the different parameters in the different organs. Thus the method used for the statistical analysis of the data seemed to be acceptable.

Results and discussion. Except for cholesterol content (mg/g organ) in the liver which is significantly correlated with body weight ($r = +0.71$; $p < 0.01$), no significant correlation was found in the different organs studied between the different parameters measured and the body weights.

The accompanying table shows that the cholesterol content of the adrenals, the liver, the lungs, the spleen and the kidneys is higher than in the other tissues. The cholesteryl esters represent 9–14% of total cholesterol in most organs, being higher, however, in the liver and overall in the adrenals. There is a significant correlation between the cholesterol content of each organ and the percentage of esterified cholesterol in the same organ ($r = +0.73$; $p < 0.02$). Cholesteryl ester is considered as a form of storage from which it can be mobilized for several structural and metabolic functions[3]. The high cholesteryl ester content of the liver and the adrenals can be related to their high metabolic rate directed mainly to lipoprotein synthesis and steroidogenesis.

Norum and coworkers have demonstrated the presence of a coenzyme A-dependant esterification mechanism for cholesterol in intestinal mucosa from guinea pigs[13]; likewise, ACAT activity was shown to be present in the liver[10], the kidneys, the spleen and the adrenal glands of guinea pigs[14].

As it is shown in the table, the present work demonstrates that cholesterol esterifying activity is present in most organs studied.

The FER is found to be highest in the adrenals; the spleen, the liver, the proximal part of the small intestine, the kidneys and the colon have lower but still substantial cholesterol esterifying activity. On the other hand, the middle and the distal parts of the small intestine, the stomach, the aorta and the lungs have only slight ACAT activity. For each organ, the values of FER are significantly correlated with the values of the percentage of esterified cholesterol of the same organ ($r = +0.86$; $p < 0.001$). This latter finding suggests that ACAT could play a major role in determining the level of tissue ester cholesterol content. However, care must be taken before firm conclusions can be drawn. Indeed, more precise information ought to be obtained by taking into account the possible specific requirements of each organ when measuring its ACAT activity[15], and the possible role of a recently-described sterol carrier protein (SCP_2) necessary for effective esterification of cholesterol by the hepatic microsomes. Moreover, it must also be noted that differences in ACAT activity between tissues can mean either differences in the quantity of enzyme or differences in the cholesterol content of the microsomes. Finally, a cholesterol esterase was demonstrated by Drevon in several organs of the guinea pigs[14]; its activity has not been evaluated in our study.

Nevertheless, in support of an important role of ACAT in cholesterol metabolism and cholesterol disposition is the demonstration that in cholesterol-fed guinea pigs the increase in the cholesteryl ester content of various organs is relatively well correlated with the increase of the ACAT activity in these organs[17].

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Acute ^{60}Co-gamma irradiation of rats decreases the inhibitory effect of succinate on the lipid peroxidation of liver mitochondria

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Summary. Succinate inhibits NADPH-dependent lipid peroxidation of liver mitochondria. This effect of succinate decreased 12 h after whole-body ^{60}Co-gamma irradiation, depending on the dose of irradiation.

Key words. Rat liver; liver, rat; mitochondria; irradiation, ^{60}Co-; lipid peroxidation; succinate.

Lipid peroxidation is a consequence of free radical production in biological systems, which may cause irreversible damage. Protection against free radicals is generally attributed to the superoxide dismutase-catalase system and to glutathione. Lipid peroxidation is known to occur in mitochondria in the presence of NADPH and physiological quantities of ferric ions[1]. The increase of lipid peroxidation is considered to be an indirect, deleterious effect of ionizing radiation[2,3,4] which can be demonstrated in mitochondria 2–3 days after irradiation in vivo[5,6,7].

Mészáros and co-workers[8] have observed that succinate inhibits NADPH-dependent (ADP-Fe stimulated) lipid peroxida-

tion of liver mitochondria by 80%. Microsomal lipid peroxidation also decreases in the presence of mitochondria and succinate. In the present experiments the inhibitory effect of succinate on lipid peroxidation is investigated in liver organelles prepared from irradiated rats.

Methods. Hybrid male rats ($R/AxLE/H/F_1$ purchased from LATI, Gödöllő) weighing 120–150 g were irradiated by 1.0, 5.0 or 15.0 Gy using a ^{60}Co-gamma irradiation apparatus; the dose rate was 0.365 Gy/min. The animals were decapitated 12 h after irradiation, then mitochondria and microsomes were prepared as described previously[8]. Lipid peroxidation was assessed by the measurement of malondialdehyde (MDA), a product of peroxidation, applying the thiobarbituric assay[9]. The volume of incubation mixture was 1 ml in each assay, containing 0.3 mM NADPH, 3 mM ADP, 15 μM $FeCl_3$ and NADPH regenerating system consisting of 6 mM glucose-6-phosphate and 0.4 unit of glucose-6-phosphate dehydrogenase, in 0.15 M Tris-HCl buffer, pH 7.4 at 37 °C. The amount of added mitochondrial and microsomal protein is indicated in the figures. Each experimental result was obtained from a group of 10 animals.

