Physiol. Res. 44: 241–247, 1995

The Influence of Essential Phospholipids (ESSENTIALE) on Liver Regeneration in Gamma Irradiated Rats

K. KROPÁČOVÁ, E. MIŠÚROVÁ

Department of Cellular and Molecular Biology, Šafárik University, Košice, Slovak Republic

Received January 2, 1995
Accepted March 24, 1995

Summary

Gamma irradiation with a dose of 5.7 Gy within 30 min before partial hepatectomy (PHE) caused latent damage in the intact rat liver. This was expressed in the course of proliferation induced in the liver remnant by inhibition of the regenerative process, which was indicated by a decreased mitotic index and cellularity, an increased ratio of metaphases/prophases and a high chromosomal aberration frequency. The preparation of essential phospholipids (ESSENTIALE) that was injected in a dose of 360 mg/kg (i.p.) either 24 h before irradiation or 30 min after irradiation or repeatedly before and after irradiation, markedly stimulated the process of liver regeneration after PHE in both nonirradiated and irradiated rats. It moderated all the alterations induced by irradiation, especially changes in cellularity. The most effective was the repeated administration of ESSENTIALE whereas its single administration before irradiation was more effective than that after irradiation. Our results suggest that ESSENTIALE has not only a stabilizing effect on cell membranes, but also mitigates damage of genetic material induced by irradiation.

Key words

ESSENTIALE – Essential phospholipids – Irradiation – Regenerating rat liver – Cell proliferation – Chromosomal aberrations

Introduction

Phospholipids which constitute 50–80 % of cell membranes have a high content of saturated fatty acids. However, nonsaturated, so-called essential fatty acids are extraordinarily important for a number of metabolic processes. In the preparation ESSENTIALE, highly nonsaturated fatty acids, especially linolic (approximately 70 %), linoleic and oleic acids are substituted for saturated fatty acids. The essential phospholipids are transported mainly to hepatocytes where they are incorporated in an unchanged form into the cell membranes. The nonsaturated phospholipids in preparation ESSENTIALE are combined with other important substances such as vitamins, because their need in damaged cells is enhanced.

ESSENTIALE is recommended for treatment of various liver diseases such as hepatitis, cirrhosis and fatty degeneration of different etiology (Loginov *et al.* 1986, Hanták *et al.* 1990, Gordienko 1990). The essential phospholipids incorporated into cell membranes contribute to liver tissue regeneration (Holeček *et al.* 1985, Šimek *et al.* 1989). In patients with alcoholic cirrhosis they have an inhibitory effect on the peroxidation of lipids in the liver. In experiments on animals, it was ascertained that the administration of essential phospholipids can also mitigate the disturbance of lipid metabolism caused by tetrachlormethane administration (Vengerovskij *et al.* 1987) and can stabilize cell membranes including the membranes of lysosomes. This is expressed by a decrease of acid DNAse activity and of other lysosomal enzymes (Fomichenko and Ermakov 1986). In the rat liver regenerating after partial hepatectomy (PHE), ESSENTIALE administration decreases triglycerides concentration and increases mitotic activity (Holeček *et al.* 1992).

In this study we investigated the influence of ESSENTIALE on the development of the so-called latent radiation-induced damage of the rat liver. Latent damage also arises in other moderately proliferating tissues and interferes mainly with the genetic material of the cell. Such damage is expressed after stimulation of cells to divide (for example, after partial resection of a tissue, chemical or viral injury) by different biochemical and cytological changes including inhibition of DNA synthesis and mitosis and cytogenetic alterations in the regenerating liver remnant (Albert and Bucher 1960, Kropáčová and Mišúrová 1981, Mišúrová *et al.* 1987).

In previous studies, we found that it was possible to alleviate the development of latent radiation-induced liver damage by administration of radioprotective substances (Cysteamin, Adeturon, Gamafos) and also by some hepatoprotective substances (Flavobion, Thioctacid), which are used for prevention and therapy of various hepatic lesions (Kropáčová *et al.* 1980, Kropáčová and Mišúrová 1985, 1990, 1992). In the present experiments, the effect of the hepatoprotective substance ESSENTIALE forte on rat liver damage induced by irradiation was evaluated by weighing the organ and assessing its cellularity, mitotic index, ratio of metaphase to prophase numbers and frequency of chromosomal abnormalities in the liver remnant regenerating after partial hepatectomy (PHE).

