0.57 Gy for 90 days and 0.96 Gy for 35 days; when the mortality had increased massively, the irradiation was stopped at 0.57 Gy and 0.96 Gy daily dose. A group of animals was irradiated continuously for 7 days with daily exposure rates of 0.55 Gy (a total of 3.63), 1.1 Gy (a total of 7.66 Gy), 1.6 Gy (a total of 11.49 Gy), 2.19 Gy (a total of 15.32 Gy) and 2.74 Gy (a total of 19.15) of gamma rays. All analyses were performed during and at the end of the exposure. After irradiation and transfer in a laboratory and after subsequent 16-hour fasting the animals were killed by rapid decapitation. TG was assessed according to Eggstein and Kreutz (1966) and PL from lipid phosphorus in thymus homogenates was determined according to Bartlett (1959). Animals in the experimental field, shielded from irradiation, and intact animals from the vivarium (only with the 0.57 Gy daily dose) served as the controls.

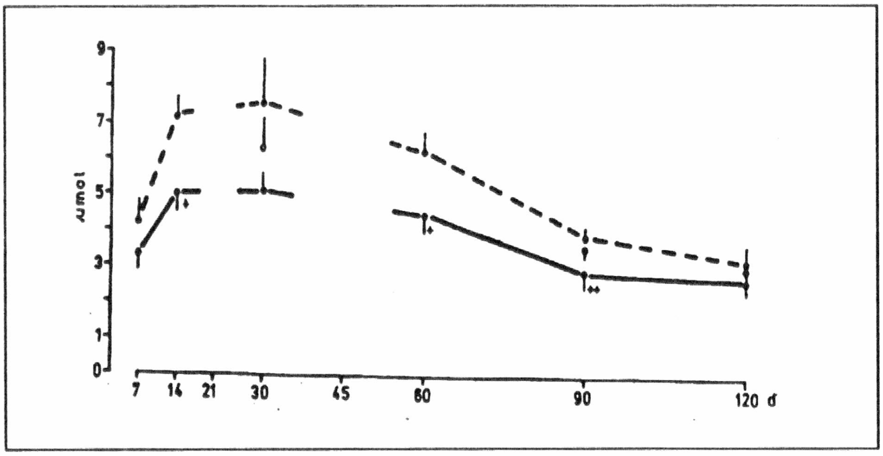

Fig. 1
The content of phospholipids in the thymus of rats irradiated continuously for 120 days (d) with a daily dose of 0.19 Gy, analyzed during and at the end of the exposure. Full line - irradiated, interrupted line - control animals. Empty circles: values of intact (vivarial) animals. Values given as M ± S.E.M., the significance of differences expressed as + (P < 0.05) or ++ (P < 0.01).

Statistically the experiment was evaluated by t-test for non-paired values (irradiated animals vs field controls).

Results

Daily rate of 0.19 Gy. Neither the concentration of TG and PL (not shown) nor TG content (not shown) were changed. A decreased PL content was noted on day 14, persisted until day 120 when only a nonsignificant difference was found compared with the field controls (Fig. 1).

Daily rate of 0.57 Gy. The TG concentration became sharply elevated between irradiation days 7-14, continued to increase, though slightly, to a peak on irradiation day 60. Between days 7-21 of exposure the values of field controls were also increased as compared with initial values and then they became stabilized at this level till the end of the experiment. Values in intact rats rose between days 30 and 90 (Fig. 2.).

On the other hand, the TG content was nonsignificantly lower between days 30 and 60 and it was not until irradiation day 90 that it was significantly lower (not shown). Though the PL concentration did not change markedly (not shown), the PL content diminished considerably already after 3 days of exposure and then sharply decreased to a minimum on day 21 and tended to stabilize at this level (Fig. 3).

Daily rate of 0.96 Gy. An increased TG concentration was already found on day 3 of exposure with a maximum during irradiation days 15–35 (Fig. 4).

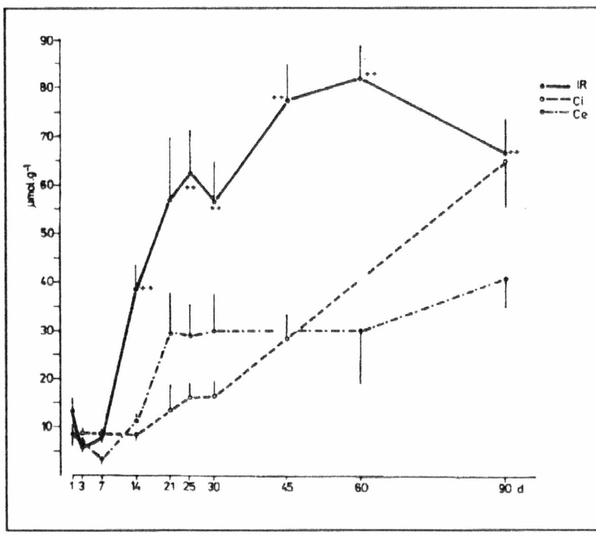

Fig. 2
The concentration of triacylglycerols in the thymus of rats, irradiated continuously for 90 days with a daily dose of 0.57 Gy, analyzed during and at the end of the exposure. IR – irradiated group, Ce – control animals from the experimental field, Ci – intact animals from the animal house. Values are given as M ± S.E.M., the significance of differences (IR vs Ce) expressed as + (P < 0.05) or ++ (P < 0.01).

