

MATERIALS AND METHODS

Labeled compounds

^{14}C -Methylene chloride (specific activity 2.7 mCi/mmol) of 99% purity by gas-liquid radiochromatography and ^{14}C -chloroform (specific activity 2.0 mCi/mmol) of 98% purity by gas-liquid radiochromatography were obtained from New England Nuclear, Dreieichenhain, FRG. ^{14}C -Carbon tetrachloride (specific activity 6.94-7.95 mCi/mmol) 98% pure by gas-liquid radiochromatography was obtained from the Radiochemical Centre, Amersham, England.

Animals

NMRI-mice weighing about 25 g were used. Experiments with methylene chloride and chloroform were performed with male mice whereas both male and female mice were used in the study of carbon tetrachloride. The animals were kept at room temperature and given a commercial pellet diet (AB Astra-Ewos, Södertälje, Sweden) and tap water *ad libitum*.

Autoradiography

^{14}C -Methylene chloride was diluted with unlabeled, analytical grade methylene chloride to a final solution of 1.25 $\mu\text{Ci}/\mu\text{l}$. ^{14}C -Chloroform was diluted with unlabeled analytical grade chloroform to a final solution of 4.0 $\mu\text{Ci}/\mu\text{l}$. ^{14}C -Carbon tetrachloride was diluted with unlabeled analytical grade carbon tetrachloride to a final solution of 2.0 $\mu\text{Ci}/\mu\text{l}$. Mice were administered 12.5 μCi (10 μl) ^{14}C -methylene chloride, 20 μCi

(5 μ l) ^{14}C -chloroform or 10 μCi (5 μ l) ^{14}C -carbon tetrachloride 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 studies

^{14}C -Methylene chloride was diluted with unlabeled analytical grade methylene chloride to a final solution of 0.144 $\mu\text{Ci}/\mu\text{l}$. ^{14}C -Chloroform and ^{14}C -carbon tetrachloride were diluted with unlabeled analytical grade chloroform and carbon tetrachloride to final solutions of 0.265 $\mu\text{Ci}/\mu\text{l}$ and 0.261 $\mu\text{Ci}/\mu\text{l}$ respectively. Mice were administered 10 μl ^{14}C -methylene chloride, 5 μl ^{14}C -chloroform or 5 μl ^{14}C -carbon tetrachloride by inhalation for 10 minutes as described in Chapter I. The elimination of radioactivity was followed for 8 hours in the metabolic cage after which time the remaining radioactivity levels in several organs were determined as described in Chapter I.

RESULTS AND DISCUSSION

Autoradiography

Methylene chloride. Low-temperature autoradiography showed that methylene chloride was rapidly and evenly distributed in the body immediately after inhalation but a remarkably high

uptake of radioactivity was noted in the white matter of the brain, body fat, blood, liver and kidney. Autoradiograms obtained from dry, evaporated whole-body sections immediately after inhalation showed quite a high amount of radioactivity in the liver but also in the bronchi and kidney. The radioactivity registered in the fatty tissues, blood and brain may thus be assumed to be due to methylene chloride itself. No volatile radioactivity could be registered in the brain by low-temperature autoradiography at 30 minutes or at any longer survival time. However, volatile radioactivity was present in the body fat still 1 hour after inhalation (Fig. IV:1). Non-volatile radioactivity in the liver, kidney and bronchi had increased considerably 30 minutes after inhalation and was still high 1 hour after inhalation (Fig. IV:1). From 30 minutes after inhalation and at all longer survival times radioactivity was also registered in tissues with a rapid cell turnover such as the bone marrow, thymus and gastro-intestinal mucosa and in tissues with a high rate of protein synthesis such as the spleen, exocrine pancreas and salivary glands. A high level of radioactivity was registered by low-temperature autoradiography in the blood up to 4 hours after inhalation after which time the autoradiograms obtained at -80°C and -20°C showed the same distribution picture. Probably, the high level of radioactivity in the blood observed by low-temperature autoradiography at survival times when methylene chloride itself had left fatty and nervous tissues, but not by autoradiography of dried, evaporated sections, represented $^{14}\text{COHb}$, which during the extensive handling of the thin sections was equilibrated with atmospheric oxygen whereas this did not occur during the

