Placental transfer and tissue distribution of $^{14}$C-styrene: an autoradiographic study in mice

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ABSTRACT The distribution of $^{14}$C-styrene was studied in the pregnant mouse using a whole body low-temperature autoradiographic technique. In unsectioned tissues studied by liquid scintillation techniques, the concentrations of styrene and its metabolites in maternal and fetal blood and organs and in the placenta and amniotic fluid were determined. The organs which had higher concentrations of volatile styrene were maternal lung, kidney, liver, adipose tissue, and brain in mice killed shortly after injection. Non-volatile metabolites were localised in the lung, liver, kidney, gall bladder, and intestine. There were considerable amounts of radioactivity in the fetuses, though the concentrations were not as high in the maternal tissues. Fetal tissue levels were almost the same as maternal brain in mice killed from one to six hours after injection. The concentrations of styrene and its metabolites in placenta and amniotic fluid were about twice those in the fetal tissues. The placenta seems to play the part of a barrier for the fetus.

Because of their extensive use, organic solvents have received much attention in the past decade as possible factors in causing embryotoxic effects in man. One of these, styrene, plays an important commercial part as it has been used in the production of polymers, copolymers, and reinforced plastics that have been used as food packaging materials.¹² Styrene has been reported to be mutagenic in many experimental systems when metabolically activated; toxicity and teratogenicity have been reported in chick embryos,² but there is only one report on the fetal uptake and distribution of styrene.⁵

In view of the current interest in the toxicology of styrene and the lack of quantitative data on its distribution in the fetus we have studied the distribution of $^{14}$C-styrene using a whole body low temperature autoradiographic technique to assess the fetal uptake and distribution after injection to dams.

Methods

Autoradiography

$[^{14}]$C-Styrene with a specific activity of 2-2 m Ci:mmol was purchased from the Radiochemical Centre, Amersham, UK, and dissolved in sufficient styrene to obtain a concentration of 1 $\mu$Ci/$\mu$l. The mice (CD-1,crj), in their 16th day of pregnancy, were injected with 5 $\mu$Ci styrene in a tail vein with 0.025 ml of corn oil. The animals were killed by cervical dislocation after one minute, 30 minutes, one hour, two hours, or six hours and immersed in n-hexane cooled by carbon dioxide (-78°C). The frozen animals were embedded on a microtome stage with 5% aqueous sodium carboxy methyl cellulose gel. The autoradiographic technique of Ullberg was used with modifications to prevent evaporation of the volatile styrene.⁴ The frozen and embedded mice were cut into 70 $\mu$m thick sections, pressed against x ray film, and kept at -70°C for from five to 15 weeks. The autoradiograms showed the distribution of both the volatile styrene and its metabolites. Other sections from the same parts were freeze dried overnight and heated at 40°C for 48 hours, allowing the volatile radioactivity to evaporate before exposure. The autoradiograms showed the distribution of only the non-volatile metabolites. In this way distribution pictures were obtained for styrene plus metabolites and for the non-volatile metabolites alone.

Quantitation of radioactivity in tissues and excreta (liquid scintillation spectrophotometry)

In the unsectioned tissues with $[^{14}]$C-styrene studies, by liquid scintillation the concentration of styrene and its metabolites in maternal and fetal blood and organs.

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and in the placenta and amniotic fluid were quantitatively determined. A 3 μmole amount of the styrene with 3 μCi of radioactivity was given to each pregnant mouse as described above.

Results

RADIOAUTOGRAPHY

One minute after injection, there were high levels of volatile radioactivity (non-metabolised styrene) in the lung, adipose tissue, and the blood vessels of the head. Non-volatile radioactivity was present only in the lung at this early phase (figs 1a, b).

