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Nuclear and chromatin lipids: metabolism in normal and γ-irradiated rats

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Abstract

The data on nuclear and chromatin lipid metabolism are reviewed. The amount of neutral lipids and phospholipids in nuclei of rat thymus, liver and neocortex neuron as well as the amount of lipids in rat thymus and liver chromatin are described. The metabolic responses of nuclear and chromatin lipids from thymus to different doses and dose rates of γ-irradiation of rats are discussed. In most cases, the nuclear and chromatin lipid responses are distinct. Changes in nuclear and chromatin lipid metabolism in response to γ-irradiation are suggested to connect with the signal transduction pathway and the regulation of the transcriptional and replicative chromatin activity. The influence of β-carotene and picrotoxin on rat liver nuclear lipids and neocortex neuronal nuclear lipids, respectively, was analyzed. The possible involvement of the lipid traffic in the chromatin lipid responses to γ-irradiation and other agents is suggested.
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1. Introduction

The involvement of phospholipids in cell signaling is extensively studied [1]. The participation of nuclear lipids in the different stimulus response events was widely discussed [2] and the role of cholesterol in the signal transduction was suggested [3]. The involvement of mammalian cell chromatin lipids in metabolic cell responses has not been adequately investigated. At present, it is clear that chromatin phospholipids play a role in genomic structures and functions of animal and plant cells [4]. Yet, in earlier work, it was shown that active chromatin is much richer in phospholipids and cholesterol than is repressed chromatin. The lipids of active chromatin have a much more active metabolism as measured by incorporation of 1,3-^{14}C-glycerol into the lipids of isolated nucleus [5]. Synthesis of the chromatin-associated phospholipids from rat hepatocyte nuclei was investigated as compared with that of the lipids associated with nuclear and microsomal fractions in the rat injected with [^{32}P]orthophosphate. The results indicated that there exists transport of phospholipids from the microsomes to the chromatin [6]. In early studies, we showed that chromatin phospholipids and cholesterol of rat tissues were labeled with 2-^{14}C-acetate in vivo much more actively than those in nuclei [7]. Simultaneously, the synthesis of phospholipids found in microsomes, nuclei and chromatin has been studied in rat liver after partial hepatoectomy. ^{32}P-incorporation in phospholipids of the chromatin (specific activity) over a period of 30 h was much more than that in the microsomes. It is concluded that chromatin phospholipids increase their metabolism in relation to the S-phase of the cell cycle [8]. Over a period of 40 min after γ-irradiation of rats at a lethal dose of 10 Gy and 2-^{14}C-acetate injection, the specific activity of chromatin cholesterol was much higher than that in microsomes of rat liver [9]. It was shown that liver and thymus chromatin phospholipids and cholesterol take part in animal radiation response [10]. A penetration of exogenous cholesterol in chromatin was investigated. [^{3}H]cholesterol was found in chromatin proteins when mouse spleen cell suspension was incubated with tritiated cholesterol for various time intervals. The number of cholesterol molecules

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bound per nucleus was estimated to be on the order of 1000, which is in the same range as the number reported for steroid hormone receptors [11].

2. The lipid content in nuclei and chromatin of rat tissues

The chromatin was abundantly isolated from nuclei devoid of the nuclear envelope upon treatment with Triton X-100 [6,8,11]. This detergent extracted large amount of proteins and lipids from the nucleus [12]. We isolated nuclei from rat liver according to Ref. [13], and thymus nuclei by the method described in Ref. [14]. Chromatin was isolated as described in Ref. [15]. In our experiments, the purity of nuclei was determined by marker enzyme activity and by electron microscopy. The native distribution of lipids between nucleus compartments remained unchanged by this treatment. Tables 1 and 2 list the amount of lipids in nuclei and chromatin liver and thymus and neocortex neuronal nuclei of rats. It is seen that the lipid composition of chromatin is not distinguished significantly from the lipid composition of nuclei in rat thymus and liver. The quantity of nuclear and chromatin lipids varied widely. Determination of lipid content of Ehrlich ascites tumor cell nuclei devoid of the nuclear envelope and chromatin isolated from them revealed that the cholesterol content in nuclei and in chromatin is 8 and 10.3 μg/mg protein, respectively, and that the phospholipid content in nuclei and in chromatin is 10.5 and 4.25 μg/mg protein, respectively. Hence, the amount of cholesterol in chromatin of tumor cells is as much as twice the amount of phospholipids [24]. If liver nuclei are denuded by 0.3–1.0% Triton X-100, the amount of phospholipids is 10% of the whole nuclei. [25]. The amount of phospholipids in chromatin from hepatocyte denuded nuclei is 5.77 ± 0.9 μg/mg protein [26]. Chromatin isolated from denuded hepatocyte nuclei is enriched in sphingomyelin (SM) [27]. A distribution of chromatin lipids between DNA and chromatin protein was investigated using labeled glycerol and cholesterol in Ehrlich ascites tumor cells. Incorporation of the lipid precursors was shown in nonhistone proteins, histones and DNA-bound lipids [28]. Enzymatic destruction of chromatin RNA results in the liberation of lipids from the chromatin [29].

