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BEHAVIOR OF ACETALDEHYDE TRANSPORTED IN BLOOD

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Abstract—An exact determination of acetaldehyde in blood was carried out using Conway units. The sample was deproteinized in airtight Conway units to avoid the loss of acetaldehyde in the course of deproteinization. Acetaldehyde that diffused into circulating blood after the oxidation of ethanol in tissues did not exist free in the plasma for long and was carried mainly by the red blood cells. When erythrocytes were saturated completely with acetaldehyde, the maximum amount of acetaldehyde bound to the erythrocytes was equivalent to 1.2 μmoles/ml of blood.

The oxidation of ethanol to acetaldehyde in animal tissues has been reported by several authors (see Ref. 1). The principal enzyme responsible for the oxidation of ethanol is hepatic alcohol dehydrogenase. Ethanol is also oxidized by catalase [2, 3] and by a microsomal component rich in cytochrome P-450 [4–6]. Acetaldehyde, the first metabolic derivative of ethanol, is rapidly oxidized in the liver, and its level in blood is low even during the period of ethanol oxidation [7, 8].

To elucidate the biological action of alcohol, it seemed important to obtain detailed biological information about the acetaldehyde in blood. Several methods have been used thus far for the determination of acetaldehyde in blood, including gas chromatographic and enzymatic methods, which have received much recognition as accurate and specific methods. On the other hand, on the basis of the findings of Evans and Gillam [9], who studied the absorption spectra of a number of semicarbazones, Burbridge *et al.* [10] have developed a spectrophotometric method for the determination of acetaldehyde. The present paper proposes a simple and exact method, which is a modification of the procedure recommended by Burbridge. In addition, some characteristics of acetaldehyde transport in blood have been described. Some results relating to this study have been presented in a preliminary report [11].

MATERIALS AND METHODS

Preparation of red blood cell suspensions. Female albino rats of the Wistar strain, weighing 150–200 g, were given a standard laboratory diet and water *ad lib.* Blood was withdrawn from the ventral aorta, using a syringe containing a trace of heparin (1000 units/ml), and red blood cells were harvested by centrifuging at 800 rpm for 15 min. Red blood cells remaining after the removal of plasma were washed three times with about 5 vol. of physiological saline solution. Care was taken to remove the buffy coat. Finally, the washed red blood cells were resuspended in physiological saline solution in such a way that the total volume corresponded to the original blood volume. A human erythrocyte–saline suspension was also prepared as above.

Preparation of tissue homogenate. Tissues were homogenized (¼, w/v) in ice-cold physiological saline solution, using a Teflon homogenizer.

Standard curve. As shown in Fig. 1, a linear relationship exists between absorbance and concentration of acetaldehyde over a wide range. The exact contents of the acetaldehyde in the original stock (Merk, Darmstadt, Germany) were previously analyzed according to the titration method of Siggia and Maxcy [12]. The standard curve obtained by this method coincides well with that drawn by directly mixing semicarbazide solution with acetaldehyde solution.

Determination of acetaldehyde. A 0.5 ml sample

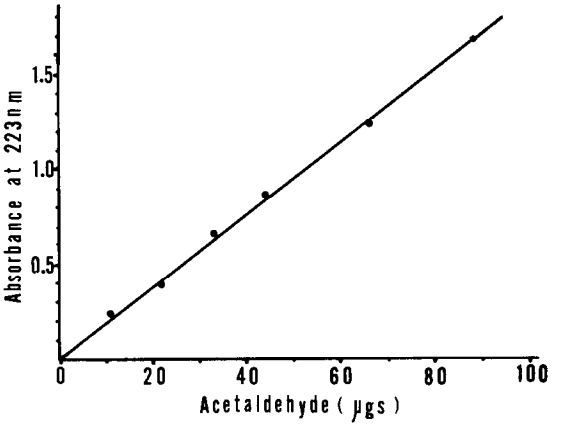

Fig. 1. Standard curve for acetaldehyde. A 1.0 ml aliquot of an aqueous solution containing acetaldehyde was added to the outer chamber of a Conway unit. A 0.5 ml sample of 10 mM semicarbazide was pipetted into the center chamber. After incubation for 30 min at 37°, 0.02 ml of the semicarbazone solution was adjusted to 0.52 ml with distilled water and the absorbance at 223 nm was measured.

