

Early studies of styrene biotransformation in animals reported the formation of benzoic acid from styrene excreted as hippuric acid in the urine (413) but also the excretion of glucuronides after administration of styrene or styrene oxide (398). Danishefsky and Willhite (89) noted the rapid and extensive excretion of radioactivity in rat urine after subcutaneous injection of radiolabeled styrene; 71% of the dose being recovered in the urine within 24 hours. The feces contained 2.62% of the dose. El Masri *et al.* (117) identified the metabolites of styrene in the urine of orally dosed rabbits as hippuric acid, mandelic acid and phenylglycol glucuronide. Styrene oxide gave rise to hippuric acid and mandelic acid but no phenylglycol. Direct administration of phenylglycol gave hippuric acid, mandelic acid and phenylglycol glucuronide. The formation of a mercapturic acid after administration of styrene was also noted. It was proposed that styrene oxide and phenylglycol were two possible intermediates in styrene biotransformation. James and White (197) compared the metabolites of styrene and styrene oxide in the urine of rabbits and rats administered the two compounds. Styrene, 1.4 mmoles/kg fed to rabbits, was excreted as a glucuronide (6% of the dose) suggested to be a conjugate of phenylglycol, hippuric acid (40%), mandelic acid (32%) and hydroxyphenethylmercapturic acid (4.9%). Styrene oxide, 1.7 mmoles/kg orally, was excreted mainly as mandelic acid (30%) but also as hippuric acid (23%), glucuronide (20%) and hydroxyphenethylmercapturic acid (1.2%). Phenylglycol, 1.4 mmoles/kg orally, gave glucuronide (24%), mandelic acid (24%) and hippuric acid (7%). Rats receiving styrene and styrene oxide excreted traces of phenethyl-

mercapturic acid in the urine. It was suggested that styrene oxide was an intermediate in styrene biotransformation. Ohtsuji and Ikeda (304), investigating the biotransformation of styrene and several of its known metabolites in the rat, established the formation of styrene oxide as an initial step in styrene biotransformation and the hydrolysis of styrene oxide to phenylglycol, which is then converted to mandelic acid further transformed to phenylglyoxylic acid or benzoic acid. The oxidation of styrene to phenylglycol was shown to take place in rabbit and rat liver microsomes (248). The actual formation of styrene oxide from styrene has also been established in rabbit liver microsomes (249) and isolated perfused rat livers have been shown to metabolize styrene oxide rapidly to phenylglycol, mandelic acid and S-(1-phenyl-2-hydroxyethyl)glutathione (356). Bakke and Scheline (29) demonstrated three additional minor metabolites of styrene, 4-vinylphenol, 1-phenylethanol and 2-phenylethanol, in the 48 hour urine of rats fed 100 mg/kg styrene. The biotransformation of styrene in humans differs somewhat from the biotransformation in animals. Stewart *et al.* (423) found no significant increases of hippuric acid in the urine of exposed humans. Bardodej and Bardodejova (31) found mandelic acid and phenylglyoxylic acid to be the main urinary metabolites in humans exposed to 22 ppm styrene for 8 hours. The presence of hippuric acid in human urine has been established only after exposure to high (50-200 ppm) atmospheric concentrations of styrene (190).