The distribution of radioactivity after 30 minutes exposure at −70°C is shown in fig 2a. Low temperature autoradiography shows an especially high concentration of radioactivity in the lung and in the kidney. About equal concentrations were found in the liver and adipose tissue and the concentration was also high in the tissue surrounding the eyeball. A comparison with autoradiograms of dried and evaporated sections from the same mouse (fig 2b) showed that non-volatile radioactivity, representing styrene metabolites, was localised in the lung, liver, kidney, and intestine, whereas lower radioactivity was visible in body fat and the nervous system. There were considerable amounts of radioactivity in the fetuses, though the concentrations were not so high as in the maternal tissues. As may be seen by comparing the two pictures (figs 2a, b), only styrene itself seems to have an affinity for body fat.

Figures 3a and b show the radioautograms one hour after injection. A comparison with autoradiograms of dried and evaporated sections from the same mouse showed that volatile radioactivity (styrene itself) was localised in adipose tissue, whereas both styrene and styrene metabolites were localised mainly in the liver, kidney, and lung. In the radioautogram one hour after injection, the radioactivity of the placenta was considerably higher than that of the fetal tissues.

Non-volatile radioactivity in the lung, liver, kidney, and intestine was high up to one, two, and six hours after injection. Two hours after injection, the concentrations in the gall bladder and intestine exceeded those of lung and liver. Radioactivity was also present in the fetus. At these survival times the concentrations...
in the blood and the brain of the mother were low, being almost the same level as in the corresponding tissues of the fetus (figs 4a, b).

By looking at the different distribution pictures of the autoradiograms obtained from the frozen sections and freeze-dried evaporated sections from the same animal, we can compare the distribution of volatile and non-volatile substances. The difference was considerable at the shorter survival time—for instance, within 30 minutes or one hour after injection. At six hours after injection, however, there was practically none as by then almost all the volatile styrene had been converted into its metabolites, suggesting that the metabolic rate of styrene is rather fast. In dried and evaporated sections of mice six hours after injection a high radioactivity was localised in the gall bladder, liver, kidney, and intestine (figs 5a, b).

LIQUID SCINTILLATION SPECTROPHOTOMETRY

From the quantitative liquid scintillation spectrophotometric data it was determined that radioactivity was distributed quickly throughout the body. The organ which first had the highest concentration of styrene and its metabolites was the maternal lung in mice killed shortly after injection. In mice killed five minutes after injection the radioactivities of the lung, gall bladder, liver, kidney, and adipose tissue were 24.36, 4.22, 1.49, 0.94, and 2.01 μmole styrene equivalent/g tissue, respectively. The lung was shown to be an important storehouse even in animals administered styrene by intravenous injection. Later, maternal gall bladder also showed high levels of activity if mice killed one, two, or six hours after the injection (table 1). Radioactivity in the urine was also high in mice killed two or six hours after injection.
The radioactivity passed the placenta at all the times studied (table 2). The radioactivity in the fetal tissues was lower than the highly distributed radioactivity in the maternal tissues such as lung, gall bladder, liver, and kidney in mice killed five minutes, 30 minutes, or one hour after injection. In mice killed 30 minutes after injection maternal blood, brain, and liver were 0.22, 0.27, and 1.37 μmole styrene equivalents/g tissue, respectively. By contrast, the placenta, amniotic fluid, fetal brain, and fetal liver contained 0.14, 0.04, 0.03, respectively.