Materials and Methods

Adult male Wistar (SPF) rats, weighing 240–280 g, were used in the present experiments. Animals were fed a standard laboratory diet LD in pellets (Velaz Prague) and tap water was supplied *ad libitum*. The animals were divided into 6 groups consisting of 6 to 8 animals each.

Group C (controls) – saline was injected 24 h before PHE,

Group E – ESSENTIALE was injected 24 h before PHE,

Group I – single dose of 5.7 Gy 30 min before PHE,

Group E+I – ESSENTIALE was injected 24 h before irradiation and PHE,

Group I+E – ESSENTIALE was injected 30 min after irradiation (at the end of PHE),

Group E+I+E – ESSENTIALE was injected 24 h before irradiation and 30 min after irradiation.

Animals were irradiated with a single whole body dose of 5.7 Gy from a ^{60}Co gamma-ray source (Chisostat, Czech Republic) at a dose rate of 256 mGy . min^{-1}.

One capsule of ESSENTIALE forte ("SBS" – RO Bosnalijek Nattermann, Sarajevo, Bosna) contains 300 mg phosphatidylcholine, 6 mg pyridoxine hydrochloride, 6 μg cyanocobalamine, 30 mg nicotinamide, 6 mg thiamin nitrate, 6 g riboflavine and 6 mg α-tocoferol acetate. The preparation was administered intraperitoneally in saline at a dose of 360 mg/kg of body weight, 24 h before irradiation and/or 30 min after irradiation.

Animals of all the groups were submitted to 2/3 PHE (middle and left lateral lobes were removed) in ether anaesthesia according to the standard method (Higgins and Anderson 1931) always in the morning hours (08.00–10.00 h) and were examined in the time of maximum mitotic activity, i.e. 30 h after PHE.

Squash preparations were prepared from the tissue of regenerating liver stained using the Feulgen method. On examining about 50 000 cells in each experimental group, all the mitotic figures and chromosomal abnormalities in the postmetaphase were recorded. On the basis of these data, the mitotic index (MI) (i.e. the number of mitotic figures corresponding to 1000 cells), and the ratio of the number of metaphases to prophases (M/P) were calculated. The genetic damage was evaluated on the basis of chromosomal bridges and excentrical fragments in postmetaphase cells. In each group 400–500 postmetaphases were evaluated. The extent of damage was expressed as the percentage of aberrant cells of all recorded postmetaphase cells. Tissue cellularity, i.e. the number of cells per g of tissue, and the total cellularity of liver were estimated using a Coulter counter. The increment of regenerating liver weight was calculated on the basis of the rest of the liver weight which presented 30 % of the whole liver at the time of the operation (since about 70 % of the total liver was excised during PHE) and the weight of the regenerating liver at the time of examination.

The statistical significance was evaluated by Peritz' F-test (Harper 1984). The results concerning the regenerated livers are given in the figures as the mean values ± S.E.M. of 6 to 8 animals. Results of intact livers are not given.

Results

Cellularity

Tissue cellularity (cells per gram of wet weight) was increased in the regenerating liver of control animals (C) and animals with ESSENTIALE administered before PHE (E) when compared with the values in intact livers taken during partial hepatectomy which amounted to $10.19 \pm 0.45 \times 10^7$ cells/g w.w. (Fig. 1). In irradiated rats, tissue cellularity decreased by 25 % in the intact livers and by 50 % in the regenerating livers. ESSENTIALE has moderated the decrease of tissue cellularity after single and especially after repeated administration.

