ABNORMAL OXIDATION OF ^{14}C-FORMALDEHYDE TO $^{14}CO_2$ IN ERYTHROCYTES OF ALCOHOLICS AND NONALCOHOLICS AFTER CONSUMPTION OF ALCOHOLIC BEVERAGES

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The continuous-flow ionization chamber method has achieved widespread use for the measurement of substrate oxidation in isolated tissues in vitro (*1*). We used this method for continuous measurements of enzyme activities directed toward the development of in vitro radioisotope techniques for biochemical (*2,3*) and clinical investigations (*4,5*), and also for early diagnosis of folic acid deficiency (*6*), hyperphenylalaninemia, maple-syrup urine disease, and glucose-6-phosphate dehydrogenase deficiency in man (*7*). Interestingly, we showed that this method was also a useful tool for continuous measurement of nonenzymatic oxidation of several substrates, i.e., DL-3,4-dihydroxyphenylalanine (*8*), and DL-histidine (*9*). The present study is a further evaluation of this method for measurement of $^{14}CO_2$ production from ^{14}C-formaldehyde incubated with erythrocytes obtained from alcoholics and nonalcoholics after consumption of alcoholic beverages. Studies on the incorporation of formaldehyde into human erythrocytes incubated with and without ethanol have also been included.

TABLE 1. EXPERIMENTAL SUBJECTS (MEDIAN VALUES AND RANGE)

	No. of subjects	Age (yr)	Duration of alcoholism (yr)
Normal volunteers	5	28 (22–36)	None
Alcoholics 0–12 hr, 12–24 hr and then 2–5 days after consumption of alcoholic beverages (Group A)	4	43.2 (40–47)	7.3 (4–10)
Alcoholics 3–21 days after consumption of alcoholic beverages (Group B)	5	46.8 (33–65)	5 (1–10)

MATERIALS AND METHODS

$^{14}CO_2$ production study. Subjects for this study were selected from workers and patients of the Clinic at the Centre Hospitalier Universitaire in Sherbrooke, Quebec. The five normal subjects had not consumed alcohol before this study. The diagnosis of alcoholism among the nine alcoholic subjects was judged fairly certain by specialists at the Alcoholic Clinic. Table 1 summarized historical aspects of the patients' disease.

For experimental procedures, anticoagulated fresh blood was withdrawn from normal volunteers and alcoholics. Details for the method of preparing cell suspension have been described previously (*6*). In each study, human erythrocytes in Gey's balanced salt solution were incubated at 37°C in the presence of 1.0 μCi ^{14}C-formaldehyde (specific activity: 0.333 mCi/mg, New England Nuclear Corp.) and gassed with 95% O_2 and 5% CO_2 during 180 min. The $^{14}CO_2$ production from ^{14}C-labeled formaldehyde was measured continuously by an in vitro ionization chamber method as described previously (*1,2,6*). Experiments were carried out in five normal volunteers. Repeat studies were then made with three of these normal subjects immediately after consumption of 24–74 oz. of beer containing 6 degrees of alcohol. Another series of experiments were performed with four alcoholics (Group A) 0–12 hr after consumption of 1–5 liters of beer containing 10 degrees of alcohol and some other alcoholic beverages (e.g., whisky, gin, etc.). Repeat studies were made with three of these patients 12–24 hr and then 2–5 days later. Finally, studies were made with five alcoholics (Group B) within 3–21 days after the consumption of alcohol.

