Histol Histopathol (2003) 18: 49-54
DOI: 10.14670/HH-18.49

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Styrene hepatotoxicity in rats treated by inhalation or intraperitoneally: a structural investigation

P. De Piceis Polver[1], C. Fenoglio[1], R. Nano[1], T. Coccini[2], V. Bertone[1], R. Vaccarone[1] and G. Gerzeli[1]

[1]Department of Animal Biology, University of Pavia, Italy and

[2]Toxicology Research Center, University of Pavia, and IRCCS S. Maugeri Foundation, Pavia, Italy

Summary. The purpose of this study was to investigate the toxicity of styrene in the liver of adult rats treated either by inhalation of styrene vapour (300 ppm, 6 h/d, 5 d/wk, for 2 wk) or intraperitoneally with different styrene doses (4, 40, 400 mg/Kg) for 3 consecutive days. Using a light microscope, some alterations of liver parenchyma and sinusoid dilation were noticed, more marked in the group treated with the intraperitoneal administration of the chemical. Using an electron microscope, some additional changes were observed (once again, more marked in the latter group of rats): a) an increase in the content of lipids inside hepatocytes, and b) the rise of intracytoplasmic, intercellular and perisinusoidal collagen fibres. Therefore, cell damage and functional disturbance of sinusoids due to perisinusoidal fibrosis are apparent in the liver of both groups of rats exposed to styrene treatment, but these changes are definitely more significant in those subjected to intraperitoneal administration.

Key words: Styrene, Rat, Liver, Structural alterations

Introduction

The chemical styrene (ethenylbenzene) is widely used in the manufacture of resins, reinforced plastics, polymers and in the boat-building industry. It is also present in the air due to automobile exhaust, cigarette smoke and industrial emission (Miller et al., 1994).

Acute styrene-toxicity in humans and laboratory animals produces irritation of the skin and respiratory tract, and has a depressive effect on the central nervous system (Bond, 1989; Pahwa and Kaira, 1993; Gram, 1995). Styrene is metabolized in the liver, where it is transformed to styrene oxide - a toxic, mutagenic and potentially carcinogenic form. Studies have therefore been carried out also on styrene hepatotoxicity mainly from a metabolic point of view (Katoh et al., 1989; Sumner and Fennel, 1994; Gadberry et al., 1996), taking into account the different susceptibilities of rat and mouse (Cruzan et al., 1997, 1998, 2001; Sumner et al., 1997) and that of different mouse strains (Morgan et al., 1993b, 1995; Carlson, 1997).

In a previous research, some of us studied the modifications in the rat trachea and lung following either styrene subchronic inhalation or intraperitoneal administration of the chemical (Coccini et al., 1997). On that occasion, we observed some alterations, mostly regarding ciliated and secretory cells of the trachea and type II pneumocytes of the lung, while the alveolar wall showed a thickening due to an increase in collagen fibrils. Damage to the respiratory tract was similar in rats treated either parenterally or by inhalation, but the effects after injections tended to be more severe.

Using the same experimental conditions of the lung, the purpose of the present study was to observe the histomorphological changes of the liver after styrene-treatment of rats either by inhalation (the normal way of exposure for man) or intraperitoneal injections with different doses (which lead styrene to the liver in a more direct manner).

Materials and methods

Styrene (99% pure) was purchased from Aldrich Chemical, Milan, Italy. Adult male Sprague-Dawley rats were divided randomly into groups and housed two per cage under constant temperature and humidity conditions and adequate photocycle for two weeks before starting the investigation. All experiments were performed in compliance with the European Community Guide for the Care and Use of Laboratory Animals.