3. Nuclear and chromatin lipid metabolism in the tissues of rats irradiated at a sublethal dose of ionizing radiation

Ionizing radiation causes mitosis inhibition, induces apoptosis or cell repair. It is of great interest to investigate changes in lipid metabolism in nuclei and chromatin of cells and tissues from γ-irradiated animals. γ-Irradiation leads to nonmonotonic metabolic and functional response in animal cells and tissues [30]. Thus, ionizing radiation makes it possible to observe different stages of damage and repair using one type of cell and tissue. The value of the body and organ mass and the intensity of total RNA and total protein synthesis were used as criteria of the metabolic response. An activity of enzyme ornithin decarboxylase (ODC), the key enzyme of polyamine synthesis, was used as a marker of the cell activity [31].

We used fractionated γ-irradiation of rats at a dose of 2 Gy × 3 in a weekly interval up to a total dose of 6 Gy. The chromatin and nuclear lipids were isolated and determined in compliance with Refs. [18,23]. By the third irradiation, thymus mass was 1/3 of normal. The ODC activity decreased within 1 h after irradiation and sharply elevated

Table 1
The amount of lipids isolated from nuclei of liver, thymus and neocortex neuron of rats (μg lipid/mg protein)

Lipids	Liver nuclei	Thymus nuclei	Neocortex neuronal nuclei
Cholesterol	13.4 ± 1.1 [16]	5.6 ± 0.9 [18]	–
	3.5 ± 0.3 [17]	8.8 ± 2.3 [19]	
		9.8 ± 1.1 [20]	
		9.1 ± 0.9 [21]	
Fatty acids	12.8 ± 1.7 [17]	21.3 ± 1.8 [18]	163 ± 21 [22]
	61 ± 10 [16]	12.3 ± 0.5 [19]	
		29.7 ± 3.6 [20]	
Diacylglycerides	6.8 ± 0.8 [17]	6.0 ± 0.6 [20]	
Total phospholipids	43.1 ± 6.0 [17]	35.4 ± 3.3 [23]	71.3 ± 2.5 [22]
Phosphatidylcholine	14.5 ± 1.8 [16]	17.5 ± 4.0 [21]	
		25.3 ± 0.6 [20]	
Phosphatidylethanolamine	5.5 ± 1.0 [16]	3.7 ± 0.5 [18]	
	6.9 ± 1.2 [17]	6.5 ± 1.5 [20]	
Sphingomyelin	1.7 ± 0.3 [17]	1.1 ± 0.2 [20]	
	4.3 ± 0.8 [16]		
Cardiolipin	2.0 ± 0.9 [16]	1.9 ± 0.3 [18]	
	1.2 ± 0.3 [17]	3.1 ± 0.1 [20]	
Phosphatidylinositol	3.2 ± 0.9 [17]	3.0 ± 0.9 [21]	
Phosphatidylserine	0.9 ± 0.1 [17]	2.5 ± 0.7 [21]	
Monoacylglycerides	–	5.0 ± 1.4 [19]	

Values are mean ± S.E. of four independent experiments.

Table 2
The amount of lipids isolated from liver and thymus chromatin

Lipids	Liver chromatin	Thymus chromatin
Cholesterol	10.6 ± 2.2 [16]	4.7 ± 0.8 [19]
		5.9 ± 0.5 [18]
		5.2 ± 0.6 [21]
Fatty acids	59 ± 10 [16]	18.4 ± 1.2 [18]
		23.0 ± 3.3 [21]
Phosphatidylcholine + phosphatidylserine	21.1 ± 1.4 [16]	22.7 ± 1.0 [23]
Phosphatidylcholine	–	17.1 ± 3.0 [21]
Total phospholipids	52 ± 5 [7]	37.9 ± 0.2 [23]
Phosphatidylethanolamine	5.6 ± 0.3 [16]	4.9 ± 0.3 [23]
		5.4 ± 1.3 [21]
Sphingomyelin	4.8 ± 0.5 [16]	1.1 ± 0.5 [23]
		1.1 ± 0.3 [21]
Cardiolipin	5.3 ± 0.9 [16]	0.9 ± 0.2 [23]
	3.5 ± 0.7 [7]	1.0 ± 0.1 [21]
Phosphatidylinositol	–	1.6 ± 0.4 [21]
Phosphatidylserine	–	1.9 ± 0.4 [21]
Monoacylglycerides	–	2.3 ± 0.8 [19]

Other details are as in Table 1.

