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Acetaldehyde, A Product of Deoxynucleoside Metabolism in Human Erythrocyte Ghosts.* (29457)

FABIAN J. LIONETTI, NORMAND L. FORTIER AND JUDITH A. JEDZINIAK
(Introduced by E. R. Loew)
Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts

Acetaldehyde has been reported in human red blood cells(1,2) as well as in the blood of several mammalian species(3). The significance of its presence is unknown. Its reactivity and low concentration (40-100 μg/ml) render it difficult to detect in extracts of whole blood made with common protein precipitants such as tricholoracetic acid, tungstic acid, or zinc hydroxide. However, it may be distilled quantitatively from blood due to its high vapor pressure in aqueous solutions at 37°C(4,2).

Human erythrocyte ghosts contain no acetaldehyde as the small endogenous concentration of intact cells is removed during hemolysis and washing. However, when ghosts are incubated with deoxynucleosides or deoxyribosephosphate a catabolic pathway may be described in which acetaldehyde is produced along with several phosphate esters.

Experimental. Ghost suspensions were made and equilibrated as described previously(5). They were reconstituted with physiological buffer to give cell counts of 5×10^6 cells per mm^3. Thirty-five ml of ghost suspension were incubated with deoxyinosine or deoxyadenosine at 37°C in incubation flasks kept under an atmosphere of 5% CO_2-95% O_2 for 5 hours at shaking rates of 50-60 oscillations per minute. Phosphate buffer (pH 7.4) was added to a final concentration of 4.0 mM. In several experiments hemolysates were made by freezing and thawing twice washed, packed erythrocytes and incubated (5.0 ml total volume) with deoxyribose-5-P.

Samples were withdrawn at intervals and precipitated with cold 30% metaphosphoric acid, centrifuged, the precipitate washed with 2% metaphosphoric acid, the combined supernatants adjusted to a pH 6.8 and diluted (final concentration metaphosphoric acid 4%). Acetaldehyde was determined with yeast alcohol dehydrogenase(6) and confirmed colorimetrically with semicarbazide. The extracts from the incubations were gassed at 37°C with nitrogen in a small train and the vaporized acetaldehyde trapped in a buffered solution of semicarbazide (pH 7.0). The acetaldehyde semicarbazone chromophore was then estimated from its absorbancy at 224 mμ(2).

The magnitude of deoxynucleoside metabolized was measured by comparing the hypoxanthine produced(7) with the diminution

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TABLE I. Acetaldehyde from Deoxyinosine in Ghosts.

Incubation hr	Acetaldehyde in extract (μmoles/10^{12} cells) Alcohol dehydrogenase method	Semicarbazide method
0	0	3.0
1	48	42.
2	18	22.
3	21	26.

Ghost suspensions were incubated with deoxyinosine (10 mM) and inorganic phosphate (4 mM) at a pH of 7.0. Aliquots (1.0 ml) of the incubation mixture were diluted to 5.0 ml with water and the acetaldehyde trapped in 3.0 ml of semicarbazide reagent. Aliquots were also precipitated with metaphosphoric acid and assayed with alcohol dehydrogenase.

of diphenylamine reactive chromophore(8). Both were approximately 1200 μMoles per 10^{12} cells in 4 hours.

Results and discussion. Acetaldehyde was found in the extracts with deoxyinosine as substrate (Table I). Little difference in rates of accumulation was found between deoxyinosine or deoxyadenosine. Considerable variability was found among blood samples from different donors. In all blood samples a maximum accumulation occurred, which varied from one to three hours of incubation with nucleoside (approximately 45 μMoles/10^{12} cells). The aldehyde must be derived from the carbon of the substrate as none was found in ghosts without deoxynucleosides. Nor was acetaldehyde detected when nondeoxy ribose derivatives such as inosine, or ribose-5-phosphate were used as substrate. A separate 50 ml volume of ghost suspension containing 10 mM deoxyinosine was incubated for 1 hour, the contents gassed with nitrogen, and the volatile aldehyde trapped in 2,4 dinitrophenylhydrazine in HCl. Crystals of 2,4 dinitrophenylhydrazones were obtained which were recrystallized twice from a diluted sample of redistilled acetaldehyde similarly gassed and trapped in reagent. Both melted at 166-168°C.