Treatments

Inhalation-exposure

Rats (250 g) were exposed to 300 ppm (7.9 μmol/L) styrene, 6 h/d (from 8 a.m. to 2 p.m.), 5 d/wk, for 2 wk using a dynamic exposure chamber of 1 m^3. Saturated styrene vapour was diluted with room air and the styrene

Offprint requests to: Prof. Paola De Piceis Polver, Università di Pavia, Dipartimento di Biologia Animale, Piazza Botta 10, 27100 Pavia, Italy. e-mail address: depiceis@unipv.it

concentration in the chamber was continuously monitored with an infrared spectrometer (Miran 1 A, Wilks Scientific Corp., wavelength 11 μm, slit 0.5 μm, path length 12.75 m). Control rats breathed conditioned air in an identical exposure chamber. Animals were individually housed and deprived of food during exposure, while drinking water was available *ad libitum*. In the inhalation chamber, temperature was 22±1 °C, humidity 55±5%, and styrene concentration 326±47 ppm. Six animals in both the styrene and control groups were sacrificed by decapitation under CO_2-narcosis (immediately after cessation of the last exposure to styrene or air).

Intraperitoneal administration

Rats (250 g) were randomly divided into four groups of six animals each. Treatments were performed at 9 a.m. for 3 consecutive days. Styrene was dissolved in corn oil and injected intraperitoneally (4, 40, 400 mg/Kg body weight) using a volume of 2 ml/Kg body weight. Controls received daily injections of 2 ml/Kg corn oil. Rats fasted overnight and were sacrificed under CO_2-anaesthesia 24 h after the last administration.

Morphological studies

The right lobe of the liver was excised, divided into 1x1 mm cubes and processed for morphological analysis at electron microscopy. The pieces were fixed by immersion in cacodylate-buffered 2.5% glutaraldehyde at 4 °C for 3 h, washed for 1 h in 0.1 M cacodylate buffer (pH 7.4) and then postfixed for 1 h in buffered 1% osmium tetroxide, dehydrated in graded ethanol and embedded in Epon 812. Semithin sections (1 μm thick) were stained with 1% toluidine blue and haematoxylin-eosin. Thin sections (500 Å thick) were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 900 electron microscope at 50 kV.

Results

The amount of styrene inhaled by the rats during the daily exposure of 300 ppm was estimated to be equivalent to 60-120 mg styrene/rat (230-460 mg/Kg body weight), with 70-90% taken up in the body (Vainio et al., 1979). The animals receiving styrene by inhalation (300 ppm) tended to be slightly somnolent with a crouched posture and closed eyes during the treatment, but these effects rapidly disappeared in the postexposure period. The rats treated intraperitoneally with styrene (4, 40, 400 mg/Kg body weight) showed dose-dependent changes such as sedation and reduced motor activity lasting a few hours in the postadministration period. There were no differences in body weight gain between styrene- treated and control rats.

At light microscopy, following the animals' exposure to styrene inhalation, alterations of liver structure and reactivity were not noticed, except for an increase in number and volume of intracellular lipid droplets (data not shown). Following intraperitoneal treatment of rats with different doses of styrene, a dilation of sinusoids and an increase in lipid droplets were observed already with the lowest dose (4 mg/Kg body weight) (Fig. 1A,B). With the highest dose (400 mg/Kg body weight), we observed disorganized cellular plates, hepatocytes showing a pale cytoplasm, clusters of connective tissue cells near centrilobular veins and further sinusoid dilation (Fig. 1C,D).

At electron microscopy, in rats treated with styrene either by inhalation or intraperitoneally, discontinuous alterations of liver parenchyma and sinusoids were noted, while extensive areas of necrosis and signs of wide fibrosis were not visible. Hepatocytes contained more numerous lipid droplets, some dilated smooth endoplasmic reticulum (SER) tubules and a few small, dark mitochondria following styrene inhalation (Fig. 2B). Besides normal mitochondria, enlarged mitochondria with irregular cristae were observable after intraperitoneal administration (Fig. 2D). The occasional presence of one or more intracellular erythrocytes (Fig. 2C) and numerous intracytoplasmic bundles of collagen fibres (Fig. 3A,B) were noticed following both treatments. An increase in intercellular and perisinusoidal collagen fibres was evident. Abundant collagen bundles were occasionally present not only in the space of Disse, but also as a subendothelial layer forming a thickening of the sinusoidal wall (Fig. 3A). Sinusoids often contained, besides hematic cells, spheroidal amorphous material frequently enclosing either rough endoplasmic reticulum (RER) cisternae or mitochondria (data not shown).

