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THE METABOLIC FATE OF FORMALDEHYDE-^{14}C INTRAPERITONEALLY ADMINISTERED TO THE RAT

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Abstract—When formaldehyde-^{14}C was given intraperitoneally to the rat 82% of the dose was found in the expired air in the form of $^{14}CO_2$. The urine contained 13–14% of the isotope in the form of methionine, serine, and an adduct formed from cysteine and formaldehyde. The last material formed spontaneously by mixing normal urine and labeled formaldehyde.

WE HAVE been interested for some time in the action of formaldehyde on microorganisms.[1–3] During the course of this study we became involved in the metabolism of this 1-carbon compound by bacteria. Our finding of 1,3-thiazane-4-carboxylic acid in *Aerobacter aerogenes* treated with formaldehyde[2] prompted the present investigation of the metabolism of formaldehyde by the rat. While the fate of formaldehyde has been studied quite thoroughly in various preparations (see for example Berg,[4] Nakao and Greenberg,[5, 6] and Stevens and Sakami[7]), little or no work has been performed on the intact animal. As a sidelight to the present study, it was felt that the findings might elucidate the question of the active ingredient in methanol intoxication.[8–10]

EXPERIMENTAL

Adult white female rats weighing 130–150 g were used for each experiment. They were injected i.p. with a solution (1 ml) containing sufficient formaldehyde to give a final concentration in the range of 70 mg/kg. The total ^{14}C activity of each dose was 10μc. In another set of experiments the dose of formaldehyde was reduced to 7 mg/kg while maintaining the ^{14}C activity at 10 μc. Immediately after injection the animal was placed in an all-glass metabolism chamber equipped for collection of feces and urine.

Collection of $^{14}CO_2$

Incoming air was purified by passing through tubes containing Drierite (W. A. Hammond, Drierite Co., Xenia, Ohio) before entering the chamber. After the air left the chamber, it was passed through another column of Drierite and a saturated solution of $NaHSO_3$ before entering the ionization chamber in which radioactive counts were made with a Cary model 31 vibrating reed electrometer (Applied Physics Corp.). The electrometer was attached to a Brown recorder (Minneapolis–Honeywell) for continuous measurements of the $^{14}CO_2$. After leaving the ionization chamber

the air was pulled through a train consisting of two glass traps which contained 1 N NaOH (25 ml). Air was drawn through this system at a rate of 0·5 l/min. The traps were changed at 1- to 2-hour intervals. For longer collection periods a trap containing 200 ml of 1 N NaOH was used.

Determination of $^{14}CO_2$ in the sodium hydroxide traps

The method of Possmann *et al*[11] was modified by D. N. Robertson of this laboratory for the determination of low-level radioactivity in the expired CO_2 from rats. The modification consisted of making an enlarged Warburg flask containing a center well big enough to hold a Wheaton liquid scintillation vial. Hyamine 10-X[trademark of Rohm and Hass Chemical Corp., for *p*-(diisobutylcresoxyethyl)dimenthyl benzyl ammonium hydroxide] was purchased as 1 M solution in methanol from the Packard Instrument Co. An aliquot (5 ml) of the above solution was placed in a scintillation vial. The vial was placed in the modified Warburg vessel along with 5 ml of the $Na_2C^{14}O_3$ solution from the traps. Sulfuric acid (2 ml of a 25% solution) was added to the side arm of the flask. After sealing the entire system, the acid was tipped into the carbonate solution, and the flasks were shaken on a rotating table shaker for 2 hr to ensure complete absorption of the released CO_2 into the Hyamine solution. The vials were then filled with a standard toluene scintillator solution (15 ml), capped, and counted in an automatic liquid scintillation spectrometer (Packard Instrument Co.)

Identification of metabolites in the urine

In the identification of ^{14}C-labeled metabolites in the urine, the following techniques were used.

Paper chromatography. Paper strips one inch wide were spotted and chromatographed in the following solvents: (1) 80% phenol; (2) alcoholic ammonium hydroide, (100:1); (3) the top layer from *n*-butanol–acetic acid–water (4:1:5). After development in the particular solvent system, the chromatograms were scanned in a model C-100-A actigraph II (Nuclear-Chicago Corp.) with a windowless D-47 gas-flow counter (Nuclear-Chicago Corp.).

Column chromatography. The solutions were chromatographed on a Dowex 50-H^+ (200–400 mesh, X—12, 0·9 × 40 cm) with 1 N HCl as the eluting solvent. Fractions (5 ml) were collected and 0·4 ml used for liquid scintillation counting.

Estimation of formaldehyde in the urine. Formaldehyde-^{14}C activity in the urine was determined by conversion of the formaldehyde to formaldimethone.[12] This material was then dissolved in hot ethanol made up to 10 ml and an aliquot (0·4 ml) counted by means of liquid scintillation.

