

## An Acetaldehyde Dehydrogenase from Germinating Seeds<sup>1</sup>

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**Summary.** An acetaldehyde dehydrogenase from germinating peanut cotyledons has been purified and its properties have been studied. At the highest purification achieved the preparation is free of alcohol dehydrogenase activity.

The enzyme is specific toward diphosphopyridine nucleotide, and can oxidize a variety of aldehydes. The highest reaction rate is obtained with acetaldehyde, which is oxidized to acetate. All the attempts to demonstrate the formation of an energy-rich acetyl derivative during the course of the reaction failed. The enzyme is inhibited by aldol; it is sensitive toward sulfhydryl reagents, including arsenite. Reduced glutathione stabilizes the enzyme, while cysteine, mercaptoethanol, and coenzyme A are inhibitory.

Acetaldehyde dehydrogenase is activated by phosphate and inhibited by fatty acyl-CoA derivatives. It appears to be activated by the substrate, as was deduced from the shape of the plot of reaction velocity against acetaldehyde. These properties suggest that the enzyme is an allosteric protein.

The plot of reaction velocity against substrate concentration is anomalous. The shape of this plot seems to reflect the presence of 2 different enzymatic activities, one with extremely high apparent affinity for acetaldehyde. The 2 activities may reflect 2 conformational states of a single enzyme or 2 separate enzymes.

Experiments with tissue slices indicate that the reaction catalyzed by this enzyme is a step in the oxidation of ethanol to acetyl-CoA. This enzyme may also participate in the oxidation of pyruvate to acetyl-CoA in certain tissues.

Besides the glyceraldehyde-3-phosphate dehydrogenases of the glycolytic and photosynthetic pathways 3 aldehyde dehydrogenases have been described in higher plants thus far. A long chain fatty aldehyde dehydrogenase discovered by Martin and Stumpf (15), catalyzes the oxidation of long chain aldehydes to the corresponding acids in the  $\alpha$ -oxidation system. That enzyme was first shown in the microsomes of germinating peanuts. It is DPN specific, and has high affinity for long chain aldehydes. Its physiological role is apparently in the degradation of fatty acids.

Another aldehyde dehydrogenase was found in pea mitochondria by Davies (3). This enzyme appears to be the glycolaldehyde dehydrogenase described by Davies later (4). In both cases the enzyme was present in extracts of mitochondria of pea seedling epicotyls, and was found to reduce either DPN or TPN and to require cysteine for activity. Although the glycolaldehyde dehydro-

genase is capable of oxidizing acetaldehyde, its primary physiological role seems to be the oxidation of glycolaldehyde in the biosynthesis of glycine (4). This was suggested because glycolaldehyde was oxidized faster than acetaldehyde and because experiments with spinach leaves, where the dehydrogenase is also present, showed that glycolaldehyde-<sup>14</sup>C was converted to glycine-<sup>14</sup>C in that tissue.

The third enzyme is the one described in this work. It is present in the soluble fraction of germinating peanut cotyledons. This enzyme is specific toward DPN, oxidizes acetaldehyde faster than glycolaldehyde, and is incapable of oxidizing long chain aldehydes. Its physiological role seems to be in the metabolism of acetaldehyde, ethanol, and pyruvate, and by implication in the oxidation of carbohydrates.

Evidently these are 3 enzymes which differ in their intracellular localization and in their properties, and possess distinct physiological roles.

In a previous paper (14) we have described the purification of the acetaldehyde dehydrogenase from germinating peanut cotyledons; the properties of this enzyme have been investigated further and form the object of this report.

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## Materials and Methods

Formaldehyde, a 36% reagent grade aqueous solution was obtained from Allied Chemicals; acetaldehyde from Eastman; butyraldehyde, caproic aldehyde and aldol, from Matheson Coleman and Bell; benzaldehyde from Baker and Adamson; Na glyoxylate from Nutritional Biochemicals, and palmitoyl coenzyme A from Sigma. Other reagents and materials were obtained as reported previously (14).