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of a 10 mM semicarbazide solution were pipetted into the center chamber of a Conway microdiffusion unit (Shibata 6031-04). A 1.0 ml aliquot of the sample containing acetaldehyde was added to the outer chamber which had been equipped previously with a small cup that contained a deproteinizing agent. After the unit was set to be airtight, the cup in the outer chamber was tumbled when desired. The sample, which was deproteinized, was mixed by gently rotating the unit.

About 1 ml of a 1 M $HClO_4$ solution was used as a deproteinizing agent, except in recovery experiments when 1 ml of $ZnSO_4$ and $Ba(OH)_2$ solutions (Nelson reagent) [13] was used. Then the unit was placed in an incubator at 37° for 30 min. After cooling at room temperature, 0.02 ml of the semicarbazone solution in the center chamber of the unit was added to 0.5 ml of distilled water, and the absorbance was measured at 223 nm in a Hitachi spectrophotometer model 204 against a blank containing 0.02 ml of 10 mM semicarbazide and 0.5 ml of distilled water.

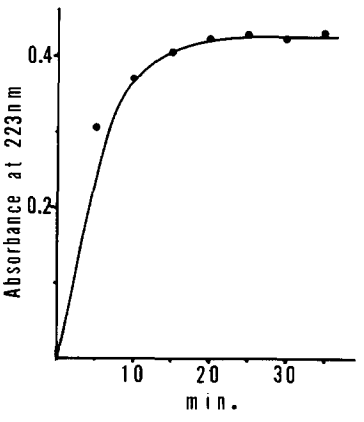

Fig. 2. Time-course of recovery of acetaldehyde from whole blood. The sample was prepared by mixing 1 mM acetaldehyde–saline solution with whole blood in a volume ratio of 1:1. One ml of the sample was added to the outer chamber of a Conway unit. After the sample was deproteinized, the unit was placed in an incubator at 37° for the times shown on the abscissa.

RESULTS AND DISCUSSION

Acetaldehyde levels in blood were estimated simply and rapidly in Conway units by measuring the absorbance of semicarbazone. Furthermore in airtight Conway units the loss of acetaldehyde in the course of estimation was minimized by deproteinizing the samples in them, as described in Materials and Methods. All of the acetaldehyde that was present in the outer chamber of the Conway unit had diffused completely into the semicarbazide solution in the central well by heating at 37° for 30 min after deproteinization (Fig. 2).

Acetaldehyde was recovered almost completely from saline solution, 5% bovine albumin solution, and plasma simply by heating at 37° for 30 min without deproteinization; complete recovery of aldehyde from whole blood and red cell suspension, however, could be achieved only after the deproteinization (Table 1). These findings indicate a certain binding, between blood cells and acetaldehyde molecules, is cleaved by deproteinization. $HClO_4$ solution was superior to the Nelson reagent as a deproteinizing agent with respect to simplicity. As shown in Table 2, no significant production of acetaldehyde occurred during deproteinization by $HClO_4$ at 37° for 30 min, even in the presence of ethanol.

The acetaldehyde resulting from the oxidation of ethanol is oxidized further to acetic acid in most tissues, but it is also apparent that acetaldehyde is removed from the body by way of the lung [14] or

Table 1. Recovery of acetaldehyde from saline solution, 5% bovine albumin solution, plasma, whole blood, and erythrocyte suspension*

	Deproteinizing agent	Aldehyde recovered		% Error
		μg	%	
Saline	$HClO_4$	21.68	98.5	−1.5
	Nelson reagent	22.20	100.9	+0.9
	0	22.08	100.5	+0.5
5% Bovine albumin	$HClO_4$	22.14	100.6	+0.6
	Nelson reagent	20.07	100.3	+0.3
	0	21.67	98.5	−0.3
Plasma	$HClO_4$	22.17	100.8	+0.8
	Nelson reagent	22.66	103.0	+3.0
	0	20.47	93.1	−6.9
Whole blood	$HClO_4$	22.03	100.1	+0.1
	Nelson reagent	21.23	96.5	−3.5
	0	1.03	4.7	−95.3
Erythrocyte suspension	$HClO_4$	22.40	101.8	+1.8
	Nelson reagent	22.19	100.9	+0.9
	0	1.46	6.6	−93.4

* Samples were prepared by mixing 1 mM acetaldehyde–saline solution (44 μg/ml) with saline solution, 5% bovine albumin, plasma, whole blood, or erythrocyte suspension in a volume ratio of 1:1. One ml from each sample was pipetted into the outer chamber of a Conway unit.