<table>
<thead>
<tr>
<th>Time after Injection</th>
<th>Maternal Blood (μmole/g tissue)</th>
<th>Fetal Blood (μmole/g tissue)</th>
<th>Maternal Brain (μmole/g tissue)</th>
<th>Fetal Brain (μmole/g tissue)</th>
<th>Maternal Liver (μmole/g tissue)</th>
<th>Fetal Liver (μmole/g tissue)</th>
<th>Maternal Placenta (μmole/g tissue)</th>
<th>Fetal Placenta (μmole/g tissue)</th>
<th>Amniotic Fluid (μmole/g tissue)</th>
<th>Amnion (μmole/g tissue)</th>
<th>Uterus (μmole/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i min</td>
<td>0.27 ± 0.17</td>
<td>0.49 ± 0.35</td>
<td>1.49 ± 0.78</td>
<td>0.21 ± 0.13</td>
<td>0.06 ± 0.02</td>
<td>0.03 ± 0.02</td>
<td>0.03 ± 0.02</td>
<td>0.06 ± 0.03</td>
<td>0.11 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>1 min</td>
<td>0.22 ± 0.06</td>
<td>0.27 ± 0.14</td>
<td>1.37 ± 0.88</td>
<td>0.14 ± 0.04</td>
<td>0.06 ± 0.04</td>
<td>0.04 ± 0.03</td>
<td>0.04 ± 0.03</td>
<td>0.06 ± 0.04</td>
<td>0.21 ± 0.17</td>
<td>0.10 ± 0.09</td>
<td>0.20 ± 0.09</td>
</tr>
<tr>
<td>h</td>
<td>0.31 ± 0.16</td>
<td>0.20 ± 0.19</td>
<td>1.40 ± 0.76</td>
<td>0.16 ± 0.07</td>
<td>0.13 ± 0.15</td>
<td>0.10 ± 0.09</td>
<td>0.20 ± 0.09</td>
<td>0.20 ± 0.09</td>
<td>0.20 ± 0.09</td>
<td>0.20 ± 0.09</td>
<td>0.20 ± 0.09</td>
</tr>
<tr>
<td>1 h</td>
<td>0.16 ± 0.05</td>
<td>0.08 ± 0.05</td>
<td>0.31 ± 0.13</td>
<td>0.09 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.20 ± 0.07</td>
<td>0.20 ± 0.07</td>
<td>0.20 ± 0.07</td>
<td>0.20 ± 0.07</td>
</tr>
<tr>
<td>h</td>
<td>0.24 ± 0.18</td>
<td>0.04 ± 0.01</td>
<td>0.22 ± 0.10</td>
<td>0.12 ± 0.06</td>
<td>0.11 ± 0.03</td>
<td>0.09 ± 0.06</td>
<td>0.27 ± 0.12</td>
<td>0.27 ± 0.12</td>
<td>0.27 ± 0.12</td>
<td>0.27 ± 0.12</td>
<td>0.27 ± 0.12</td>
</tr>
<tr>
<td>h</td>
<td>0.03 ± 0.03</td>
<td>0.03 ± 0.02</td>
<td>0.41 ± 0.43</td>
<td>0.03 ± 0.03</td>
<td>0.07 ± 0.06</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
</tbody>
</table>

The results are expressed as μmole styrene equivalents per gram tissue (mean ± SE).
and 0.05 μmole styrene equivalents/g tissue, respectively. The concentrations of styrene and its metabolites in the placenta and uterus were about three times those in the fetal blood and tissues. The placenta thus seemed to be playing the part of a barrier for the fetus.

Fetus blood and tissue concentrations reached somewhat higher levels when mice were killed at one hour after injection compared with those killed at five or 30 minutes after injection. At two hours after injection, fetal tissue concentrations were almost the same as the maternal placenta or brain. At 24 hours after injection, activity was still apparent in both maternal and fetal tissues. While the activities of the maternal tissues decreased remarkably, fetal concentrations were almost the same as maternal blood concentration.

Table 2  Concentration of radioactivity (styrene plus metabolite) in various tissues of the mouse

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Cortex</th>
<th>Cerebellum</th>
<th>Midbrain</th>
<th>Lung</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min (n = 5)</td>
<td>0.44 ± 0.34</td>
<td>0.53 ± 0.24</td>
<td>0.57 ± 0.52</td>
<td>24-36 ± 11.19</td>
<td>1.15 ± 0.34</td>
</tr>
<tr>
<td>30 min (n = 3)</td>
<td>0.29 ± 0.12</td>
<td>0.28 ± 0.14</td>
<td>0.21 ± 0.18</td>
<td>28.02 ± 25.46</td>
<td>0.75 ± 0.52</td>
</tr>
<tr>
<td>1 h (n = 6)</td>
<td>0.18 ± 0.16</td>
<td>0.18 ± 0.12</td>
<td>0.31 ± 0.44</td>
<td>18.53 ± 8.32</td>
<td>1.08 ± 0.16</td>
</tr>
<tr>
<td>2 h (n = 5)</td>
<td>0.09 ± 0.05</td>
<td>0.13 ± 0.09</td>
<td>0.03 ± 0.01</td>
<td>9.31 ± 4.53</td>
<td>0.61 ± 0.33</td>
</tr>
<tr>
<td>6 h (n = 5)</td>
<td>0.06 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>24.19 ± 26.10</td>
<td>0.34 ± 0.28</td>
</tr>
<tr>
<td>24 h (n = 5)</td>
<td>0.02 ± 0.02</td>
<td>0.02 ± 0.03</td>
<td>0.03 ± 0.03</td>
<td>19.61 ± 22.29</td>
<td>0.22 ± 0.31</td>
</tr>
</tbody>
</table>