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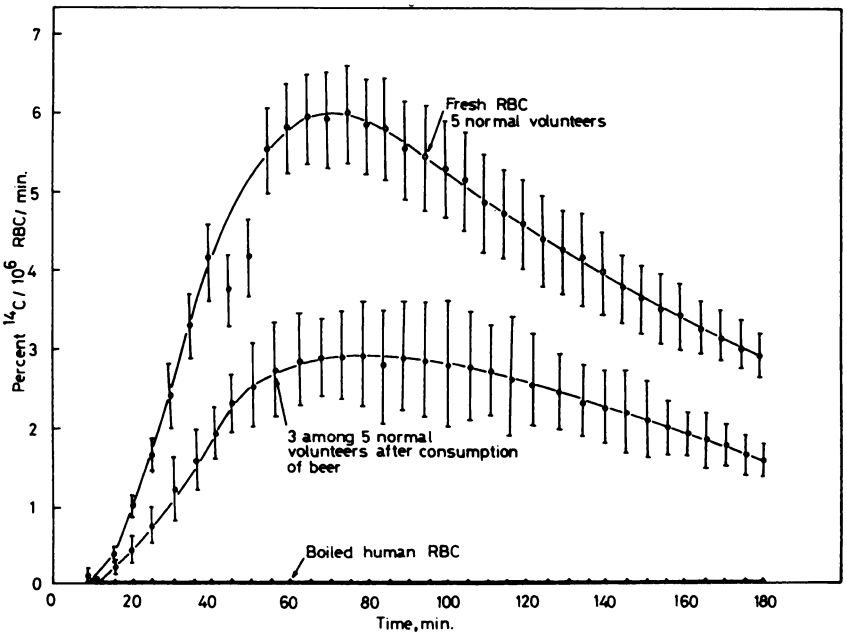

FIG. 1. Composite data of rates of $^{14}CO_2$ production from ^{14}C-formaldehyde by boiled erythrocytes of three normal volunteers, fresh erythrocytes of five normal volunteers, and fresh erythrocytes of three of these five normal volunteers immediately after consumption of alcoholic beverages. Ordinate represents percent of incubated ^{14}C produced as $^{14}CO_2$ per minute per 10^6 erythrocytes, and abscissa represents time in minutes after administration of ^{14}C-formaldehyde. Each point represents mean of $^{14}CO_2$ production for each group of experiments at given time, and length of vertical bar through each point represents ± 1 standard error of mean.

Formaldehyde uptake by erythrocytes. Details for the method of preparing cell suspension and study of radioactivity uptake have been described previously (*10*). In each experiment, cell suspension was prepared from blood of normal volunteers. Approximately 0.3 ml of the cell suspension was delivered to one of a series of tubes containing 3.2 ml of basal medium of Eagle, pH 7.6 (Grand Island Biological Co., Grand Island, N.Y.) and 0.3 μCi ^{14}C-formaldehyde with and without 0.1–1.0 λ of absolute alcohol. Volume of incubation was 3.5 ml in all experiments. After 45 min of incubation at 37°C, 5 ml of iced saline was added to the culture. Erythrocytes were washed, isolated, and then digested in Nuclear-Chicago Solubilizer (*10*). Their radioactivity was measured by a Nuclear-Chicago Mark II liquid scintillation counter.

RESULTS

Figure 1 shows composite data of changes in rates of $^{14}CO_2$ production from ^{14}C-formaldehyde in boiled and fresh erythrocytes of normal volunteers

