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Toxicokinetics and Molecular Interaction of [^{14}C]-Formaldehyde in Rats

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Abstract. The excretion, tissue distribution, and binding of [^{14}C]-formaldehyde were studied at different time intervals in male rats following a single intraperitoneal injection of 72 mg CH_2O (14.7 μCi)/kg body weight. Within 30 min, 10% of the total dose was recovered in expired air as $^{14}CO_2$ and by the end of 72 hr, 41% of the administered dose was eliminated through expired air. The total elimination of $^{14}CH_2O$ activity in urine and feces in 72 hr was 15%. Erythrocytes retained significant amounts of radioactivity, even at the end of 72 hr. Substantial levels of radioactivity were detected in most tissues one hr after administration, indicating a fast absorption and rapid distribution. Subcellular fractionation of the tissues showed that the highest levels of relative percent binding was in the microsomal fraction, whereas cytosol fractions contained lowest levels of bound radioactivity. DNA, RNA, protein and lipid fractions of liver and spleen tissues showed significantly elevated levels of ^{14}C-incorporation as compared to other tissues. The *in vivo* incorporation of ^{14}C-activity showed an increased association of $^{14}CH_2O$ with RNA in all the tissues. The maximum registration of radioactivity in RNA was at 48 hr after administration. Significantly higher amounts of ^{14}C-activity were registered in DNA of all tissues. The maximum registration of radiolabel in DNA of most tissues was at 12 hr after the $^{14}CH_2O$ administration. The liver DNA showed maximal levels at 3 hr with a second peak at 48 hr.

Substantial amounts of bound radioactivity in nucleic acids of all the tissues were observed even 72 hr after dosing. The relationship between macromolecular association and formaldehyde toxicity has been discussed.

Aldehydes are widely distributed in living matter and play important roles in biochemical and biological functions. Formaldehyde (CH_2O) is the most reactive aldehyde of the alkanal series. It is an important intermediate with a wide variety of industrial applications including plastics, resins, plywood, molding, treatment of papers and leather, and home insulations. It is also commonly used for embalming and preservation of tissues. Industrial exposure to formaldehyde from various sources such as vehicle exhaust, smog, smoking, and certain types of home insulations are widespread. In 1985, formaldehyde ranked 24th among the organic compounds produced in the United States (Chem Eng News 1986).

Concentrations of formaldehyde present in the occupational and environmental atmospheres have recently created concern over the possible adverse health hazards. Previous reports (Glass 1961; Engel and Calnan, 1966; Kitchens *et al.* 1976; Dally *et al.* 1981) and reviews (Ulsamer *et al.* 1984; Bealls and Ulsamer 1984; Farooqui 1983) have shown allergic dermatitis as well as severe irritation of mucosal membranes of the eyes and upper respiratory tract are caused by the formaldehyde exposure. The primary symptoms in humans are eye irritation, burning eyes, runny nose, dry or sore throat, headache, cough and upset stomach (Dally *et al.* 1981). Distribution studies in experimental animals have shown retention of [^{14}C]-formaldehyde in blood and nasal mucosa following inhalation (Heck *et al.* 1983, 1984). Exposure to formaldehyde by inhalation has also been shown to cause ulceration of

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nasal epithelium and nasal tumors in rats (Swenberg *et al.* 1980; Heck *et al.* 1983, 1984) and in humans (Halperin *et al.* 1983). Recently, Dalby (1982) has shown that formaldehyde could serve as a cofactor in the incidence of respiratory tumor formation in hamsters.

According to various reports (Kitchens *et al.* 1976; NRCC 1980), a detailed animal study of formaldehyde toxicity by different routes of exposure has been strongly recommended. While partial disposition of formaldehyde following oral, intraperitoneal and inhalation exposure have been studied in some animal species (Neely 1964; Ntundulu *et al.* 1976; Johansson and Tjalve 1978; Heck *et al.* 1982; Mashford and Jones 1982), no detailed toxicokinetic investigation of $^{14}CH_2O$ distribution and molecular interaction following intraperitoneal administration is currently available in the literature. The present study was initiated to elucidate the toxicokinetics of intraperitoneally administered $^{14}CH_2O$ and to provide quantitative assessment of the extent of its molecular interaction with macromolecules as well as its metabolic incorporation.

Materials and Methods

Chemicals

[^{14}C]-Formaldehyde (specific activity 51.5 mCi/mmol) was obtained from New England Nuclear, Boston, MA. The specific activity of the dosing solution was adjusted by dilution with unlabeled 1.8% solution of formaldehyde (Fisher Scientific Co, Fair Lawn, NJ) in 0.9% sodium chloride solution. The radioactivity in the dosing solution was determined in terms of disintegrations/minute (dpm) in Scinti Verse™ (Fisher Scientific Co, Fair Lawn, NJ), using a Searle Model Mark IV (Searle Analytic Inc, Chicago, IL) liquid scintillation counter. All other chemicals were reagent grade. All experiments were conducted in well-ventilated hoods.

Animals and Treatments

Male Sprague-Dawley rats (220–250 g) (Timco Co, Houston, TX), were housed in groups of three in metabolic cages with free access to drinking water and pellet food (Purina Rat Chow). Individual rats from each group were injected intraperitoneally with 1 ml of freshly diluted solution of [^{14}C]-formaldehyde containing 72 mg CH_2O (14.7 μCi)/kg body weight. Immediately following [^{14}C]-formaldehyde administration, the rats were placed individually in 3.5 L air-tight sealed all glass metabolism chambers (Delmar Roth, Maywood, IL) with free access to food and drinking water.

Collection of Expired Air

For the separate estimation of $^{14}CO_2$, expired air was collected as described by Farooqui and Ahmed (1982). Compressed dried air was passed through the chamber at 1 L/min. Air leaving the chamber was bubbled through a CO_2 trapping fluor Carbosorb® (Packard Instrument Co, Downers Grove, IL). The trapping solutions were collected under cold conditions at different time intervals, and the radioactive content determined.