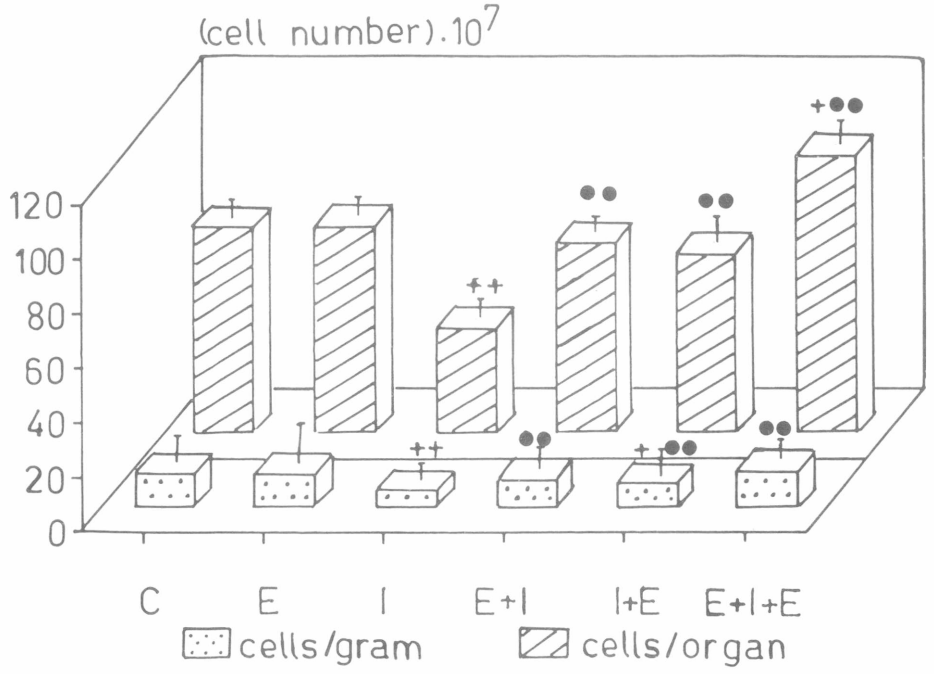

Fig. 1
Cellularity of the tissue and total cellularity of the regenerating rat liver. C – controls which had received 1 ml of saline/kg b.w., E – ESSENTIALE was injected 24 h before PHE; I – whole-body gamma irradiation with the dose of 5.7 Gy 30 min before PHE; E+I – ESSENTIALE was injected 24 h before irradiation and PHE. I+E – ESSENTIALE was injected 30 min after irradiation (at the end of PHE); E+I+E – ESSENTIALE was injected 24 h before irradiation and 30 min after irradiation. x $P<0.05$, xx $P<0.01$ compared with the controls (C). o $P<0.05$, oo $P<0.01$ compared with unprotected irradiated animals (I).

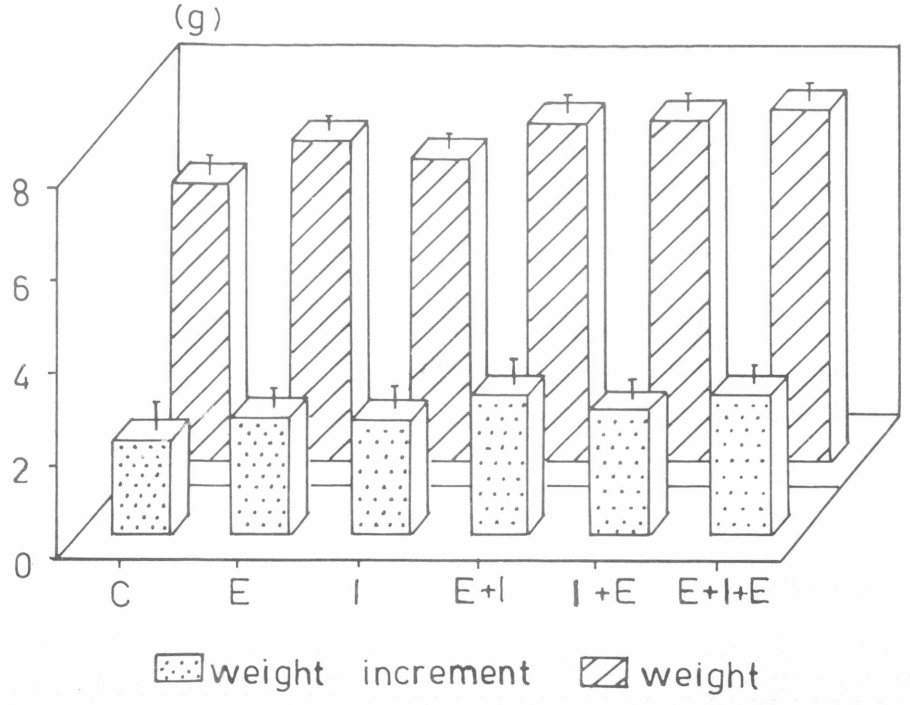

Fig. 2
Weight and weight increment of the regenerating rat liver. For legend see Fig. 1.

