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Distribution and elimination of 2-[^{14}C]-acetone in mice after inhalation exposure

by Ewa Wigaeus, BSc, Agneta Löf, BSc, Marianne Nordqvist, PhD[1]

WIGAEUS E, LÖF A, NORDQVIST M. Distribution and elimination of 2-[^{14}C]-acetone in mice after inhalation exposure. *Scand j work environ health* 8 (1982) 121—128. This study was undertaken to determine the tissue distribution and elimination of acetone and its metabolic radioactive fragments in mice after exposure to about 1,200 mg/m^3 (500 ppm) of 2-[^{14}C]-acetone vapor. The tissue concentrations of acetone seemed to reach steady state plateaus within 6 h of exposure. In the adipose tissue the maximal concentration was about one-third of that in the highly perfused nonadipose tissues, in which acetone was rather evenly distributed. The contents of radioactivity also reached a plateau within 6 h of exposure in all tissues except the liver and brown adipose tissue. In these tissues the radioactivity increased during exposures up to 24 h. Prolonging the exposure time from 6 h to 6 h/d for three and five consecutive days gave no or only a small additional accumulation of radioactivity in all tissues except adipose tissue. The half-times of acetone after 6 h of exposure were between 2 and 5 h in all tissues. Almost equal amounts of acetone were excreted via the lungs unmetabolized or metabolized to carbon dioxide. In all tissues endogenous levels of acetone were reached within 24 h after exposure. Thus, acetone did not accumulate after prolonged or repeated exposure to concentrations of 1,200 mg/m^3.

Key terms: acetone, expired air, metabolites, tissue concentration.

Acetone is a widely used industrial solvent, and occupational human exposure occurs with its use (18). About 45 % of the inhaled acetone vapor is taken up in the human body (21), and most of it is excreted via the lungs as unmetabolized acetone and metabolically formed carbon dioxide (3, 18).

Early tracer studies with animals have indicated that acetone is metabolized to acetate and formate and a 3-carbon intermediate which can enter the glycolytic cycle. Radiolabeled carbon from acetone has been found in endogenous material such as liver glycogen and various amino acids (15, 16). More recent studies have demonstrated that radioactivity from ^{14}C-acetone is present in plasma glucose, lipids, and proteins of fasting humans (17).

Acetone is often used in combination with other solvents, especially styrene. In our laboratory tissue distribution studies of styrene and other lipophilic organic solvents have shown an accumulation of the solvents in adipose tissue (4, 5, 6, 7). The elimination of these solvents from adipose tissue in man is relatively slow (1, 8, 9, 10, 11, 12), a phenomenon indicating a potential risk of prolonged exposure to such lipophilic solvents.

In the present study mice were exposed to acetone, as it is of interest to compare the distribution and elimination pattern of the highly water-soluble acetone (19) with those of the earlier studied lipophilic organic solvents.

Material and methods

Radioactively labeled acetone (2-[^{14}C]) (The Radiochemical Centre, Amersham, England, radiochemical and chemical purity 99 %) with a specific activity of 2.18 M Bq/μmol was diluted with regular acetone (Merck, Darmstedt, Federal Republic of Germany, analytical purity) to a mean specific activity of 403 Bq/μmol. The vapor of this solution was mixed with air in a polyester-laminated aluminium-foiled

[1] Research Department, National Board of Occupational Safety and Health, Solna, Sweden.

Reprint requests to: Ms E Wigaeus, Research Department, National Board of Occupational Safety and Health, S-171 84 Solna, Sweden.

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bag to a concentration of 1,200 mg/m³. Male mice (Naval Medical Research Institute, white, 25—30 g) were exposed to the acetone vapor in a metabolism cage (2.8 l) (4). The cage was placed in a cooled water bath (15—17°C) to keep the absolute humidity at a tolerable level (14). The acetone concentration was controlled regularly by gas chromatography (Porapak Q, 1.0 m, 170°C, nitrogen flow 30 ml/min, flame ionization detector, Antec 464 LP) and adjusted when necessary. The time-weighted average concentration was 1,239 (SD 54) mg/m³.