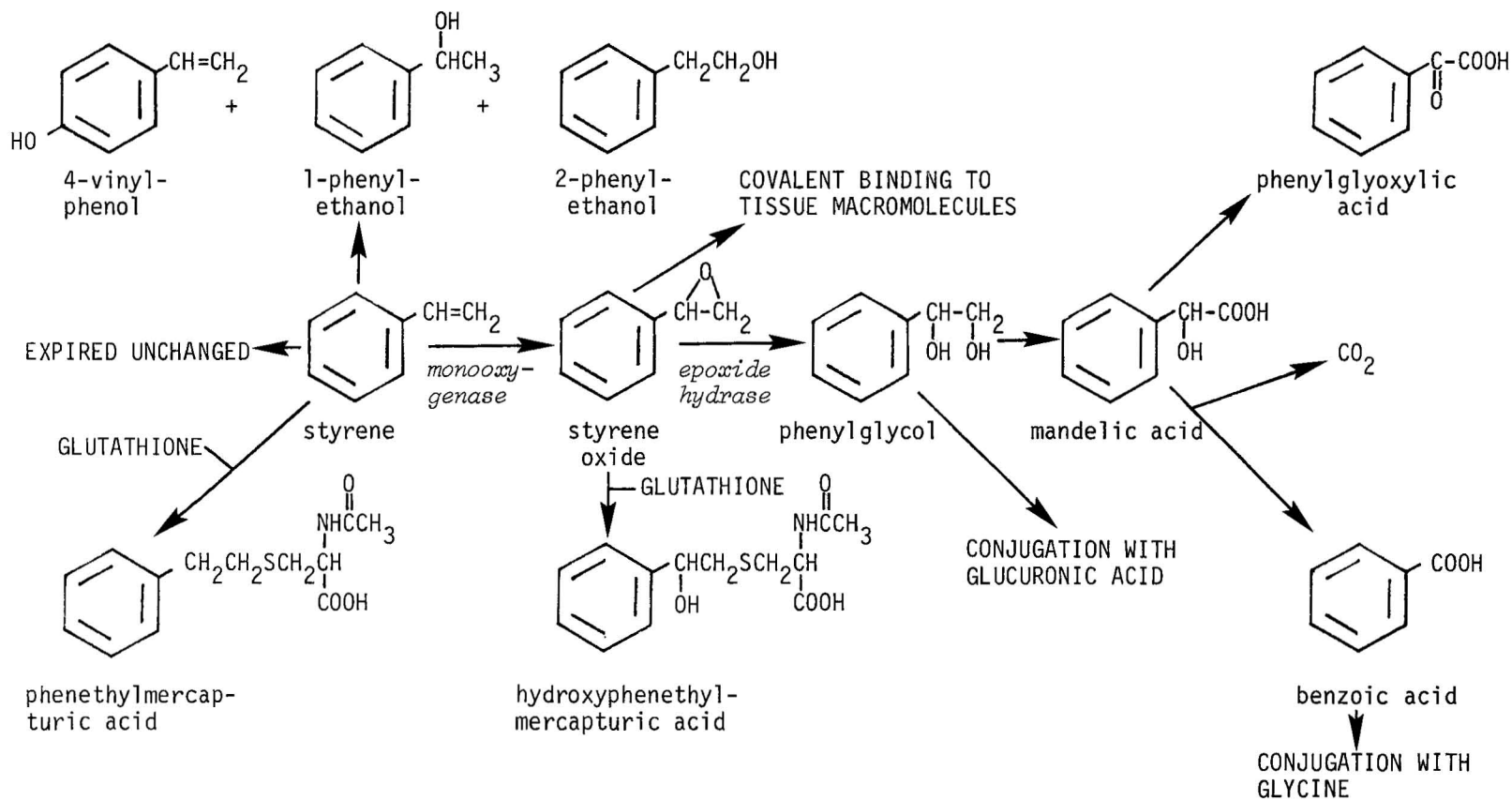


Fig.III:1. The biotransformation of styrene.

MATERIALS AND METHODS

Labeled compounds

Styrene-8- ^{14}C (specific activity 0.34 mCi/mmol) and styrene-ring- ^{14}C (specific activity 0.47 mCi/mmol), both stabilized with 1% p-methoxyphenol, were obtained as gas-liquid radiochromatographically 99% pure from New England Nuclear, Dreieichenhain, FRG.

Animals

Male NMRI-mice, weighing about 25 g, were used. The animals were kept at room temperature and given a commercial pellet diet (AB Astra-Ewos, Södertälje, Sweden) and tap water *ad libitum*.

Autoradiography

Styrene-8- ^{14}C and styrene-ring- ^{14}C were diluted with unlabeled, analytical grade styrene (stabilized with 10 ppm 4-butylpyrocatechol) to final solutions of 3.15 $\mu\text{Ci}/\mu\text{l}$ and 3.12 $\mu\text{Ci}/\mu\text{l}$, respectively. Mice were administered 10 μl of the solutions by inhalation for 10 minutes as described in Chapter I. The animals were killed by CO_2 anoxia at 0 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h and 48 h after the inhalation and subject to whole-body autoradiography in three steps as described in Chapter I.