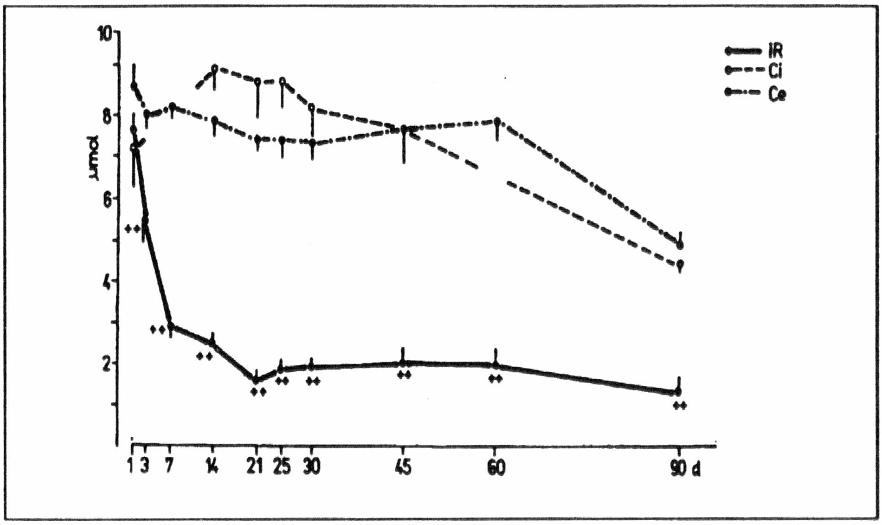

Fig. 3
The content of phospholipids in the thymus of rats irradiated for 90 days with a daily dose of 0.57 Gy. For details see Fig. 2.

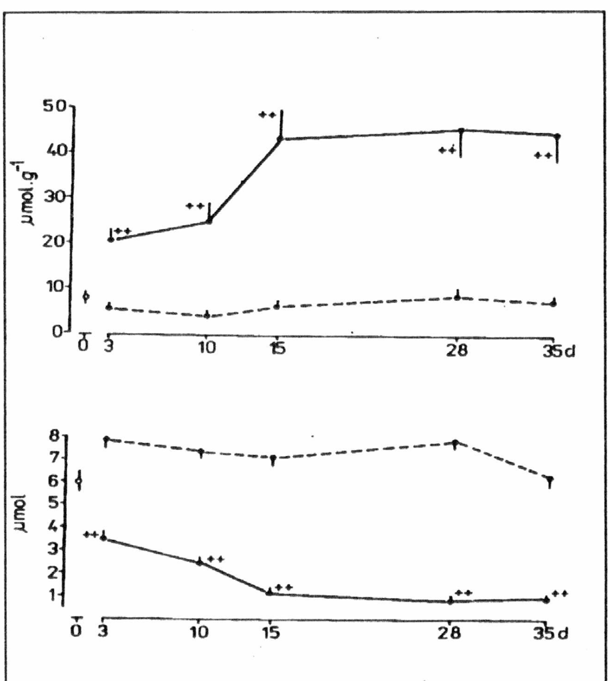

Fig. 4
The concentration of triacylglycerols in the thymus of rats irradiated continuously for 35 days with a daily dose of 0.96 Gy (upper part). The content of phospholipids in the thymus of rats irradiated continuously for 35 days with a daily dose of 0.96 Gy (lower part). For details see Fig. 1. The weight of the thymus in rats irradiated continuously for 7 days up to total doses of 3. 83-19.15 Gy. Values given as M ± S.E.M. (upper part).

PL concentration and TG content were little affected (not shown). However, the PL content decreased markedly on day 3 to a minimum on irradiation day 15 and remained decreased throughout the experiment (Fig. 4).

Continuous 7-day irradiation with graded doses of gamma radiation (3.83 – 19.15 Gy). As follows from Fig. 5, the descending curve of thymus weight appeared to fall exponentially. TG and PL contents were already diminished sharply at the total dose of 3.83 Gy. As the accumulated dose became higher, TG content oscillated and PL content further decreased (Fig. 5).

Discussion

Exposure to ionizing radiation causes damage of the genetic material to thymocytes, cortical lymphocytes being more sensitive than medullary cells (Aoyama *et al.* 1972). The epithelial cells (so-called thymic nurse cells) that are in fact cells of the thymus stroma are a relatively radioresistant population. They do not originate in the bone marrow (Sharp and Thomas 1977) and, in the thymus, they are responsible for early stages of lymphopoiesis; they may play a role in "self-distinguishing" the differentiating T-lymphocytes. The regenerative process also takes place in lethally irradiated animals, e. g. the mouse (Sharp and Thomas 1975). When reparation begins, the increase in epithelial stroma cells parallels the increase in radioresistant thymocytes (Houben-Defresne *et al.* 1984). It is well known that the regenerative processes have a biphasic course, for example, in thymus weight

