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INHIBITION OF FORMATE AND FORMALDEHYDE OXIDATION BY ETHANOL AS AN INDEX FOR ESTIMATING ALCOHOL CONCENTRATION IN ISOLATED TISSUES

Ngo Tran and Etienne LeBel

Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada

The presence of enzymes, possibly tetrahydrofolate, involved in the oxidation of both formaldehyde and formate in human or rat RBC and rat livers, but not in rat brains, has been demonstrated. The inhibition of oxidation of either ^{14}C-formate or ^{14}C-formaldehyde to $^{14}CO_2$ by various concentrations of ethanol in rat livers may be considered as an index for estimating alcohol concentrations in human isolated tissues without radiation danger. Immediate and accurate results obtained demonstrated also that this in vitro ionization chamber method with ^{14}C-labeled formate or formaldehyde would surpass spectrophotometric procedures because of its rapidity and reliability.

In a recent paper we reported that the oxidation of ^{14}C-formaldehyde was abnormal in red blood cells (RBC) of alcoholics and nonalcoholics after consumption of alcoholic beverages (*1*). This study is a further evaluation of such a finding. Our experiments were performed with two ^{14}C-labeled monocarbon fragment precursors, such as formaldehyde and formate, in human or rat RBC, and rat livers and brains, incubated with or without ethanol.

METHODS AND MATERIALS

Preparation of human or rat RBC. Anticoagulated fresh blood was withdrawn from normal volunteers or Wistar male rats (Canadian Breeding Laboratories) weighing 250–300 gm. RBC were isolated and washed twice with concentrated cold saline. Suspension of washed RBC in cold Pingee's solution and Gey's balanced salt solution (each 100 ml contains 700 mg NaCl, 37 mg KCl, 15 mg $Na_2HPO_4 \cdot 2H_2$), 3 mg KH_2PO_4, 21 mg $MgCl_2 \cdot H_2O$, 17 mg $CaCl_2$, 100 mg glucose, and 227 mg $NaHCO_3$; (Grand Island Biological Co., Grand Island, N.Y.) pH 4.5–5.0 was then used for experiments.

Preparation of rat livers and brains. Wistar male rats weighing 250–300 gm were killed by decapitation. Individual livers and brains were removed immediately and stored in an ice-water bath. The liver or brain tissue was homogenized in the cold in 0.1 *M* phosphate buffer (each 100 ml contains 1,360 mg KH_2PO_4 and 1,420 mg Na_2HPO_4), pH 7.0, in a glass homogenizing tube equipped with a Teflon pestle.

$^{14}CO_2$ production study. Details of the apparatus devised for an instantaneous and continuous measurement of $^{14}CO_2$ production from a ^{14}C-labeled biochemical in vitro have been published previously (*2,3*).

For experimental procedures, human or rat RBC and rat brain homogenates were incubated respectively with 1.0 μCi ^{14}C-formate (SA:52 mCi/m*M*, Amersham/Searle) or 1.0 μCi ^{14}C-formaldehyde (SA:9.9 mCi/m*M*, New England Nuclear, Boston, Mass.), with or without various concentrations of absolute ethanol (Consolidated Alcohol, Toronto, Ontario) in 0.1 *M* phosphate buffer, pH 7.0, or concentrated Gey's balanced salt solution, pH 4.5–5.0, within 120–180 min. Compressed gas with 95% O_2 and 5% CO_2 passed continuously through the incubation chamber at a constant flow rate of 100 cc/min. The flow of gases then passes across a Triton-755C (Johnston Laboratories, Inc., Cockeysville, Md.), which consists of a 1.2-liter ionization chamber connected to an electrometer. Continuous graphing plotting of the $^{14}CO_2$ curves was achieved by a chart recorder.

RESULTS

Figure 1 represents composite data of rates of $^{14}CO_2$ production from ^{14}C-formate incubated with rat or human RBC and rat brains, respectively. It is

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For reprints contact: Ngo Tran, Dept. of Nuclear Medicine, Centre Hospitalier Universitaire, Universite de Sherbrooke, Sherbrooke, Quebec, Canada.