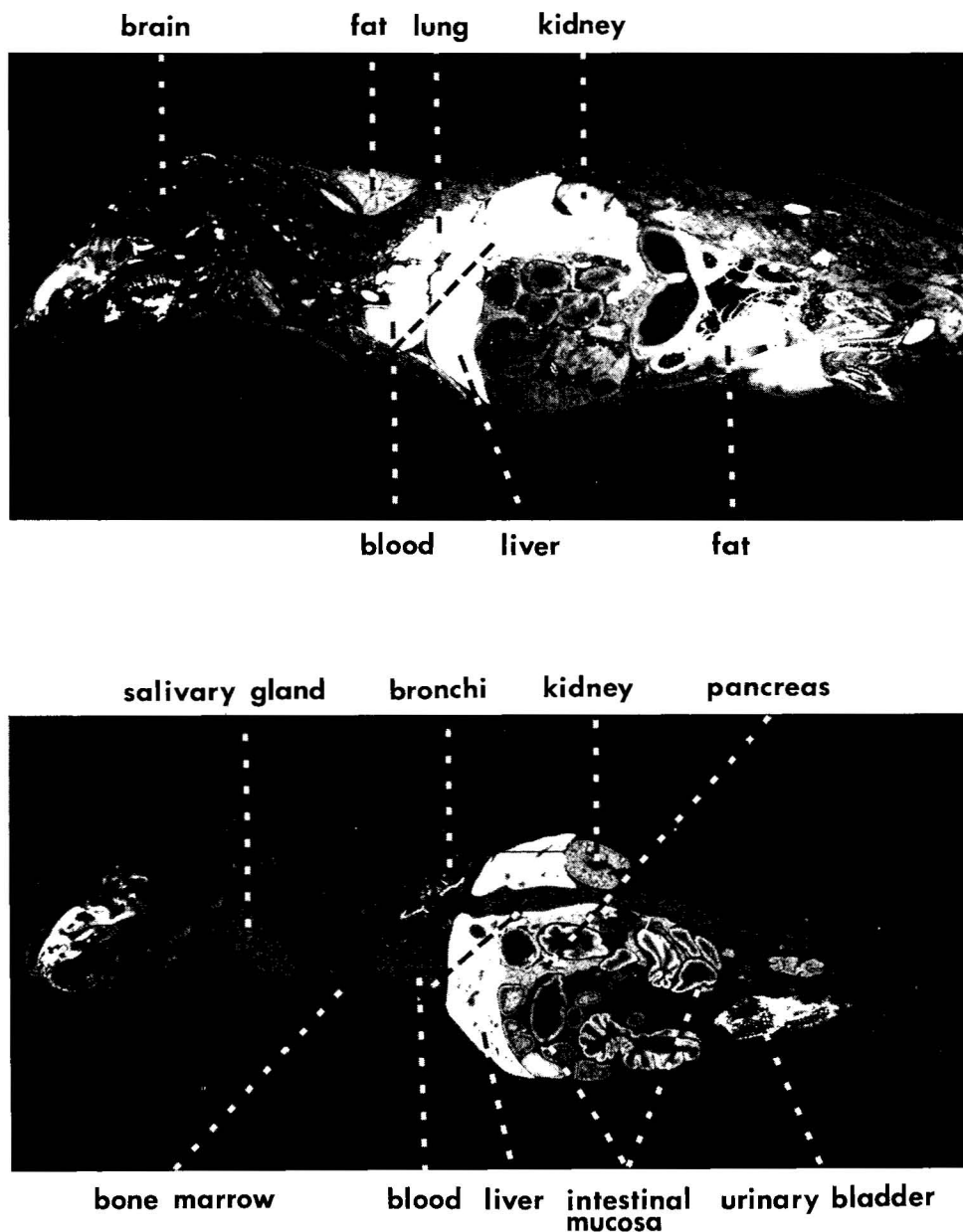


Fig. IV:1. Whole-body autoradiograms of mice 1 h after inhalation of $10\ \mu\text{l}\ ^{14}\text{C}$ -methylene chloride for 10 min. Hemi-section exposed at -80°C (upper) and dried, evaporated section exposed at -20°C (lower). Non-metabolized methylene chloride is still present in body fat. Non-volatile radioactivity is seen in the bronchi, liver, kidney and in tissues with a rapid cell turnover or a high rate of protein synthesis.