We did not find any appreciable differences in the regenerating liver weight between controls and the experimental groups of rats (Fig. 2). Therefore, the total organ cellularity changes (cells per organ weight – Fig. 1) were similar to those of the tissue cellularity per g w. w. Total cellularity of the regenerating liver 30 h after 70 % hepatectomy was lower in all groups than the cellularity of the total intact liver which amounted to $13.53 \pm 0.67 \times 10^8$ cells/organ. In irradiated rats, the total cellularity increase in the regenerating liver at this time lagged behind that in non-irradiated controls by approximately 50 %. Single administration of ESSENTIALE before or after irradiation moderated the lag of the total cellularity increment in the regenerating liver, and its repeated administration stimulated it above the level of control values.

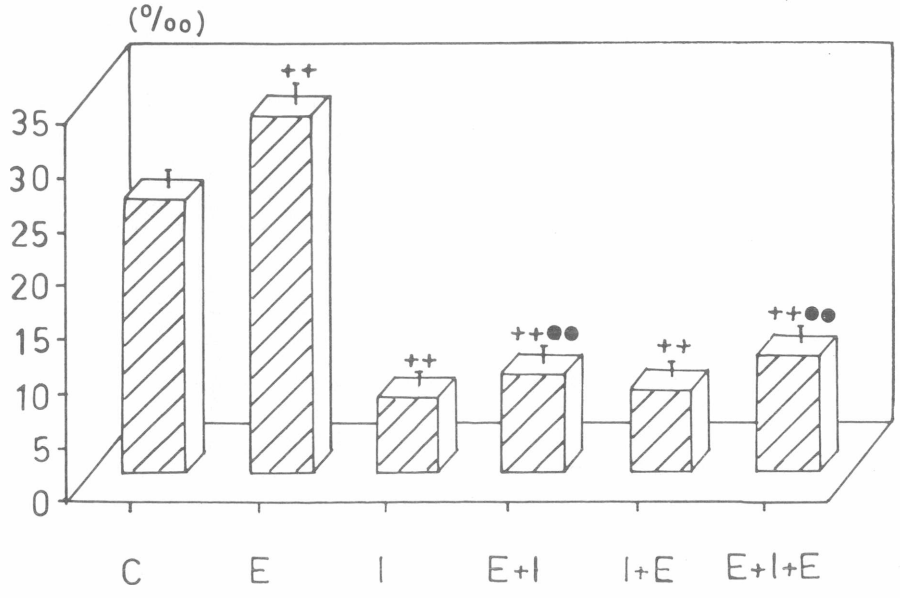

Fig. 3
Mitotic index in the regenerating rat liver. For legend see Fig. 1.