For the determination of the tissue distribution of acetone, groups of four mice were exposed to acetone for 1, 3, 6, 12, and 24 h or 6 h/d for 1, 3, and 5 consecutive days. The mice were killed by luxation immediately after exposure. The rate of elimination was determined from groups of four mice killed 1, 3, 6, 12, or 24 h after a 6-h exposure period. Different tissues were sampled for the determination of unchanged acetone by gas chromatographic analysis and for the analysis of the total amount of radioactivity by liquid scintillation counting.

Blood was obtained by decapitation. The mice were kept cold on ice until the other tissue samples were taken and sealed in preweighed cold glass vials. Samples of about 100 mg for the determination of unchanged acetone were stored at —70°C in sealed glass vials until the analysis. The acetone was evaporated from all tissue samples except blood with the use of a continuous headspace technique (to be published) that is a modified version of a method previously used in our laboratory for the analysis of organic solvents in adipose tissue (11). The amount of acetone was analyzed by gas chromatography (0.4 % Carbowax 1500 on Carbopack A, 2 m, 70°C, nitrogen flow 25 ml/min, flame ionization detector, Varian 3700 with a rebuild double injection port). Blood samples were analyzed by a previously described headspace method (21). The in vitro ratio of blood to air was determined to be 248 at 37°C. The tissue samples (about 100 mg) for the radioactivity determination were solubilized in Soluene 350®, decolored when necessary, and mixed with scintillation liquids (4). The radioactivity was assayed in a Packard TriCarb model 3375 spectrometer with external standardization. Whenever possible duplicate samples were taken, and the error of the method was calculated according to

$$SD = \{[\Sigma D^2 - (\Sigma D)^2/n]/2(n-1)\}^{1/2},$$

where D is the difference in concentration between duplicate samples.

During a 12-h elimination period after 6 h of exposure, expired air from four mice was collected in different absorption fluids and analyzed by scintillation counting for its contents of radioactive acetone, carbon dioxide, and carbon monoxide (2, 13). Hopcalite (40 % CuO + 60 % Mn_2O) was used as a catalyst for the oxidation of carbon monoxide to carbon dioxide.

Table 1. Method error as the percentage of the mean value of the radioactivity determinations.

Tissue	Method error (% of mean value)
Blood	14.1
Lung	10.9
Liver	9.8
Kidney	6.6
Brain	8.3
Muscle	17.4
Intraperitoneal adipose tissue	20.1
Subcutaneous adipose tissue	26.0
Brown adipose tissue	7.4

Results

The method error of the radioactivity determinations is given in table 1.

The accumulation of unmetabolized acetone and the total radioactivity in different tissues (means and standard deviations of the values of four mice) after up to 24 h of exposure are given in fig 1. A general feature was an increase in the tissue concentration of acetone and total radioactivity during the first 6 h of exposure. A prominent part of the total radioactivity seemed to represent metabolic fragments. Exposure times longer than 6 h gave no further accumulation in any tissues except the liver and brown adipose tissue. In these tissues the concentration of radioactivity increased during the exposures up to 24 h. At that time the concentration of radioactivity in the liver was 4.8 (SD 0.6) μmol/g, significantly higher ($p \leq 0.05$, Mann-Whitney test) than in any

other tissue. Only about 10 % of this amount was unchanged acetone. The concentration of acetone in the adipose tissue was very low compared with all the other tissues studied. Intraperitoneal and subcutaneous adipose tissues exhibited the lowest levels ($p \leq 0.01$, Mann-Whitney test), about 0.2 μmol after 24 h of exposure.

When mice were exposed 6 h/d for 3 and 5 consecutive days, most tissues showed no or only a small additional increase in radioactivity after more than 1 d of exposure (fig 2). The concentration

