low-molecular-weight DNA has potential value as a basis for laboratory methods of analysis in connection with the diagnosis of moderately severe radiation damage.

LITERATURE CITED

1. A. S. Belokhvostov, S. N. Lebedev, and S. S. Sherlina, Radiobiologiya, **27**, No. 4, 505 (1987).
2. T. Maniatis, E. Fritsch, and J. Sambrook, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor (1982).
3. A. S. Belokhvostov and N. K. Zelenkova, Cancer Lett., **5**, No. 6, 351 (1978).
4. D. B. P. Goodman and J. E. Puzas, Analyt. Biochem., **86**, No. 1, 50 (1978).
5. S. W. Distelhorst, K. Cramer, and J. C. Rogers, J. Clin. Invest., **61**, No. 5, 1204 (1978).
6. T. C. Hamilton, A. G. Smith, and C. A. Griffin, Klin. Wschr., **57**, No. 9, 451 (1979).
7. R. S. Jose and S. S. Potter, Nucleic Acids Res., **13**, No. 3, 1027 (1985).
8. S. A. Leon, B. Shapiro, D. M. Sklaroff, et al., Cancer Res., **37**, No. 3, 646 (1977).
9. A. R. Neurath, N. Strick, K. Miller, et al., J. Virol. Meth., **8**, No. 1, 73 (1984).
10. B. Shapiro, M. Chacraborty, E. M. Cohn, et al., Cancer Res., **51**, No. 11, 2116 (1983).
11. C. R. Steinman, J. Clin. Lab. Invest., **73**, No. 3, 832 (1984).
12. M. Stroun, P. Anker, J. Lyautey, et al., Eur. J. Cancer Clin. Oncol., **23**, No. 6, 707 (1987).

EFFECT OF HEPATOPROTECTIVE AGENTS THIOCTACID AND FLAVOBION ON HISTONES IN THE INTACT AND REGENERATING LIVER OF IRRADIATED RATS

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As the principal protein components of eukaryotic chromosomes histones are of fundamental importance in the stabilization of DNA structure in chromatin at different phases of the cell cycle and they play an important role in the regulation of gene expression [1].

Changes induced in histones by irradiation in vivo in different organs [2-5] or induced by partial hepatectomy [6, 7] have received comparatively little study. There is only sporadic information on the effect of irradiation on histones in the regenerating liver [8], and in combination with the use of hepatoprotective agents flavobion and thioctacid, we have no data as yet. Flavobion has many positive effects on the damaged liver: it stabilizes membranes, stimulates synthesis of ribosomal RNA [9], and, according to some data [10], its use leads to a reduced risk of development of toxic liver damage in patients. Thioctacid is a coenzyme of the Krebs cycle and is recommended as a preparation for use against liver damage in radiation sickness [11]. The aim of the investigation was to discover changes in some cytological parameters and in nucleic acid levels, the total content of extractable histones, and relative replacement of histone fractions after exposure to radiation and correction by hepatoprotective agents.

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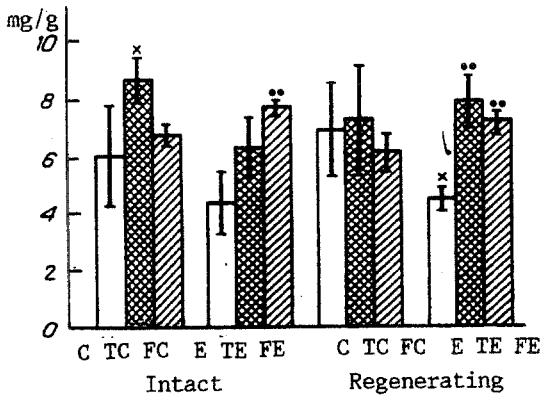

Fig. 1. Concentration of extractable histones in intact and regenerating liver of unirradiated control rats (C), irradiated rats (5.7 Gy – E), and after application of hepatoprotectives: thioctacid (T) and flavobion (F). *p < 0.05, **p < 0.01, °p < 0.05, °°p < 0.01.