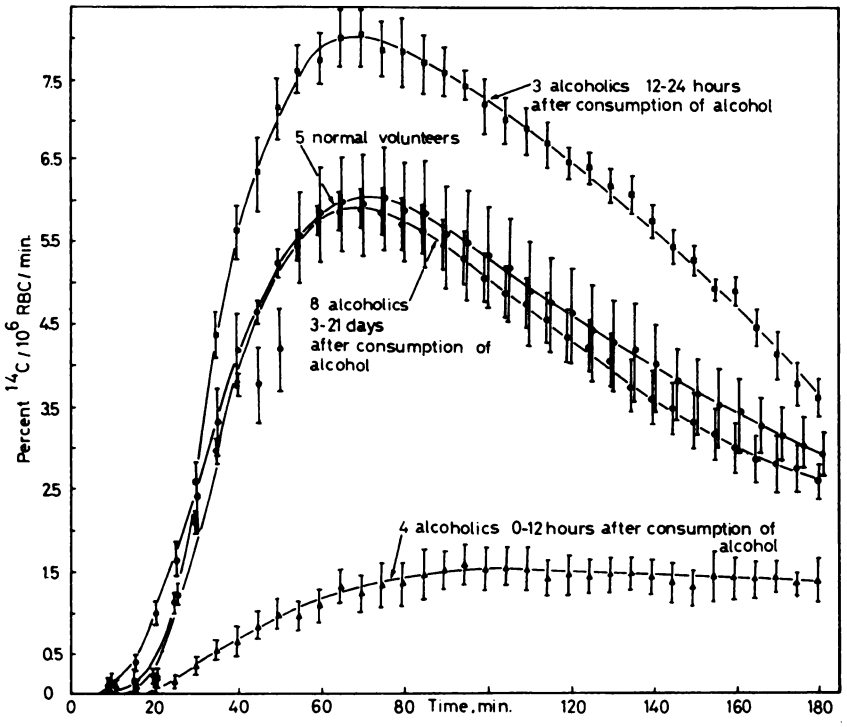

FIG. 2. Composite data of rates of $^{14}CO_2$ production from ^{14}C-formaldehyde by fresh erythrocytes of five normal volunteers, four alcoholics 0–12 hr after consumption of alcoholic beverages, three alcoholics 12–24 hr after consumption of alcoholic beverages, and eight alcoholics 3–21 days after consumption of alcoholic beverages. Ordinate represents percent of incubated ^{14}C produced as $^{14}CO_2$ per minute per 10^6 erythrocytes, and abscissa represents time in minutes after administration of ^{14}C-formaldehyde. Each point represents mean of $^{14}CO_2$ production for each group of experiments at given time, and length of vertical bar through each point represents ± 1 standard error of mean.

before and after consumption of 24–72 oz. of beer. There is no $^{14}CO_2$ production detected in boiled human erythrocytes. A qualitative difference between the oxidation of formaldehyde in erythrocytes of normal volunteers before and after consumption of alcoholic beverages is clearly seen in this figure.

Figure 2 shows composite data of changes in rates of $^{14}CO_2$ production from ^{14}C-formaldehyde in alcoholics and nonalcoholics with and without consumption of alcoholic beverages. There is a qualitative difference in the oxidation of ^{14}C-formaldehyde in alcoholics 0–12 hr after consumption of alcohol as compared with the control. An over-recovery of this oxidation can be seen in the group of alcoholics 12–24 hr later. Formaldehyde oxidation returns to the normal within 3–21 days of the consumption of alcohol. As shown in Table 2, there is a difference in $^{14}CO_2$ production in normal volunteers before and immediately after consumption of beer ($p < 0.05$). Similar results were obtained in alcoholics 0–12 hr after consumption of alcoholic beverages as compared with the controls ($p < 0.01$). A significantly increased $^{14}CO_2$ production ($p < 0.05$) is found 12–24 hr later in a repeat study. Then $^{14}CO_2$ production returns to the normal 3–21 days after consumption of alcohol ($p > 0.05$). There is no change in uptakes of formaldehyde by human erythrocytes incubated with ethanol ($p > 0.05$), as shown in Table 3.

TABLE 2. CUMULATIVE $^{14}CO_2$ PRODUCTION DURING INITIAL 180 MIN FROM ^{14}C-FORMALDEHYDE IN ERYTHROCYTES OF NORMAL VOLUNTEERS AND ALCOHOLICS WITH AND WITHOUT CONSUMPTION OF ALCOHOLIC BEVERAGES

Category name of experimental subjects	^{14}C production in 180 min Mean (% ^{14}C/10^6 erythrocytes) ± s.e.
Normal volunteers (5)	
VA	0.06545
GB	0.08718
ML*	0.08078
NT*	0.07802
ND*	0.06381
	0.07506 ± 0.00450
Normal volunteers immediately after consumption of 24–74 oz. of beer (3)	
ML*	0.04504
NT*	0.05086
ND*	0.04795
	0.04761 ± 0.04807 ($p < 0.05$)
Alcoholics 0–12 hr after consumption of alcoholic beverages (4, Group A)	
VB*	0.02418
VS*	0.02167
RV*	0.01145
BS	0.02481
	0.02053 ± 0.00310 ($p < 0.01$)
Alcoholics 12–24 hr after consumption of alcoholic beverages (3, Group A)	
VB*	0.11257
VS*	0.11708
RV*	0.09378
	0.10781 ± 0.00713 ($p < 0.05$)
Alcoholics 3–21 days after consumption of alcoholic beverages (3, Group A; 5, Group B)	
VB*	0.06328
VS*	0.08437
RV*	0.08317
LG	0.05789
RG	0.06922
ML	0.05865
RL	0.06049
RL	0.06347
	0.06757 ± 0.00374 ($p > 0.05$)

Number of subjects is noted in parentheses.
* Subject used in more than one experiment.