Table 2. Recovery of acetaldehyde contained in ethanol solution

Additions	Recovery			
	Deproteinization with $HClO_4$		Deproteinization with Nelson reagent	
	μmoles	%	μmoles	%
1 ml Acetaldehyde (0.5 mM)	0.515	103.0	0.489	97.8
1 ml Ethanol (150 mg/100 ml)	0	0	0	0
0.5 ml Acetaldehyde (1 mM) + 0.5 ml ethanol (100 mg/100 ml)	0.515	103.0	0.489	97.8

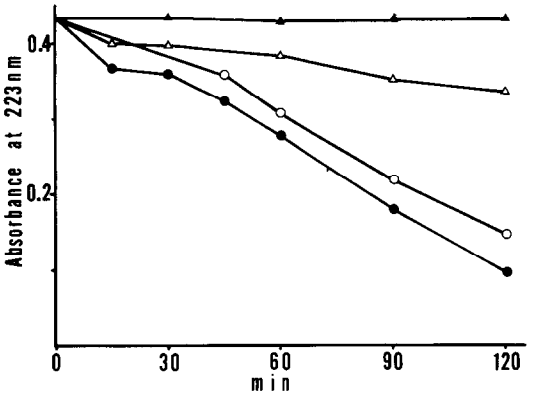

Fig. 3. Time-course of recovery of acetaldehyde from whole blood, plasma, and erythrocyte suspension, under a liquid paraffin layer. The samples were prepared by mixing 1 mM acetaldehyde–saline solution with saline solution, whole blood, plasma or erythrocyte suspension in a volume ratio of 1:1, covered with liquid paraffin, and then incubated at 37°. One ml from each sample was pipetted, at time shown on the abscissa, into the outer chamber of Conway units, and the amount of acetaldehyde was determined. Key: (——▲——) saline, (——△——) plasma, (——○——) erythrocyte suspension, and (——●——) whole blood.

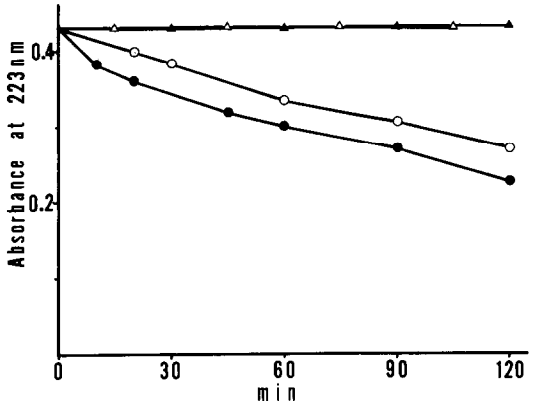

Fig. 4. Time-course of recovery of acetaldehyde from whole blood, plasma, and erythrocyte suspension in Conway units. One ml from each sample prepared as in Fig. 3 was pipetted into the outer chamber of a Conway unit, incubated at 37°, and then deproteinized, at the times shown. The center chamber contained 0.5 ml of a 10 mM semicarbazide solution. Key: (——▲——) saline, (——△——) plasma, (——○——) erythrocyte suspension, and (——●——) whole blood.

skin. The above experiments strongly indicated that the acetaldehyde carriers in blood were mainly red blood cells, even if some acetaldehyde was also metabolized by them (Fig. 3).

Matthies [15] reported that red blood cells of several animal species and of humans exhibited aldehyde dehydrogenase activity. To investigate acetaldehyde metabolism in plasma, erythrocyte suspension, and whole blood, each was mixed with acetaldehyde–saline solution. The mixtures were incubated in Conway units and in Erlenmeyer flasks in which the mixtures were placed under free atmosphere or layered liquid paraffin (Figs. 3–5).