(—) No specimens were obtained. *Adipose tissue 1: mesenterium adipose tissue. †Adipose tissue 2: subcutaneous adipose tissue. The results expressed as μmole styrene equivalents per gram tissue (mean ± SE).
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Fig 5a, b Whole body autoradiograms of pregnant mice six hours after injection of $^{14}$C-styrene; hemisection at $-70^\circ$ = (a), dried and evaporated section. (b) Film exposure times: (a) 9 weeks, (b) 9 weeks. Abbreviations, see figs 2 and 4.

Discussion

Whole body autoradiography, like any other tracer technique, does not supply any information about the nature of the radioactivity detected, which may belong both to the administered substance or to its metabolites, or both. When performing autoradiography with organic solvents, however, advantage may be taken of their volatility to distinguish between the solvents themselves and their metabolites. Low temperature whole body autoradiography registers the total radioactivity—that is, both volatile and non-volatile radioactivity. Conventional whole body autoradiography of tape fastened, freeze dried sections registers only non-volatile radioactivity.7

In the present study even 30 minutes after the injection a high concentration of non-volatile material was found in the lung, kidney, and intestinal and

<table>
<thead>
<tr>
<th>Gall bladder</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Adipose tissue 1*</th>
<th>Adipose tissue 2†</th>
<th>Faeaces</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.22 ± 3.38</td>
<td>0.35 ± 0.12</td>
<td>0.94 ± 0.45</td>
<td>0.94 ± 0.55</td>
<td>2.01 ± 0.60</td>
<td>(—)</td>
<td>(—)</td>
</tr>
<tr>
<td>7.2 ± 2.35</td>
<td>0.16 ± 0.07</td>
<td>0.82 ± 0.29</td>
<td>1.17 ± 0.71</td>
<td>1.57 ± 1.35</td>
<td>(—)</td>
<td>(—)</td>
</tr>
<tr>
<td>23.77 ± 16.36</td>
<td>0.29 ± 0.20</td>
<td>0.91 ± 0.23</td>
<td>1.73 ± 1.42</td>
<td>1.30 ± 0.62</td>
<td>(—)</td>
<td>(—)</td>
</tr>
<tr>
<td>13.48 ± 6.91</td>
<td>0.09 ± 0.03</td>
<td>0.50 ± 0.16</td>
<td>0.39 ± 0.07</td>
<td>0.22 ± 0.03</td>
<td>0.02 ± 0.01</td>
<td>89.45</td>
</tr>
<tr>
<td>27.61 ± 11.37</td>
<td>0.22 ± 0.27</td>
<td>0.32 ± 0.18</td>
<td>0.16 ± 0.08</td>
<td>0.17 ± 0.13</td>
<td>2.02 ± 2.46</td>
<td>50.13 ± 33.29</td>
</tr>
<tr>
<td>1.32 ± 0.69</td>
<td>0.04 ± 0.01</td>
<td>0.39 ± 0.54</td>
<td>0.07 ± 0.07</td>
<td>0.08 ± 0.09</td>
<td>0.64 ± 0.43</td>
<td>15.14 ± 13.25</td>
</tr>
</tbody>
</table>
gastric mucosa, as well as the liver. This indicates rapid metabolism and consequently a high concentration of enzyme in all these tissues. The endoplasmic reticulum of the liver cell is the most important site of drug metabolism, but extrahepatic tissues such as the lungs, kidneys, gastrointestinal tract, and skin may also contribute.\textsuperscript{6,10}