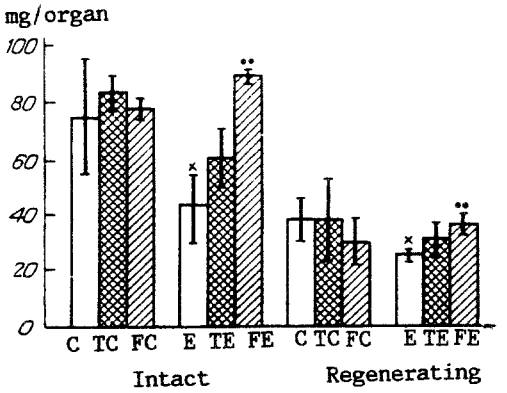

Fig. 2. Content of extractable histones in intact and regenerating liver of unirradiated control rats (C), irradiated rats (5.7 Gy – E), and after application of hepatoprotectives: thioctacid (T) and flavobion (F). Legend as to Fig. 1.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats weighing 270-300 g initially. The animals were fed on a standard laboratory diet (LD) and were given water to drink ad lib. Flavobion (Spofa) was applied perorally through a tube and thioctacid (Asta Pharma AG) was given intraperitoneally in a dose of 70 mg/kg or 15 mg/kg respectively [11]. Some of the rats were irradiated after 1 h in a total dose of 5.7 Gy of γ-rays (^{60}Co). During the 30 min after irradiation the animals underwent partial hepatectomy, at which, by a standard method [11], about 70% of the weight of the liver was removed (groups of irradiated unprotected and protected animals — E, FE, and TE). Meanwhile control unirradiated animals underwent a similar operation (groups of control animals – C, FC, and TC). All the operations were performed at the same time of day, during the morning (8-10 a.m.). Histones were studied in the intact liver (in loci removed at operation i.e., 90 min after application of the hepatoprotective agents and/or 60 min after irradiation) 30 h after partial hepatectomy. Nuclei were isolated and histones extracted (with 0.2 M sulfuric acid) by the method in [12]. The total concentration of extract of histones was determined by the method in [13], in which bovine serum albumin was used as the standard and the results were calculated in milligrams histones per gram of tissue (concentration) and per weight of the organ (content). Histones

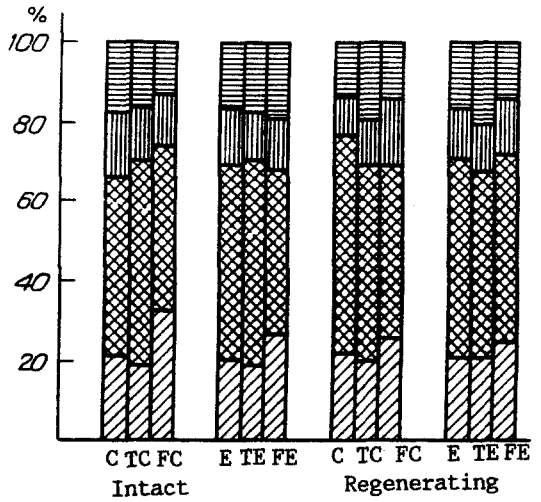

Fig. 3. Relative content of individual histone fractions in intact and regenerating liver of unirradiated control rats (C), irradiated rats (5.7 Gy – E), and after application of hepatoprotectives: thioctacid (T) and flavobion (F). Legend as to Fig. 1.

were separated on 15% acrylamide gel by the method in [14] on the GE11 instrument (Pharmacia). Individual histone fractions were determined by subjecting the gels to densitometry on the Shimadzu CS-930 instrument.

The results were subjected to statistical analysis [17].

EXPERIMENTAL RESULTS

The substances tested had a marked effect on the concentration and total content of extracted histones. After administration of thioctacid alone the concentration of the histones in the intact rat liver increased but the total content of histones changed less demonstratively (Figs. 1 and 2). After administration of flavobion alone, the increase was not statistically significant.

At partial hepatectomy about 70% of the mass of the liver was removed and the histone content in the organ fell by approximately 30% although the concentration was unchanged. Until 30 h after the operation the histone content in the regenerating part of the liver rose by 50% of the initial values. Use of the hepatoprotective agents did not affect the concentration or total content of histones in the regenerating liver.

Whole-body irradiation with γ-rays in a dose of 5.7 Gy led to a marked fall in the histone concentration in the unprotected rats. Thioctacid, given 1 h before the operation, weakened these changes in the intact and regenerating liver. The protective effect of flavobion was more marked than that of thioctacid. In the intact and regenerating liver of irradiated rats protected by flavobion, the concentration and content of histones lay within the limits of the values obtained for control unirradiated rats. The quantitative changes described in the histones in the regenerating and, especially, in the intact liver of the irradiated rats were more marked than changes in the DNA concentration and content. More demonstrative changes in histones than in DNA have been observed in the rat thymus after irradiation with various doses of x-rays [4, 5], as shown by a temporary fall of the H/DNA ratio. Since histone synthesis is closely linked with DNA synthesis [16] and since a marked fall of the histone content takes place immediately after irradiation (in the intact liver, as early as 60 min after irradiation) while the DNA content remains unchanged, it can be postulated that quantitative changes in histones correspond to changes in extractability of the histones from chromatin after irradiation and to their more rapid degradation.