rapid handling and exposure at very low temperature of hemisections. Excretion of methylene chloride metabolites took place mainly via the kidney as evidenced by a high amount of non-volatile radioactivity in the urinary bladder between 30 minutes and 8 hours after inhalation. A small amount of non-volatile radioactivity was also registered in the intestinal contents between 2 hours and 8 hours after inhalation. The kidney, bronchi and liver non-volatile radioactivity levels decreased slightly between 2 hours and 8 hours after inhalation whereafter an almost constant amount of radioactivity was present in these organs up to 48 hours after inhalation. A small part of the non-volatile radioactivity registered in the liver, kidney and bronchi immediately after inhalation was not removed by the extraction procedure described in Chapter I. At all other survival times non-extractable radioactivity was present in the liver, kidney, bronchi and in the tissues with a rapid cell turnover or high rate of protein synthesis. Fig. IV:2 shows the distribution of non-volatile and non-extractable radioactivity from ^{14}C -methylene chloride 24 hours after inhalation. A remarkably high concentration of methylene chloride metabolites is still present in the liver.

Chloroform. Autoradiography of chloroform at -80°C immediately after inhalation showed a very high uptake of radioactivity in the body fat, spinal cord, spinal nerves, meninges, white matter of the cerebrum, cerebellar cortex, lung, liver, blood and kidney. Autoradiography of dried, evaporated sections immediately after inhalation revealed that non-volatile chloroform metabolites were already present in the bronchi,

