Acetaldehyde Metabolism by the Rat Heart (37621)

G. W. FORSYTH,[1] H. T. NAGASAWA, AND C. S. ALEXANDER
Departments of Medicine and Medicinal Chemistry, University of Minnesota 55455 and Veterans Administration Hospital, Minneapolis, Minnesota 55417

Acute ethanol intoxication is characterized by elevated circulating levels of acetaldehyde as well as ethanol. Since chronic ethanol consumption can induce alcoholic cardiomyopathy (1, 5) a knowledge of the metabolism of these two compounds by the heart is of definite importance. Ethanol has been thoroughly studied, and it does not appear to be metabolized in the heart (6, 9, 11). On a molar basis, acetaldehyde is considerably more toxic than ethanol, having a recognized sympathomimetic activity (2, 13). Pharmacological effects on the heart occur with acetaldehyde at concentrations found in the blood during acute ethanol intoxication in man (6, 8, 16). Dietrich (4) has measured aldehyde dehydrogenase activity in rat heart using indoleacetaldehyde as a substrate, but there is no information in the literature about *in vivo* or *in vitro* acetaldehyde oxidation by the heart.

We have perfused isolated beating rat hearts with [1,2-^{14}C]acetaldehyde, and quantitatively measured $^{14}CO_2$ production in order to evaluate the oxidation of acetaldehyde as a detoxifying mechanism in the heart. The role of heart aldehyde dehydrogenase in this $^{14}CO_2$ production was investigated by pretreating animals with the aldehyde dehydrogenase inhibitor tetraethyl dithiuram disulfide (disulfiram). Finally, the metabolism of sodium [1-^{14}C]acetate to $^{14}CO_2$ by the isolated heart was determined and the relative rates of utilization of acetate and acetaldehyde under these conditions were compared.

Materials and Methods. [1-^{14}C]acetate and [1,2-^{14}C]acetaldehyde were purchased from New England Nuclear. The ^{14}C acetaldehyde was transferred into aqueous solution at neutral pH by vacuum distillation, the specific activity adjusted to 2.0 μCi/μmole by addition of freshly distilled unlabeled acetaldehyde, and the stock solutions stored in sealed vials at 4°.

The supplier of the [1,2-^{14}C]acetaldehyde assumed a radiochemical purity of greater than 98% based on a gas liquid chromatography step used to separate [1,2-^{14}C]acetaldehyde from [1,2-^{14}C]acetylene. We tested the ^{14}C acetaldehyde for the presence of radioactive impurities having vapour pressure significantly different from acetaldehyde. Vacuum distillation of 1 μCi of [1,2-^{14}C] acetaldehyde (510 μg) from aqueous solution into a $NaHSO_3$ trap gave identical transfer of mass and radioactivity within the limits of our analytical technique. Acetaldehyde concentrations were determined by both gas chromatography (15) and colorimetry (14), with a variability of less than ± 2% for results obtained by the two analytical procedures.

Hearts of male Sprague–Dawley rats weighing 225–275 g were excised under pentobarbital anaesthesia and their beating was arrested by submerging them in physiological saline chilled to 0°. Beating resumed when retrograde perfusion was begun at 37° under 80 cm pressure of oxygenated Krebs–Ringer solution (3) buffered with a final concentration of 35.5 m*M* Tris HCl, pH 7.6 or 8.3 m*M* piperazine-*N*-*N*′-bis(2-ethane sulfonic acid) (PIPES), pH 7.4. The glucose concentration was 5.0 mM. After 10 min of equilibration [1,2-^{14}C]acetaldehyde was added to the medium (5×10^{-5} *M*, 2 μCi/μmole). Fifteen ml of perfusate was passed through the beating heart before collecting 10 ml of

[1] Present address: Department of Veterinary Physiology, University of Saskatchewan, Saskatchewan Suskatoon, Canada.

perfusate (average coronary flow of 5.6 ± 0.7 ml/min) for the $^{14}CO_2$ measurement.