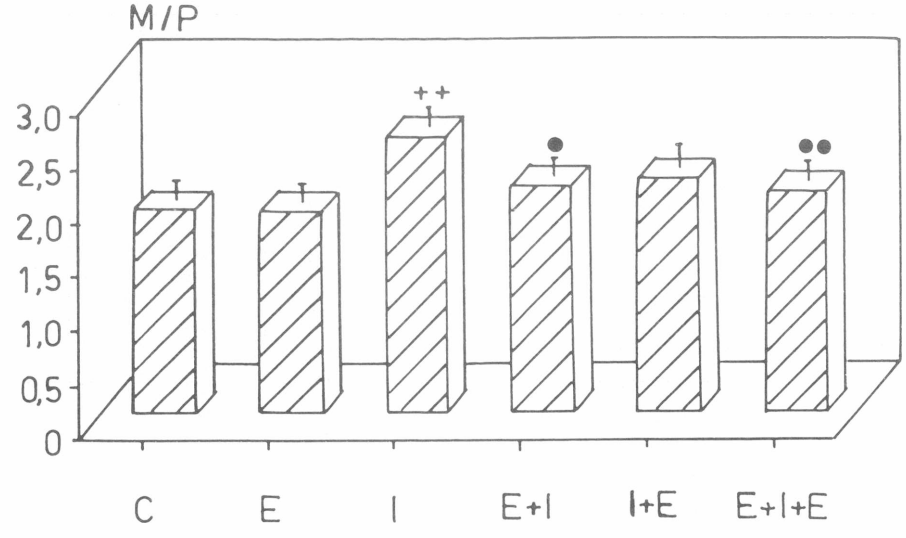

Fig. 4
Metaphase to prophase ratio in the regenerating rat liver. For legend see Fig. 1.

Mitotic index

In non-irradiated animals, MI increased from 0.015 ± 0.003 % to 2.525 ± 0.043 % in the course of liver regeneration (Fig. 3). In the regenerating liver of animals administered ESSENTIALE (E), the increase of MI was even higher compared with the untreated controls (C). Irradiation with the dose of 5.7 Gy (I) inhibited cell proliferation. This was expressed in approximately 3.5 times lower counts of mitotic figures

when compared with the controls. The inhibition of mitotic activity induced by irradiation was moderated by ESSENTIALE administration. If ESSENTIALE was administered before irradiation (E+I), or repeatedly before and after irradiation (E+I+E), the difference was statistically significant if compared with the non-protected animals (I). The values of MI in the group of E+I+E were also significantly higher if compared with groups of animals which were administered ESSENTIALE in a single dose before or after irradiation (E+I, I+E).

Ratio of the metaphase/prophase numbers

Values of the ratio M/P in control animals and animals which had received ESSENTIALE 24 h before the partial hepatectomy were on the same level (Fig. 4). The ratio M/P markedly increased after the irradiation. The administration of ESSENTIALE moderated the increase of the ratio M/P induced by radiation in all groups of protected rats.

Chromosomal aberrations

In the regenerating liver of control animals and animals administered ESSENTIALE before the operation about 3 % of cells with chromosomal aberrations were noted (Fig. 5). Irradiation with the dose of 5.7 Gy increased the number of chromosomal aberrations to 91 %. The administration of the hepatoprotective substance decreased the frequency of chromosomal aberrations in all three groups of protected rats markedly when compared with the non-protected irradiated animals.

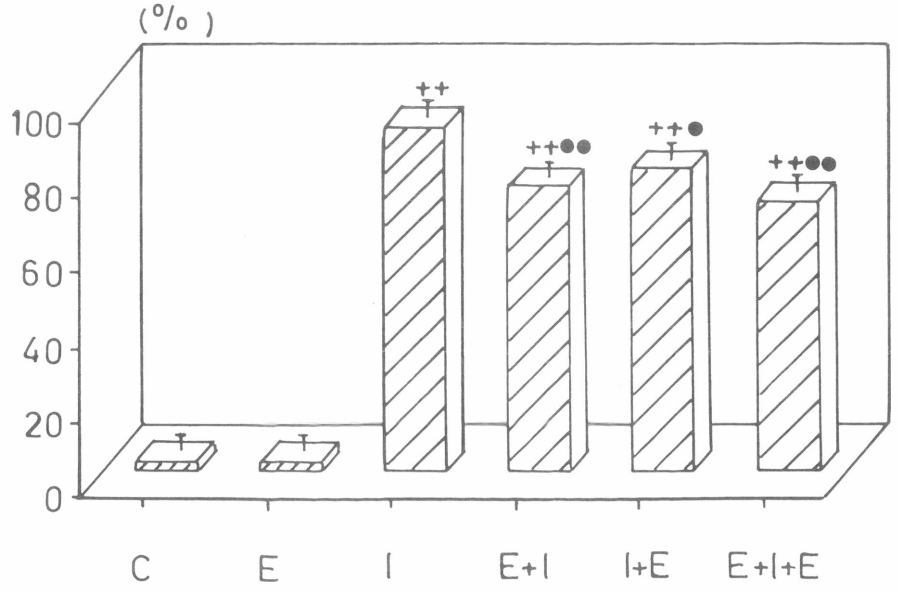

Fig. 5
Aberrant cells in the regenerating rat liver. For legend see Fig. 1.