Excretion, Distribution and Binding Studies Sample Collection

At various time intervals following the administration of [^{14}C]-formaldehyde, uncontaminated urine and feces of individual rats were collected in separate plastic vessels of metabolic cages under properly cooled conditions to avoid the loss of any volatile radioactivity. At designated times following administration of formaldehyde, the animals were anesthetized with ether, and blood samples from the portal vein were collected in heparinized tubes. Organs, glands and tissues were also removed, cleaned and rinsed in cold saline, blotted with filter paper, and immediately frozen on dry ice. The heparinized blood was centrifuged for 10 min immediately after collection at 1,500 g at 4°C. The plasma and buffy coat were separately removed by suction. The packed erythrocytes were washed three times with equal volumes of cold 0.9% sodium chloride solution. The red blood cells were subjected to hypotonic lysis in 5 mM phosphate buffer pH 8.0. The heme and globin were separated as described by Farooqui and Ahmed (1982) by treatment of the hemolysate with ten volumes of 1.5% hydrochloric acid in cold acetone.

Chemical Fractionation of Tissues

The weighed tissues were minced and homogenized in 10 volumes of 0.25M sucrose using a motor driven teflon Potter-Elvehjem homogenizer. Subcellular fractionation of tissue homogenates was carried out by the standard differential centrifugation method and the purity of individual subcellular fraction was determined by using standard markers in the usual manner (de Duve *et al.* 1955; de Duve 1971).

Total radioactivity in tissue homogenates and subcellular fractions was determined with a Biological Sample oxidizer model B 306 (Packard Instrument Co., Downers Grove, IL). In most cases, the determined radioactivity was converted to μg-equivalent formaldehyde, based on the specific activity of the administered dose. To determine the bound radioactivity, known aliquots of whole homogenates and subcellular fractions were precipitated overnight with cold 10% (w/v), trichloroacetic acid (TCA). After centrifugation, the precipitates were washed twice with 5% TCA, twice with ethanol-ether (1:3) and finally with 70% ethanol to remove any unbound or loosely bound radioactivity. The final dried precipitates were dissolved in 0.1N NaOH and suitable aliquots were counted after mixing with Aquasol® to determine the bound radioactivity. Due to variations in the stability of $^{14}CH_2O$ and/or its metabolite(s) binding to macromolecules, all conditions and treatment procedures were kept similar so that in all tissues the remaining radioactivity could be considered as firmly bound. Thus, all the binding studies in the present work are comparable among the various tissues tested.

Fractionation of ^{14}C-Activity in Macromolecules

Known amounts of tissue homogenates were precipitated with 10% (w/v) TCA, centrifuged and the residues washed twice with

Table 1. Levels of ^{14}C-activity eliminated in expired air, urine and feces

Time (hr)	μg-equivalent [^{14}C]-formaldehyde[a]			
	Expired air ($^{14}CO_2$)	Urine	Feces	Total
0–0.5	1848.8 ± 161 (10.3)	—	—	1848.8 (10.3)
0.5–1	1675.0 ± 121 (9.3)	491.2 ± 36 (2.7)	0.7 ± 0.05 (0.004)	2166.9 (12.0)
1–3	3245.9 ± 241 (18.0)	567.6 ± 49 (3.2)	0.6 ± 0.03 (0.004)	3814.1 (21.2)
3–6	453.3 ± 31 (2.5)	494.2 ± 33 (2.8)	0.8 ± 0.05 (0.005)	948.3 (5.3)
6–12	124.4 ± 9 (0.6)	400.5 ± 37 (2.2)	2.2 ± 0.07 (0.012)	527.1 (2.9)
12–24	72.8 ± 7 (0.4)	365.1 ± 26 (2.1)	6.2 ± 0.3 (0.034)	444.1 (2.5)
24–48	35.0 ± 4 (0.2)	231.4 ± 19 (1.3)	11.7 ± 0.8 (0.061)	243.1 (1.4)
48–72	22.6 ± 2 (0.1)	176.0 ± 9 (1.0)	6.5 ± 0.7 (0.036)	205.1 (1.1)
Total	7477.7 (41.4)	2726.0 (15.3)	28.7 (0.16)	10232.4 (56.9)

[a] Values are means ± S.D. of three animals. Numbers in parentheses are percent of dose administered

5% (w/v) TCA and further separated in lipid, DNA, RNA, and protein fractions as follows:

Lipids: The precipitate obtained after TCA washing was extracted first with ethanol-ether (1:3) and then with methanol-chloroform (1:2) for the extraction of total lipids. Phospholipids were further precipitated from the total lipid extracts by mixing equal volumes of chilled acetone with the lipid extract, the supernatant being considered as a neutral lipid fraction. The residue after centrifugation, containing phospholipids, was washed twice with cold acetone to remove any unbound radioactivity or other contaminations. Total lipid and phospholipid contents were estimated by weighing the residue obtained after evaporating the extract to dryness under vaccum.

Nucleic Acids and Proteins: The residue after total lipid extraction was digested with deoxyribonuclease I (type CL, Sigma Chemical Co, St. Louis, MO) in 0.1 M Tris-HCl, pH 7.5, 5 mM $MgCl_2$ at 37°C for 1 hr. After incubation, an equal volume of 10% TCA was added to the mixture and centrifuged at 2,200 g for 20 min. The supernatant was designated as the DNA fraction. The pellet was suspended in 0.1 M Tris-HCl, pH 7.5, and digested with ribonuclease A (type XII, Sigma Chemical Co, St. Louis, MO) at 37° for 2 hr. The incubation was terminated by the addition of an equal volume of 10% TCA and the mixture centrifuged at 2,200 g for 30 min. The supernatant was designated as the RNA fraction and the remaining pellet was the protein fraction. The radioactivity and the concentration of each macromolecule were determined.

Other Determinations

DNA was determined by the method of Burton (1968) using calf-thymus DNA as the standard. RNA was determined by the orcinol method, using yeast RNA as the standard (Schneider 1957). Protein was estimated by the method of Bradford (1976) with bovine serum albumin as the standard. The radioactivity determined in macromolecular fractions was converted to nmol-equivalent per mg macromolecule, based upon the specific activity of the dose administered. Statistical comparisons were carried out by Student t-test ($p < 0.01$).