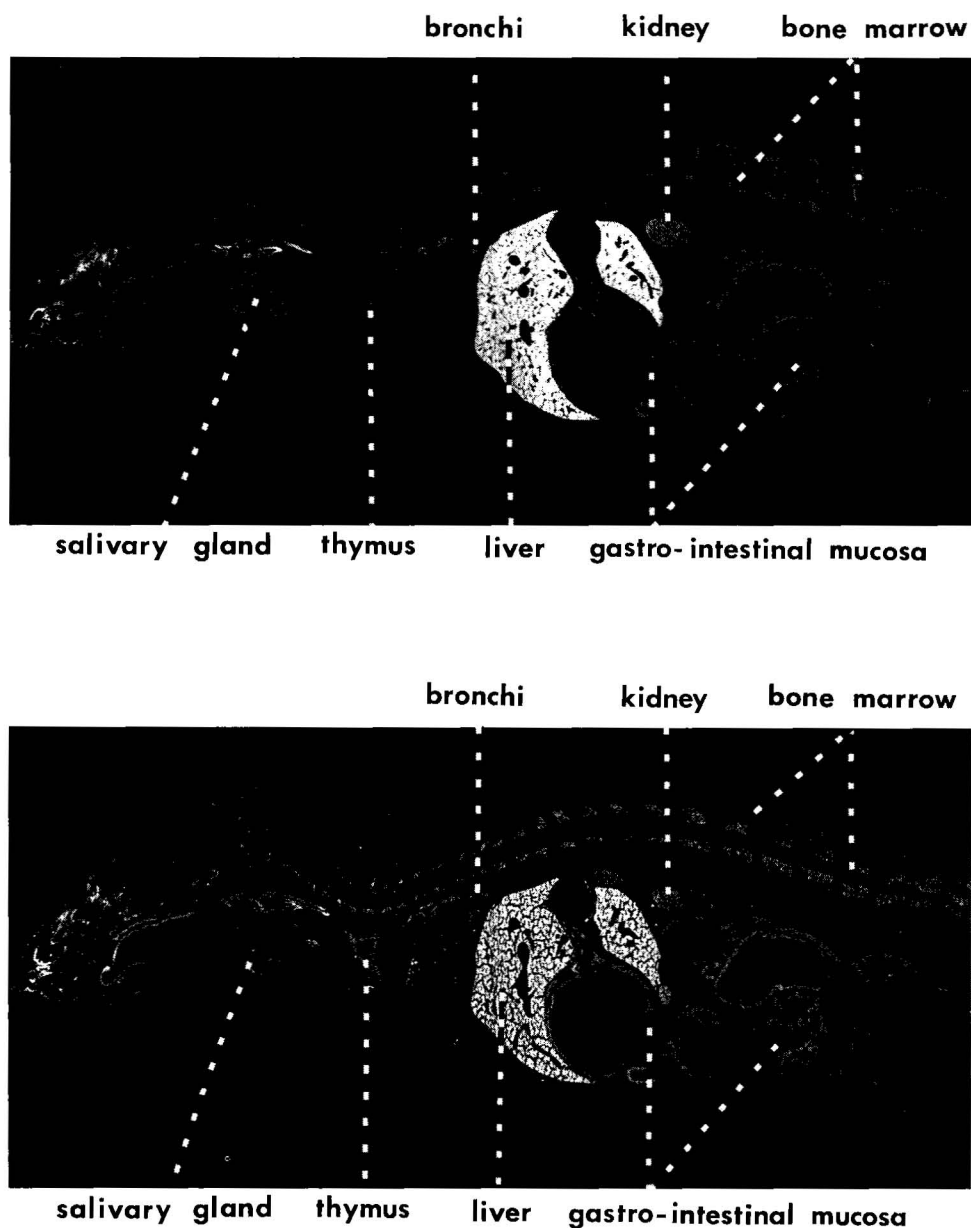


Fig. IV:2. Whole-body autoradiograms of mice 24 h after inhalation of 10 μ l ^{14} C-methylene chloride for 10 min. Dried, evaporated section (upper) and dried, evaporated and extracted section (lower). A high amount of non-extractable metabolites is still present in the liver. Radioactivity is also seen incorporated into tissues with a rapid cell turnover or a high rate of protein synthesis. Note the even distribution of radioactivity within the liver.

liver, kidney, salivary gland and in the contents of the duodenum. The non-volatile radioactivity in the liver was unevenly distributed within the organ. Thus, chloroform itself showed a high affinity for fatty and nervous tissues where radioactivity could be registered only by exposure of hemisections at low temperature. By low-temperature autoradiography radioactivity could be registered in the body fat up to 2 hours after inhalation (Fig. IV:3) with traces of radioactivity still present at 4 and 8 hours after inhalation. In contrast to the relatively rapid disappearance of volatile radioactivity from the adipose tissues and also from the cerebrum and spinal cord, which were largely cleared of radioactivity at 2 hours (Fig. IV:3), chloroform showed a very high affinity for the cerebellar cortex, spinal nerves and meninges, which were all heavily labeled still 48 hours after inhalation. A high amount of radioactivity was also registered in the liver, lung and kidney by exposure of hemisections at -80°C , which, compared to autoradiograms obtained from dry, evaporated sections represented an increasing amount of non-volatile radioactivity. The level of non-volatile radioactivity in the kidney cortex was slightly higher than that in the liver. Non-volatile chloroform metabolites were excreted in the urine but also in the bile as evidenced by radioactivity registered in the intestinal contents up to 8 hours after inhalation. From 30 minutes after inhalation there was also a slight incorporation of radioactivity into tissues with a rapid cell turnover or a high rate of protein synthesis. This radioactivity could not be completely extracted from the dried and evaporated sections (Fig. IV:3). A large part of the radio-