Besides patterns of altered parenchyma, normal hepatocytes were still visible after both types of treatment.

Discussion

The liver of rats exposed to styrene by inhalation (300 ppm/6h/d/2wk) did not show serious injuries at light microscope examination, while Morgan and co-workers (1993a) have described disorganization of hepatic cords or centrilobular cytoplasmic degeneration and basophilia in mice, which had been exposed to 500 ppm of styrene for the same amount of time. Analysis at the electron microscope level revealed some additional changes: the appearance of small, dark mitochondria, which may be a sign of cellular damage, and an increase in collagen perisinusoidal fibres, which could cause a capillarization of sinusoids; their wall probably becomes a barrier obstructing the material exchange between blood and hepatocytes, contributing to a disfunction of the hepatic cells (Witte et al., 1992; Bianchi and Gudat, 1994; Hall, 1994).

The liver of rats treated intraperitoneally with different doses of styrene (4, 40, 400 mg/Kg body weight) for three days showed structural alterations from the lowest through to the highest doses of styrene. Signs

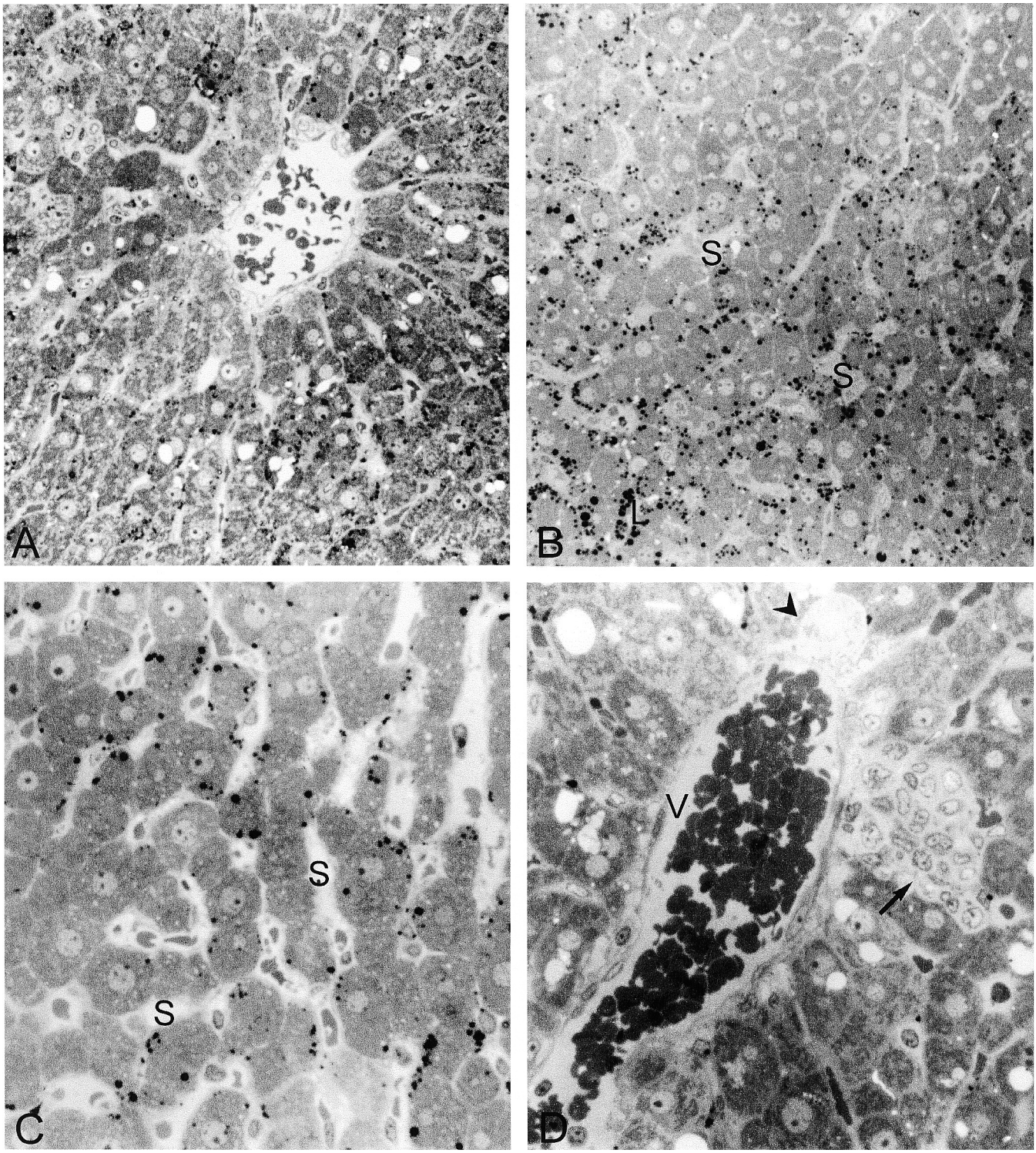

Fig. 1. Rat liver stained with toluidine blue. **A.** Control rat: parenchyma around a centrilobular vein. x 300. **B.** Rat treated intraperitoneally with 4 mg/Kg of styrene: dilated sinusoids (S) and numerous lipid droplets (L). x 300. **C and D.** Rat treated intraperitoneally with 400 mg/Kg of styrene: dilated sinusoids (S), hepatocytes with rarified cytoplasm (arrowhead), connective tissue cells (arrow) gathered near a centrilobular vein (V). x 500