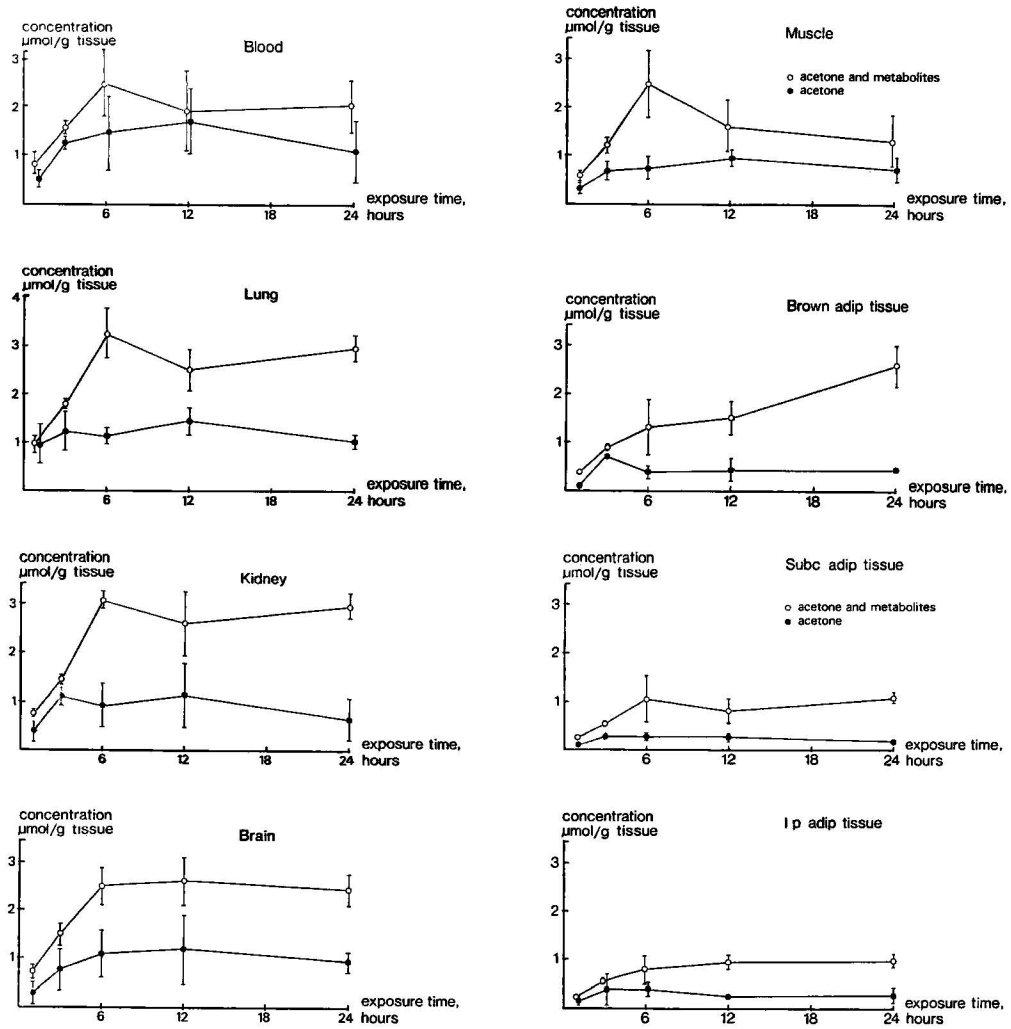

Fig 1. Tissue concentrations of acetone and its radioactive metabolic fragments (o) and those of unmetabolized acetone (●) after exposure to a ^{14}C-acetone concentration of about 1,200 mg/m^3 for different time periods. The concentration of radioactivity is expressed as micromoles of acetone equivalents per gram of wet tissue based on the specific activity of the ^{14}C-labeled acetone used. The mean values and standard deviations of four mice are given. The background levels, mainly endogenously formed acetone, were 0.02—0.10 μmol/g of tissue. (adip = adipose, ip = intraperitoneal, subc = subcutaneous)

in adipose tissue, however, increased significantly with increased exposure time up to 5 d. The concentration in brown adipose tissue reached 5.5 (SD 0.4) μmol/g, which was about twice as high as after 24 h of exposure and four times as high as after 6 h of exposure.