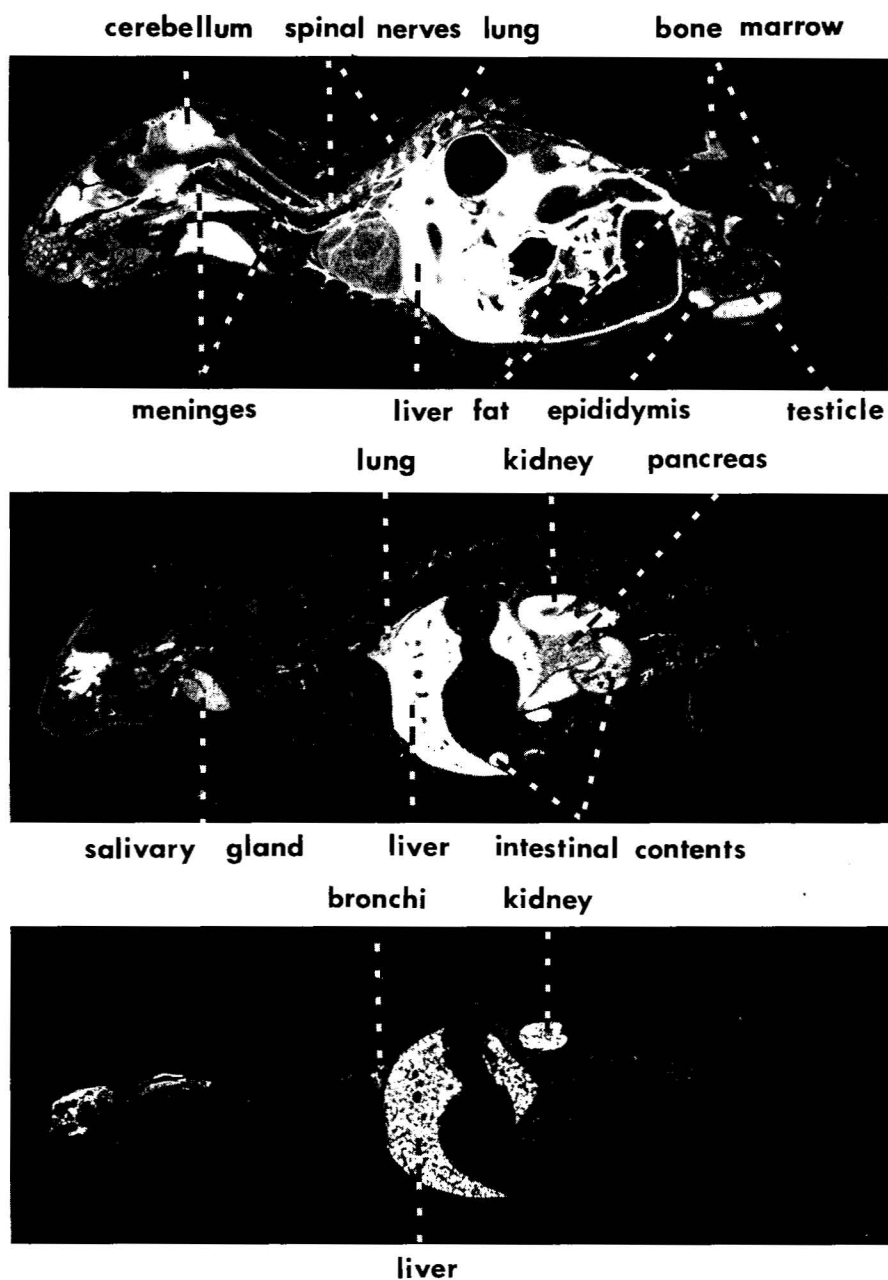


Fig. IV:3. Whole-body autoradiograms of mice 2 h after inhalation of 5 μ l ^{14}C -chloroform for 10 min. Hemisection exposed at -80°C (upper), dried, evaporated section exposed at -20°C (middle) and dried, evaporated and extracted section (lower). Note the high level of non-metabolized chloroform in the cerebellum, spinal nerves and meninges. Non-extractable metabolites are present in the bronchi, liver and kidney. Note the irregular distribution of firmly bound radioactivity within the liver.

activity in the liver, bronchi and kidney cortex was likewise not extractable (Fig. IV:3). The non-volatile radioactivity in the liver always had a mottled appearance, which was even more pronounced after extraction of the sections, indicating a localized, presumably centrilobular distribution of firmly bound metabolites. Radioactivity was found to be firmly bound in the liver, kidney cortex and bronchi still 48 hours after inhalation. Radioactivity was also registered in the epididymis and in the testis with an interstitial localization. The radioactivity in the epididymis was volatile to a large extent whereas the radioactivity in the testes was non-volatile but extractable.

Carbon tetrachloride. Low-temperature autoradiography of carbon tetrachloride immediately after inhalation showed an even distribution of radioactivity but with a remarkably high uptake of radioactivity in the body fat, brain, spinal cord, spinal nerves and bone marrow. High levels of radioactivity were also noted in the liver, kidney, lung, blood and gastro-intestinal mucosa. By comparison with autoradiograms obtained from dried, evaporated sections all the radioactivity in the fatty and nervous tissues was found to be volatile whereas non-volatile radioactivity was registered in the bronchi, liver, kidney and gastro-intestinal mucosa. The non-volatile radioactivity in the liver showed a mottled distribution pattern similar to that obtained after inhalation of chloroform. Non-volatile radioactivity in the gastro-intestinal mucosa showed a strikingly high accumulation in the mucosa of the glandular part of the stomach and in the mucosa of the colon and rectum. At 30 minutes

after inhalation (Fig. IV:4) radioactivity could be registered only by low-temperature autoradiography in the white matter of the cerebrum and cerebellum as well as in the spinal cord, spinal nerves and in the body fat. Non-volatile radioactivity was present in the liver, kidney, salivary gland, bronchi and in the gastro-intestinal mucosa with remarkably high levels noted in the mucosa of the glandular part of the stomach, colon and rectum. The radioactivity in the kidney is low compared to that in the liver. Like methylene chloride and chloroform carbon tetrachloride itself thus seems to have a high affinity for nervous and fatty tissues. However, carbon tetrachloride was registered for a considerably longer period of time in body fat; still 8 hours after inhalation volatile radioactivity was present in the fat depots of the body (Fig. IV:5). All volatile radioactivity had disappeared from the adipose tissues at 24 hours after inhalation. Carbon tetrachloride was registered in nervous tissues as long as 4 hours after inhalation. At 30 minutes after inhalation non-volatile radioactivity had already reached a high level in the liver which remained unchanged and largely non-extractable up to 48 hours after inhalation. The level of non-volatile radioactivity in the kidney was always lower than that in the liver. The radioactivity in the kidney cortex could not be extracted at any survival time. A small, non-extractable amount of carbon tetrachloride metabolites in the bronchi was noted at all survival times. A large part of the radioactivity in the gastro-intestinal mucosa was likewise non-extractable (Fig. IV:5) at all survival times. In female mice non-volatile radioactivity was registered in the vaginal and uterine mucosa