In the mixtures layered under liquid paraffin, the rates of acetaldehyde oxidation increased according to the following series: saline, plasma, erythrocyte suspension, and whole blood, indicating that the system for the oxidation of acetaldehyde occurred mainly in red blood cells, but also slightly in plasma (Fig. 3). It is indicated in Fig. 4 that the acetaldehyde

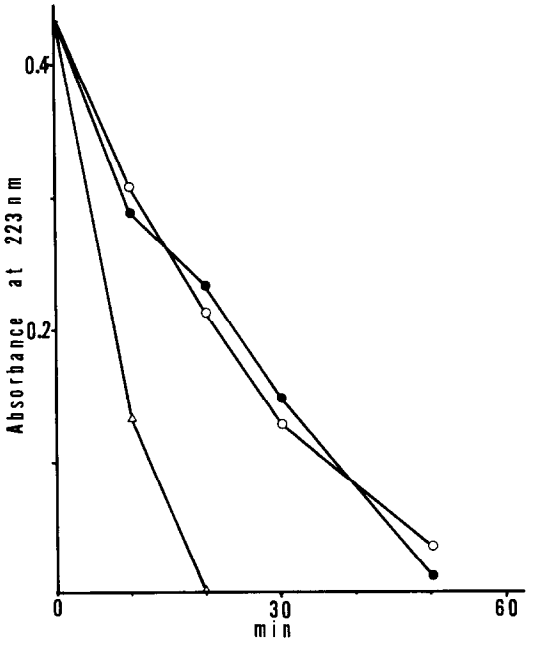

Fig. 5. Time-course of recovery of acetaldehyde from whole blood, plasma and erythrocyte suspension. Excepting that the liquid paraffin was omitted, experiments were carried out as described in the legend of Fig. 3. Key: (——△——) plasma, (——○——) erythrocyte suspension, and (——●——) whole blood.

in plasma rapidly evaporated at 37° before oxidizing in the medium and diffusing into the semicarbazide solution in the center chamber of the Conway units. This finding was in marked contrast to the acetaldehyde in blood and red cell suspension. This means that acetaldehyde did not exist free for long in the plasma, under biological conditions. The rapid decreases of acetaldehyde were also observed in several tissue homogenates under a liquid paraffin layer (Fig. 6). It is apparent that the rapid disappearance of acetaldehyde added to tissue, especially liver and kidney, is dependent on an aldehyde dehydrogenase in them. These observations imply that acetaldehyde generated in the liver by the oxidation of ethanol partially diffuses into blood and is carried mainly by red blood cells to the peripheral tissues where it is excreted or metabolized further to acetic acid.

In the recovery experiments from whole blood and red cell suspension, the recoveries of acetaldehyde from the samples in Conway units were found to be slightly higher than those from the samples that were covered with liquid paraffin (Figs. 3 and 4), suggesting that some acetaldehyde evaporated, without being oxidized prior to deproteinization, from the samples kept at 37° in Conway units, and diffused into the semicarbazide solution in the central well. Acetaldehyde disappeared rapidly from whole blood, red cell suspension, and plasma under the atmosphere (Fig. 5), indicating enhanced evaporation and oxidation.

To elucidate the acetaldehyde carrying power of the blood corpuscles, 1 ml of a mixture composed of equal volumes of a red blood cell suspension and a saline solution containing one of several acetaldehyde concentrations was applied to a Conway unit (Fig. 7). At an acetaldehyde concentration of less than 0.4 μmole/ml, the acetaldehyde in the mixture was not detectable as semicarbazone without deproteinization. At an acetaldehyde concentration of about 1 μmole/ml or above, however, the amount of acetaldehyde released after deproteinization, i.e. bound to blood cells, remained the same, no matter

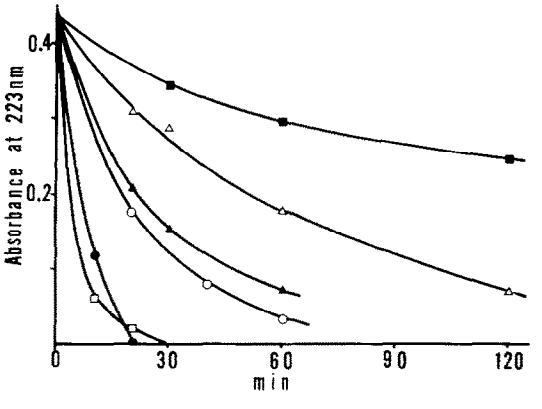

Fig. 6. Time-course of recovery of acetaldehyde from tissue homogenates of rat. Experiments were carried out as described in the legend of Fig. 3 using 25% tissue homogenates. Key: (——■——) muscle, (——△——) brain, (——▲——) spleen, (——○——) heart, (——●——) liver, and (——□——) kidney.