Repeated preliminary studies following the $^{14}CH_2O$ administration, showed no decrease in concentration of radioactivity upon drying aliquots of the urine, feces, and tissue homogenates. Thus, the radioactivity present in each case was considered to be an accurate representation of nonvolatile products.

Results

General Animal Behavior

Within 20–30 min following the single intraperitoneal administration of formaldehyde, rats developed some cholinomimetic signs, such as reddening of nose, salivation, and lacrimation. The animals were sluggish and dull and remained in this condition for about 4 hr. The animals returned to their normal condition in 4 to 6 hr. At the end of 6 hr of dosing, all the animals had mild diarrhea, which disappeared by the end of 12 hr.

Elimination of ^{14}C-Activity from the Animal Body

The elimination of radioactivity derived from [^{14}C]-formaldehyde in expired air, urine, and feces following a single intraperitoneal administration is shown in Table 1. Within the first half hour, 10% of

the dose was recovered in expired air trapped as $^{14}CO_2$. The expired air was not analyzed for unchanged formaldehyde, because it is unlikely that such a reactive aldehyde will remain free to be exhaled (Ulsamer *et al.* 1984). By 3 hr post-$^{14}CH_2O$ administration, a total of 40% was exhaled as $^{14}CO_2$. Elimination of ^{14}C-activity in expired air declined sharply after 3 hr and comparatively little was exhaled by the animals after that. At the end of 72 hr, the cumulative percent of radioactivity eliminated by expired air was 41% of the total dose.

No volatile radioactivity was detectable in the urine and feces. Urinary excretion of ^{14}C-activity over time was fairly constant although a slight peak occurred between 1 and 3 hr of dosing, which decreased gradually with time. The total elimination of the $^{14}CH_2O$ activity via the urine in the 72 hr post-administration was about 15%. The major amount of urinary excretion (13%) occurred within the first 24 hr period. The total excretion of radioactivity in feces over 72 hr was only 0.2% of the administered dose. In 72 hr following $^{14}CH_2O$ administration, the total elimination of radioactivity in expired air, urine, and feces was 57% of the dose.

Levels of Radioactivity in Total Blood, Red Blood Cells, and Plasma

The incorporation of radioactivity derived from $^{14}CH_2O$ in blood was studied as a function of time (Figure 1). As depicted in Figure 1A, the highest levels of ^{14}C in blood were attained within the first hour. Plasma ^{14}C levels declined consistently with time, whereas, in red blood cells, there was a decline until 6 hr, which was followed by a significant elevation. The radioactivity was retained in significant amounts in red blood cells, even at the end of 72 hr after the single administration. Most of the radioactivity of erythrocytes was associated with hemoglobin. On further fractionation of hemoglobin, the globin fraction showed a higher retention of ^{14}C-activity as compared to the heme fraction (Figure 1B). The association of ^{14}C-activity in the globin fraction increased with time, whereas, that in the heme fraction decreased concomitantly.

Distribution of Radioactivity in Rat Tissues

The levels of radioactivity in various rat tissues as a function of time in units of μg-equivalents of $^{14}CH_2O$/g tissue are shown in Table 2. Following a single administration of $^{14}CH_2O$, high concentrations of radioactivity were found in liver, kidney,

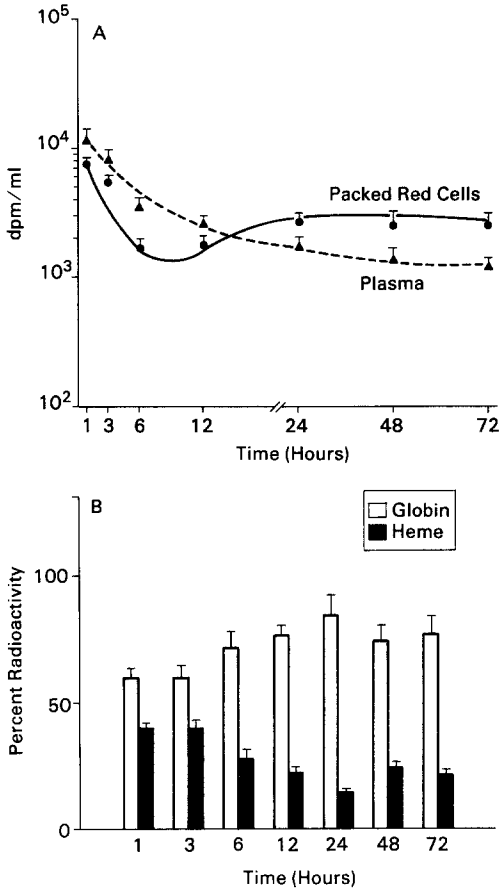

Fig. 1. Time-course of [^{14}C]-activity derived from $^{14}CH_2O$ following a single intraperitoneal administration [72 mg (14.7 μCi)/kg]. (A) in red cells and plasma (B) percent radioactivity in hemoglobin fractions. The values are mean ± S.D. of three animals

and gastrointestinal (GI) tract in the first 6 hr, followed by a gradual decline with the time. Spleen, thymus, lung, and trachea also had considerably higher amounts of ^{14}C-activity in the first 3 hr. In all the tissues, peak levels occurred between 1 and 3 hr. Brain tissue had the lowest levels at the time periods studied. Although radioactivity declined with time, significant amounts were measurable in all the tissues, even 72 hr after treatment. The kinetics of absorption and elimination ($t_{½}Ka$, $t_{½}oC$ and $t_{½}\beta$) are shown in Table 3. The rate of uptake in all the tissues studied was very rapid with the $t_{½}\beta$ of absorption rate (Ka) being less than 1 hr. Elimination rate varied somewhat between tissues. The tissues with slow elimination rates were the brain, heart and kidney with $t_{½}\beta$ values of 288.7, 187.3 and 121.6 hr, respectively. The $t_{½}\beta$ for all the other tissues ranged between 50 and 100 hr.