for the period of 7 days [32]. The amount of thymus chromatin and nuclear lipids changed (Table 3). The amount of fatty acids increased in nuclei and chromatin. The content of phosphatidylcholine + phosphatidylserine (PC+PS) and phosphatidylethanolamine (PE) declined; the amount of SM and cardiolipin (CL) remain unchanged. The amount of fatty acids and phospholipids in whole thymocytes was also the same [18,23]. Phospholipid disruption under the action of phospholipase A can result in the formation of fatty acids. The results of calculation show that existing hydrolysis level of PC + PS and PE in nuclei and chromatin after irradiation is not responsible for the observed growth of the fatty acids. Apparently, irradiation may alter the traffic of fatty acids between nucleus and cytoplasmic compartments. Sublethal doses of γ-radiation induced an activation of the lipid synthesis in rat thymus within 1 h after irradiation [33]. By using a cytochemical method for detecting phospholipids inside the nucleus of intact cells at the electron microscopic level, the changes were analyzed in intranuclear phospholipids in DNA replicating versus resting cells, which are both present in the same sample of regenerating liver after partial hepatoectomy. The data obtained indicate a significant reduction in the phospholipids detectable inside the nucleus in all steps of the S-phase. The authors believed that this could depend on an increased nuclear phospholipid hydrolysis, the products of which should activate some of the enzymes involved in the control of DNA replication [34]. The decrease of PC + PS and PE in chromatin of the irradiated rats is of great interest. Changes of chromatin organization induced by PC and PE were shown on isolated rat liver nuclei. The results obtained indicate that PC and PE increased chromatin condensation and impaired RNA maturation and transport [35]. Decrease in PC and PE in liver chromatin of irradiated rat may depend on the activity of PC-dependent phospholipase C. The enzymatic PC hydrolysis forms diacylglycerol and phosphorylcholine. Diacylglycerol exerts many various effects and activates protein kinase C [2].

Table 4
The amount (μg lipid/mg protein) of phospholipids and fatty acids in the rat neocortex homogenate and neuronal nuclei 30 min after the intraperitonial injection of picrotoxin (4 μg/kg body mass)

Lipids	Homogenate		Nuclei	
	Control	Picrotoxin	Control	Picrotoxin
Total phospholipids	348 ± 20	388 ± 23	71.3 ± 2.5	52.9 ± 3.3 *
Fatty acids	170 ± 14	156.4 ± 17.5	163 ± 21	216 ± 9 *

Each value is the mean ± S.E. of four to five independent experiments.
* Difference is significant under paired comparison at $p < 0.05$.

The existence of the PC-dependent phospholipase C in rat liver chromatin was shown. This enzyme differs with respect to nuclear membrane in pH and K_m [36]. Table 4 demonstrates the increase in fatty acid content and decrease of phospholipids observed in nucleus of rat neocortex neurons after injection of picrotoxin to rat [22]. Picrotoxin is a glutamate receptor inhibitor that induces convulsive reaction in rat. Consequently, the decrease of total phospholipids or PC and PE and the increase of fatty acids are registered after damage in proliferating and nonproliferating cells. To clarify the significance of the change in fatty acid and phospholipid contents in the cell nucleus, the effects of the chronic irradiation were explored at a low dose rate. The influence of continuous supply of diet with β-carotene was

Table 3
The amount (μg lipid/mg protein) of fatty acids, cholesterol and phospholipids in the thymus homogenate, nuclei and chromatin in an hour after γ-irradiation of rats at a dose of 6 Gy (2 Gy × 3 in a week)

Lipids	Homogenate		Nuclei		Chromatin	
	Control	6 Gy	Control	6 Gy	Control	6 Gy
Fatty acids	63 ± 2	74 ± 8	21 ± 2	50 ± 6 *	18.4 ± 1.2	35 ± 2 *
Cholesterol	10.5 ± 0.1	10.8 ± 0.2	5.6 ± 0.2	5.3 ± 0.3	5.9 ± 0.5	5.4 ± 0.1
Sphingomyelin	1.3 ± 0.2	1.4 ± 0.4	1.2 ± 0.3	1.1 ± 0.4	1.1 ± 0.5	0.8 ± 0.1
Phosphatidylcholine + phosphatidylserine	20.2 ± 1.7	18.0 ± 1.0	16.2 ± 1.5	10.1 ± 0.1 *	22.7 ± 1.0	15.4 ± 0.5 *
Phosphatidylethanolamine	5.9 ± 0.5	4.6 ± 0.5	3.7 ± 0.5	2.5 ± 0.2 *	4.9 ± 0.3	3.2 ± 0.1 *
Cardiolipin	1.8 ± 0.5	1.4 ± 0.4	1.9 ± 0.3	1.4 ± 0.5	0.9 ± 0.2	1.0 ± 0.2