Discussion

The onset and development of the regenerative process in the liver after injury or partial hepatectomy is regulated by several hormones and factors (Šimek and Sobotka 1983, Sobczak and Duguet 1986, Keppler 1987, Bucher 1991). It can also be influenced by the administration of hepatoprotective substances, for example Flavobion (silymarin) or Thioctacid, which interfere in metabolic processes (Fassati and Fassati 1973, Kožurková *et al.* 1992, Kropáčová and Mišúrová 1992, Haková and Mišúrová 1993).

Stimulation of liver regeneration after partial hepatectomy can also be achieved by the administration of phospholipid preparations INTRALIPID (Vitrum AB Sweden) and ESSENTIALE (Holeček *et al.* 1985, 1992). Since INTRALIPID and ESSENTIALE both have a high content of essential fatty acids, which are important components of cell membranes, the positive effect of these substances on liver regeneration can be based on the same mechanisms. These probably include direct incorporation of the essential fatty acids into the cell membranes (Samochowiec and Wojcicki 1977, Müting and Reikowski 1977, Fomichenko and Ermakov 1986) and activation of ATP synthesis for supplying the increased energy demands of the regenerating liver. After partial hepatectomy, ATP is predominantly produced by oxidation of fatty acids (Šimek *et al.* 1989). The supply of fatty acids to hepatocytes of the regenerating liver after PHE is also used for the

synthesis of cholesterol which is necessary for the formation of membrane structures (Barbason *et al.* 1983).

The group of vitamins including vitamin E is the second component of ESSENTIALE. Vitamin E as an antioxidant has an inhibitory effect on the peroxidation of lipids. In this way it also directly protects the phospholipids of cell membranes against their oxidative degradation (Loginov *et al.* 1986, Del Maestro 1991, Chow 1991). Excessive oxidative processes which develop after irradiation not only damage the cell membranes but also other important cell components including DNA which is the main molecular target of damage caused by irradiation (Chapman and Allalunis-Turner 1991). Free radicals derived from oxygen usually initiate these oxidative processes and contribute not only to the radiation damage of the organism but also to ageing and tumour development. Vitamin E has the ability to scavenge free radicals and in this way to delay the beginning of ageing changes and to protect against the development of some tumours and effects of ionizing radiation (Kolosov *et al.* 1977, Del Maestro 1991, Mišúrová *et al.* 1991).

The results of our experiment suggest that, after irradiation with a sublethal dose (5.7 Gy) of gamma radiation, the positive effect of ESSENTIALE on the liver is mediated more by the stabilization of cell membranes than by the protection of genetic material. This suggestion is based on the fact that the administration of ESSENTIALE under all three regimens moderated the cellularity changes more intensively (evaluated according to the number of non-disrupted cells) than the changes of the mitotic index or the frequency of chromosomal aberrations. In all indices, the preparation was more effective when administered before irradiation than after it. This indicates that it contributes to the mitigation of the indirect effect of irradiation caused by short living radicals. On the other hand, a certain positive influence of ESSENTIALE after single postirradiation or repeated administration especially on the cellularity of regenerating liver is considered to be an indication of the stabilizing and stimulating effect of the preparation on cell membranes.

The moderation of radiation-induced changes of mitotic activity, the duration of the mitotic cycle phases (manifested by an increase in the ratio metaphase/prophase) and frequency of chromosomal aberrations in the regenerating rat livers treated with ESSENTIALE indicates that this preparation is effective not only by stabilizing cell membrane systems and activating of metabolism but also partially by protecting the genetic apparatus of cells.

The effect of ESSENTIALE on the genetic material can act by preventing the increased activity of acid nucleases in consequence of the stabilization of lysosomal membranes (Fomichenko and Ermakov 1986) or by activating the production of prostaglandins which could act radioprotectively (Walden *et al.* 1987, Bucher 1991).