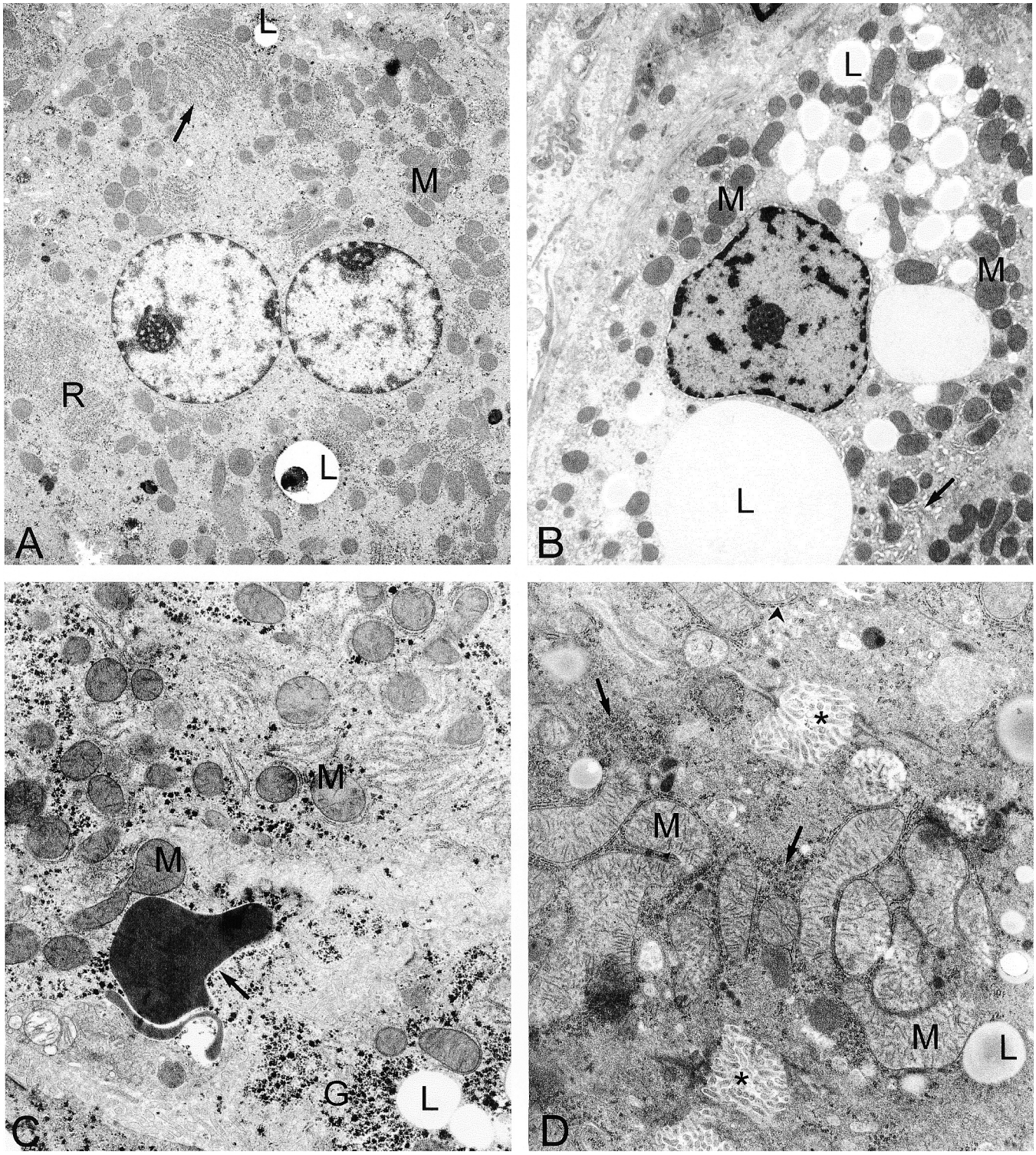

Fig. 2. Electron micrographs of rat liver. **A.** Control rat: binuclear hepatocyte with numerous mitochondria (M), parallel RER cisternae (arrow), several free ribosomes (R), few lipid droplets (L). x 4,200. **B.** Rat treated with styrene by inhalation: numerous lipid droplets of different size (L), dilated SER tubules (arrow), dark mitochondria (M). x 4,200. **C.** Rat treated with styrene by inhalation: erythrocyte penetrated into one hepatocyte (arrow). G: glycogen particles; M: mitochondria; arrowhead: RER cisternae; L: lipid droplet; asterisk: microvilli of two hepatocyte plasmalemma. x 8,500. **D.** Rat treated intraperitoneally with styrene (4 mg/Kg): dilated mitochondria with irregular cristae (M) and numerous bile canaliculi (asterisk). L: lipid droplet; arrow: packed polyribosomes; arrowhead: RER cisternae. x 10,000

of cellular degeneration, congestion and inflammatory reaction were already visible in centrilobular areas already at light microscopy level. This coincides with the fact that xenobiotic metabolism occurs predominantly in the perivenous zone (Jungermann and Kietzmann, 1996). Furthermore, centrilobular hepatocytes contain half the quantity of glutathione in comparison with periportal cells (Kaplowitz and Ookhtens, 1985): as styrene oxide and other styrene metabolites can be detoxified by conjugation with GSH (Ryan and Bend, 1977), perivenous hepatocytes are consequently more sensitive to these electrophilic