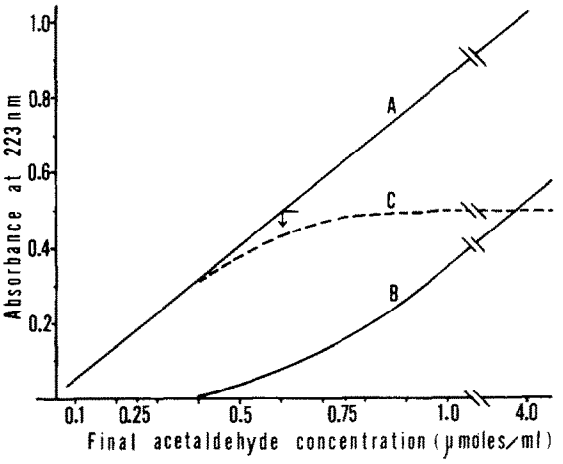

Fig. 7. Effect of deproteinization on the release of acetaldehyde in rat erythrocyte–saline suspension. An erythrocyte–saline suspension was mixed with an acetaldehyde solution (0.2 to ~8 μmoles/ml) in a volume ratio of 1:1. One ml from each sample was pipetted into the outer chamber of a Conway unit and the amount of acetaldehyde was determined. (A), after deproteinization (total amount of acetaldehyde); (B), without deproteinization (amount of acetaldehyde free in solution); and (C) A − B (amount of acetaldehyde bound to erythrocytes).

how high the applied aldehyde content was, suggesting that the red blood cells were already saturated at this concentration with acetaldehyde molecules (curve C in Fig. 7).

This finding indicates that the amount of acetaldehyde that can be transported by red blood cells is limited. The result shown in Fig. 7, together with the results that the aldehyde in plasma was removed easily by heating at 37° (Table 1 and Fig. 4), and that the aldehyde was rapidly oxidized in many tissues (Fig. 6), suggests that the acetaldehyde, which exceeded about 1 μmole/ml in final concentration, cannot exist in blood for long, and is rapidly excreted or oxidized in many peripheral tissues, as discussed above.

The maximum amount of acetaldehyde bound to 1 ml of rat erythrocyte suspension was double the acetaldehyde concentration calculated by extrapolating the maximal O.D. value of curve C to curve A in Fig. 7, which is identical with the standard curve in Fig. 1, that is 1.2 μmoles/ml (52.8 μg/ml).

The usual blood levels of acetaldehyde that have been reported thus far [16–18] are far less than this saturation value. Even if a larger amount of acetaldehyde diffuses into the blood from the liver, the amount of acetaldehyde bound to erythrocytes is limited, and the acetaldehyde in the plasma will be easily removed by excretion and oxidation in the course of peripheral circulation as discussed above. Acetaldehyde is also oxidized in the blood. Therefore, the acetaldehyde in the blood remains at remarkably low levels. As shown in Table 3, the release of acetaldehyde without deproteinization from a human erythrocyte-saline suspension was higher than that of the rat. Further studies may be required to elucidate the more detailed relationship

Table 3. Recovery of acetaldehyde from rat and human erythrocyte–saline suspension*

	Aldehyde added (μmoles)	Deproteinizing agent	Recovery	
			μmoles	%
Rat erythrocyte suspension	0.75	$HClO_4$	0.730	94.2
		0	0.272	36.3
	0.5	$HClO_4$	0.483	96.6
		0	0.028	5.6
Human erythrocyte suspension	0.75	$HClO_4$	0.775	103.3
		0	0.401	53.5
	0.5	$HClO_4$	0.503	105.5
		0	0.051	10.2

* Samples were prepared by mixing acetaldehyde–saline solution with rat and human erythrocyte–saline suspension in a volume ratio of 1:1. One ml from each sample was pipetted into the outer chamber of Conway units.

between the acetaldehyde molecules and blood corpuscles, ghosts, or hemoglobin with respect to transport of acetaldehyde in blood.

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