Elimination study

Styrene-8- ^{14}C was diluted with unlabeled, analytical grade styrene to a final solution of 0.169 $\mu\text{Ci}/\mu\text{l}$. Mice were administered 10 μl of the solution by inhalation for 10 minutes. The elimination of radioactivity by various routes was followed for 8 hours in the metabolic cage whereafter the remaining radioactivity levels in several organs were determined as described in Chapter I.

RESULTS AND DISCUSSION

Autoradiography

Autoradiograms obtained after inhalation of styrene-8- ^{14}C and styrene-ring- ^{14}C are similar at short survival times. Figs. III:2 and III:3 show autoradiograms obtained immediately after inhalation of styrene labeled in the side chain and in the aromatic ring, respectively. Low-temperature autoradiography shows a high uptake of radioactivity in the adipose and nervous tissues but also in the blood, liver, kidney and lung. A comparison with autoradiograms of dried, evaporated sections reveals that styrene metabolites are localized to the bronchi, liver and lung already immediately after the inhalation whereas the adipose and nervous tissues show no radioactivity. Thus, the conclusion may be drawn that the radioactivity in these tissues is due to non-metabolized styrene. As shown by low-temperature autoradiography, styrene is cleared from nervous tissues in 1 hour but is retained in adipose tissues for 24