metabolites. Moreover, treatment with styrene (400 mg/Kg or 300 ppm) causes depletion of hepatic as well pulmonary GSH content, while even greater depletion of GSH stores occurs after injection of styrene oxide (200 mg/Kg) (Coccini et al., 1997). At electron microscopy, symptoms of cellular alteration - in the form of some enlarged mitochondria with irregular cristae - were identifiable. An increase in the number of collagen fibre bundles, observed both in the space of Disse and inside the hepatocytes, indicate an increase in collagen production or, at least, an unbalance between collagen synthesis and deposition, and collagen degradation (Rojkind, 1994). Hepatocytes, Ito cells, fibroblasts and endothelial cells in the liver may contribute to collagen production (Friedman, 1990), but the main cell involved in this process is the Ito cell (Greenwel et al., 1994). The increase in collagen fibres and amorphous material observed in the space of Disse may give rise to a reduction in sinusoid functionality. Furthermore, sinusoids showed a thickening of the wall - in the form of a subendothelial membrane - and fenestrae reduction; this structural alteration may reduce endothelial permeability. Sinusoid dilation, more evident in the liver of rats treated with 400 mg/Kg of styrene, is correlated with the phenomenon of hepatic congestion observed also by Morgan and co-workers (1993a) in mice treated by styrene inhalation with increasing doses of the chemical reaching a maximum of 500 ppm.