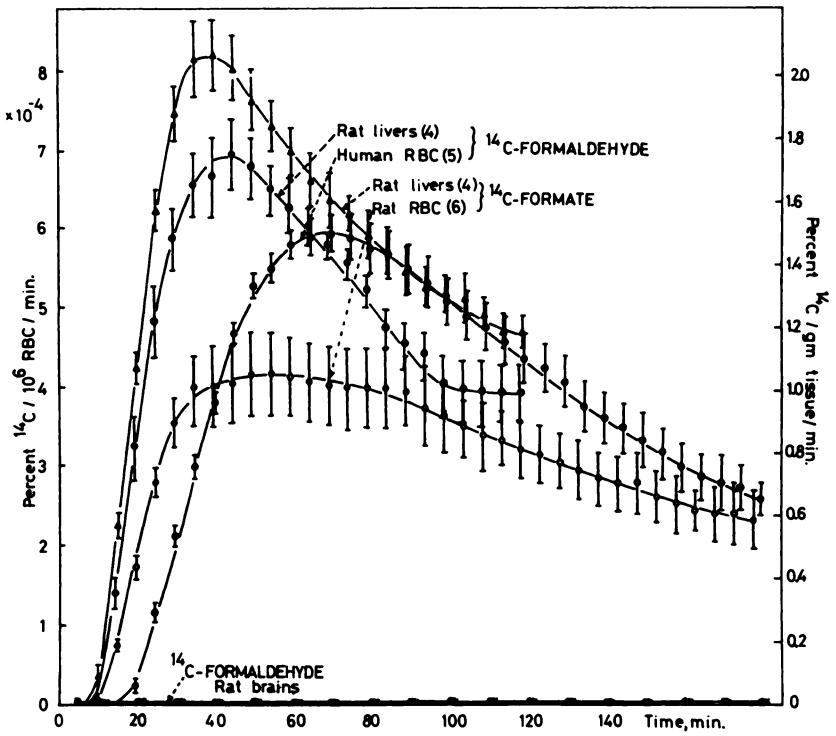

FIG. 1. Composite data of rates of $^{14}CO_2$ production from ^{14}C-formaldehyde or ^{14}C-formate incubated with human or rat RBC, rat livers, and rat brains. Ordinate represents percent of incubated ^{14}C produced as $^{14}CO_2/10^6$ RBC/min or percent ^{14}C/gm tissue/min, and abscissa represents time in min after incubation of either ^{14}C-formaldehyde or ^{14}C-formate. 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.

clearly seen that there is no $^{14}CO_2$ production from ^{14}C-formaldehyde incubated with rat brains.

Table 1 summarized cumulative percent ^{14}C appearing as $^{14}CO_2$ presented in Fig. 1.

Figures 2 and 3 represent composite data of rates of $^{14}CO_2$ production from ^{14}C-formaldehyde and ^{14}C-formate incubated with rat liver homogenates, respectively, with or without various concentrations of ethanol. As shown in Table 2, an inhibition in the oxidation of both formaldehyde and formate by ethanol was clearly seen.

Figure 4 shows an inhibition (expressed as percentage per gram of tissue) of formaldehyde and formate oxidations by ethanol. It appeared that degrees of such an inhibition were proportionally related to ethanol concentrations.

TABLE 1. CUMULATIVE $^{14}CO_2$ PRODUCTION PER GRAM TISSUE OR 10^8 RBC*

Category	$^{14}CO_2$/gm tissue during 120 min (% ± s.e.)	$^{14}CO_2/10^8$ RBC during 180 min (% ± s.e.)
^{14}C-formaldehyde		
Human RBC (5)	—	7.50 ± 0.40
Rat livers (4)	13.77 ± 0.37	—
Rat brains (4)	0	—
^{14}C-formate		
Rat livers (4)	16.62 ± 0.27	—
Rat RBC (6)	—	2.04 ± 0.20

* Production from ^{14}C-formate and ^{14}C-formaldehyde incubated with human or rat RBC, rat livers, and rat brains. The number of experiments is noted in parentheses.