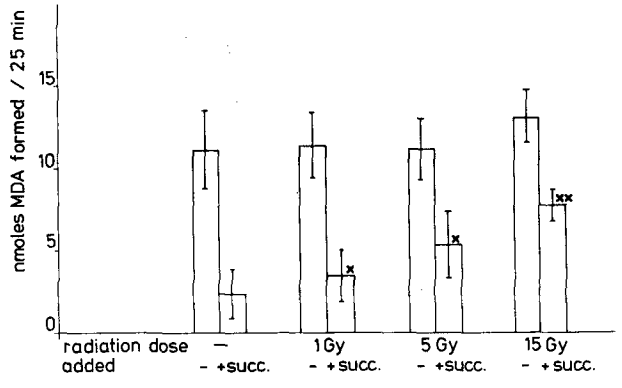

Figure 1. Effect of succinate on the NADPH-dependent malondialdehyde production of mitochondria 12 h after whole-body irradiation. Incubation was carried out as described in Methods; 8 mM Na-succinate was added as indicated. Mitochondrial protein concentration was 1.7 mg. Results are given as mean ± SE; n = 10. The extent of succinate inhibition in the control and irradiated groups was compared; ×, $p < 0.05$, ××, $p < 0.01$.

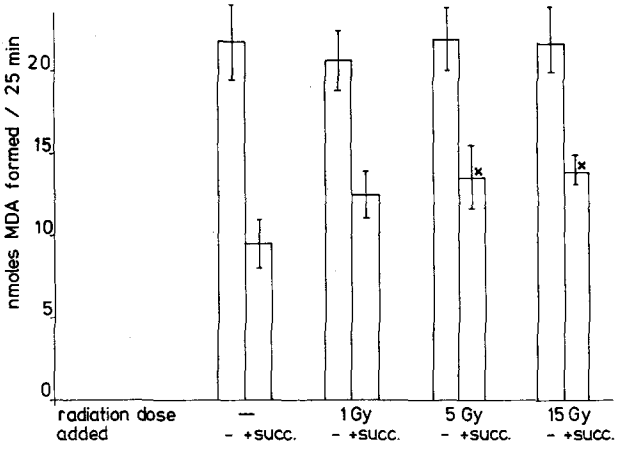

Figure 2. Effect of succinate on the NADPH-dependent malondialdehyde production in a reconstituted system containing microsomes and mitochondria, 12 h after whole-body irradiation. Incubation was carried out as described in Methods; 8 mM Na-succinate was added as indicated. The concentration of microsomal protein was 2.0 mg/ml; mitochondrial protein was 1.7 mg/ml. Results are means ± SE; n = 10. The extent of succinate inhibition in the control group and the irradiated groups was compared; ×, $p < 0.05$.

Results. Mitochondria were isolated from irradiated and untreated animals, and lipid peroxidation was assayed in the absence and presence of 8 mM succinate. As shown in figure 1 the rate of lipid peroxidation (without succinate) remained unchanged 12 h after whole body irradiation. However, the inhibitory effect of succinate (which was about 80% in the control group) diminished after irradiation in a dose-dependent manner. Even a 1-Gy dose of gamma irradiation decreased the succinate effect significantly. After a dose of 15 Gy the succinate inhibition was only about 40%.

Isolated microsomes showed lipid peroxidation at a rate of 20.3 ± 2.8 nmol MDA/25 min, which did not change after irradiation. Microsomal lipid peroxidation was not inhibited by succinate (not shown). However, in a reconstituted system containing microsomes and mitochondria (fig. 2) even microsomal lipid peroxidation was reduced by succinate, in agreement with our earlier results[8]. Figure 2 shows that in the case of organelles prepared from irradiated animals the inhibitory effect of succinate was significantly diminished, depending on the dose of irradiation. In the latter system, containing mitochondria and microsomes, the change caused by irradiation was less marked than in the case of mitochondria alone.

Discussion. The results presented above demonstrate that whole-body ^{60}Co-gamma irradiation decreases the sensitivity of mitochondrial lipid peroxidation to the inhibitory effect of succinate in a dose-dependent manner. This change is less marked in the reconstituted system containing mitochondria and microsomes. These observations indicate that the inhibition of lipid peroxidation described by Mészáros and coworkers[8] may play an important role in the defense against free radicals, as well as in other defense systems. Irradiation reduces the efficiency of the inhibition of lipid peroxidation, which may explain the accumulation of MDA afterwards.

Succinate inhibition of lipid peroxidation appears to be a very specific phenomenon, since it can be relieved by malonate[8]. The protective effect of succinate, a respiratory substrate, may be mediated by some antioxidant compound which can be reduced in the mitochondria, such as ubiquinone[10], vitamin E[11] or α-tocopherol-quinone[12]. This system apparently includes some highly radiation-sensitive component[13]. Though the molecular mechanism of this protective system is not known, the present observations indicate its importance in the defense against lipid peroxidation and, in turn, radiation-induced changes may provide a clue to the elucidation of its mechanism.

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