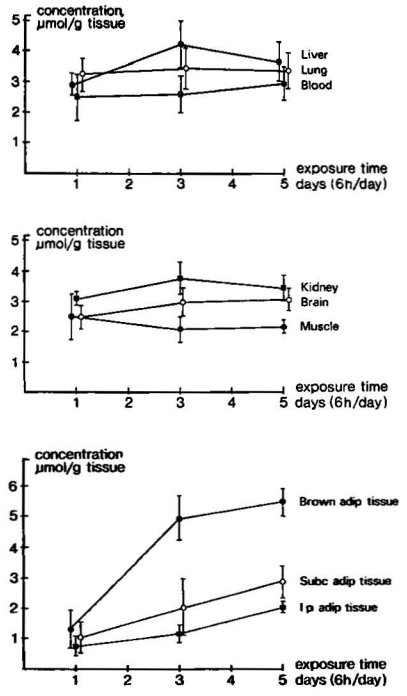

Fig 2. Tissue concentrations of acetone and its radioactive metabolic fragments after exposure for 6 h/d to a ^{14}C-acetone concentration of about 1,200 mg/m^3 for one, three, and five consecutive days. The concentration of radioactivity is expressed as micromoles of acetone equivalents per gram of wet tissue based on the specific activity of the ^{14}C-labeled acetone used. The mean values and standard deviations of four mice are given. (adip = adipose, ip = intraperitoneal, subc = subcutaneous)

Similar to most tissues depicted in fig 1 and 2 pancreas, spleen, thymus, heart, testis, and vas deferens showed their maximal radioactive contents after 6 h of exposure. The concentrations ranged between 2.1 (SD 0.7) μmol/g (testis) and 3.6 (SD 0.7) μmol/g (pancreas).

The ratio of the acetone concentration in the sampled tissues to that in the blood was less than one for all the sampled tissues at all exposure times with the exception of the lungs, the site of administration (fig 3). The lung : blood ratio was almost 2 after 1 h of exposure. Subcutaneous adipose tissue had the lowest ratios, about 0.2 for all exposure periods. When the ratios of acetone equivalents are compared, the picture is quite different (fig 4). After 1 and 3 h of exposure only the lungs had a ratio higher than 1, whereas also the ratios of the kidneys and liver exceeded 1 after 6 h of exposure. After 24 h of exposure only the muscles and the subcutaneous and intraperitoneal adipose tissue had ratios of less than 1. The liver and brown adipose tissue ratios rose continuously with increased exposure time and were 2.4 and 1.4, respectively, after 24 h of exposure.

The elimination of radioactivity and unmetabolized acetone following a 6-h exposure period is shown in fig 5. The blood, kidneys, lungs, brain, and muscles showed the fastest elimination of acetone with half-times of about 2—3 h during the first 6 h after exposure. The slowest elimination was seen from subcutaneous adipose tissue with a half-time slightly longer than 5 h. Twenty-four hours after expo-

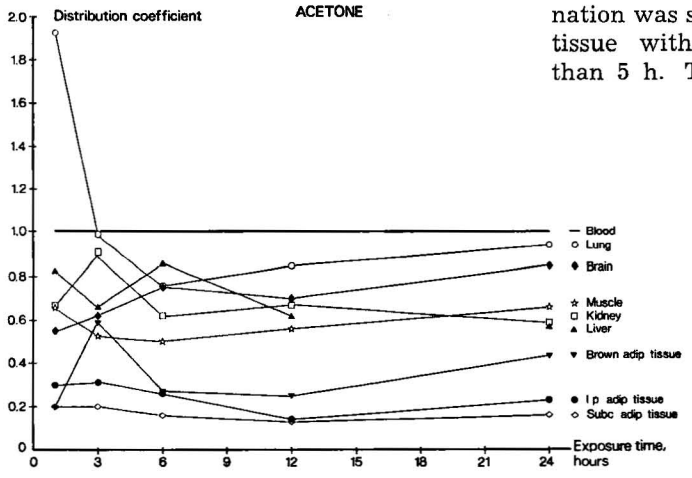

Fig 3. Tissue : blood concentration ratios of acetone after exposure to a ^{14}C-acetone concentration of about 1,200 mg/m^3 for different periods of time. (adip = adipose, ip = intraperitoneal, subc = subcutaneous)

sure the acetone concentration had reached the endogenous levels (0.02—0.10 μmol/g tissue) in all tissues. There were, however, still detectable amounts of metabolites left in all tissues except the blood and muscles.

The tissue : blood ratio of acetone increased with increased time of elimination. Immediately after 6 h of exposure the acetone concentration of all the tissues was lower than that of blood (fig 3 & 5), but after 6 h of elimination most tissues had about the same concentration as that of blood, except the liver, which had a concentration about 3.5 times higher.