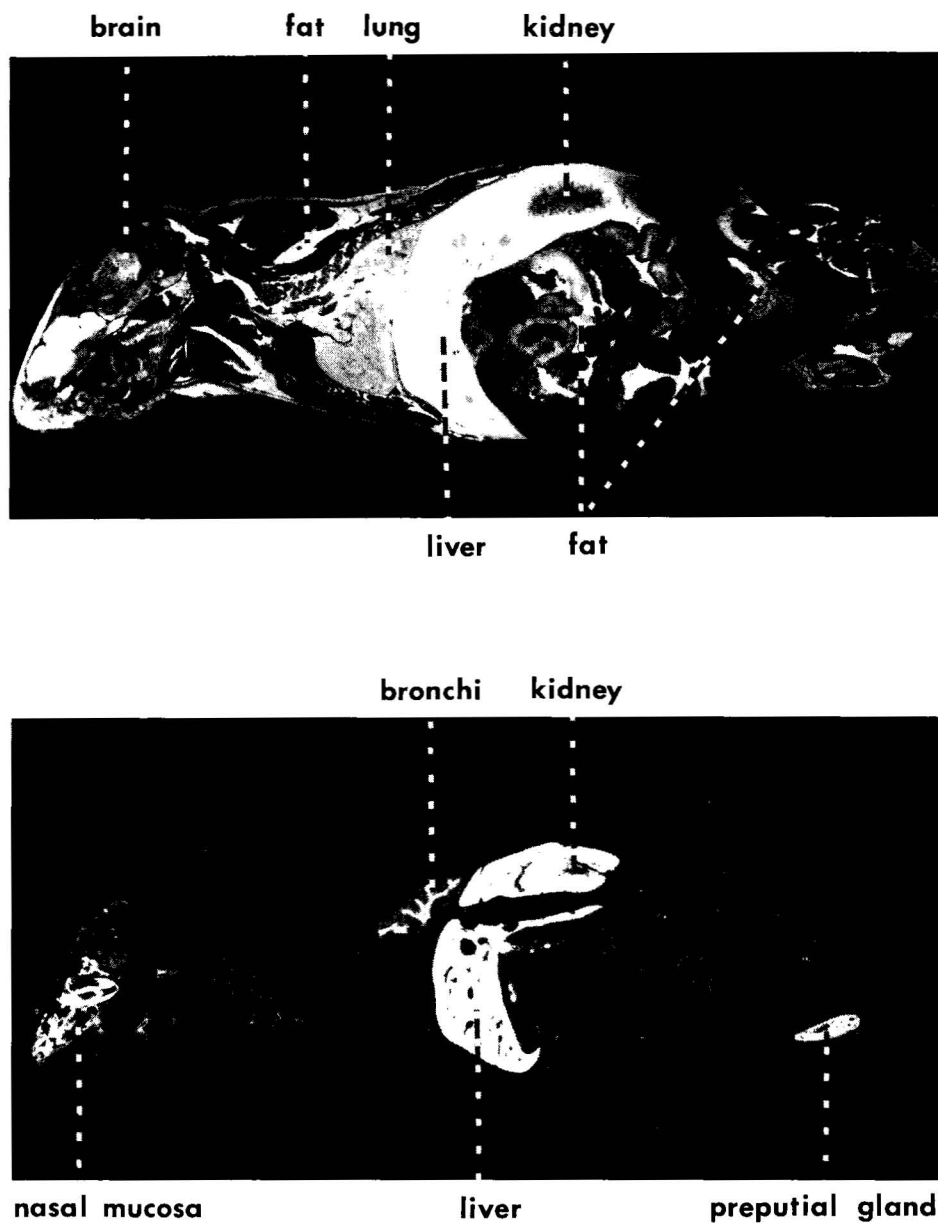


Fig. III:2. Whole-body autoradiograms of mice immediately after inhalation of 10 μ l styrene-8- ^{14} C for 10 min. Hemisection exposed at -80°C (upper) and dried, evaporated section exposed at -20°C (lower). Note the high uptake of non-metabolized styrene in adipose and nervous tissues. Metabolites are visible in the bronchi, liver and kidney.