DISCUSSION

In the present study, we observed the oxidation of formaldehyde to CO_2 in fresh human erythrocytes. The fact that $^{14}CO_2$ production occurred in fresh erythrocytes but not in boiled erythrocytes suggests the presence of enzyme processes involved in the oxidation of this substrate in this human tissue. Similar results were reported with another monocarbon fragment, i.e., formate, in rat (*6*) and human (*9*) erythrocytes.

In the next series of studies, we observed a decreased $^{14}CO_2$ production from ^{14}C-formaldehyde in erythrocytes of alcoholics and nonalcoholics 0–12 hr after consumption of alcoholic beverages. This altered catabolism of formaldehyde may not be due to a delayed physical transport of this substrate across the cell membrane since no changes in uptakes of formaldehyde by erythrocytes of normal volunteers were found when these cells were incubated with ethanol. Therefore the result obtained may be due to an alteration in erythrocyte metabolism, such as the inhibition of tetrahydrofolate activity involved in the oxidation of formaldehyde (Fig. 3). Unfortunately, since tetrahydrofolate is extremely labile, little direct information appears to be available to test the validity of this finding. There have been reports showing a delay in the oxidation of ^{14}C-formate, a monocarbon fragment attached to tetrahydrofolate, in erythrocytes of folic acid-deficient rats in vitro (*6*), and a decrease in the excretion of $^{14}CO_2$ after intravenous administration of ^{14}C-formate to folic acid-deficient rats (*11*) and patients (*12*) as well as to irradiated rats (*13*) in vivo. The latter result was

TABLE 3. UPTAKES OF ^{14}C-FORMALDEHYDE BY ERYTHROCYTES OF NORMAL VOLUNTEERS INCUBATED WITH OR WITHOUT ETHANOL DURING 45 MIN

Category	No. of experiments	Radioactivity uptake in 45 min (dpm ± s.e.)/10^9 erythrocytes
Control	5	5.778 ± 0.858
1 λ of ethanol	5	4.565 ± 0.611 ($p > 0.05$)
10 λ of ethanol	5	5.441 ± 0.504 ($p > 0.05$)

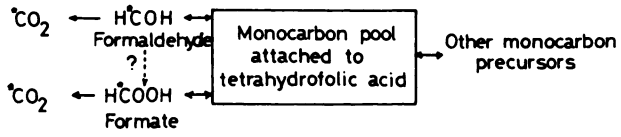

FIG. 3. Metabolic fate of carbon of formaldehyde and formate in monocarbon pool attached to tetrahydrofolate.

possibly due to an inhibition of tetrahydrofolate by ionizing radiation (*13,14*).

An increased $^{14}CO_2$ production from ^{14}C-formaldehyde 12–24 hr later suggests an over-recovery of enzyme activity. Repeat $^{14}CO_2$ studies showed that formaldehyde oxidation returned to the normal when measured 3–21 days after consumption of alcoholic beverages. These observed abnormalities in the oxidation of formaldehyde, possibly caused by inhibition of tetrahydrofolate by alcohol, may support the previous result showing that ethanol suppresses hematopoiesis, perhaps by directly affecting folate metabolism (*15*).

The fact that both inhibition and over-recovery of enzyme activities were found in very short periods of time may allow an explanation for contradictory results on serum folate levels in alcoholics. Indeed, high mean serum folate was reported by some authors (*16*), whereas others have found a low value of this vitamin level among alcoholics (*17*).

SUMMARY

We report observations on alterations in oxidative catabolism of formaldehyde in erythrocytes of alcoholics and nonalcoholics 0–12 hr after consumption of alcoholic beverages. These results are consistent with an inhibition of tetrahydrofolate activity by alcohol. An increased $^{14}CO_2$ production from ^{14}C-formaldehyde in repeat studies 12–24 hr later indicates an over-recovery of the formaldehyde oxidizing system. The oxidation of formaldehyde returned to normal when measured 3–21 days after the consumption of alcohol. These observed abnormalities in the oxidation of formaldehyde may support the previous suggestion that ethanol suppresses hematopoiesis, perhaps by directly affecting folate metabolism.

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