In order to assess the absorption and distribution of $^{14}CH_2O$, the distribution coefficients (ratio of

Table 2. Tissue levels of radioactivity following intraperitoneal administration of [^{14}C]-formaldehyde

Tissue	μg-equivalent [^{14}C]-formaldehyde/g tissue						
	1 hr	3 hr	6 hr	12 hr	24 hr	48 hr	72 hr
Blood[a]*	21.4 ± 1.4	14.8 ± 1.1	5.5 ± 0.4	4.9 ± 0.3	3.6 ± 0.3	3.4 ± 0.2	3.0 ± 0.2
	(0.12 ± 0.01)	(0.08 ± 0.01)	(0.03 ± 0.0)	(0.03 ± 0.0)	(0.02 ± 0.0)	(0.02 ± 0.0)	(0.02 ± 0.0)
Liver	34.8 ± 3.0	5.26 ± 4.9	26.8 ± 3.0	23.2 ± 2.1	22.0 ± 1.6	12.8 ± 1.3	15.6 ± 1.4
	(0.20 ± 0.02)	(0.29 ± 0.03)	(0.15 ± 0.01)	(0.13 ± 0.01)	(0.12 ± 0.01)	(0.07 ± 0.01)	(0.09 ± 0.01)
Spleen	27.7 ± 2.3	30.8 ± 3.3	19.8 ± 2.0	25.9 ± 2.1	22.6 ± 1.9	15.4 ± 1.8	9.2 ± 0.5
	(0.16 ± 0.01)	(0.17 ± 0.02)	(0.11 ± 0.01)	(0.14 ± 0.02)	(0.13 ± 0.01)	(0.09 ± 0.01)	(0.05 ± 0.0)
Kidney	34.1 ± 4.6	28.4 ± 3.1	20.0 ± 1.6	18.5 ± 1.5	17.0 ± 1.2	13.0 ± 1.3	15.4 ± 1.6
	(0.20 ± 0.01)	(0.16 ± 0.02)	(0.11 ± 0.01)	(0.10 ± 0.01)	(0.10 ± 0.01)	(0.07 ± 0.01)	(0.09 ± 0.01)
Thymus	31.0 ± 3.5	33.2 ± 4.0	24.6 ± 2.6	25.8 ± 2.6	38.7 ± 4.2	17.6 ± 2.0	22.7 ± 3.0
	(0.17 ± 0.02)	(0.18 ± 0.02)	(0.14 ± 0.01)	(0.14 ± 0.01)	(0.22 ± 0.02)	(0.09 ± 0.01)	(0.13 ± 0.01)
GI Tract	35.4 ± 3.3	46.0 ± 4.8	38.1 ± 4.1	31.9 ± 3.4	21.6 ± 2.6	14.1 ± 1.3	11.7 ± 1.0
	(0.21 ± 0.02)	(0.26 ± 0.03)	(0.22 ± 0.02)	(0.18 ± 0.01)	(0.12 ± 0.01)	(0.07 ± 0.01)	(0.01 ± 0.0)
Heart	17.8 ± 1.3	15.0 ± 1.8	11.2 ± 1.0	13.2 ± 1.4	8.6 ± 0.6	9.5 ± 0.7	9.3 ± 1.0
	(0.09 ± 0.01)	(0.08 ± 0.01)	(0.06 ± 0.0)	(0.07 ± 0.01)	(0.05 ± 0.0)	(0.06 ± 0.0)	(0.06 ± 0.01)
Lung	22.2 ± 2.0	17.2 ± 1.5	13.4 ± 1.1	13.0 ± 1.3	13.2 ± 1.0	8.8 ± 0.7	11.5 ± 0.9
	(0.13 ± 0.01)	(0.09 ± 0.01)	(0.07 ± 0.01)	(0.07 ± 0.00)	(0.07 ± 0.01)	(0.05 ± 0.0)	(0.06 ± 0.01)
Trachea	19.8 ± 1.6	21.1 ± 2.0	11.9 ± 0.9	11.2 ± 1.0	14.1 ± 1.1	7.3 ± 0.5	6.8 ± 0.5
	(0.11 ± 0.01)	(0.12 ± 0.01)	(0.06 ± 0.0)	(0.06 ± 0.0)	(0.07 ± 0.10)	(0.04 ± 0.0)	(0.04 ± 0.0)
Brain	14.7 ± 1.1	14.3 ± 1.0	8.8 ± 0.6	8.2 ± 0.6	7.3 ± 0.6	7.7 ± 0.7	8.8 ± 0.7
	(0.07 ± 0.01)	(0.07 ± 0.0)	(0.05 ± 0.0)	(0.05 ± 0.0)	(0.04 ± 0.0)	(0.04 ± 0.0)	(0.05 ± 0.0)
Skin	15.8 ± 1.3	16.1 ± 1.1	8.2 ± 0.7	10.3 ± 0.8	11.5 ± 0.8	7.7 ± 0.5	8.8 ± 0.7
	(0.08 ± 0.01)	(0.09 ± 0.01)	(0.05 ± 0.0)	(0.06 ± 0.0)	(0.06 ± 0.0)	(0.04 ± 0.0)	(0.05 ± 0.0)

[a] Values are means ± S.D. of three animals. Values reported in parenthesis are percent of dose administered/g tissue. *μg-equivalent/ml

Table 3. Apparent half lives of radioactivity in various tissues[a]

Tissues	$t_{\frac{1}{2}}K_a$[b] (hr)	$t_{\frac{1}{2}}\alpha$[c] (hr)	$t_{\frac{1}{2}}\beta$[c] (hr)	Area under the curve (μg/g · hr)
Blood	0.56	0.85	99.00	731.5
Liver	0.83	2.65	86.63	3329.5
Spleen	0.52	0.66	47.14	1907.9
Kidney	0.48	0.99	121.58	3514.0
Heart	0.24	3.84	187.30	3032.1
Lung	0.26	1.71	101.91	2218.3
Trachea	0.61	1.20	67.28	1337.1
Brain	0.41	2.77	288.75	3635.6

[a] The apparent rate constants K, α and β and their respective intercepts were estimated using nonlinear least squares fit of data by NONLIN (Metzler *et al.*, 1976). The fitted values were subsequently used to calculate half lives and areas under the curve

[b] K_a is the apparent rate (hr^{-1}) constant of absorption of the radioactivity in the tissue

[c] α and β are, respectively, the apparent rapid and slow elimination rate (hr^{-1}) constants of radioactivity from each tissue

μg-equivalent $^{14}CH_2O$ in tissue to that in blood) of major tissues (Table 4) was also determined. The blood contained less radioactivity than most of the tissues at various time intervals. The distribution coefficient of most of the tissues was maximum at 24 hr after dosing.