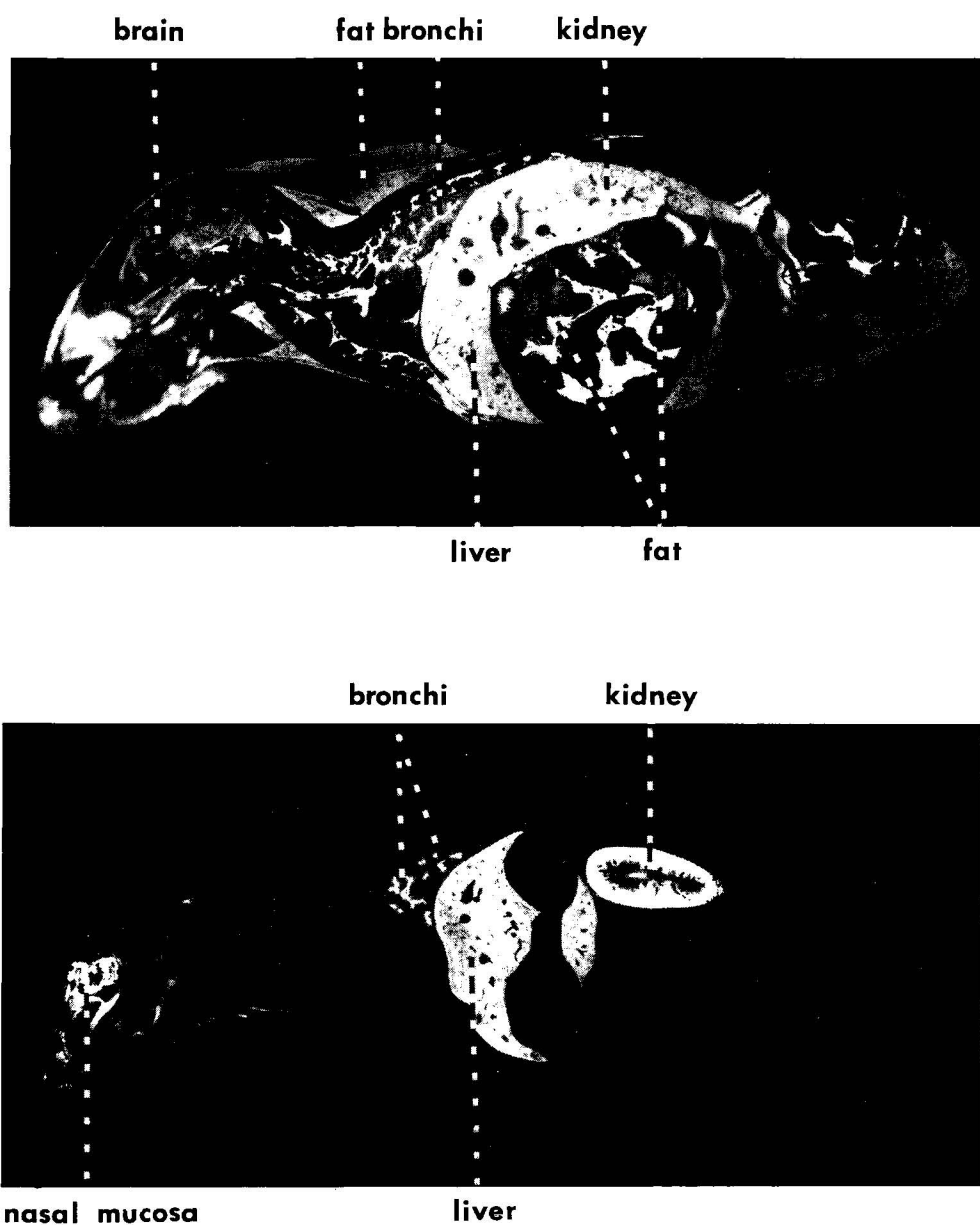


Fig. III:3. Whole-body autoradiograms of mice immediately after inhalation of 10 μl styrene-ring- ^{14}C for 10 min. Hemisection exposed at -80°C (upper) and dried, evaporated section exposed at -20°C (lower). There is a high level of non-metabolized styrene in adipose and nervous tissues. Metabolites are seen in the bronchi, liver and kidney. The autoradiograms are similar to those shown in Fig. III:2.

hours with traces of radioactivity still visible in fat 48 hours after inhalation. Autoradiograms obtained from dried, evaporated sections 30 minutes after inhalation show an increased amount of radioactivity in the liver and kidney which remains high up to 2 hours after inhalation. At 4 hours the liver radioactivity has dropped considerably but then remains at a fairly constant level up to 48 hours. The kidney radioactivity is still high 8 hours after inhalation but falls off to a constant level at 24 hours. The radioactivity registered in the bronchi remained up to 48 hours after inhalation. There was a large excretion of styrene metabolites not only via the kidneys but also in the bile, which was evident by radioactivity appearing in the intestinal contents 1 hour after inhalation where it was still visible at 8 hours, being cleared at 24 hours after inhalation. Autoradiography of dried, evaporated and extracted sections revealed that some of the radioactivity present in the kidney, liver and bronchi was non-extractable from 30 minutes after inhalation up to the last survival time 48 hours. Autoradiography of styrene-8- ^{14}C , as compared to styrene-ring- ^{14}C , showed an increasing labeling from 2 hours after inhalation of tissues with a rapid cell turnover such as the bone marrow, thymus and gastro-intestinal mucosa and tissues with a high rate of protein synthesis such as the exocrine pancreas, spleen and salivary glands. At 24 hours after inhalation the distribution patterns of styrene metabolites appear very different after administration of styrene-8- ^{14}C (Fig. III:4) or styrene-ring- ^{14}C (Fig. III:5). It is known that carbon atom 8 is split off to a certain extent during the biotransformation of styrene resulting in the