The 10 ml perfusate sample plus 0.5 ml of 5% $NaHCO_3$ solution added as carrier were "shell-frozen" at —78° in a 50-ml boiling flask. Fifty microliters of 10 *N* H_2SO_4 were added to the frozen perfusate, and the temperature reduced to —196° with liquid nitrogen. The sample, an acetaldehyde trap containing 5 ml of 5% $NaHSO_3$ and a carbon dioxide trap of 5 ml saturated $Ba(OH)_2$ solution, were placed on a vacuum manifold. $^{14}CO_2$ was released from the perfusate sample by thawing and then transferred through the bisulfite trap to the $Ba(OH)_2$ trap by vacuum distillation using the temperature differential between solid carbon dioxide and liquid nitrogen. The barium carbonate was collected by filtration, washed successively with 5 ml of water, ethanol and ether, and transferred to a vial for liquid scintillation counting.

Results. Blank determinations were carried out as described above except that the perfusate was not passed through the heart before analysis for $^{14}CO_2$ content.

The identity and purity of the barium [^{14}C]carbonate was verified by acidification to release CO_2 and separation of the $^{14}CO_2$ from other possible contaminating ^{14}C compounds by gas chromatography. This separation was carried out on a Beckman GC-M instrument with a 4.5 ft × 0.25 in. column of Poropak Q at 55° using a thermal conductivity detector at 120°. The helium carrier gas (60 cm^3/min) was passed from the thermal conductivity detector into the scrubbing column of a Packard Tri-Carb sample oxidizer. CO_2 was collected from the carrier gas by charging the scrubbing column with ethanolamine for the duration of the appearance of the CO_2 peak plus 1 min. The contents of the column were then flushed into a vial for liquid scintillation counting according to the normal program sequence of the sample oxidizer. Recovery of ^{14}C from the barium carbonate precipitates showed that 94 ± 2.7% of the radioactivity was present as $^{14}CO_2$.

The stability of acetaldehyde in the presence of O_2 was established by assay of ^{14}C acetate (7) in the oxygenated perfusate containing [^{14}C]acetaldehyde. Less than 0.2% of the ^{14}C was present as [^{14}C]acetate at zero time, and this value did not increase after 15 min of exposure to the perfusate.

The oxidation of acetaldehyde to CO_2 in the perfused rat heart was consistent and reproducible (Table I). *In vivo* administration of disulfiram before sacrificing the rats produced a significant 60–70% inhibition of this oxidation. Acetate oxidation to CO_2 was compared by perfusing hearts with sodium [1-^{14}C]acetate (3×10^{-5} *M*, 2 μCi/μmole). As suggested by Lindeneg *et al.* (9) acetate was readily utilized by the heart. Averaging the results of two experiments, 124 ± 11 nmoles of CO_2 were produced per 500 nmoles of acetate entering the heart.

The rate of oxidation of acetaldehyde to CO_2 was calculated to be 422 ± 44 nmoles CO_2/hr/g wet weight. The metabolism of acetaldehyde to compounds other than CO_2 was estimated by measuring ^{14}C remaining

TABLE I. Acetaldehyde Oxidation to CO_2 by the Isolated Rat Heart.[a]

	nmoles $^{14}CO_2$ produced per 500 nmoles acetaldehyde entering the heart		
Krebs-Ringer solution buffer	Control[b]	Disulfiram	*p*
Tris–HCl, pH 7.6	10.34 ± 0.86 (5)	3.62 ± 0.64 (2)	<0.001
Na PIPES, pH 7.4	10.72 ± 0.82 (4)	2.38 ± 0.72 (3)	<0.001

[a] Hearts were perfused with medium containing [1,2-^{14}C]acetaldehyde (5×10^{-5} *M*, 2 μCi/μmole). Oxidation was determined by fractional distillation of $^{14}CO_2$ from 10 ml samples of perfusate, and trapping the $^{14}CO_2$ in a solution of barium hydroxide. All values were corrected for a 74% recovery of CO_2 obtained under the experimental conditions. Disulfiram treated rats were injected ip with 300 mg/kg disulfiram 24 and 4 hr before sacrifice. Disulfiram was suspended in physiological saline at 120 mg/ml with 5 mg/ml of carrageenin.

[b] Means ± SD with number of observations and statistical significance.

in the hearts after a 60-sec wash with buffer lacking [^{14}C]acetaldehyde. Adding this value to the rate of CO_2 production gave an acetaldehyde oxidation rate of 1017 ± 146 nmoles/hr/g wet weight. Lindros *et al.* (10) measured higher rates of metabolism in perfused rat liver, but the lowest acetaldehyde concentration which they studied (4×10^{-4} *M*) is probably 5 × the maximum blood levels occurring in acute ethanol intoxication (15).