Molecular Interactions of [^{14}C]-Formaldehyde

The macromolecules from tissue homogenates were precipitated and exhaustively extracted as mentioned earlier. The *in vivo* association of [^{14}C]-formaldehyde in various tissues in terms of ng-equivalent/mg protein are represented in Table 5. The total concentrations of ^{14}C derived from $^{14}CH_2O$ in most of the tissues were highest in the first hour after dosing. However, liver, trachea, and skin tissues showed peak levels at the end of 3 hr. There was a persistent decline in ^{14}C concentration with time in all the tissues. Most of the tissues had at least 18% of the respective total ^{14}C-activity firmly bound to the macromolecules in the first hour, whereas, thymus, spleen, and liver tissues showed exceptionally higher binding (27–36%) in the first hour. Although the total radioactivity from $^{14}CH_2O$ in tissues declined with time, the relative percent binding to macromolecules increased. By the end of 72 hr after initial dosing, most of the tissues showed 45–50% binding with respect to their total levels with the exceptions of brain and skin tissues whereas only 36% incorporation was observed at the end of 72 hr. Substantial binding (77%) was observed in the case of spleen at the end of 72 hr.

Table 4. Distribution coefficient of [^{14}C]-formaldehyde following intraperitoneal administration[a]

Tissue	Time (hr)						
	1	3	6	12	24	48	72
Liver	1.63 ± 0.14	3.45 ± 0.33	5.05 ± 0.44	4.93 ± 0.31	6.01 ± 0.51	4.26 ± 0.35	4.90 ± 0.44
Spleen	1.34 ± 0.01	2.08 ± 0.18	3.64 ± 0.32	5.19 ± 0.43	6.20 ± 0.55	4.33 ± 0.35	3.07 ± 0.30
Kidney	1.54 ± 0.18	1.82 ± 0.14	3.64 ± 0.33	3.78 ± 0.03	4.75 ± 0.43	3.72 ± 0.33	4.93 ± 0.46
Thymus	1.45 ± 0.14	2.14 ± 0.16	4.37 ± 0.40	5.27 ± 0.53	9.45 ± 0.89	5.08 ± 0.48	6.87 ± 0.59
Heart	0.83 ± 0.07	1.09 ± 0.09	2.04 ± 0.21	2.69 ± 0.21	2.70 ± 0.22	2.79 ± 0.22	2.94 ± 0.23
Lung	1.04 ± 0.09	1.26 ± 0.10	2.49 ± 0.19	2.65 ± 0.23	3.17 ± 0.27	2.59 ± 0.24	3.63 ± 0.30
Trachea	0.93 ± 0.07	1.43 ± 0.12	2.06 ± 0.17	2.29 ± 0.18	3.32 ± 0.31	2.35 ± 0.20	2.27 ± 0.21
Brain	0.73 ± 0.05	0.97 ± 0.07	1.57 ± 0.11	1.67 ± 0.12	2.03 ± 0.17	2.32 ± 0.19	2.93 ± 0.24

[a] Distribution coefficient is the ratio of μg equivalent formaldehyde/g tissue to μg equivalent/g blood. Values are means ± S.D. of three animals

Table 5. Recovery of total and protein bound radioactivity from [^{14}C]-formaldehyde in tissue homogenates[a]

Tissue	[^{14}C]-formaldehyde, ng-equivalent/mg protein									
	1 hr		3 hr		6 hr		24 hr		72 hr	
	Total	Bound	Total	Bound	Total	Bound	Total	Bound	Total	Bound
Liver	589 ± 43	165 ± 18 (27%)	665 ± 76	228 ± 19 (34%)	462 ± 40	209 ± 16 (45%)	454 ± 39	202 ± 21 (44%)	286 ± 25	131 ± 14 (46%)
Spleen	554 ± 46	171 ± 16 (31%)	466 ± 41	213 ± 19 (46%)	319 ± 29	169 ± 16 (53%)	449 ± 42	257 ± 19 (57%)	181 ± 13	140 ± 16 (77%)
Kidney	758 ± 61	141 ± 15 (19%)	391 ± 30	145 ± 10 (37%)	306 ± 19	124 ± 11 (41%)	358 ± 21	151 ± 14 (42%)	328 ± 26	134 ± 14 (41%)
Thymus	724 ± 62	264 ± 21 (36%)	635 ± 50	375 ± 28 (59%)	528 ± 48	315 ± 31 (60%)	853 ± 78	432 ± 37 (51%)	655 ± 61	379 ± 25 (58%)
GI Tract	978 ± 80	208 ± 16 (21%)	1198 ± 96	336 ± 27 (28%)	1301 ± 101	487 ± 37 (38%)	523 ± 47	264 ± 21 (51%)	306 ± 30	151 ± 15 (49%)
Heart	489 ± 40	87 ± 7 (18%)	229 ± 19	82 ± 11 (36%)	340 ± 24	145 ± 11 (43%)	218 ± 18	116 ± 9 (53%)	208 ± 17	101 ± 8 (49%)
Lung	773 ± 62	117 ± 11 (15%)	386 ± 31	129 ± 19 (33%)	400 ± 36	177 ± 11 (44%)	317 ± 22	119 ± 12 (38%)	307 ± 28	153 ± 14 (50%)
Trachea	849 ± 73	161 ± 13 (19%)	947 ± 82	205 ± 12 (22%)	701 ± 52	187 ± 14 (27%)	564 ± 44	151 ± 15 (27%)	483 ± 33	211 ± 13 (44%)
Brain	410 ± 31	77 ± 8 (19%)	423 ± 33	112 ± 9 (26%)	279 ± 21	101 ± 9 (36%)	223 ± 21	87 ± 6 (39%)	235 ± 19	84 ± 7 (36%)
Skin	571 ± 41	115 ± 13 (20%)	866 ± 58	235 ± 23 (27%)	495 ± 41	172 ± 15 (35%)	440 ± 33	167 ± 14 (38%)	414 ± 34	149 ± 13 (36%)