The occasional presence of intracellular erythrocytes observed is difficult to interpret: it recalls the internalization of lymphocytes by emperipolesis found in some epithelial cells of different animals (Trowell, 1958) and in the human liver following alcoholic chronic hepatitis (Bianchi and Gudat, 1994).

The increase in number of lipid droplets inside hepatocytes after both styrene treatments is comparable to fatty change developed in liver cells following either alcohol consumption (McGee, 1992) or drug taking (Hall, 1994).

Therefore, even if we did not observe signs of

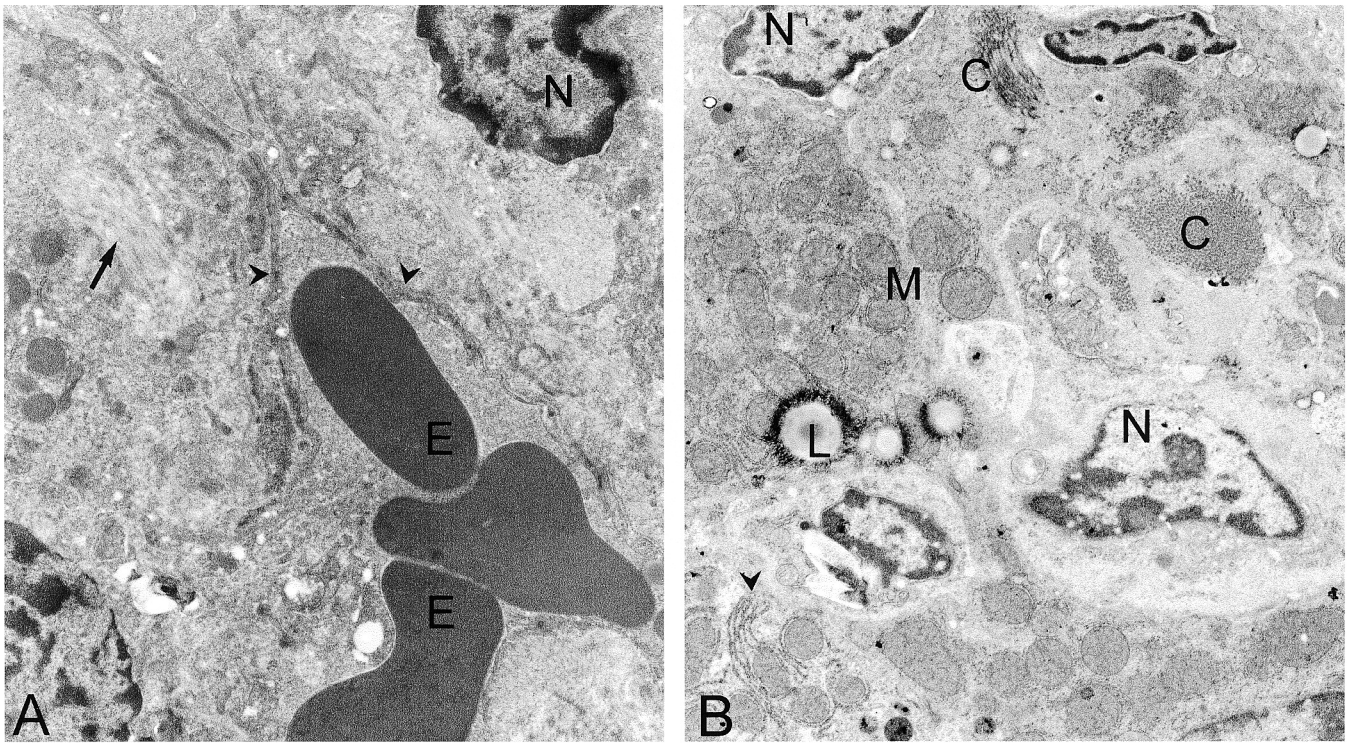

Fig. 3. Electron micrographs of rat liver. **A.** Rat treated intraperitoneally with styrene (40 mg/Kg): thickening of the sinusoid endothelial wall (arrowhead) and intracytoplasmic bundles of collagen fibres (arrow). N: nonparenchymal cell nuclei; E: erythrocytes. x 7,500**. B.** Rat treated intraperitoneally with styrene (400 mg/Kg): several bundles of intracytoplasmic collagen fibres **(C)**, cut both longitudinally and transversally; M: mitochondria; L: lipid droplets; arrowhead: RER cisternae; N: nonparenchymal cell nuclei. x 6,000

extensive necrosis or perivenular fibrosis, we did find that styrene, injected intraperitoneally at different doses, causes structural alterations at different levels in the liver of rats, already at the lowest dose. This damage is greater than that induced by exposure to styrene by inhalation, probably because the intraperitoneal route leads styrene directly to the liver.

Acknowledgements. This work was supported by a grant from the Ministry of the University and Scientific Research, Italy.

References

Bianchi L. and Gudat F. (1994). Chronic hepatitis. In: Pathology of the liver. Third ed. Mac Sween R.N.M., Anthony P.P., Scheuer P.J., Burt A.D. and Portmann B.C. (eds). Churchill Livingstone. Edinburgh, London, Madrid, Melbourne, New York and Tokyo. pp 349-396.