Values are means ± S.E. of three independent experiments. For each experiment, three to five animals were used. Statistical significance was calculated by Student's *t*-test.
* Difference is significant under paired comparison at $p < 0.05$.

also investigated. If diet is enriched with β-carotene, it accumulates in the liver and increases the adaptation of an animal to environmental conditions [37]. We used γ-irradiation at a dose rate of 3 cGy/day up a dose of 2 Gy [17]. Irradiation of rats at a dose rate of 1–3 cGy/day during the whole lifetime did not shorten the mean life span [38]. In the liver of irradiated rats, an amount of glycogen changed [39]. As seen from Table 5, both these agents induce the increase of fatty acids in liver nuclei. The total phospholipid content did not change. An amount of PC was elevated both in the nuclei of irradiated rats and in β-carotene fed supplied rats. At the same time, PE enhances only in nuclei of irradiated rats. In nuclei of β-carotene fed rats, the amount of PS increased. In both cases, an amount of cholesterol diminished and an amount of diacylglycerides increased. Therefore, certain levels of metabolism are accompanied with the increase of nuclear fatty acids without the decrease of nuclear phospholipids. An increase in DAG is of great interest. DAG was shown to be an activator of protein kinase C (PKC), which enters the nuclei by a signal transduction pathway [2]. Agonists induce rapid redistribution of the different PKC isozymes to distinct subcellular structures. Under this condition, PKC_{ε} associated with nuclear membranes in NJH 3T3 cells [40]. DAG plays an important role in a number of agonist-induced signal transduction pathways. For instance, α-trombin induced in cultured fibroblasts a rapid increase in the level of DAG mass in the nuclei and a selective increase in nuclear PKC-α. The molecular species profiles of the nuclear DAG generated resemble the species profiles of PC. In response to this agonist, nuclear PE was altered in a dramatic manner [41]. These results demonstrate that PC hydrolysis is the predominant source of the agonist-induced nuclear DAG in this case.

Differentiation of murine erythroleukaemia cells with the chemical agent DMSO leads to a cessation of proliferation and to a decrease in the level of nuclear DAG [42]. The decrease of nuclear DAG in thymocytes of chronic irradiated rats is accompanied with the fall of the ODC activity in thymus of chronic γ-irradiated rats [27,43].

DAG generated by a phosphatidylcholine-specific phospholipase C is a well established activator of the important signaling system protein kinase C. DAG appears to mediate various cellular responses to TNF and IL-1. It has been suggested that there may be more than one distinct pool of DAG in the nucleus and that these pools appear to be spatially separated and derived from different sources. These species of DAG have different functions within the nucleus [2]. In all these cases, we may speculate that the changes in lipids result from the variation of lipid traffic from the cytoplasm in nuclei and chromatin. An elevation of PE in liver nuclei is of great interest under the chronic low dose rate irradiation of the rats, but the significant PE elevation is obscure.

An increase of nuclear PS is suggested to have an effect on RNA polymerase activity [44]. PS can release the H1 histone from intact nuclei. The DNA template availability increased and DNA synthesis is activated [45]. It is suggested that β-carotene can influence transcriptional activity of the liver nuclei through the increase in PS.

Table 5
The amount (μg lipid/mg protein) of lipids isolated from the liver nuclei of rats irradiated at a dose rate 3 cGy up to a dose of 2 Gy and from liver nuclei of rats fed with β-carotene (3 mg/kg body mass daily)

Lipids	Control $n=6$[a]	3 cGy/day $n=3$[a]	β-carotene $n=3$[a]
Fatty acids	12.2 ± 1.7	19.6 ± .1.5 *	23.0 ± 2.3 *
Cholesterol	3.5 ± 0.3	1.4 ± 0.3 *	2.3 ± 0.3 *
Diacylglycerides	6.8 ± 0.8	13.1 ± 2.2 *	17.7 ± 4.1 *
Total phospholipids	43.1 ± 6.0	40.8 ± 7.4	51.9 ± 6.1
Phosphatidylcholine	14.5 ± 1.8	22.9 ± 1.3 *	22.0 ± 2.0 *
Phosphatidylethanolamine	6.9 ± 1.2	10.2 ± 0.3 *	8.6 ± 1.6
Phosphatidylserine	1.9 ± 0.1	1.3 ± 1.2	6.5 ± 0.6 *
Phosphatidylinositol	3.2 ± 0.9	5.0 ± 0.3	5.0 ± 1.2
Sphingomyelin	1.7 ± 0.3	2.7 ± 0.7	2.0 ± 0.3
Cardiolipin	1.2 ± 0.3	1.4 ± 0.2	1.4 ± 0.3
Body mass (g)	258 ± 8	190 ± 7	252 ± 5

Other details are as in Table 3.
[a] Number of experiments.
* Difference is significant at $p<0.05$.