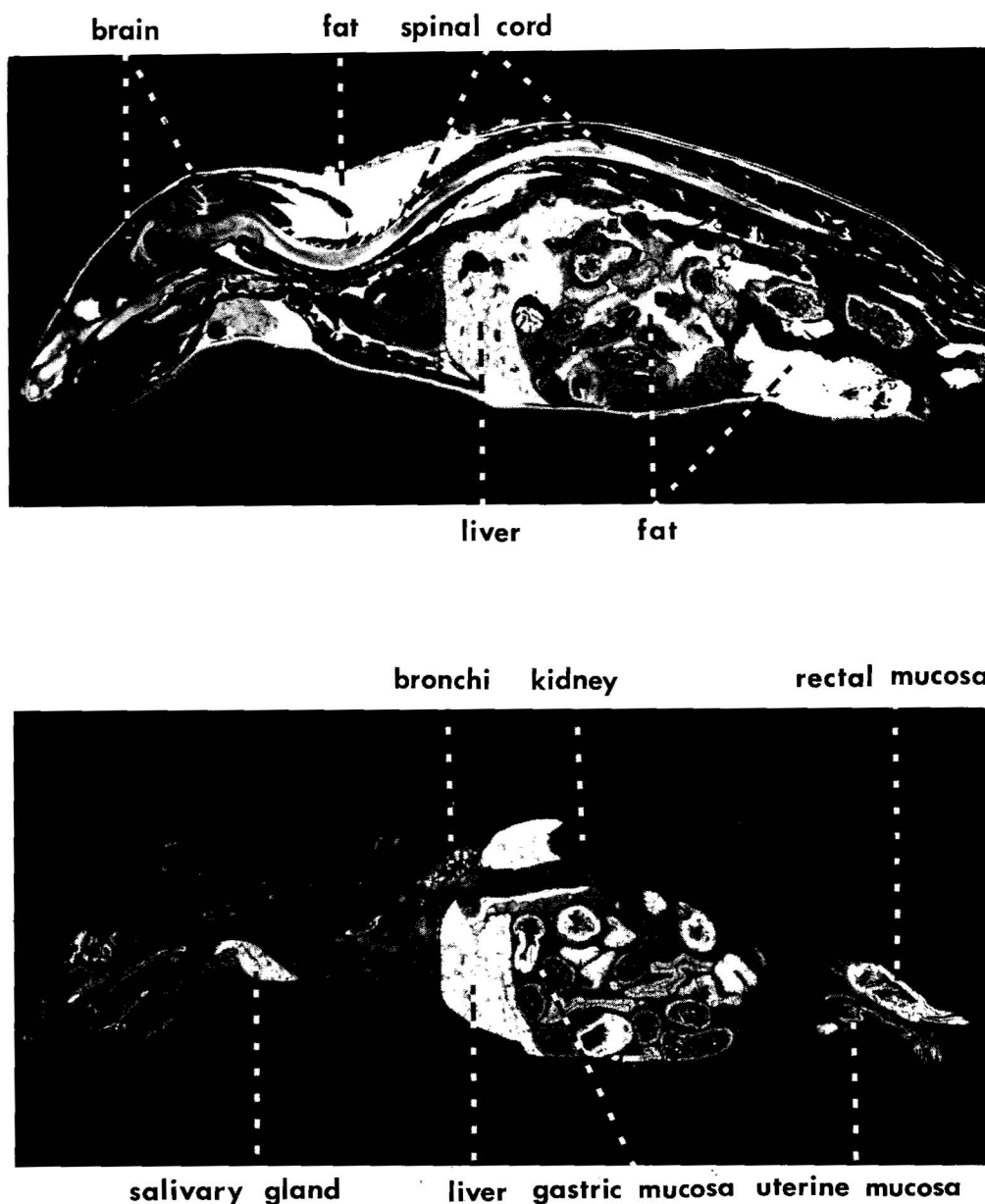


Fig. IV:4. Whole-body autoradiograms of a female mouse 30 minutes after inhalation of $5\text{ }\mu\text{l}$ ^{14}C -carbon tetrachloride for 10 min. Hemisection exposed at -80°C (upper) and dried, evaporated section exposed at -20°C (lower). A high concentration of non-metabolized carbon tetrachloride is present in the body fat, white matter of the brain, spinal cord and spinal nerves. Note the high level of carbon tetrachloride metabolites in the gastro-intestinal mucosa and in the liver where an uneven distribution of radioactivity can be seen.