The tissue : blood ratio of radioactivity also increased with prolonged elimination time. Immediately and 1 h after exposure the radioactive contents of all the tissues except adipose tissue were about the same as that of the blood (fig 4 & 5). Thereafter the ratio rose with increased time of elimination. Twelve hours after exposure the liver had the highest concentration of radioactivity, 10 times higher than the blood concentration, while the muscles and the subcutaneous and intraperitoneal adipose tissue only had slightly higher concentrations than that of the blood.

The cumulative excretion of radioactive acetone and carbon dioxide in the expired air up to 12 h after a 6-h exposure period is shown in fig 6. No detectable amounts of radioactive carbon monoxide was found in the expired air. Forty-two micromoles of radioactive acetone was eliminated from a mouse via the expired air during 12 h with 95 % of the amount recovered within the first 6 h. The elimination of carbon dioxide during 12 h corresponded to 37 μmol of acetone; 85 % of the amount was eliminated within the first 6 h.

Discussion

The present distribution study confirms the hypothesis that acetone is not selectively absorbed in any tissue but is more evenly distributed in the body water. This situation is quite different from that of more lipophilic solvents, which according to our earlier studies accumulate and reach high concentrations in subcutaneous adipose tissue (4, 5, 6, 7). In this study the lowest solvent concentrations were found in the adipose tissue, the very lowest occurring in subcutaneous adipose tissue. This phenomenon can, apart from the poor perfusion in these tissues, be explained by the hydrophilic character of acetone (19). After 3—6 h of exposure the increase of the acetone concentration in the tissues studied leveled off to a steady state plateau that indicated that equilibrium had been obtained with the actual air concentration (fig 1).

The ratio for acetone between subcutaneous adipose tissue (μmol/kg) and the inspiratory air (μmol/l) was about 5 after 1 h, and it rose to about 15 after 6 h of exposure. More lipophilic solvents show much higher ratios after comparable exposures. When rats were exposed to 1,014 mg/m³ of styrene the fat : air ratio was about 64 after 1 h and 197 after 4 h of exposure (4). The corresponding ratios for rats exposed to 1,117 mg/m³ of p-xylene

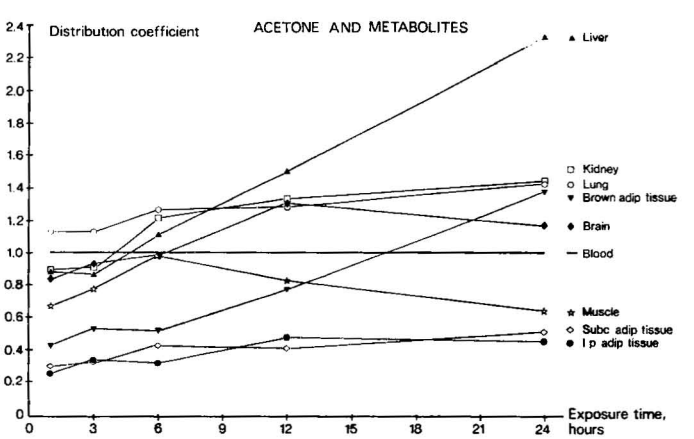

Fig 4. Tissue : blood concentration ratios of radioactivity after exposure to a ^{14}C-acetone concentration of about 1,200 mg/m³ for different periods of time. (adip = adipose, ip = intraperitoneal, subc = subcutaneous)