[a] Values are means ± S.D. of three animals. Values in parentheses represent percent binding of the total radioactivity in homogenates

Subcellular Localization of Radioactivity: Subcellular distribution and the incorporation of radioactivity into the macromolecules of cell organelles were studied in four major tissues at 2-time periods (Figure 2). As expected due to its reactive nature, the radioactivity derived from [^{14}C]-formaldehyde was indiscriminately distributed in all the subcellular fractions of various tissues studied. At the end of 3 hr, the cytosol fraction of liver and kidney contained the highest levels of total radioactivity. Whereas, in other tissues at both the time periods, the mitochondrial fraction showed highest levels of total radioactivity as compared to most other subcellular fractions. The only exception was the lung at 3 hr, where the microsomal fraction had the highest content of total radioactivity. In the subcellular fractions of most tissues, the levels of unbound radioactivity were decreased, whereas the bound radioactivity was slightly increased over a period of 24 hr. The highest levels of relative percent binding were observed in the microsomal fraction whereas cytosol fractions contained lowest levels of bound radioactivity.

Macromolecular Incorporation of Radioactivity: The homogenates of five major tissues were frac-

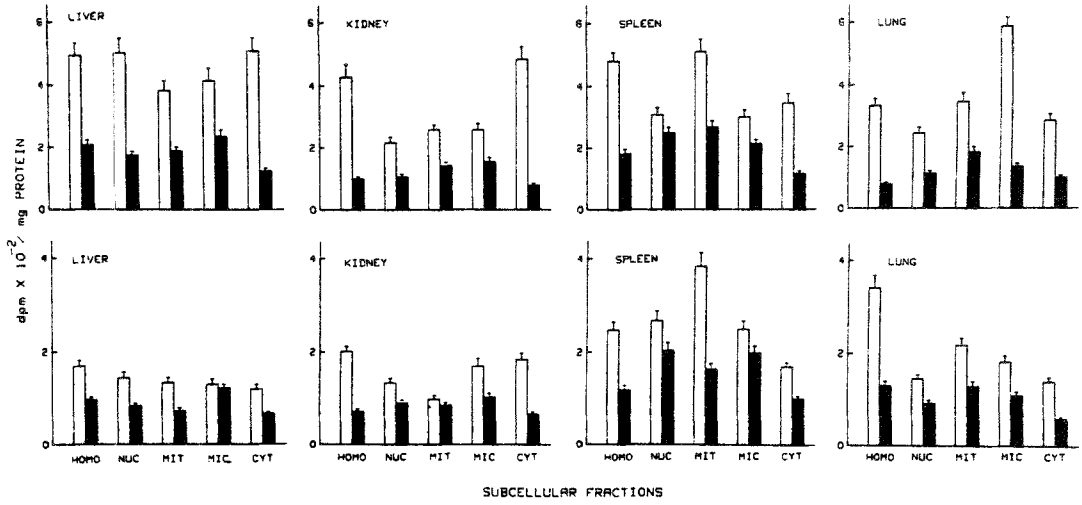

Fig. 2. Content of bound ^{14}C-activity from $^{14}CH_2O$ in various subcellular fractions 3 hr (top row) and 24 hr (bottom row) after a single intraperitoneal administration [72 mg (14.7 μCi)/kg]. Open bars represent total radioactivity and solid bars, bound ^{14}C-activity. HOMO, whole homogenate; NUC, nuclear; MIT, mitochondrial, MIC, microsomal and CYT, cytosol fractions. Each bar represent mean ± S.D. of three animals.

Table 6. Macromolecular incorporation of radioactivity following intraperitoneal administration of [^{14}C]-formaldehyde

Tissue	Time (h)	μg-equivalent [^{14}C]-formaldehyde/g tissue				
		DNA	RNA	Protein	Lipid	Total[a]
Liver	3	1.16 ± 0.08	0.68 ± 0.08	19.72 ± 1.30	0.80 ± 0.06	22.36 (43%)
	12	0.46 ± 0.04	0.40 ± 0.03	12.37 ± 1.00	0.04 ± 0.02	13.63 (59%)
	48	0.32 ± 0.02	0.37 ± 0.05	7.58 ± 0.60	0.19 ± 0.01	8.46 (66%)
Spleen	3	1.04 ± 0.10	0.93 ± 0.10	15.38 ± 1.42	0.37 ± 0.02	17.72 (58%)
	12	1.26 ± 0.11	1.01 ± 0.10	14.65 ± 1.41	0.32 ± 0.03	17.24 (67%)
	48	0.38 ± 0.05	0.41 ± 0.03	8.64 ± 0.72	0.22 ± 0.01	9.65 (63%)
Kidney	3	0.46 ± 0.05	0.43 ± 0.04	11.50 ± 1.05	0.41 ± 0.03	12.80 (45%)
	12	0.43 ± 0.05	0.42 ± 0.04	9.33 ± 0.85	0.32 ± 0.03	10.50 (57%)
	48	0.39 ± 0.04	0.33 ± 0.03	6.20 ± 0.58	0.23 ± 0.01	7.15 (55%)
Heart	3	0.40 ± 0.03	0.33 ± 0.04	5.87 ± 0.36	0.19 ± 0.01	6.79 (45%)
	12	0.34 ± 0.04	0.36 ± 0.03	4.66 ± 0.30	0.15 ± 0.01	5.51 (42%)
	48	0.30 ± 0.03	0.32 ± 0.02	4.68 ± 0.36	0.16 ± 0.02	5.46 (57%)
Lung	3	0.43 ± 0.05	0.39 ± 0.04	6.28 ± 0.52	0.36 ± 0.03	7.45 (43%)
	12	0.36 ± 0.04	0.32 ± 0.03	6.01 ± 0.55	0.26 ± 0.02	6.94 (53%)
	48	0.33 ± 0.02	0.30 ± 0.03	4.00 ± 0.31	0.21 ± 0.01	4.84 (55%)

[a] Values in the parentheses are the percent macromolecular incorporation of the total μg equivalent ^{14}C-formaldehyde/g tissue. Values are the mean ± S.D. of three animals

tionated into DNA, RNA, protein, and lipid fractions as described earlier and the incorporation of ^{14}C-activity determined in each macromolecular fraction. The *in vivo* association of ^{14}C-activity in these fractions in terms of μg-equivalent [^{14}C]-formaldehyde per gram tissue is shown in Table 6. Radioactivity in the tissues decreased with time, whereas the bound levels increased. DNA, RNA, protein, and lipid fractions of liver and spleen tissues showed higher levels of ^{14}C-incorporation as compared to the other tissues, whereas, the heart tissue contained the lowest levels.