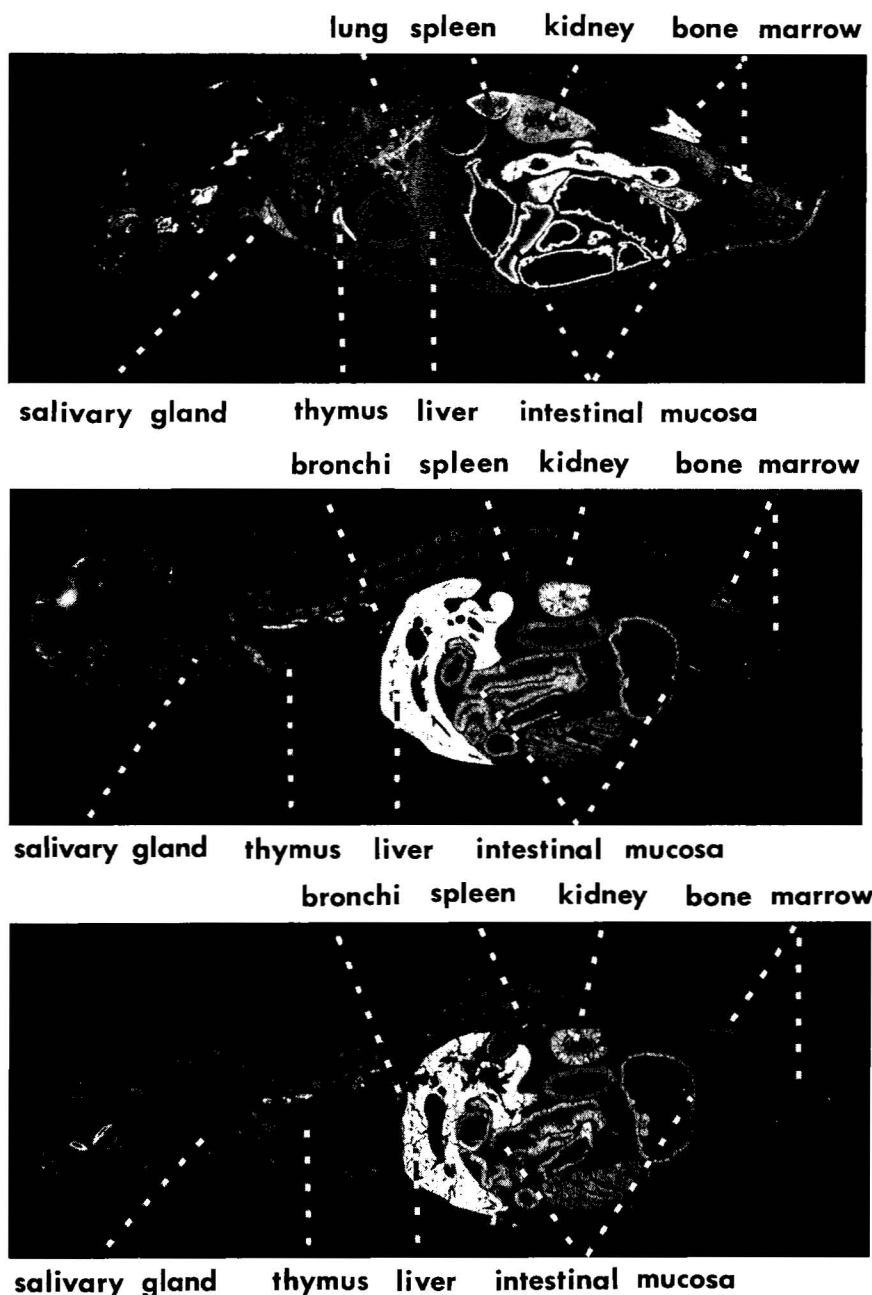


Fig. III:4. Autoradiograms of mice 24 h after iv injection of 25 μ l ^{14}C -formaldehyde (upper) and 24 h after inhalation of 10 μ l styrene-8- ^{14}C for 10 min (middle: dried, evaporated section, lower: dried, evaporated and extracted section). The highest levels of radioactivity from styrene-8- ^{14}C are seen in the kidney and liver whereas the bone marrow and gastro-intestinal mucosa show the highest levels of radioactivity after administration of ^{14}C -formaldehyde.