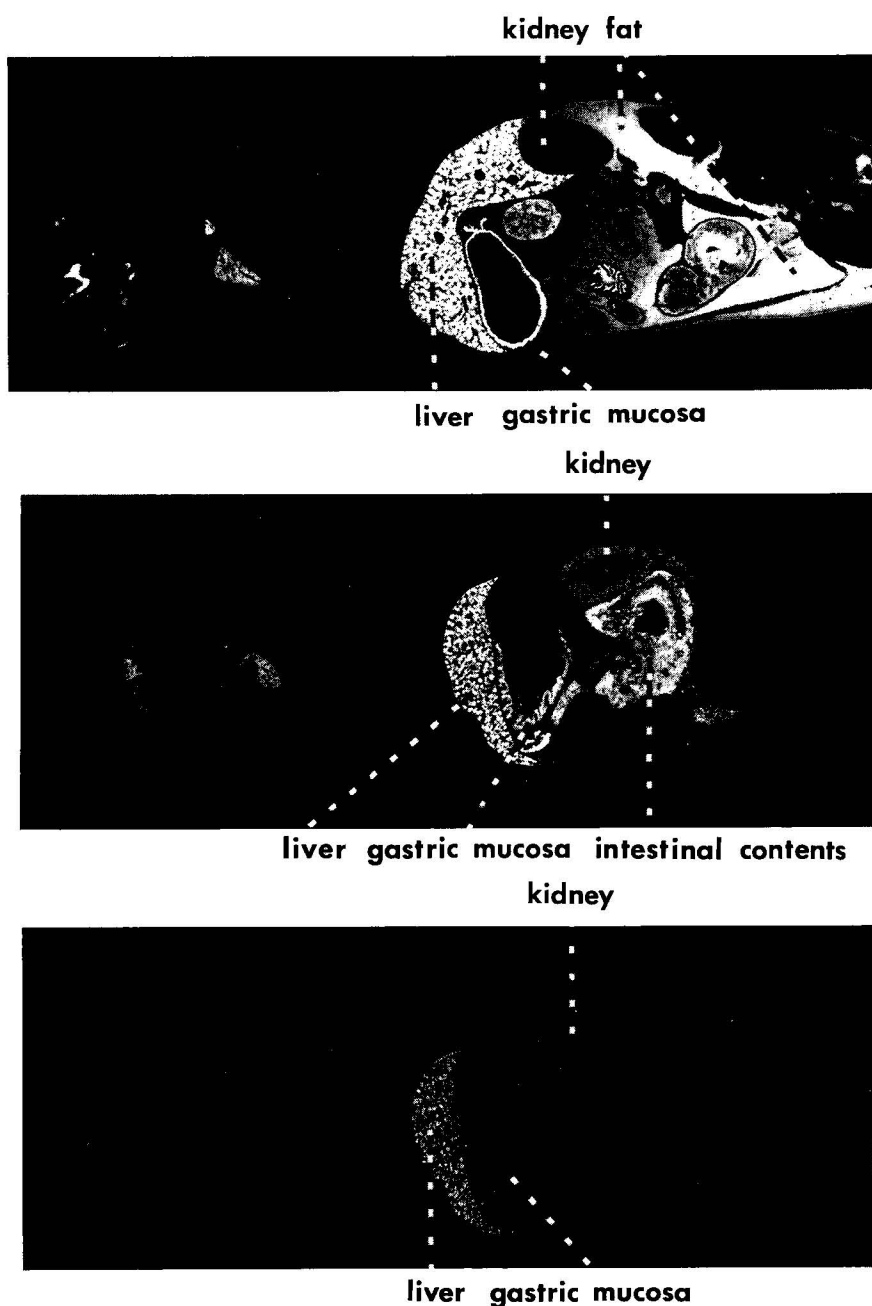


Fig. IV:5. Whole-body autoradiograms of female mice 8 hours after inhalation of 5 μ l ^{14}C -carbon tetrachloride for 10 min. Hemisection exposed at -80°C (upper), dried, evaporated section (middle) and dried, evaporated and extracted section (lower). A considerable amount of non-metabolized carbon tetrachloride is still present in body fat. Non-extractable metabolites are seen in the liver, kidney cortex and gastric mucosa.