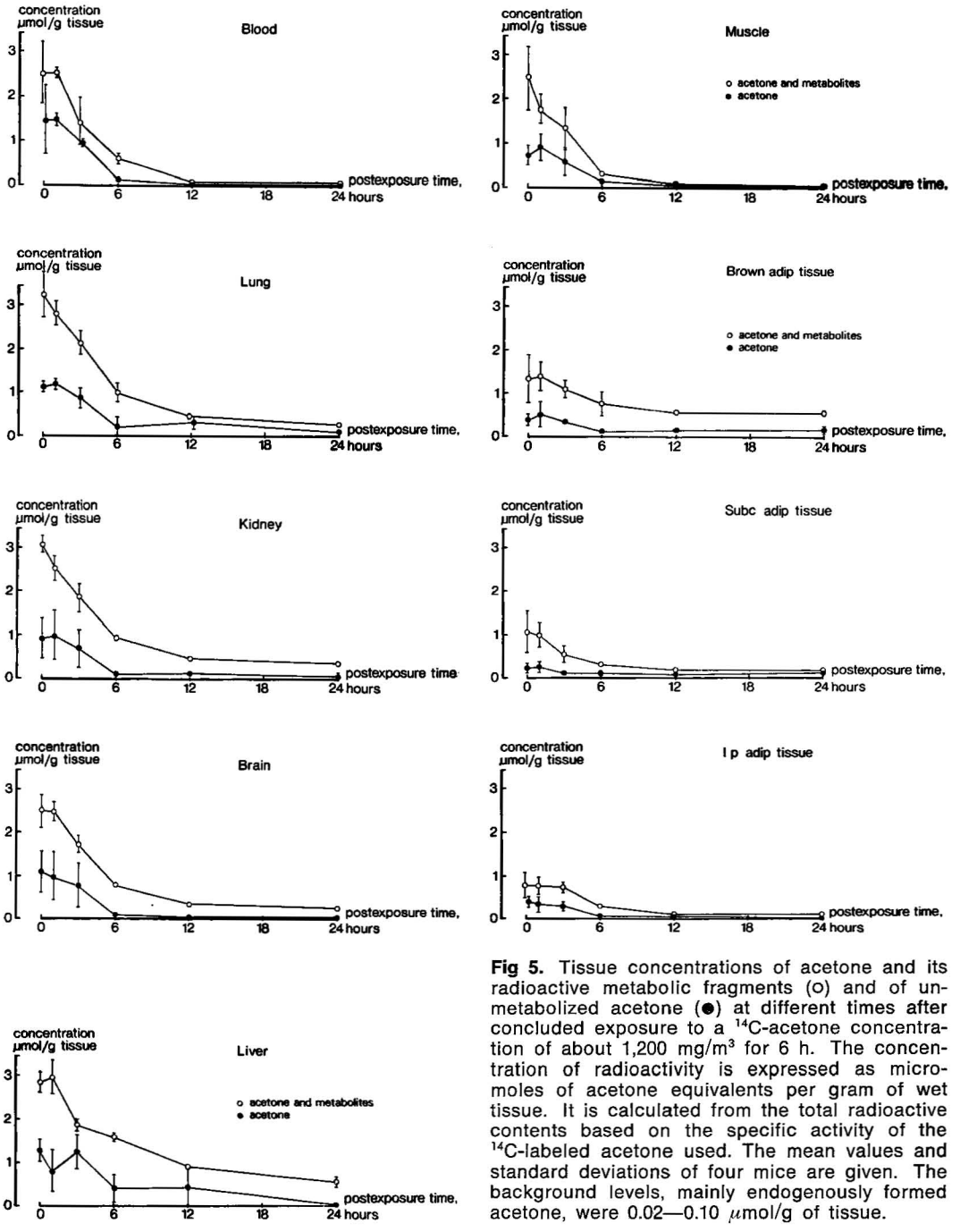

Fig 5. Tissue concentrations of acetone and its radioactive metabolic fragments (o) and of unmetabolized acetone (●) at different times after concluded exposure to a ^{14}C-acetone concentration of about 1,200 mg/m^3 for 6 h. The concentration of radioactivity is expressed as micromoles of acetone equivalents per gram of wet tissue. It is calculated from the total radioactive contents based on the specific activity of the ^{14}C-labeled acetone used. The mean values and standard deviations of four mice are given. The background levels, mainly endogenously formed acetone, were 0.02—0.10 μmol/g of tissue.

were 85 after 1 h, 204 after 4 h, and 526 after 8 h of exposure (5). The subcutaneous adipose tissue : blood ratio (fig 3) stayed fairly constant at about 0.2 throughout the entire exposure period. In the styrene and p-xylene study this ratio increased with an increased exposure time and was 36 and 35, respectively, after 4 h of exposure.