Acknowledgements

This work was partially supported by a grant from the Ministry of Education and Science of Slovakia, Project No. 1/239/93. We thank Mrs. Katarína Miklušová for preparation of the illustrations and Miss Jana Parošová for her excellent technical assistance.

References

ALBERT M.D., BUCHER N.L.R.: Latent injury and repair in rat liver induced to regenerate at intervals after X-radiation. *Cancer Res.* **20**: 1514–1523, 1960.

BARBASON H., RASSENFOSSE C., BETZ E.H.: Promotion mechanism of phenobarbital and partial hepatectomy in DENA hepatocarcinogenesis cell kinetics effect. *Br. J. Cancer* **47**: 517–525, 1983.

BUCHER N.L.R.: Liver regeneration: an overview. *J. Gastroenterol. Hepatol.* **6**: 615–624, 1991.

CHAPMAN J.D., ALLALUNIS-TURNER M.J.: Cellular and molecular targets in normal tissue radiation injury. In: *Radiation Injury to the Nervous System*. P.H. GUTIN, S.A. LEIBEL, G.E. SHELINE (eds), Raven Press, New York, 1991, pp. 3–15.

CHOW C.K.: Vitamins and related dietary antioxidants. In: *Trace Elements, Micronutrients, and Free Radicals.* I.E. DREOSTI (ed.), Humana Press, Totowa, NJ, 1991, pp. 129–147.

DEL MAESTRO R.: Free radicals as mediators of tissue injury. In: *Trace Elements, Micronutrients, and Free Radicals.* I.E. DREOSTI (ed.) Humana Press, Totowa, NJ, 1991, pp. 25–51.

FASSATI P., FASSATI M.: Silymarin and some problems of current therapy of chronic hepatopathies (in Czech). *Čas. lék. čes.* **112**: 865–869, 1973.

FOMICHENKO K.V., ERMAKOV V.I.: The effect of Essential and Vitohepat which can stabilize the membrane of liver lysosomes (in Russian). *Vopr. Med. Chim.* **32**: 21–24, 1986.

GORDIENKO A.D.: Pharmacological and biochemical effects of nonsaturated phospholipids (in Russian). *Farmacol. Toksikol.* **53**: 78–81, 1990.

HAKOVÁ H., MIŠÚROVÁ E.: The effect of silymarin and gamma radiation on nucleic acids in rat organs. *J. Pharm. Pharmacol.* **45**: 910–912, 1993.

HANTÁK J., BOČA M., MIKULECKÝ M., STANŠEK D.: Essential phospholipids in the treatment of chronic infection with the hepatitis B virus (in Slovak). *Vnitř. lék. (Prague)* **36**: 1164–1171, 1990.

HARPER J.F.: Basis program of a robust multiple comparison test for statistical analysis of all differences among group means. *Comput. Biol. Med.* **14**: 437–445, 1984.

HIGGINS G.M., ANDERSON R.M.: Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. *Arch. Pathol.* **12**: 187–202, 1931.

HOLEČEK M., ŠIMEK J., VOŠVRDOVÁ H., PLASOVÁ J., ULYBIN B., ZADÁK Z., SOBOTKA L.: Effect of glucose, INTRALIPID and Nutramine on the onset of liver regeneration during the early period after partial hepatectomy. *Physiol. Bohemoslov.* **34**: 426–433, 1985.

HOLEČEK M., MRÁZ J., KOLDOVÁ P., SKOPEC F.: Effect of polyunsaturated phosphatidylcholine on liver regeneration onset after hepatectomy in the rat. *Arzneimittelforsch.* **42**: 337–339, 1992.

KEPPLER D.: Biochemical aspects of liver regeneration. In: *Liver Cirrhosis.* J.L. BOYER, L. BIANCHI (eds), MTP Press, Lancaster, 1987, pp. 19–25.

KOLOSOV A.V., ITKINA L.D., BULATOVA V.M.: The use of ESSENTIALE in patients with ischaemic heart disease in advanced stages and in senile patients in geriatric practice. *Essential Phospholipids in the Treatment of Liver Disease*, Symposium, Moscow, 1977, pp. 70–72.