Bond J.A. (1989). Review of the toxicology of styrene. Crit. Rev. Toxicol. 19, 227-249.

Carlson G.P. (1997). Comparison of mouse strains for susceptibility to styrene-induced hepatotoxicity and pneumotoxicity. J. Toxicol. Environ. Health 51, 177-187.

Coccini T., Fenoglio C., Nano R., De Piceis Polver P., Moscato G. and Manzo L. (1997). Styrene-induced alterations in the respiratory tract of rats treated by inhalation or intraperitoneally. J. Toxicol. Environ. Health 52, 63-77.

Cruzan G., Cushman J.R., Andrews L.S., Granville G.C., Miller R.R., Hardy C.J., Coombs D.W. and Mullins P.A. (1997). Subchronic inhalation studies of styrene in CD rats and CD-1 mice. Fundam. Appl. Toxicol. 35(2), 152-165.

Cruzan G., Cushman J.R., Andrews L.S., Granville G.C., Johnson K.A., Hardy Coombs D.W., Mullins P.A. and Brown W.R. (1998). Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks. Toxicol. Sci. 46, 266-281.

Cruzan G., Cushman J.R., Andrews L.S., Granville G.C., Johnson K.A., Bevan Hardy C.J., Coombs D.W., Mullins P.A. and Brown W.R. (2001). Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. J. Appl. Toxicol. 21, 185-198.

Friedman S.L. (1990). Cellular sources of collagen and regulation of collagen production in liver. Semin. Liver Dis. 10, 20-29.

Gadberry M.G., DeNicola D.B. and Carlson G.P. (1966). Pneumotoxicity and hepatotoxicity of styrene and styrene oxide. J. Toxicol. Environ. Health 48, 273-294.

Gram T.E. (1995). Metabolic activation and toxicity of some chemical agents to lung tissue and cells. Biochem. Pharmacol. 49, 1721-1727.

Greenwel P., Geerts A., Ogata I., Solis-Herruzo J.A. and Rojkind M. (1994). Liver fibrosis. In: The liver biology and pathobiology. Third ed. Arias I.M., Boyer J.L. , Fausto N., Jacoby W.B., Schachter D.A. and Schafritz D.A. (eds.). Raven Press. New York. pp 1367-1381.