The *in vivo* incorporation curve of ^{14}C-activity associated with nucleic acids from [^{14}C]-formaldehyde showed a higher association of $^{14}CH_2O$ with RNA in all the tissues studied (Figure 3). The radioactivity in RNA of most tissues increased with time. The maximum registration of radioactivity of RNA was at 48 hr after the administration, followed by a decline at 72 hr in all the tissues. Considerably higher registration of radioactivity in RNA in all the tissues was observed even at the end of 72 hr (Figure 3A). Significantly higher amounts of ^{14}C-activity were registered in DNA from all the tissues.

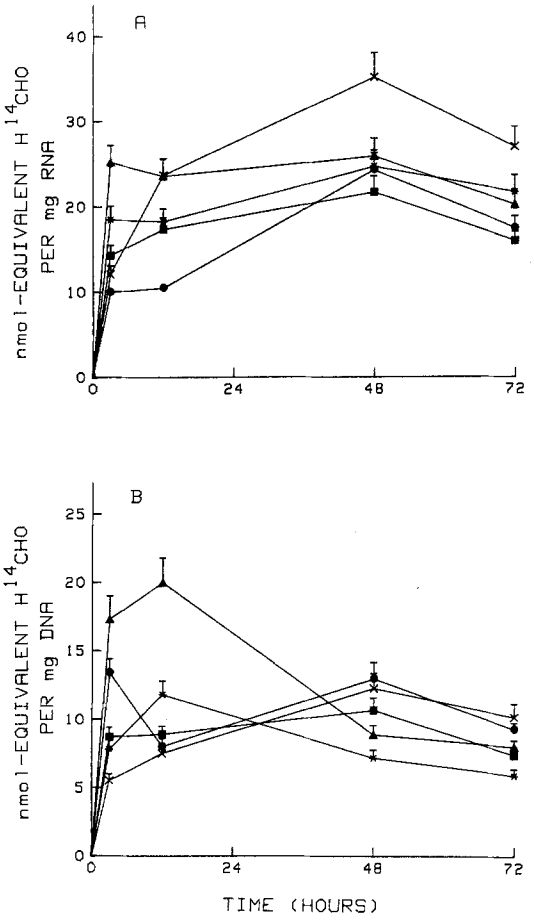

Fig. 3. ^{14}C-activity content in [A] RNA and [B] DNA of various rat tissues as a function of time following single intraperitoneal administration of formaldehyde [72 mg (14.7 μCi)/kg]. (—●—) liver; (—▲—) spleen; (—■—) kidney; (—X—) heart; and (—*—) lung. Each value represents the mean ± S.D. of three animals

The liver DNA showed maximal levels of radioactivity at 3 hr with a second peak at 48 hr, whereas DNA from other organs, with the exception of the heart, showed maximal levels at 12 hr after the $^{14}CH_2O$ administration. The radioactivity in heart DNA increased with time until 48 hr, and was followed by a slight decline. All the tissues studied displayed significantly higher amounts of ^{14}C-activity even at the end of 72 hr following the initial administration (Figure 3B).

Table 7 shows the incorporation of radioactivity in phospholipid fractions of various tissues. Of the total ^{14}C-activity incorporated into the lipid fractions of various tissues at different time intervals, over 55% was registered in the phospholipid fractions. Lung phospholipid fractions had highest levels at all time periods compared to other tissues. The ^{14}C-activity of total lipid fraction declined with time, whereas the incorporation into the phospholipids increased concomitantly.

Table 7. Incorporation of radioactivity in phospholipids after [^{14}C]-formaldehyde administration

	μg-equivalent [^{14}C]-formaldehyde/g tissue[a]		
Tissue	3 hr	12 hr	48 hr
Liver	0.50 ± 0.05 (63%)	0.24 ± 0.02 (60%)	1.10 ± 0.01 (53%)
Spleen	0.17 ± 0.02 (47%)	0.17 ± 0.01 (53%)	0.12 ± 0.01 (55%)
Kidney	0.23 ± 0.01 (57%)	0.19 ± 0.02 (58%)	0.14 ± 0.01 (62%)
Heart	0.11 ± 0.01 (60%)	0.09 ± 0.01 (63%)	0.10 ± 0.01 (63%)
Lung	0.22 ± 0.02 (61%)	0.17 ± 0.01 (67%)	0.15 ± 0.02 (71%)

[a] Values are the mean ± S.D. of three animals. Values in parentheses are the percent of total lipid fraction

Discussion

Formaldehyde, a one-carbon compound is a highly reactive chemical which undergoes extensive biotransformation in various biological systems (Neely 1964; Kitchens *et al.* 1976). In many tissues of various species, formaldehyde is metabolized rapidly to formic acid followed by further oxidation to carbon dioxide and water (Malorny *et al.* 1965; McMartin *et al.* 1979).

The results of our study indicated that $^{14}CO_2$ exhalation was the major route of formaldehyde elimination from the animal. A total of 41% of the administered dose was exhaled by rats in 72 hr. Another 15% of the total ^{14}C-activity was eliminated in urine and feces. Neely (1964) has reported an 82% elimination of $^{14}CO_2$ in expired air within 48 hr of $^{14}CH_2O$ administration. This difference in the rate of elimination of formaldehyde could be due to the different animal sex and strains employed. Mashford and Jones (1982) also suggested that the rates of elimination of formaldehyde could be different in various strains of rats. However, our findings were very similar to those of Heck *et al.* (1982), although the route of administration was by inhalation. The present study indicates that substantial radioactivity remains in the rat tissues 72 hr after initial i.p. administration. Due to its reactive nature, entry of the carbon atom of $^{14}CH_2O$ into a C_1-pool makes it a precursor for a large number of biological compounds. Thus, the high levels of residual radioactivity could be due to metabolic incorporation either by binding to macromolecules or perhaps by forming unexcretable conjugates.