DISCUSSION

In the present study we demonstrate the oxidation

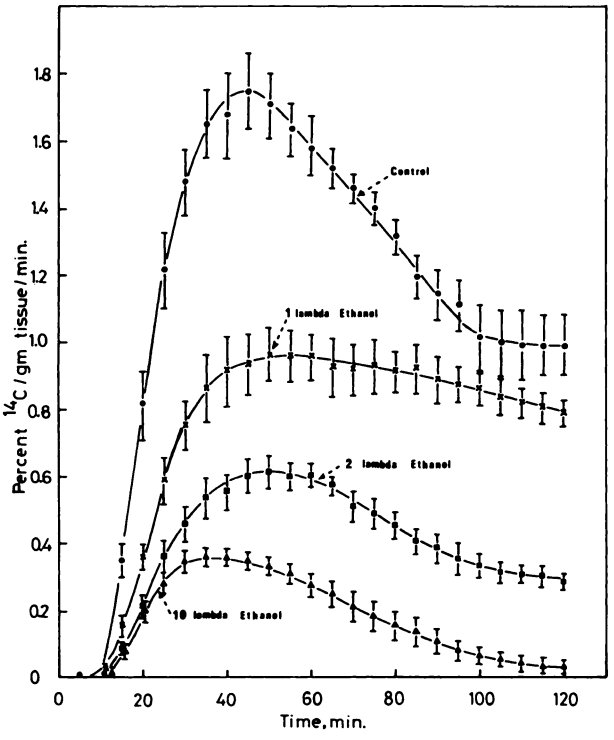

FIG. 2. Composite data of rates of $^{14}CO_2$ from ^{14}C-formaldehyde incubated with rat liver homogenates with or without various amounts of ethanol. See Fig. 1 for details.

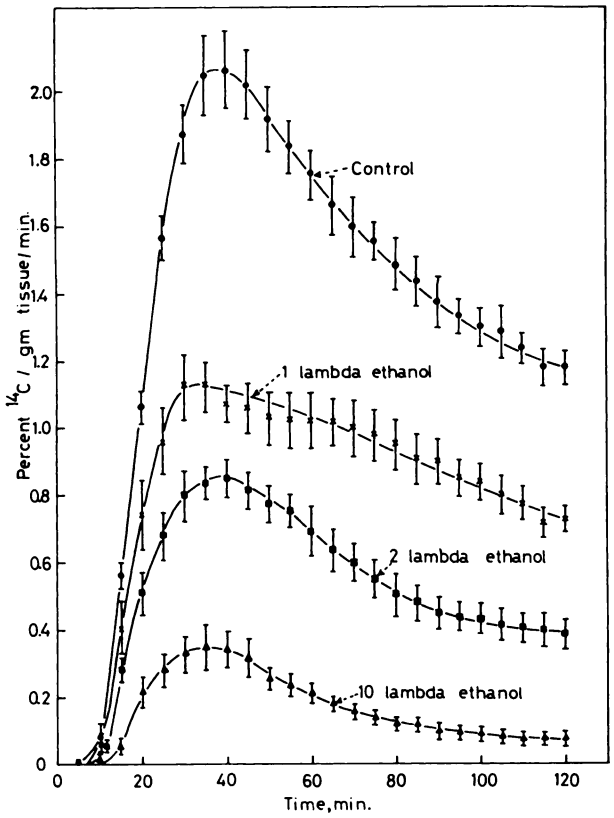

FIG. 3. Composite data of rates of $^{14}CO_2$ from ^{14}C-formate incubated with rat liver homogenates, with or without various amounts of ethanol. See Fig. 1 for details.