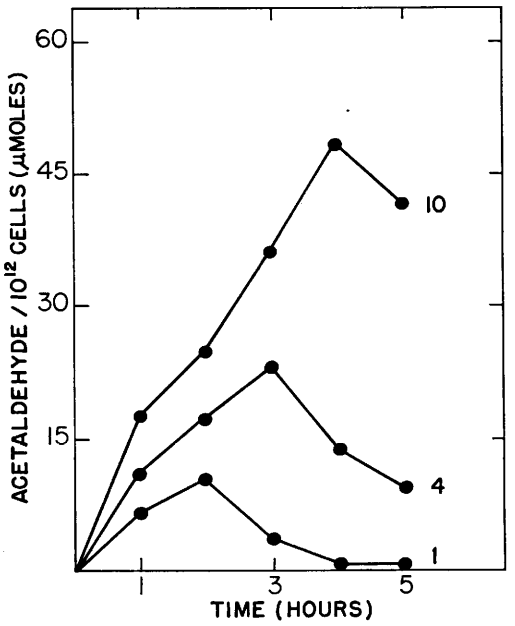

FIG. 1. Dependence of acetaldehyde accumulation on deoxyinosine concentration. The points represent acetaldehyde (enzyme assay) in metaphosphoric extracts of ghost suspensions incubated with 1,4, and 10 mM deoxyinosine. Inorganic phosphate in all cases was 4 mM.

The highest quantities of acetaldehyde were found at one hour of incubation in the experiment of Table I which suggested a turnover of the aldehyde in ghosts. That this is so is seen in Fig. 1. The kinetic maximum varied from 1-3 hours dependent on the ghost preparation and dependent on exogenous substrate. Triosephosphates† (G-3-P plus DHAP(9)) were likewise found to accumulate in the extracts, accumulating in ghosts to several orders of magnitude greater than the acetaldehyde. The amounts at the kinetic maxima (approximately 2 hours) for 1,4 and 10 mM deoxyinosine were 15, 40, and 120 μMoles/10^{12} respectively. The TrP did not turn over in the tissue as did the acetaldehyde as the steady state concentrations remained equilibrated similar to experiments reported with inosine(10).

Preliminary evidence suggests the presence of a deoxyribosephosphate aldolase in erythrocytes as an enzyme of the deoxynucleoside pathway. When dR-5-P, made from dAMP (11) was incubated with hemolysates made by twice freezing and thawing erythrocytes,

† Abbreviations are: glyceraldehyde-3-P, G-3-P; dihydroxyacetone-P, DHAP; deoxyribose-5-P, dR5P; deoxyadenylic acid, dAMP; triosephosphates, TrP; fructosediphosphate, FDP; diphosphopyridine-nucleotide, NAD^+.

TABLE II. Metabolism of dR-5-P by Hemolysates.

Incubation hr	dR-5-P used	Acetaldehyde	TrP
		μmoles/10^{12} RBC	
0	0	0	0
1	149	120	74
2	259	193	163

Incubation consisted of 5.0 ml containing 1.0 ml of a thrice frozen-thawed 25% cell suspension in .015 M phosphate buffer (pH 7.4) and dR-5-P (1.0 mM).

both acetaldehyde and triose-P were produced (Table II). At the low substrate level of the dR-5-P incubation with hemolysates, less TrP than acetaldehyde accumulated. This we believe results from considerably greater FDP aldolase activity in hemolysates than in ghosts. Likewise ghosts contain only 10-20% of the erythrocyte triosephosphate dehydrogenase and little endogenous NAD^+ (10). TrP would thus be more stable in ghosts.

From nucleoside concentrations (10 mM) yielding maximum rates, it was estimated that 5% of substrate deoxypentose was found in the acetaldehyde and TrP fractions at their kinetic maxima. The turnover of the acetaldehyde nevertheless suggests that a substantial portion of deoxypentose may be split by the aldolase. It has been observed that acetaldehyde added to blood is rapidly metabolized(4). Extensive accumulation from deoxynucleosides would thus not occur. The finding by Jaffe(12) that deoxyinosine is effective in preventing the oxidation of hemoglobin to methemoglobin may find explanation in the fact that acetaldehyde, a good reducing agent, is produced when erythrocytes metabolize deoxynucleosides.

Summary. Acetaldehyde was found to be a product of the catabolism of deoxyinosine and deoxyribose-5-P in human erythrocyte ghosts. The simultaneous production of triosephosphates indicates the presence in erythrocytes of deoxyribosephosphate aldolase.

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Role of Renal Tissue Calcium in Alteration of Urinary Specific Activity.* (29458)

E. J. MILLER† AND W. F. NEUMAN (Introduced by I. Zipkin)
Department of Radiation Biology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Although urine and blood calcium specific activities in the dog are generally the same following intravenous administration of radioactive calcium, it has previously been reported(1) that under certain circumstances large discrepancies between their specific activities may be observed. Specific activity differences were noted following intraperi-

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† Present address: Nat. Inst. Dental Research, Nat. Inst. Health, Bethesda, Md.