(Blomgren and Revesz 1968;Takada *et al.* 1969). Secondary involution of the thymus after irradiation

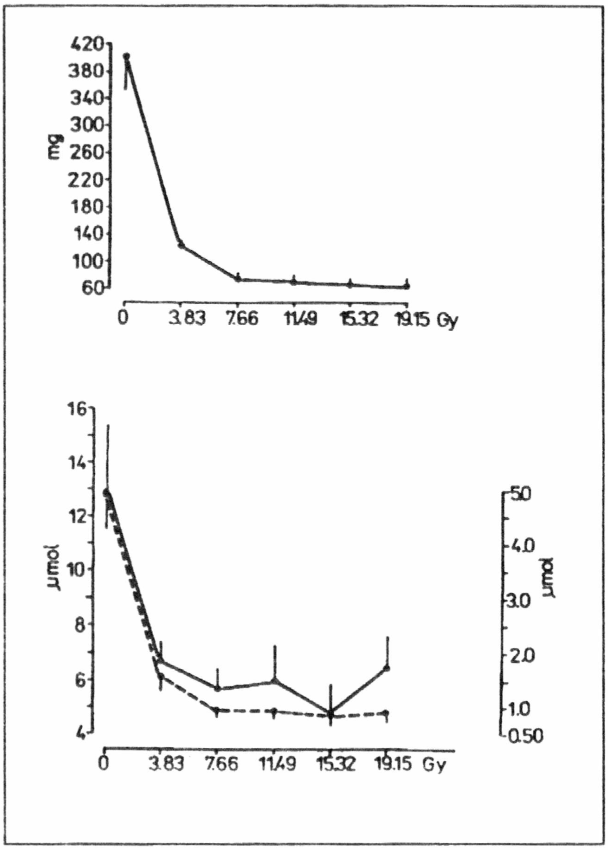

Fig. 5
The content of triacylglycerols (full line, left scale) and of phospholipids (broken line, right scale) in the thymus of rats irradiated continuously for 7 days up to a total accumulated dose 3.83 – 19.15 Gy. Values are given as M ± S.E.M. (lower part).

can be explained by exhaustion of the protothymocyte pool resulting in a decreased proliferation capacity of these cells, accompanied concomitantly by bone marrow failure to form thymocyte precursors (Watkins and Sharp 1979; Boersma *et al.* 1981).

Data relating to thymus lipid changes appear only sporadically. Miras *et al.* (1968a) found that sublethal gamma irradiation decreases fatty acid oxidation in isolated thymocytes and/or in isolated thymus mitochondria of rabbits. The decrease in fatty acid oxidation as well as the increase in glyceride synthesis contributes to the increase in TG concentration. After sublethal gamma irradiation of rabbits, the incorporation of ^{14}C-palmitate into total lipids in the thymus was enhanced; concomitantly an increased TG concentration in the thymus was found (Miras *et al.* 1968b). In both lethally (14.4 Gy) and sublethally (6 Gy) gamma irradiated rats, Sedláková *et al.* (1988a) found a temporary increase in the incorporation of ^{14}C-glucose and $^{3}H_2O$ into total lipids of the thymus; the incorporations of labelled glucose recovered by day 28 after exposure while $^{3}H_2O$ incorporation was still enhanced. After 120-day continuous irradiation at a daily dose of 0. 6 Gy of gamma rays, Sedláková *et al.* (1988b) found that an increase in ^{14}C-acetate incorporation into total lipids of the thymus runs in parallel with an increase in TG concentration in this tissue; within 150-day continuous irradiation at a daily exposure rate of 0. 1 Gy, the incorporation of labelled acetate into total lipids did not change though an elevated TG concentration was found in the thymus between irradiation days 60 – 120.

During radiation-induced thymocyte destruction phospholipids are liberated from the cell membranes and, consequently, the PL content diminishes in parallel to the decreasing organ weight; however, the concentration of this fraction does not change appreciably. Reduced ^{32}P incorporation into thymocytes in irradiated rats was described by Ord, Stocken (1958). All this suggests that PL content is a very important parameter indicating the extent of radiation damage as well as the reparative processes in the thymus. On the other hand,the TG content is of no great diagnostic value.

An important question concerns the reparation of lipid changes in the thymus after both single and continuous irradiations. As was stated above, the restitution of radiation-induced decreased content to normal levels is thought to be a valuable indicator of reparation, A similar pattern of changes is also seen in thymus weight changes. Sedláková *et al.* (1980) have found that the PL concentration was recovered within one month after continuous irradiation of rats at a daily rate of 0.57 Gy of gamma rays, whereas for the increased TG concentration it took as long as 6 months after exposure.

The lipid content of the thymus oscillates during the day. The follow-up of the circadian rhythm showed that the TG and PL contents in the light decrease and a in the dark increase (Ahlers *et al.* 1983). It can thus be assumed that irradiation in a different part of the day may modify the response of thymus lipids. The determination of lipid concentration and/or content in the thymus, namely the PL content, are valuable indicators of the extent and recovery of radiation damage. The relation of thymus lipid changes to individual thymocyte populations after irradiations remains to be elucidated.

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