TABLE 2. CUMULATIVE $^{14}CO_2$ PRODUCTION PER GRAM TISSUE DURING INITIAL 120 MIN*

Category	Cumulative percent ^{14}C/gm tissue during 120 min (% ± s.e.)
^{14}C-formaldehyde	
Control (4)	13.775 ± 0.361
1 λ ethanol (4)	9.104 ± 0.888 ($p < 0.02$)
2 λ ethanol (4)	4.618 ± 0.316 ($p < 0.001$)
10 λ ethanol (4)	2.125 ± 0.212 ($p < 0.001$)
^{14}C-formate	
Control (4)	17.640 ± 0.720
1 λ ethanol (4)	10.233 ± 0.630 ($p < 0.001$)
2 λ ethanol (4)	6.422 ± 0.628 ($p < 0.001$)
10 λ ethanol (4)	2.057 ± 0.256 ($p < 0.001$)

* Production from ^{14}C-formate and ^{14}C-formaldehyde incubated with rat tissues, with or without ethanol, respectively. The number of experiments is noted in parentheses.

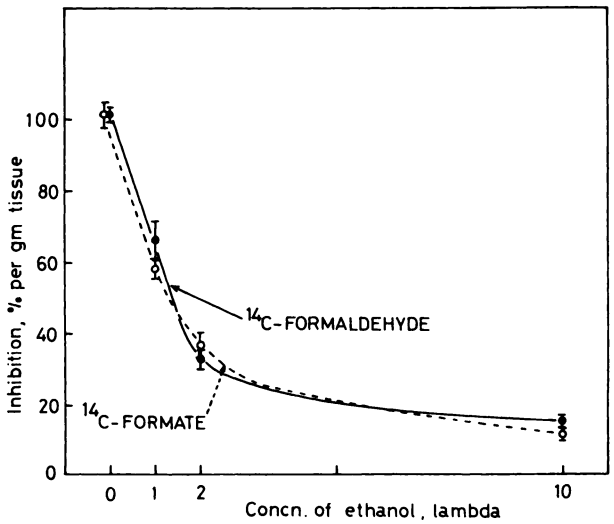

FIG. 4. Degrees of inhibition of ^{14}C-formaldehyde and ^{14}C-formate oxidation by ethanol respectively. Ordinate represents percentage of such inhibition per gram of tissue and abscissa represents concentrations of incubated ethanol.

of both formaldehyde and formate to CO_2 in rat or human RBC and rat livers but not in rat brains. The results obtained indicate therefore that there are enzymes involved in the oxidation of these labeled compounds in rat or human RBC and rat livers but not in rat brains.

As shown in Figs. 2 and 3, ethanol inhibited continuously the oxidation of both formaldehyde and formate in rat livers. This result explains the mechanism involved in an abnormal oxidation of formaldehyde in RBC of alcoholics and nonalcoholics after consumption of alcohol. Furthermore, this supports our hypothesis (*1*) suggesting an alteration in intraerythrocytic events by ethanol in alcoholics, such as an inhibition of tetrahydrofolate activity (*1,4*) involved in the oxidation of formaldehyde and formate. It is known indeed that the metabolism of these substrates is related to monocarbon pool attached to tetrahydrofolate (*5,6*). As seen in Fig. 4, degrees of such an inhibition of formaldehyde or formate oxidation, expressed as percentage per gram of tissue, appear to be dependent on various concentrations of incubated ethanol. The inhibition of either formaldehyde or formate oxidation by ethanol may be therefore considered as an index (*7,8*) for estimating alcohol concentration in isolated tissues, i.e., human blood or liver tissues obtained from the biopsy. It is of interest to note finally that abnormal oxidation of both formaldehyde and formate was found to occur at ethanol concentrations of 0.002–0.02% in isolated tissues, respectively, and that these alcohol concentrations were approximately 2–20 times smaller than those detected from regular spectrophotometric procedures (*9*).

Thus an ionization chamber with either ^{14}C-formaldehyde or ^{14}C-formate would be a reliable semi-automated method for estimating alcohol concentration in isolated tissues.

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