Periodate oxidation. Conversion of methionine to methionine sulfoxide was carried out as described by Stevens and Sakami.[7]

Estimation of metabolites in urine. A known aliquot of urine (100 μ liters) was spotted on paper and developed in the phenol solvent for 15 hr. After scanning the paper for ^{14}C activity, the corresponding sections of the paper were eluted with 1 ml water, and the resultant eluate was counted by liquid scintillation.

The ^{14}C-labeled formaldehyde (specific activity of 10 mc/mmole), was supplied by the New England Nuclear Corp., Boston, Mass.

RESULTS

The evolution of labeled CO_2 from a typical rat injected with ^{14}C formaldehyde is shown in Fig 1. The recovery data for the isotope are given in Table 1.

Identification of metabolites present in the urine

Figure 2 shows a typical scan of a sample of urine chromatographed in the phenol solvent for 15 hr. Table 2 indicates the distances that the urine metabolites and the

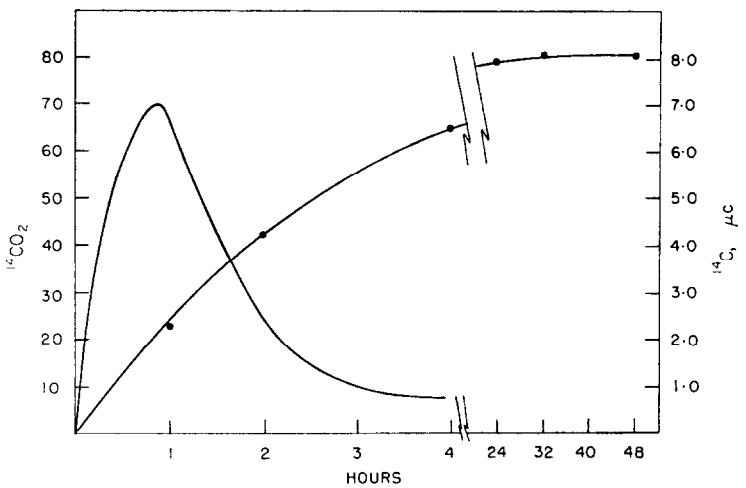

FIG. 1. $^{14}CO_2$ evolution by the rat after administration of ^{14}C formaldehyde (60·5 mg/kg). The total activity injected was 10 μc. The rate of $^{14}CO_2$ evolution is indicated by the left ordinate (the calibration is similar to that reported previously; see Ref. 1). The right ordinate represents the cumulative ^{14}C count in the CO_2 samples.

TABLE 1. RECOVERY OF ^{14}C IN THE URINE AND AIR FROM A RAT INJECTED WITH 10 μc OF LABELED FORMALDEHYDE

Total dose was 60·5 mg formaldehyde/kilo

Sample	Recovered (μc)	Initial Dose (%)
CO_2 in the expired air	8·20	82·0
Urine	1·39	13·9
Component 1,* 47%		
Component 2,† 38%		
Component 3, 7%		
Formaldehyde 1, 7%		

* Components 1, 2, and 3 refer to the three peaks found in Fig. 2 with R_f values of 0·69, 0·52, and 0·25 respectively.

† Components 2 and 3 were missing when the formaldehyde dose was 7·0 mg/kilo.

standards moved in the three solvent systems. In the following section the results of the experiments designed to establish the identity of the metabolites will be presented. *Component* 1, R_f 0·69 (*Fig.* 2). This metabolite had R_f values similar to methionine in the three solvents used. In addition it was eluted from the Dowex-50 column in a

manner analogous to methionine. On oxidation of the eluted peak from the Dowex-50 column with periodate, a material that chromatographed like methionine sulfoxide was formed (Fig. 3). A sample of urine was saturated with cold methionine, and the resulting solution was caused to crystallize. The crystals were recrystallized to a constant specific activity. The remaining urine after the methionine had been filtered off

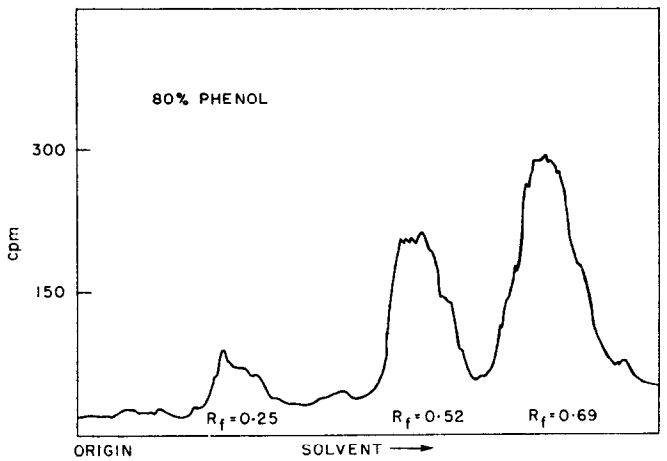

FIG. 2. A typical ^{14}C scan of 100 μl of urine from a treated animal chromatographed in the phenol solvent.