4. The influence of chronic low dose rate irradiation on the lipid metabolism in nuclei and chromatin from the tissue of irradiated rats

We have investigated the influence of the more injurious dose rate of γ-irradiation on thymocytes nuclei and chromatin. Rats were irradiated in a special γ-camera at a dose rate of 12.9 cGy/day up to a total dose of 10 Gy [43]. It has been shown that ionizing radiation at a dose rate of 10–13 cGy/day up to a total dose of 20 Gy diminishes the mean life span of rats up to two folds. The death of rats was conditioned by destruction in hemopoesis [46]. The body and thymus mass of rats exposed to the 10 Gy irradiation did not differ from control. The ODC activity decreased at the early stage of irradiation, and turned back to normal level [43]. The protein synthesis in thymocytes from irradiated rats was increased. The synthesis of the total lipids in the cells remained unchanged by 2-^{14}C-acetate incorporation. Radioactivity of both total lipids and phospholipids in the nuclei and chromatin was increased (Table 6). An amount of fatty acids in the thymocytes from irradiated rats was diminished, but no changes were registered in the nuclei and chromatin. The label incorporation in the nuclear and chromatin fatty acids was rather more intensive than that in the whole cells. The transfer of fatty acids in nuclei and chromatin was suggested to increase by γ-irradiation of rats (Table 7). The effect of chronic ionizing radiation at a low dose rate induced significant increase of the amount of PS in thymocyte nuclei. In chromatin, the amount of PS and phosphatidylinositol (PI) increased (Table 8). This is apparently due to the involvement of these lipids in signal

Table 6
Radioactivity (cpm/mg protein) of total lipid fraction and total phospholipid fraction of cells, nuclei and chromatin of the thymus of control and irradiated rats at a dose rate of 12.9 cGy/day up to dose of 10 Gy

Objects	Total lipid fraction			Total phospholipid fraction		
	Control	10 Gy	Percentage of control under paired comparison	Control	10 Gy	Percentage of control under paired comparison
Cells	9470 ± 820	9820 ± 1110	106 ± 8.5	4120 ± 750	3710 ± 350	99 ± 29
Nuclei	4070 ± 920	6940 ± 230 *	178 ± 7.9 *	1650 ± 290	3050 ± 400	190 ± 19.1 *
Chromatin	3920 ± 520	5760 ± 360 *	151 ± 9.4 *	1780 ± 190	3030 ± 630	167 ± 17.3

Other details are as in Table 3.
* Difference is significant at $p < 0.05$.

mechanism of induction of specific gene transcription. The activation of "immediate early genes" by acute low dose ionizing radiation and the activation of Hps 70 gene expression upon chronic γ-irradiation were shown [47,48]. When the cells are exposed to ionizing radiation, it leads to complex cellular responses resulting in cell death and altered proliferation status. The underlying cytotoxic, cytoprotective and cellular stress responses to radiation are mediated by existing signaling pathways. Virtually all known signaling cell pathways are involved in radiation cell response [49]. Phosphoinositides are known as mediator of nuclear responses. PI and its metabolites are hydrolyzed when mitosis is stimulated; at the same time, the amount of nuclei DAG increases and PKC in the nuclei translocates [2]. In the case of continuous γ-irradiation at a damaging dose rate, PI accumulation in chromatin is most likely to connect with inhibition of cell proliferation and DNA repair under partial normalization of a state. PI metabolites take part in the activation of DNA polymerase α. This enzyme participates in the synthesis and excision repair of DNA [50]. PS is known as an effective activator of PKC [51]. PS accumulation in nuclei and chromatin is probably due to the activation of gene transcription for the radiation damage repair. PS activated RNA polymerase and induced the chromatin decondensation on the isolated liver nuclei [35,52]. PI and PS accumulation and the rapid entering of fatty acids in chromatin of thymocytes from irradiated rats are related to the chromatin transcriptional activity and repair of DNA.

Thus, we analyzed nuclear and chromatin lipid metabolism during an adaptation to a harmful environment. Rats were irradiated at the same conditions up to a total dose of 20 Gy. The liver cholesterol content under these conditions of treatment altered inconsiderably [39]. The activation of liver cholesterol synthesis upon irradiation of animals is suggested to be a common liver tissue response to acute sublethal and lethal ionizing radiation [53]. If rats were chronic irradiated up to dose of 20 Gy, the cholesterol synthesis in liver was unchanged [54]. In the liver cells at the same conditions of rats irradiated cytologically, prenecrobiotic changes were shown [55]. The pronounced alterations in the lipid composition were observed in the nuclear fraction of liver while the lipid composition of whole liver was the same as control [56]. In mitochondria CL and cholesterol were increased [57]. The nuclear level of PE increased significantly after irradiation; the level of PC + PS was also enhanced (Table 9). The cell nuclei are known to be a site where the main regulatory signals are realized. An enhancement in the level of PC and PE after irradiation at low dose rate can be related to the involvement of these phospholipids in one of the cell signaling systems. It is important to underline that the level of nuclear cholesterol increased (Table 9). These data unambiguously imply that the nuclear cholesterol take part in responses of animal to the chronic low dose rate irradiation. It is of great interest that the level of nuclear fatty acids decreased. The fatty acids are used by nuclei as an energy source; hence, their