The ratio for acetone between blood and the inspiratory air was about 25 after 1 h of exposure, and it rose to 60 after 3 h and to about 70 after 6 h of exposure. The

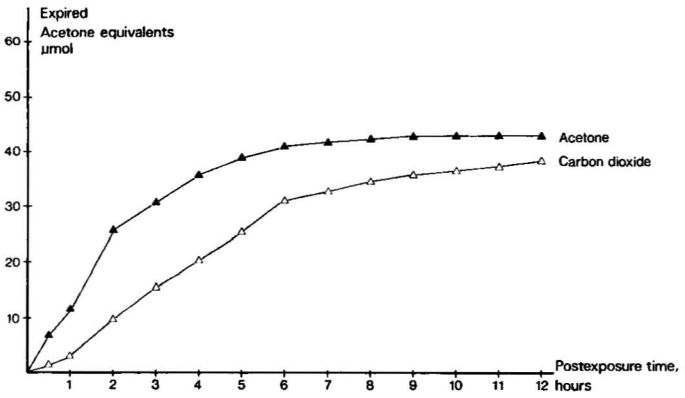

Fig 6. Cumulative excretion of ^{14}C-acetone and ^{14}C-carbon dioxide after 6 h of exposure to a ^{14}C-acetone concentration of about 1,200 mg/m^3.

blood : inspiratory air ratio in our previous study with man (mean values of arterial and venous blood concentrations) was about 6 after 1 h and 10 after 2 h of exposure at rest (21). When light physical activity (50 W on a bicycle ergometer) was introduced, the ratio rose to about 11 after 1 h and 23 after 2 h of exposure. Thus, the blood : air ratio of acetone after 1 h of exposure was about two and four times higher, respectively, for the mouse than for man exposed to about the same acetone concentration during light physical activity or at rest.

The blood : air coefficient, determined in vitro, was 248 in this study. Elsewhere it has been reported to be in the range of 245 to 275 (19, 21). It is not very probable that our relatively low in vivo blood : air coefficient of about 70 can solely be explained by systemic uptake. A contributing factor could be the very high water affinity of acetone, which results in the dissolution of acetone in saliva and in the mucous membranes in the respiratory tract and leads to a lower acetone concentration in the air reaching the alveoli.

The continued accumulation of radioactivity in the liver and brown adipose tissue in comparison with that of other tissues could be due to high metabolic turnover in these tissues and therefore to high concentrations of ^{14}C-labeled fragments from acetone. In the liver the metabolite fraction of the total radioactivity increased from 44 % after 1 h to 56 % after 6 h and 87 % after 24 h of exposure. In some tissues the concentration of acetone and total radioactivity seems to be lower at 12 and/or 24 h of exposure than at 6 h of exposure. The explanation for this difference can be food deprivation and the resulting reduced physical activity, which leads to reduced acetone uptake (21).

Expiration is the major route of elimination for acetone (3, 18, 21). The fraction of unmetabolized acetone recovered in the breath is dose dependent. This phenomenon was demonstrated in dogs as early as 1897 by Schwarz, who found that, when doses of 300—600 mg/kg (5.16—10.33 mmol/kg) were given, about 55 % was exhaled, but only 18 % was exhaled when the dose was 3.5 mg/kg (0.06 mmol/kg) (20). Further investigations with rats have shown that less than 10 % of the substance is exhaled unchanged when small doses of 1—6 mg/kg (0.02—0.10 mmol/kg) are given (16).

In our previous study on acetone in man the uptake during 2 h of exposure was 8—16 mg/kg (0.14—0.28 mmol/kg) and the amount exhaled 16—27 % of the uptake (21). In this present study with mice about 52 % of the expired radioactivity after concluded exposure was unmetabolized acetone, and the remaining part carbon dioxide. Only traces of unmetabolized acetone was exhaled 6 h after 6 h of exposure (fig 6), and within 24 h after exposure all tissue concentrations of acetone were down to the endogenous levels (fig 5). Thus, with this acetone concentration in the inspiratory air, acetone is not likely to accumulate in the body at repeated exposures. This result was also demonstrated when mice were exposed 6 h/d for 3 and 5 consecutive days. No, or only a small further, increase in total radioactivity was observed in most tissues in comparison with the level at 6 h of exposure.

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