In the present study, it was also observed that the initial higher radioactivity in erythrocytes, following single administration of $^{14}CH_2O$, is followed by a rapid decline. Subsequently, radioactivity in

erythrocytes increased again and surpassed the radioactivity in plasma. Incorporation of ^{14}C-activity in erythrocytes also indicates the bioactivation of $^{14}CH_2O$ molecule, since the ^{14}C-activity in plasma declined significantly with time.

The radioactivity from $^{14}CH_2O$ was distributed in all of the rat tissues. Liver, kidney, and gastrointestinal tract had the higher levels of ^{14}C-activity compared to other tissues. The presence of a higher ^{14}C-activity in the GI tract could be due to the continuous resecretion of $^{14}CH_2O$ and/or its metabolites in the GI tract via the bile which, in turn, could cause constant irritation of intestinal mucosa and lead to diarrhea.

Resistance to exhaustive extraction of radioactivity also indicates the incorporation of radioactive fragments into macromolecules. Thus, it suggests that the incorporation of ^{14}C could either be incorporated in the existing macromolecules or formate may be incorporated into amino acids or nucleic acid monomers (Malorny *et al.* 1965; Bowes and Cater 1968; Pruett *et al.* 1980).

In the present *in vivo* study the registration of ^{14}C-activity into macromolecules of various rat tissues was observed. The variations in the incorporation of radioactivity in nucleic acids, between the organs and with time is most likely due to different rates of RNA and DNA turnover in different tissues. Therefore, it is not surprising that nucleic acids from spleen and liver, organs characterized by a high metabolic activity, showed higher ^{14}C incorporations compared to other organs.

Formaldehyde is extensively used in structural and functional studies of nucleic acids. It undergoes several reactions with the nucleic acids and their components. *In vitro* rapid primary reaction is the formation of methylol derivatives (Fraenkel-Conrat 1954) and a slower reaction results in the formation of cross-links at both the monomer and polymer levels (Feldman 1973). Chemical agents that covalently cross-link nucleic acids are important mutagens, carcinogens, antitumor agents and other toxic substances (Dubelman and Shapiro 1977). Genetic effects of formaldehyde including mutations in microorganisms and insects are very well known and these effects are considered to be the results of such cross-linking reactions (Auerbach *et al.* 1977). In the present *in vivo* study, we observed that ^{14}C-activity from $^{14}CH_2O$ is well incorporated into the nucleic acids and higher levels of binding were prominent in various tissues even 72 hr after a single administration. On the basis of various *in vitro* studies, indicating the participation of purines and cytosine in the cross-linking of nucleic acids by formaldehyde (Auerbach *et al.* 1977; Feldman 1973; Chaw *et al.* 1980), it can be postulated that crosslinking of nucleic acids by formaldehyde and/or its metabolite(s) is possible in animals following long-term or higher levels of exposures, and could thus induce mutagenesis in animals as well as humans. Siomin *et al.* (1974) have suggested that *in vitro* mutagenic action of formaldehyde is associated with an action on the DNA by reaction products of formaldehyde with amino-containing compounds. In fact, Heminki (1982) has reported the *in vitro* formation of N^2-methylguanine, N^6-methylguanine and N^4-methylcytosine from CH_2O-treated nucleic acids. If stable cross-links are formed *in vivo* between DNA reactive sites and formaldehyde or reaction products, then these links could interfere with the replication of DNA and may result in mutations. Grafstorm *et al.* (1985) have reported inhibition of O^6-methylguanine repair in human bronchial fibroblasts which potentiated mutagenicity of an alkylating agent N-methyl-N-nitrosourea. Formaldehyde-induced mutagenicity has also been reported by Alderson (1985).

It is evident from the *in vitro* studies (Siomin *et al.* 1974) that the amino acids lead to the primary reaction of formaldehyde to form mono and dimethylol derivatives. The rate of this process is far greater than the rate of the interaction of formaldehyde with nucleic acids. Hence, the incorporation of ^{14}C-activity from $^{14}CH_2O$ should be higher in proteins than in nucleic acids. Registration of significantly higher levels of ^{14}C-activity in protein fractions of various tissues in the present study could be due to the incorporation of ^{14}C, probably as formate, into amino acids and then into proteins. Formaldehyde is more chemically active than any of its direct metabolites and its interaction with various tissue macromolecules makes it the chemical substance of most concern for carcinogenicity. The pattern of metabolic incorporation of ^{14}C or ^{3}H-CH_2O has been studied (Cassanova-Schmitz *et al.* 1984).

The subcellular fractionation studies indicated an indiscriminate registration of ^{14}C-activity in all the subcellular fractions of various tissues examined. Comparatively higher registration of radioactivity from $^{14}CH_2O$ and/or its metabolites in the microsomal fraction could suggest a major involvement of microsomal oxidation. *In vivo* association of ^{14}C-activity in nuclear fractions can directly be correlated with the formation of intermolecular cross-linking between histone molecules as also suggested by Ohba *et al.* (1979) in their *in vitro* studies of formaldehyde reactions with calf thymus nucleohistones.

The present study also shows the incorporation of radioactivity into phospholipids. Duvigneaud *et al.* (1950) have demonstrated that incorporation of

^{14}C-activity in choline following $^{14}CH_2O$ administration to rats. One possibility for the registration of radioactivity in the phospholipids, mainly phosphatidyl ethanolamine, could be from methionine. The formation of methionine from $^{14}CH_2O$ has already been demonstrated (Berg 1951; Neely 1964).

The *in vivo* incorporation of ^{14}C-activity from $^{14}CH_2O$ into the macromolecular fractions of various tissues, as shown in the experiments, may offer a molecular basis for cellular changes that might be occurring following the formaldehyde exposure.

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