Table 7
The amount (μg lipid/mg protein) and radioactivity (cpm/mg protein) of cholesterol and fatty acids of cells, nuclei and chromatin of the thymus of control and irradiated rats

Objects	Quantity			Radioactivity		
	Control	10 Gy	Percentage of control under paired comparison	Control	10 Gy	Percentage of control under paired comparison
Cholesterol						
Cells	12.3 ± 0.9	10.9 ± 1.6	87 ± 6.2	352 ± 153	415 ± 170	130 ± 29
Nuclei	9.6 ± 0.3	9.1 ± 0.9	95 ± 9.5	209 ± 63	205 ± 22	98 ± 12
Chromatin	5.2 ± 0.6	5.4 ± 0.1	107 ± 12	167 ± 23	249 ± 50	148 ± 18
Fatty acids						
Cells	63.8 ± 8.8	38.4 ± 5.4	60 ± 5.8 *	277 ± 42	349 ± 59	125 ± 2.3 *
Nuclei	46.9 ± 14	46.4 ± 12.6	99 ± 12	235 ± 12	464 ± 9 *	170 ± 10 *
Chromatin	23.0 ± 3.3	21.8 ± 2.0	97 ± 8.8	197 ± 54	391 ± 97	205 ± 18 *

Other details are as in Table 3.
* Difference is significant at $p < 0.05$.

Table 8
The amount (μg lipid/mg protein) of phospholipids in thymus cells, nuclei and chromatin after chronic γ-irradiation of rats at a dose rate of 12.9 cGy/day up to a dose of 10 Gy

Lipids	Thymocytes			Nuclei			Chromatin		
	Control	10 Gy	Percentage of control[#]	Control	10 Gy	Percentage of control[#]	Control	10 Gy	Percentage of control[#]
Sphingomyelin	1.1 ± 0.1	1.1 ± 0.1	100 ± 2	1.4 ± 0.3	1.5 ± 0.4	107 ± 8	1.1 ± 0.3	1.0 ± 0.2	93 ± 11
Phosphatidylcholine	22.7 ± 0.8	24.1 ± 3.5	105 ± 12	15.1 ± 4.8	17.5 ± 4.6	116 ± 12	17.1 ± 3.0	22.8 ± 4.2	134 ± 17
Phosphatidylserine	3.7 ± 0.1	4.3 ± 1.3	114 ± 27	1.8 ± 0.5	2.5 ± 0.7	141 ± 2 *	1.9 ± 0.4	3.0 ± 0.6	156 ± 2 *
Phosphatidylinositol	3.1 ± 0.1	3.4 ± 0.2	110 ± 10	2.0 ± 0.4	3.0 ± 0.9	139 ± 20	1.6 ± 0.4	2.8 ± 0.7	171 ± 9 *
Phosphatidylethanolamine	7.5 ± 0.4	7.7 ± 1.8	103 ± 9	4.5 ± 1.4	4.6 ± 0.8	110 ± 13	5.4 ± 1.3	5.8 ± 1.3	111 ± 16
Cardiolipin	1.7 ± 0.2	1.2 ± 0.1	92 ± 6	1.0 ± 0.2	1.1 ± 0.2	112 ± 11	1.0 ± 0.1	0.9 ± 0.1	88 ± 7

Other details are as in Table 3.
[#] Difference is significant under paired comparison at $p < 0.05$.
* Difference is significant at $p < 0.02$.

specific changes in this organelle may coincide with radiation-induced alteration in energy production. Changes in the composition of nuclear lipids can also be induced by translocation of some specific proteins from the cytoplasm to the nuclei. This type of event takes place in pathways of regulatory signal transduction [2,58]. The lipids of the nuclear envelope comprise the largest part of the nuclear lipids [19]. Our data indicated great changes in the fluidity of liver nuclear membranes after chronic irradiation of rats at a dose rate of 12.9 cGy/day up to a final dose of 20 Gy. In chromatin from the liver of irradiated rats, the amount of cholesterol showed weak tendency to increase, but the amount of fatty acids, PC + PS, PE and SM did not change in comparison to the control value. Only the amount of CL is shown to sharply decrease (Table 9). The role of chromatin and DNA-bound CL in DNA structure and cell proliferation are now discussed [59]. Thus, the correlation between the changes of genome function and the chromatin lipid composition after the effect of ionizing radiation on animal could be proposed.