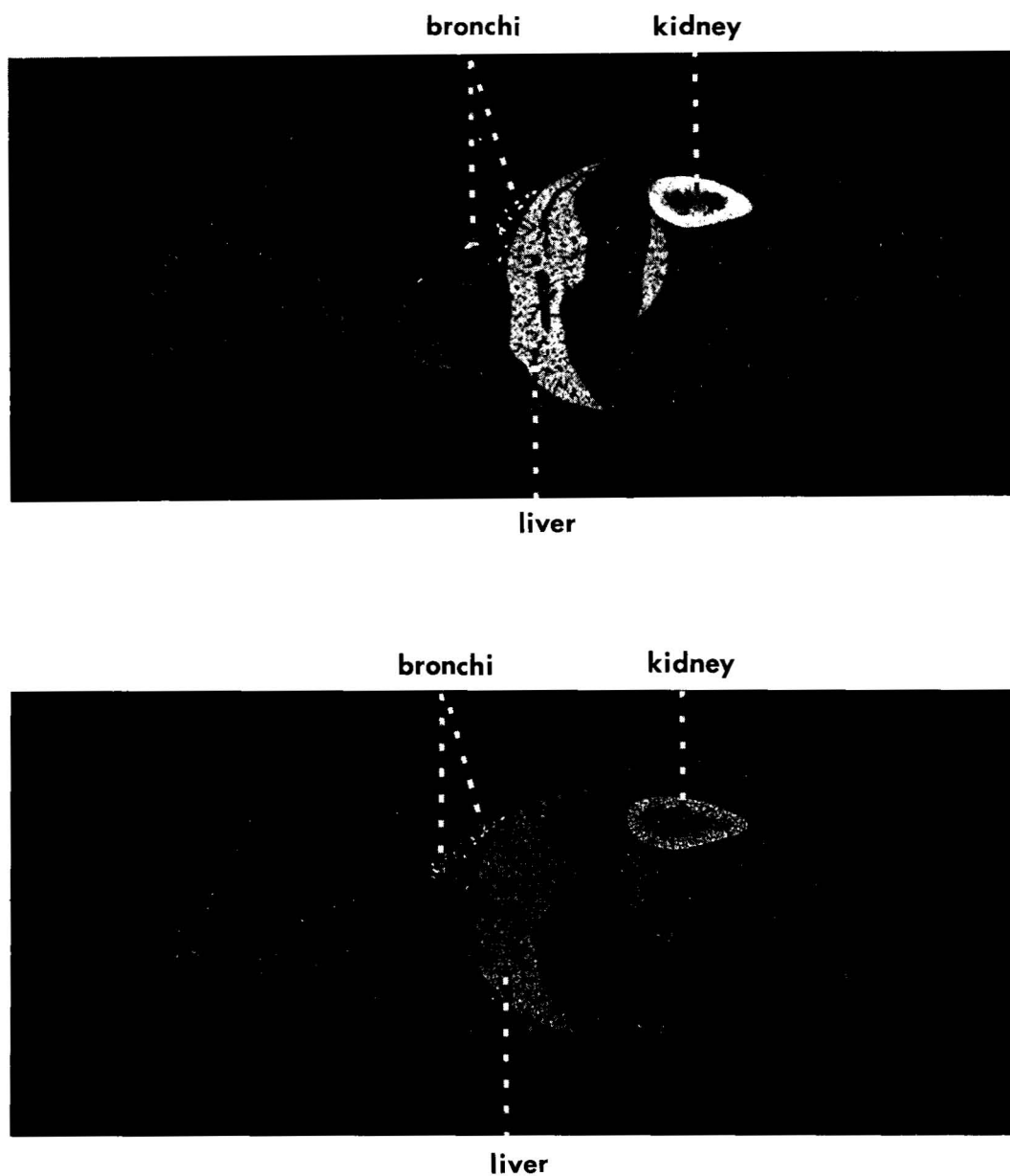


Fig. III:5. Autoradiograms of a mouse 24 h after inhalation of 10 μ l styrene-ring- ^{14}C for 10 minutes. Dried evaporated section (upper) and dried, evaporated and extracted section (lower). The autoradiograms are strikingly different from those shown in Fig. III:4. A relatively large proportion of the radioactivity in the liver, kidney and bronchi is firmly bound.

ultimate formation of CO_2 . As discussed below, it is assumed that the split off carbon may join the one-carbon pool of the body and enter different normal metabolic pathways. Styrene labeled at position C8 would therefore give a somewhat "false" distribution of radioactivity, which includes the quite normal incorporation of some of its radioactivity into the above mentioned tissues, whereas styrene labeled in the aromatic ring would give the "true" distribution of styrene and its metabolites. Metabolically incorporated radioactivity is of course firmly bound and cannot be extracted from the sections as shown in Fig. III:4. However, the radioactivity in the bronchi, liver and lung is likewise not extractable either after administration of styrene-8- ^{14}C (Fig. III:4) or styrene-ring- ^{14}C (Fig. III:5).

Low-temperature autoradiography compared to autoradiography of dried, evaporated sections showed that the radioactivity in adipose and nervous tissues was volatile at all survival times. No metabolites were registered in these tissues which is contrary to the theory of Savolainen (366) that toxic styrene intermediates reacting with nervous tissue components would be responsible for its neurotoxicity. Since no styrene metabolites could be registered in the brain, and of course also no firmly bound radioactivity, the autoradiographic results are also in contrast to the study by Savolainen and Vainio (369) where persistent labeling of brain proteins was observed in rats after administration of radiolabeled styrene. Thus, the nervous system depressant effects observed at acute exposure of animals