Discussion. Small but significant quantities of acetaldehyde were oxidized to carbon dioxide by the isolated, beating rat heart. The presence of Tris buffer in the perfusate demonstrated that acetaldehyde metabolism was not affected by excess concentrations of primary amines. Thus, Schiff's base formation with amino groups of plasma proteins may not interfere with acetaldehyde transfer into, and metabolism by, heart cells *in vivo*.

Inhibition of $^{14}CO_2$ production from [^{14}C]acetaldehyde by pretreatment *in vivo* with disulfiram was consistent with enzymic oxidation by aldehyde dehydrogenase as the first step. A comparison of the percent of acetate and acetaldehyde converted to CO_2 during a single pass perfusion suggested that aldehyde dehydrogenase was the rate limiting reaction in acetaldehyde oxidation.

Several investigators have postulated that acetaldehyde toxicity in the heart may be due to its sympathomimetic activity in the release of norepinephrine (6, 8, 12). This effect is accomplished by as little as 0.2 m*M* of acetaldehyde, a level easily reached in drinking man. Thus, the limited capacity of the heart to oxidize acetaldehyde on the one hand, and the release of norepinephrine by such small circulating levels on the other hand, combine to enhance the potential cardiotoxicity of acetaldehyde. Walsh *et al.* (17) showed that the catecholaldehyde oxidation step in norepinephrine catabolism was inhibited by acetaldehyde. Our evidence for acetaldehyde oxidation to CO_2 by the isolated rat hearts suggests that acetaldehyde could interfere with oxidative catecholamine metabolism of the heart. Thus, both release and metabolism of catecholamines should be considered in assessing the cardiotoxic effects of acetaldehyde.

Summary. Isolated beating rat hearts perfused with [1,2-^{14}C]acetaldehyde oxidize the compound to $^{14}CO_2$. Inhibition of the oxidation by pretreatment *in vivo* with disulfiram suggests the involvement of a myocardial aldehyde dehydrogenase. The dehydrogenation of acetaldehyde may be rate-limiting since acetate oxidation to carbon dioxide proceeds faster than acetaldehyde oxidation in the perfused rat heart.

We are grateful to Steve Altman for helpful discussion and technical assistance.

1. Alexander, C. S., Ann. Intern. Med. **67**, 670 (1967).

2. Asmussen, E., Hald, J., and Larsen, V., Acta Pharmacol. Toxicol. **4**, 311 (1948).

3. Cohen, P. P., *in* "Manometric Techniques" (W. W. Umbreit, R. H. Burris, and J. F. Stauffer, eds.), p. 135. Burgess, Minneapolis (1957).

4. Dietrich, R. A., Biochem. Pharmacol. **15**, 1911 (1966).

5. Evans, W., Brit. Heart J. **21**, 445 (1959).

6. Gailis, L., and Verdy, M., Can. J. Biochem. **49**, 227 (1971).

7. Huang, K. P., Anal. Biochem. **37**, 98 (1970).

8. James, T. N., and Bear, E. S., Amer. Heart J. **74**, 243 (1967).

9. Lindeneg, O., Mellemgaard, K., Fabricus, J., and Lundquist, F., Clin. Sci. **27**, 427 (1964).

10. Lindros, K. O., Vihma, R., and Forsander, O. A., Biochem. J. **126**, 945 (1972).

11. Lochner, A., Cowley, R., and Brink, A. J., Amer. Heart J. **78**, 770 (1969).

12. Moller, S. E., Acta Pharmacol. Toxicol. **30**, 449 (1971).

13. Perman, E. S., Acta Physiol. Scand. **43**, 71 (1958).

14. Sawicki, E., Hauser, T. R., Stanley, T. W., and Elbert, W., Anal. Chem. **33**, 93 (1961).

15. Truitt, E. B., Jr., Quart. J. Stud. Al. **31**, 1 (1970).

16. Walsh, M. J., Hollander, P. B., and Truitt, E. B., Jr., J. Pharmacol. Exp. Ther. **167**, 173 (1969).

17. Walsh, M. J., Truitt, E. B., Jr., and Davis, V. E., Mol. Pharmacol. **6**, 416 (1970).

Received May 25, 1973. P.S.E.B.M., 1973, Vol. 144.