KOŽURKOVÁ M., KROPÁČOVÁ K., MIŠÚROVÁ E., REXA R.: The effect of hepatoprotective preparations thioctacid and flavobion on histones in intact and regenerating liver in irradiated rats (in Russian). *Bull. Exp. Biol. Med.* **113**: 191–193, 1992.

KROPÁČOVÁ K., MIŠÚROVÁ E., PRASLIČKA M.: Influence of cysteamine and RTG irradiation on mitotic activity and chromosomal abnormalities in regenerating liver rats. *Radiobiol. Radiother.* **21**: 55–67, 1980.

KROPÁČOVÁ K., MIŠÚROVÁ E.: Mitotic activity and chromosomal aberrations in the rat regenerating liver after X-ray exposure (in Russian). *Bull. Exp. Biol. Med.* **91**: 359–361, 1981.

KROPÁČOVÁ K., MIŠÚROVÁ E.: Modification of the latent radiation-induced damage in the rat liver by adeturon (in Slovak). *Voj. zdrav. listy* **54**: 117–122, 1985.

KROPÁČOVÁ K., MIŠÚROVÁ E.: Radiation-induced latent injury in liver and its influencing by radioprotectants. *Curr. Trends Cosmic Biol. Med.* Vol. I, 1990, pp. 143–148.

KROPÁČOVÁ K., MIŠÚROVÁ E.: Modification of radiation-induced latent injury of rat liver by thioctacid and flavobion (in Russian). *Bull. Exp. Biol. Med.* **113**: 547–548, 1992.

LOGINOV A. C., DZHALAKOV K. D., BLOK J. E., CSENGIKOV E. A., AUSHEVA L. K.: The treatment of chronic diseases of the liver by catergen. (in Russian). *Terapev. Arch.* **58**: 73–76, 1986.

MIŠÚROVÁ E., KŘIVÁŇOVÁ M., KROPÁČOVÁ K., FABIANOVÁ B.: Regeneration of liver after partial hepatectomy in X-ray irradiated rats. *Radiobiol. Radiother.* **28**: 59–68, 1987.

MIŠÚROVÁ E., KROPÁČOVÁ K., BREZÁNI P.: Possibilities of modifying the effects of protracted gamma irradiation by vitamins. *Curr. Trends Cosmic Biol. Med.* Vol. II, 1991, pp. 159–165.

MÜTING D., REIKOWSKI J.: The metabolic action of essential phospholipids following intravenous and oral administration in cases of histologically proven fatty liver and cirrhosis of the liver. *Essential Phospholipids in the Treatment of Liver Disease,* Symposium, Moscow, 1977, pp. 35–42.

SAMOCHOWIEC L., WOJCICKI J.: Pharmacological properties of the essential phospholipids (EPL) with reference to liver disease. *Essential Phospholipid in the Treatment of Liver Disease,* Symposium, Moscow, 1977, pp. 9–16.

ŠIMEK J., SOBOTKA L.: Current view on the mechanisms of the liver regeneration (in Czech). *Čs. fysiol.* **32**: 13–32,1983.

ŠIMEK J., ČERVINKOVÁ Z., HOLEČEK M.: Effect of functional load in the mechanisms of liver regeneration (in Czech). *Čs. fysiol.* **38**: 219–240, 1989.

SOBCZAK J., DUGUET M.: Molecular biology of liver regeneration. *Biochimie* **68**: 957–967, 1986.

VENGEROVSKIJ A.I., CHUCHALIN V. S., PULŠ O. V., SARATIKOV A.S.: The effect of hepatoprotectants on metabolism of lipids by CCl_4-hepatitis (in Russian). *Bull. Exp. Biol. Med.* **103**: 430–432, 1987.

WALDEN T.L., PATCHEN M., SNYDER S.L.: 16,16-dimethyl prostaglandin E_2 increases survival in mice following irradiation. *Radiat. Res.* **109**: 440–448, 1987.

Reprint Requests

Dr. K. Kropáčová, Department of Cellular and Molecular Biology, Šafárik University, Moyzesova 11, 041 67 Košice, Slovak Republic.