The quantitative liquid scintillation spectrophotometric measurements of radioactivity agreed well with those obtained by autoradiography. That is to say, styrene and styrene metabolites showed a higher radioactivity in the lung. The lung is perfused by the entire cardiac blood output, whereas the liver receives a quarter of the output. It was also reported that the enzymes from the lung had much higher specific activities per unit of cytochrome P-450 concentration for organic solvents, especially the aromatic solvents.\textsuperscript{10} The presence of styrene metabolites in the lung of the mouse may result from the particularly high activity of styrene mono-oxygenases in the mouse lung.

An autoradiographic study on the uptake of \textsuperscript{14}C-styrene by mice, in which each animal inhaled the vapour from 10 µl of styrene in a small inhalation apparatus for 10 minutes was carried out by Bergman.\textsuperscript{7} The autoradiograms showed that the radioactivity was localised in the bronchi, lungs, and liver for both the styrene labelled in the side chain and the ring labelled compound. A large proportion of the radioactivity was found in the gall bladder and kidney, suggesting that these are the primary routes of excretion.

There have been some reports on the distribution of controlled doses of styrene monomer administered by different routes in mice.\textsuperscript{11-13} In our study the radioactivities of maternal gall bladder and urine were higher than those in any other tissues, especially one, two, or six hours after injection. The tissue distribution may be related to the route of administration or the site of labelling, but in the present study urine, bile, and expired air seemed to be the major routes of elimination.

From our quantitative time course of the radioactivity in various tissues of the mouse six and 24 hours after injection, the lung showed a higher radioactivity, although lung radioactivity decreased until two hours after injection. The high radioactivity in the lung after prolonged periods seems partly to originate from carbon dioxide as a metabolite. This agrees with a study by Danishefsky and Willhide.\textsuperscript{11} Results of studies on rats subcutaneously injected with [8-\textsuperscript{14}C]-styrene showed that there was rapid distribution to tissues and organs within one hour and that 85% of the radioactivity was eliminated within 24 hours; 71% of the radioactivity appeared in the urine, 12% in expired air as carbon dioxide, 3% in the faeces, and 3% as unchanged styrene in the expired air.

Although neutral, lypophilic, and xenobiotic molecules with a relatively low molecular weight are believed to pass to the fetus through the placenta by means of a passive diffusion process,\textsuperscript{14} concentrations of styrene and styrene metabolites appear to be much lower in the fetuses than in the maternal organs and tissues immediately after injection. The concentrations of styrene and its metabolites in placenta and amniotic fluid were about twice those in the fetal tissues. These results confirm those of a previous study by Withey and Karpiński which concluded that by comparison with fetal styrene concentrations the maternal blood concentrations were more than twice as high for 1000 and 2000 ppm styrene exposure.\textsuperscript{15} The placenta seems to play the part of a barrier for the fetus.

Possibly even more important was the retention of radioactivity in the fetus at a relatively constant level for at least 24 hours. The slower decrease rate of styrene metabolism in the fetus compared with maternal tissue after a single injection might be important, especially in the case of chronic exposure causing styrene to accumulate in fetal tissue. Although there has been no report comparing styrene metabolism in fetal tissues with that in the mother, the hepatic and extrahepatic capacity of the fetus to detoxify epoxides\textsuperscript{16} seems to be biologically important in view of the fact that xenobiotics traversing the placenta to a great extent reach the fetal organs directly through the venous duct without proceeding through the liver.

We gratefully thank Dr K Denpo, Dr M Yuzawa, and Ms N Kawano of the division of animal experimentation of Sapporo Medical College, and Dr K Inoue and T Tsuzuki of Hokkaido Institute of Public Health for their help.

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Destruction of manuscripts

From 1 July 1985 articles submitted for publication will not be returned. Authors whose papers are rejected will be advised of the decision and the manuscripts will be kept under security for three months to deal with any inquiries and then destroyed.