(413) and humans (423) to styrene as well as neurotoxic effects observed in chronically exposed workers (171, 252, 389) would, according to autoradiographic results, probably be caused by styrene itself. Autoradiography at -80°C showed some retention of styrene in body fat even 48 hours after exposure, which agrees with the long-time retention of styrene in body fat observed at occupational exposure (403). This is to be expected since styrene has a high fat solubility (Appendix 2) and a low vapor pressure at body temperature (Appendix 1). Styrene could therefore accumulate in the fat depots of the body during chronic exposure. The slow release of styrene from body fat would also make it available for biotransformation during an extended period of time. Biotransformation of styrene and elimination of the metabolites both via urine and bile was registered by autoradiography for as long as 8 hours after inhalation. Metabolites were still visible in the liver, kidney and bronchi 48 hours after inhalation. The long-time retention of radioactivity in the liver, lung and kidney of rats has been demonstrated after subcutaneous or intraperitoneal injection of radiolabeled styrene (89, 369). According to autoradiography there was a rapid distribution of styrene to the liver and kidney. After subcutaneous injection of ^{14}C -labeled styrene to rats, the highest levels of radioactivity were registered in the liver and kidney 1 hour after injection (89). High rat liver and kidney levels of styrene were also noted shortly after intravenous injection (474) whereafter the levels decreased rapidly. Autoradiography supports this observation but showed an increasing level of styrene metabolites in the liver and kidney at short survival times.

The study by Danishefsky and Willhite (89) is the only report found in the literature about the formation of $^{14}\text{CO}_2$ from styrene-8- ^{14}C . The different autoradiograms obtained after administration of styrene-8- ^{14}C or styrene-ring- ^{14}C clearly demonstrate the influence of the different modes of radiolabeling of styrene on the distribution of radioactivity. The mechanisms of CO_2 formation from styrene have not been investigated but cleavage of the side-chain seems probable since hippuric acid is found as a urinary metabolite of styrene. Spontaneous decarboxylation of mandelic acid may generate CO_2 in analogy with the decarboxylation of trichloroacetic acid suggested to occur during trichloroethylene biotransformation (32). It may also be that the C8 of the side-chain is split off initially as formaldehyde, a reaction known to occur at oxidative O-dealkylation (148). The formaldehyde would then be oxidized to formate, which joins the one-carbon pool of the body (306) from where it may serve as a precursor of endogenous substances or may be oxidized to CO_2 e.g. via Krebs cycle (150, 290). CO_2 may also be utilized in itself for the synthesis of amino acids and purines (150, 260, 290). The incorporation of radioactivity from ^{14}C -formaldehyde or $^{14}\text{CO}_2$ would be most obvious in tissues with a rapid cell turnover or protein synthesis. Fig. III:4 also includes an autoradiogram obtained 24 hours after intravenous injection of ^{14}C -formaldehyde (published with the permission of E. Brittebo and H. Tjälve), which, compared to the autoradiogram of a dried, evaporated section obtained 24 hours after inhalation of styrene-8- ^{14}C , shows the same high labeling of the bone marrow, thymus and gastro-intestinal mucosa. However, the

liver and kidney radioactivity levels after inhalation of styrene-8- ^{14}C are higher than after injection of ^{14}C -formaldehyde, indicating the presence of firmly bound "true" styrene metabolites in addition to possibly normally incorporated radioactivity. The bronchi also show some radioactivity after administration of ^{14}C -formaldehyde but the possibility that the observed bronchial radioactivity after inhalation of styrene-8- ^{14}C would be due to metabolically incorporated radioactivity only is ruled out by the localization of firmly bound radioactivity in the bronchi after inhalation of styrene-ring- ^{14}C , shown in Fig. III:5, which also confirms the presence of firmly bound styrene metabolites in the liver and kidney.

Acute or chronic styrene exposure has generally failed to cause any specific organ injuries in animals or man. However, reports of styrene mutagenicity after metabolic activation (93, 457), chromosome breaks in peripheral lymphocytes from occupationally exposed workers (270) and in rat bone marrow cells (269), the covalent binding of styrene oxide to rat liver macromolecules (264) and the results of this study, where autoradiographic registration of firmly bound styrene metabolites was possible not only in the liver but also in the kidney and bronchi, seem to justify the recent concern about styrene induced mutagenic and possibly carcinogenic effects. The different autoradiographic distribution pictures of firmly bound styrene metabolites with styrene-8- ^{14}C or styrene-ring- ^{14}C stress the importance of the right choice of label position in investigations of covalent binding of styrene metabolites to tissue macromolecules.