An acute irradiation of rats led to more changes in liver chromatin lipid composition than those under a low dose rate continuous irradiation. In 10 min after irradiation of rats at a lethal dose of 10 Gy, the amount of liver chromatin phospholipids decreased while the amount of cholesterol increased. The most pronounced effects under these conditions were the large decrease in CL and the increase of PC + PS [10]. At this period of time and at this radiation dose, nuclear protein phosphorylation and RNA and protein synthesis in rat liver increased [60]. Metabolic response of nuclear and chromatin lipids to irradiation is time dependent [9,20]. At present, the role of chromatin lipid changes in the metabolic and functional response of chromatin to ionizing radiation of an animal is obscure.

5. The effects a lethal dose of ionizing radiation on the nuclear and chromatin lipid metabolism in rat thymus

The effect of ionizing radiation at a dose of 10 Gy, which is absolute lethal, causes a mitosis block and induction of cell apoptosis in lymphoid tissues. At an earlier period of time after irradiation, changes in nuclear and chromatin lipid metabolism in rat thymus is of a preapoptotic significance. We have isolated thymocytes at that very moment after acute irradiation of rats at a dose of 10 Gy [61,62]. The changes in lipid amounts were very poor in the thymus nuclei (Table 10); the increase in fatty acid contents is also not much significant. The amount of cholesterol and phospholipids did not change. The absence of the transcriptional

Table 9
The amount (μg lipid/mg protein) of lipids isolated from liver nuclei and chromatin after whole-body irradiation of rats at a dose rate of 12.9 cGy/day up to a dose of 20 Gy

Lipids	Nuclei		Chromatin	
	Control	20 Gy	Control	20 Gy
Fatty acids	78 ± 20	54 ± 14 * *	59 ± 10	46 ± 14
Cholesterol	13.4 ± 1.1	19.6 ± 1.9 * *	10.6 ± 2.2	13.4 ± 0.4
Total phospholipids	94 ± 9	92 ± 7	–	–
Phosphatidylcholine + phosphatidylserine	17.1 ± 0.6	29.7 ± 0.2 *	21.1 ± 1.4	22.0 ± 2.7
Phosphatidylethanolamine	5.05 ± 0.95	13.8 ± 2.1 *	5.6 ± 0.3	7.5 ± 0.9
Sphingomyelin	4.3 ± 0.8	4.3 ± 0.4	4.8 ± 0.5	4.9 ± 1.2
Cardiolipin	2.02 ± 0.07	2.49 ± 0.76	5.3 ± 0.9	1.5 ± 0.1 *
Body mass (g)	520 ± 4	496 ± 18		

Other details are as in Table 3.
* Difference is significant at $p < 0.05$.
** Difference is significant under paired comparison at $p < 0.05$.

Table 10
The amount and specific radioactivity of lipids in nuclei and chromatin of thymus from γ-irradiated rats at a dose of 10 Gy

Lipids	Amount (μg lipid/mg protein)				Specific radioactivity (cpm/mg lipid)			
	Nuclei		Chromatin		Nuclei		Chromatin	
	Control	10 Gy	Control	10 Gy	Control	10 Gy	Control	10 Gy
Cholesterol	7.4 ± 1.1	6.8 ± 0.7	4.7 ± 0.5	4.9 ± 0.4	13 ± 5	11 ± 3	35 ± 20	31 ± 5
Fatty acids	14.3 ± 3.6	17.1 ± 0.8 * *	11.4 ± 3.0	11.6 ± 1.2	6.0 ± 1.0	4.5 ± 0.5	9.0 ± 2.0	7.0 ± 0.7
Phosphatidylcholine + phosphatidylserine	24.0 ± 6.0	28.0 ± 4.0	16.8 ± 3.9	19 ± 6	30.0 ± 5.0	24.0 ± 3.0	58 ± 17	48 ± 3 * *
Phosphatidylethanolamine	8.1 ± 1.3	7.3 ± 1.0	4.5 ± 0.1	7.2 ± 0.7 *	26 ± 8	20 ± 5	59 ± 4	42 ± 10
Sphingomyelin	1.8 ± 1.1	1.9 ± 0.9	1.0 ± 0.2	1.5 ± 0.5	8 ± 3	5 ± 2	34 ± 10	10 ± 5 * *
Cardiolipin	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	0.9 ± 0.2	10 ± 3	6.4 ± 2.1	90 ± 80	110 ± 40
Total phospholipids	54.9 ± 8.8	52.5 ± 1.8	36.8 ± 1.3	36.3 ± 6.8				

Thymocytes were isolated immediately after irradiation and were incubated for 40 min with 2-^{14}C-acetate. Values are means ± S.E. of three separate experiments.

* Difference is significant at $p < 0.05$.