As shown in Fig. 3 and Table 1, determination of the relative content of individual histone fractions revealed marked changes in histone H1. The relative content of this fraction rose after application of flavobion alone and in conjunction with irradiation in the intact and regenerating liver. In all four groups studied, an increase in substitution of H1 correlates with an increase in the histone concentration and content after administration of flavobion, but not of

TABLE 1. Relative Content of Histone Fractions in Intact and Regenerating Liver of Control and Irradiated Rats and Rats Receiving Thioctacid and Flavobion

Group/fraction	Control intact	Irradiated intact	Control regenerating	Irradiated regenerating
H1	20,44±2,3	19,75±1,06	21,87±0,22	20,90±2,2
H2A+H2B	45,31±2,88	47,55±3,04	54,89±2,08	49,73±0,96*
H3	17,18±1,12	15,16±1,48	10,36±0,28	12,46±0,27*
H4	16,48±1,79	15,46±2,02	12,61±1,88	15,24±0,93
Group/fraction	**Control intact + thioctacid**	**Irradiated intact + thioctacid**	**Control regenerating + thioctacid**	**Irradiated regenerating + thioctacid**
H1	19,35±0,61	19,77±2,41	20,22±1,44	20,7±1,24
H2A+H2B	53,77±1,75^{0}	52,19±2,39	48,79±1,96^{0}	46,74±2,88
H3	13,5±0,39^{0}	12,61±0,87	12,2±0,48^{0}	12,13±0,44
H4	6,16±1,33	17,16±3,06	18,92±4,47^{0}	20,49±1,89^{0}
Group/fraction	**Control intact + flavobion**	**Irradiated intact + flavobion**	**Control regenerating + flavobion**	**Irradiated regenerating + flavobion**
H1	34,95±3,3^{00}	28,53±5,61^{0}	26,66±1,19^{00}	25,35±1,03^{0}
H2A+H2B	42,89±2,31	41,47±4,39	43,04±2,52^{00}	46,86±1,99
H3	17,46±2,6	13,92±2,71	17,6±0,42^{00}	14,13±3,06
H4	16,97±4,38	19,06±4,15	13,7±0,46	13,64±2,24

Legend. *$p < 0.05$, **$p < 0.01$, $^{0}p < 0.05$, $^{00}p < 0.01$.

thioctacid. After only a single irradiation a low concentration and content of histones was observed, and the relative content of the H1 fraction likewise was not increased. Ashami [8] reported that incorporation of precursor into H1 rises to a maximum 27 h after partial hepatectomy, and that irradiation inhibits H1 synthesis.

The increase in the concentration and content of histones and in the relative content of histone fraction H1 in irradiated rats protected by flavobion were the same as in unirradiated rats receiving flavobion. Changes in histones of the nucleosomal core were not so uniform as changes in histone H1.

LITERATURE CITED

1. R. S. Wu, H. T. Panusz, C. L. Hatch, and W. M. Bonner, CRC Critical Rev. Biochem., **20**, 201 (1986).
2. R. D. Bauer, E. E. Oliphant, and J. R. Reeve, Radiat. Res., **62**, 119 (1975).
3. B. A. Korol', M. A. Tarshis, A. M. Kuzin, et al., Radiobiologiya, No. 3, 490 (1970).
4. J. Suliova and E. Mišurova, Radiobiol. Radiother., **23**, 667 (1982).
5. J. Suliova and E. Mišurova, Radiobiologiya, **23**, 513 (1983).
6. R. Balhorn, W. O. Rielke, and R. Chalkley, Biochemistry, **10**, 3952 (1971).
7. W. T. Garrard, G. H. Kidd, and J. Bonner, Biochem. Biophys. Res. Commun., **70**, 1219 (1976).
8. K. Ashami, Radiat. Res., **109**, 216 (1987).
9. R. Braatz, Arch. Pharmakol., **293**, Suppl. 192 (1976).
10. P. Barton, V. Krejcy, and M. Balzek, Čas. Lek. Česk., **6**, 173 (1985).
11. G. M. Higgins and R. M. Anderson, Arch. Pathol., **12**, 186 (1931).
12. H. H. Grunicke, Y. Yamada, Y. Natsumeda, W. Helliger, et al., Cancer Res. Clin. Oncol., **115**, 435 (1989).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., **193**, 265 (1951).
14. S. Panyim and R. Chalkley, Arch. Biochem. Biophys., **130**, 337 (1969).
15. J. F. Harper, Comput. Biol. Med., **14**, 437 (1984).
16. W. Marzluff and N. B. Pandey, Trends Biochem. Sci., **13**, 49 (1988).