TABLE 2. RESULTS OF CHROMATOGRAPHY OF STANDARDS AND URINE FROM RATS INJECTED WITH ^{14}C-FORMALDEHYDE

Compound	Solvent systems (distance from the origin) Butanol (mm)	Phenol (mm)	Alcoholic ammonium hydroxide (mm)
Urine			
Component 1*	110	200	128
Component 2*	45	148	55
Component 3*	45	68	25
Standards			
Formate*	not detected	130	70
1,3-Thiazane-4-carboxylic acid*, †	50	265	55
Methionine*	110	200	130
Serine‡	45	68	24
Cysteine/HCHO*, §	50	148	53
Methionine sulfoxide*, ¶	15		

* Compounds were detected by means of ^{14}C activity in the chromatograms.

† Prepared previously; see Ref. 2.

‡ Detected by means of ninhydrin reagent.

§ Reaction product formed by mixing equal molar solutions of cysteine and formaldehyde, 1 hr prior to chromatography.

¶ Periodate oxidation of ^{14}C-methionine; see Ref. 7.

exhibited no activity for component 1 when chromatographed in a phenol solvent. *Component* 2, R_f 0·52. Periodate oxidation of the urine sample did not effect the mobility of this component on chromatography. This result tended to eliminate 1,3-thiazane-4-carboxylic acid as a possible candidate, since the thiazine derivative is destroyed on periodate oxidation.[13] In addition the component did not chromatograph like 1,3-thiazane-4-carboxylic acid on paper or on the column.[7] Mixing equal molar aqueous solutions of cysteine and ^{14}C-formaldehyde caused the formation of a material which behaved chromatographically like this metabolite (Table 2). Furthermore, adding ^{14}C-formaldehyde to a sample of normal urine caused the formation of a similar type of compound.

Component 3, R_f 0·25. This material behaved like serine in all three solvent systems. In addition it was destroyed by periodate oxidation of the urine sample.

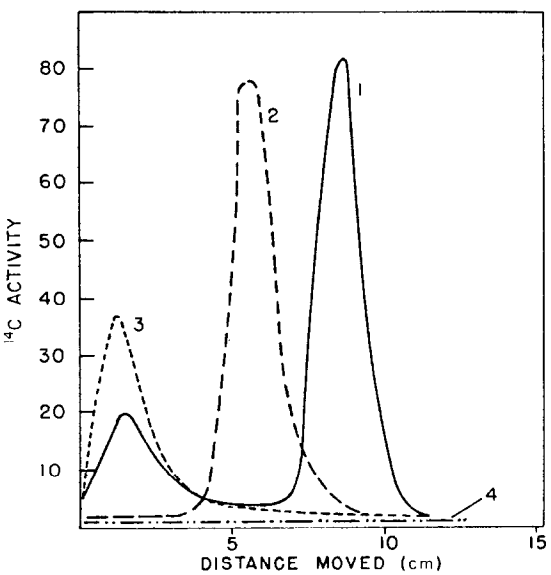

FIG. 3. Results of chromatographing various ^{14}C-labeled samples in the butanol solvent; 1 = standard ^{14}C-methionine; 2 = ^{14}C-1,3-thiazane-4-carboxylic acid; 3 = 1 after treatment with periodate; 4 = 2 after treatment with periodate. The ordinate represents per cent of full-scale deflection, where 100% = 300 cpm.

DISCUSSION

The formation of methionine as the main metabolite containing ^{14}C from the injected formaldehyde is not surprising in view of the work of Stevens and Sakami,[7] Berg,[4] and Nakao and Greenberg[5,6] on the biosynthesis of methionine by liver preparations. It must arise from a transfer of the 1-carbon compound to homocysteine by the folic acid cycle. The formation of serine would come from the same cycle in which glycine acted as the acceptor molecule. Speculating further one might visualize the formation of CO_2 from formaldehyde as occurring from transamination of serine to form pyruvate, which is a key intermediate in the Krebs cycle.

The presence of the cysteine adduct of formaldehyde is rather surprising in view of the work we performed in bacteria.[2] With bacteria, the main product was 1,3-thiazane-4-carboxylic acid which formed quite readily from homocysteine and formaldehyde. The cysteine analog was not found in the microorganisms. In the present case it appears that cysteine is a type of detoxifying agent converting the formaldehyde to a form that may be excreted by the animal. The fact that this material does not form at the lower doses of formaldehyde would indicate that the lower doses represent a level that the animal can handle without undue injury. This would correlate with the observations made on the animals—namely, at the lower dose of formaldehyde no behavioral disturbances were detected, whereas the higher dose caused a temporary paralysis of the hindquarters.

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