Hall P. de la M. (1994). Alcoholic liver disease. In: Pathology of the liver. Third ed. Mac Sween R.N.M., Antony P.P., Scheuer P.J., Burt A.D. and Portmann B.C. (eds.). Churchill Livingstone. Edinburgh, London, Madrid, Melbourne, New York and Tokyo. pp 317-348.

Jungerman K. and Kietzmann T. (1996). Zonation of parenchymal and nonparenchymal metabolism in liver. Annu. Rev. Nutr. 16, 179-203.

Kaplowitz N. and Ookhtens M. (1985). The regulation of hepatic glutathione. Annu. Rev. Pharmacol. Toxicol. 25, 715-744.

Katoh T., Higoshi K. and Inoue H. (1989). Sub-chronic effects of styrene and styrene oxide on lipid peroxidation and the metabolism of gluthatione in the rat liver and brain. J. Toxicol. Sci. 14, 1-9.

McGee J.O'D. (1992). Alcoholic liver disease. In: Oxford textbook of pathology. Vol. 2a. McGee J.O'D., Isaacson P.J., Wright N.A. (eds). Oxford University Press. Oxford, New York, Tokyo. pp 1324-1330.

Miller R.R., Newhook R. and Poole A. (1994). Styrene production, use, and human exposure. Crit. Rev. Toxicol. 24, 1-10.

Morgan D.L., Mahler J.F., O'Connor R.W., Price H.C. and Adkins B. (1993a). Styrene inhalation toxicity studies in mice. I. Hepatotoxicity in B6C3F1 mice. Fundam. Appl. Toxicol. 20, 325-335.

Morgan D.L., Mahler J.F., Dill J.A., Price H.C., O'Connor R.W. and Adkins B. (1993b). Styrene inhalation toxicity studies in mice. III. Strain differences in susceptibility. Fundam. Appl. Toxicol. 21, 326-333.

Morgan D.L., Mahler J.F., Moorman M.P., Wilson R.E., Price H.C., Richards J.H. and O'Connor R.W. (1995). Comparison of styrene hepatotoxicity in B6C3F1 and Swiss mice. Fundam. Appl. Toxicol. 27, 217-222.

Pahwa R. and Kaira J. (1993). A critical review of the neurotoxicity of styrene in humans. Vet. Hum. Toxicol. 35, 516-552.

Rojkind M. (1994). Fibrogenesis. In: The liver biology and pathobiology. Third ed. Arias I.M., Boyer J.L., Fausto N., Jacoby W.B., Schachter D.A. and Schafritz D.A. (eds.). Raven Press. New York. pp 801-809.

Ryan A.J. and Bend J.R. (1977). Metabolism of styrene oxide in the isolated perfused rat liver. Identification and quantification of major metabolites. Drug Metab. Dispos. 5, 363-367.

Sumner S.C. and Fennel T.R. (1994). Review of the metabolic fate of styrene. CRC Crit. Rev. Toxicol. 24, 11-33.

Sumner S.C., Cattley R.C., Asgharian B., Janszen D.B. and Fennel T.R. (1997). Evaluation of the metabolism and hepatotoxicity of styrene in F344 rats, B6C3F1 mice, and CD-1 mice following single and repeated inhalation exposures. Chem. Biol. Interact. 106, 47-65.

Trowell O.A. (1958). The lymphocyte. Int. Rev. Cytol. 7, 235-293.

Vainio H., Jarvisalo J. and Taskinen E. (1979). Adaptive changes caused by intermittent styrene inhalation on xenobiotic biotrasformation. Toxicol. Appl. Pharmacol. 49, 7-14.

Witte M.H., Borgs P., Way D.L., Ramirez G. Jr. Bernas M.J. and Witte C.L. (1992). Alcohol, hepatic sinusoidal microcirculation, and chronic liver disease. Alcohol 9, 473-480.

Accepted July 29, 2002