(Fig. IV:4). Non-volatile radioactivity was also present interstitially in the testis. Neither the radioactivity in the vaginal and uterine mucosa nor in the testis was extractable. Carbon tetrachloride metabolites were excreted not only in the urine but also via the bile as shown by radioactivity present in the intestinal contents from 30 minutes up to 48 hours after inhalation.

Radioactivity in fatty and nervous tissues after inhalation of ^{14}C -labeled methylene chloride, chloroform or carbon tetrachloride was registered only by low-temperature autoradiography, showing that the three solvents themselves are distributed to these tissues. The ability of the three chlorinated hydrocarbon solvents to pass into the CNS probably explains their common narcotic action. The autoradiographic results do not support the theory of Savolainen (366) that nervous system effects of these three solvents may be caused by their reactive biotransformation products since no non-volatile radioactivity was ever registered in nervous tissue. Moreover, in the case of carbon tetrachloride, Ciccoli *et al.* (73) and Villarruel *et al.* (466) found a negligible labeling of brain lipids after administration of ^{14}C -carbon tetrachloride to rats and carbon tetrachloride has also failed to cause peroxidation of rat brain lipids *in vitro* (336) and polysomal dissociation in the rat brain *in vivo* (86), both effects which are produced by carbon tetrachloride metabolites in the liver. The periods of time during which volatile radioactivity could be registered in nervous tissue by low-temper-

ature autoradiography seem to depend on the fat solubility of the three solvents, which increases in the order methylene chloride, chloroform, carbon tetrachloride (Appendix 2). Methylene chloride was thus cleared from the CNS in 30 minutes whereas chloroform, with the notable exception of its presence in the cerebellum, spinal nerves and meninges still 48 hours after inhalation, and carbon tetrachloride were registered in the CNS up to 2 hours and 4 hours respectively. The strikingly high and persistent concentration of chloroform in the cerebellar cortex, spinal nerves and meninges noted in this study was not observed in earlier autoradiographic distribution studies of chloroform in various animal species (49, 79, 81, 439), which may be due to technical errors such as film exposure of hemisections over solid carbon dioxide, which is likely to produce a fluctuating temperature, or even sectioning the animals at only -10°C to -20°C , both techniques which could make chloroform evaporate from the tissues. A high concentration of chloroform in peripheral nerves but not in the cerebellum as compared to other parts of the CNS was found in dogs anesthetized with chloroform in an early study using a chemical method of analysis (291). The affinity of chloroform for the cerebellar cortex can probably not be explained in physiological or physico-chemical terms, such as blood flow or lipid content, since these are not significantly different in the cerebellum than in other parts of the brain (79) and in which case this phenomenon would have been observed also for other organic solvents, but rather in terms of a specific interaction between chloroform and cerebellar receptors. Interestingly, addiction to chloroform has produced

neurological symptoms such as dysarthria and ataxia as well as gross and action tremor, which could suggest disturbance of the cerebellar system (84, 176).

The affinity of methylene chloride, chloroform and carbon tetrachloride for adipose tissues has been repeatedly demonstrated in earlier distribution studies (63, 79, 133, 266, 291, 409). The time during which the three solvents may be autoradiographically registered in body fat seems to follow their order of increasing fat solubility and decreasing vapor pressure at body temperature (Appendices 1 and 2). Methylene chloride was present in body fat only up to 1 hour after inhalation, which supports the results of earlier studies where a very rapid decline of radioactivity in fat was noted after inhalation of ^{14}C -methylene chloride by rats (63) and no significant amounts of radioactivity could be found in body fat of rats 2 hours after intraperitoneal injection of ^{14}C -methylene chloride (102). The short retention time of methylene chloride in fatty tissues indicates that the risk of accumulation of methylene chloride in the body during repeated exposure would be small, as suggested by Peterson (314) and DiVincenzo *et al.* (105). Carbon tetrachloride, being the most fat-soluble of the three solvents, was clearly visible in body fat up to 8 hours after inhalation. This observation together with earlier observations of high fat levels of carbon tetrachloride several hours after administration to animals (133, 409) point out the fat depots of the body as important storage sites for carbon tetrachloride at single or repeated exposure.