* * Difference is significant under paired comparison at $p < 0.05$.

or replicative activation is accompanied by the absence of serious lipid changes in preapoptotic nuclei. The sharp rise of chromatin PE in thymocytes of the irradiated rats was shown. Rat hepatocyte nuclei have an enzyme of choline base exchange. Base-exchange enzyme complex is most likely to be present in chromatin thymocytes. The presence of this base-exchange enzyme complex may allow a fast change in chromatin phospholipid composition [63]. The rise of chromatin PE amount is not accompanied with any changes of the chromatin or nuclear PC + PS amount. Thus, the interconversion of phospholipids is not shown. It is known that PE, like PC, triggered chromatin condensation [35] and inhibited endogenous RNA polymerase activity of isolated rat liver nucleus [52]. The accumulation of PE in chromatin of the lethal irradiated preapoptotic thymocytes coincides with the repression of certain genes after the lethal γ-irradiation of rats. The incorporation of the labeled acetate in the total lipid fraction of thymocytes was diminished after irradiation. No changes in specific radioactivity of fatty acids and cholesterol in nuclei and chromatin are observed after irradiation. Thus, the activation of lipogenesis after the sublethal dose observed in lymphoid cells of irradiated rats [33] was not registered under the action of the lethal dose of irradiation. ^{14}C-acetate is a primary precursor of synthesis of fatty acids and cholesterol. ^{14}C-fatty acids are incorporated in phospholipids via acylation–deacylation processes. Table 10 shows that SM and CL from chromatin are labeled more intensively than those from nuclei. A high specific radioactivity of the chromatin phospholipids may occur by active transacylation of phospholipids in chromatin or by a transfer of newly synthesized phospholipids from cytoplasmic compartments to chromatin. The traffic of highly labeled species from cytoplasm to chromatin is suggested for fatty acids and cholesterol. The investigation of 2-^{14}C-acetate incorporation in cholesterol and fatty acids of nuclei, chromatin and nuclear membrane of thymocytes supports this assumption [19]. The incorporation of labeled acetate in SM and PC + PS of chromatin is reduced; that is not observed in nuclei. SM is metabolized in chromatin. The enzyme phosphatidylcholine ceramide phosphocholine transferase or SM synthase is present in the nuclear membrane and chromatin, although with higher activity in nuclear membrane in rat liver nucleus [64]. A neutral sphingomyelinase (SMase) is present in the two nuclear fractions of rat liver cells—nuclear membrane and chromatin—and is characterized by different pH optima. The chromatin SMase activity varies during liver regeneration [26]. The amount of SM in chromatin and nuclei did not change after irradiation. Consequently, inhibition of labeled acetate incorporation in chromatin SM can be related to the inhibition of reacylation. Thus, changes in thymocyte chromatin lipid metabolism after a lethal dose of radiation differ from those in thymocyte nuclei. Changes in lipid metabolism of chromatin are coupled with preapoptotic changes in irradiated rat thymocytes.

6. Discussion

Ionizing radiation alters nuclear and chromatin lipid metabolism in the radioresistant and radiosensitive tissues of animals. Nonlethal doses of γ-irradiation and other chemicals cause changes in the amount of nuclear fatty acids. Cell repair after physical and chemical injuries is accompanied by increase in nuclear and chromatin fatty acids and decrease in nuclear and chromatin phospholipids. The chronic low dose rate irradiation leads to a significant change in phospholipid and neutral lipid metabolism in nuclei and chromatin from radiosensitive (thymus) and radioresistant (liver) tissues of rat. Under the adaptation of rat to the chronic low dose rate γ-irradiation, the increase of PI and PS in the thymocyte chromatin is associated with DNA repair. The injury of rat liver cell under irradiation at a dose rate of 12.9 cGy/day concurs with a sharp decline of the chromatin CL amount. The earlier state of rat thymocytes after a lethal dose of γ-irradiation was characterized by the increase in chromatin PE. In many cases of cell injury, changes in nuclei lipid metabolism differed from that of the chromatin lipids. The nuclear and chromatin lipid responses to the damaging agents were not completely

determined by the chromatin and the nuclear enzyme, which metabolized lipid in nuclei and chromatin. The lipid transfer from cytoplasmic compartments to nuclei and chromatin may be involved in the responses of nuclear and chromatin lipid metabolism to ionizing radiation and to other damaging agents. It was shown that acceleration of lipid metabolism in the liver of irradiated rats was concomitant with the increase in the rate of labeled cholesterol transfer in vivo to liver nuclei. In vitro, the transfer of cholesterol between microsomes and nuclei remain unchanged using lipid exchange protein. Cholesterol transfer is suggested to activate in the cells of irradiated rats via the pathway other than lipid exchange protein [65]. It shows the participation of PI transfer protein in the translocation of PI from liposomes to nucleus. [66]. The translocation of the signaling molecules to nuclei is probably coupled with the lipid traffic in nuclei and chromatin. The role of lipids in the protein translocation into the nucleus by the signal transduction pathways needs further investigation.

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