Acetaldehyde was distilled under reduced pressure, the distillate was diluted with water to yield a 1% solution which was stored at  $-15^{\circ}$ . Caproic aldehyde and benzaldehyde were dissolved in ether and the ethereal solution washed with 0.5 M  $\text{NaHCO}_3$  to remove the corresponding acids which were the major contaminants in these samples. The ether was evaporated under  $\text{N}_2$  at  $0^{\circ}$  and the residue was dissolved in  $\text{H}_2\text{O}$ . Aldol was redistilled at 4 to 6 mm Hg ( $39-44^{\circ}$ ). The receiver was cooled by a dry ice-acetone bath to prevent polymerization. The distillate was then diluted to 1 M aqueous solution and stored at  $-15^{\circ}$ . Glycolaldehyde was prepared by the method of Fenton (6). Complete conversion of dihydroxymaleic acid to glycolaldehyde was assumed for calculation of glycolaldehyde concentration. Acetyl-P was prepared as described by Stadtman (20). Acetyl-CoA and butyryl-CoA were prepared from the corresponding anhydrides (21). Salt free  $\text{NH}_2\text{OH}$  was prepared as described by Rebeiz and Castelfranco (18).

The enzyme was prepared from acetone powder of germinating peanut cotyledons as described previously (14). The purification procedure involved extraction by dilute buffer at acid pH, acetone fractionation, protamine sulfate treatment and isoelectric precipitation. The last step removed alcohol dehydrogenase as well as diaphorase and other activities responsible for the oxidation of DPNH in the cruder preparations. This enzyme was still far from pure, for instance it contained malic dehydrogenase activity, which however did not interfere with the acetaldehyde dehydrogenase assay.

An assay for acetaldehyde dehydrogenase activity based on the conversion of radioactive acetaldehyde to acetate has been described (14). A spectrophotometric assay became possible after the enzyme preparation was freed from alcohol dehydrogenase and other interferences. The enzyme (600  $\mu\text{g}$  protein) was assayed at  $35^{\circ}$  in a 1.0 cm quartz cuvette containing in a total volume of 3.0 ml: 6  $\mu\text{mole}$  acetaldehyde, 4.5  $\mu\text{mole}$  DPN and 300  $\mu\text{mole}$  of potassium phosphate buffer pH 8.6. The reaction was usually started with the addition of the enzyme. The order of addition of reactants did not affect the reaction rate significantly. The change in absorbance at 340  $\mu\text{m}$  was followed in a

Zeiss PMQ II spectrophotometer against a blank containing the enzyme, buffer and DPN. The reaction was followed for at least 6 minutes and was linear during that period except when the initial acetaldehyde concentration was less than 0.01 mM (first 3 points in fig 4), in which case there was an observable decrease in the rate as the substrate approached depletion. Initial reaction velocities were used in all the calculations. The enzyme unit was defined as the amount of enzyme which causes an absorbance change of 0.001 units per minute at 340  $\mu\text{m}$  under the conditions of the assay.

Deacylase and phosphatase were determined by incubating acetyl-CoA and acetyl-P with the enzyme preparation under conditions paralleling those of the acetaldehyde dehydrogenase assay. The reactions were carried out at room temperature in 0.1 M K phosphate buffer pH 8.6. The substrate concentration was 1 mM. Aliquots of the reaction mixture were assayed for active acetate derivatives at intervals (13).

Inorganic phosphate was determined by the method of Fiske and SubbaRow (12).

Proteins were determined by the biuret method (11) with bovine serum albumen as standard.

## Results

*Stoichiometry.* In studies on the stoichiometry of the reaction it was found that 1  $\mu\text{mole}$  DPNH was formed for each  $\mu\text{mole}$  of acetaldehyde added to the incubation mixture (table I). These results

Table I. *Stoichiometry of the Reaction*

Incubations were carried out in the Zeiss spectrophotometer as described in Materials and Methods. In each case the reaction was allowed to proceed to completion, and the total amount of DPNH formed was determined.

Acetaldehyde added $\mu\text{mole}$	DPNH formed $\mu\text{mole}$
18	17.4
30	28.5
60	56.1

were obtained with freshly distilled acetaldehyde but could not be reproduced quantitatively with acetaldehyde which had been stored as a 1% aqueous solution for several weeks at  $-15^{\circ}$ . Evidently even under these storage conditions some losses of acetaldehyde occur because of polymerization, volatility or oxidation. The other product of the reaction was previously identified as acetate (14).

*Kinetic Studies.* The reaction velocity was linear with protein concentration (fig 1).

The plot of reaction velocity versus DPN concentration followed a typical Michaelis-Menten behavior (fig 2,3). From this plot a  $K_m$  value

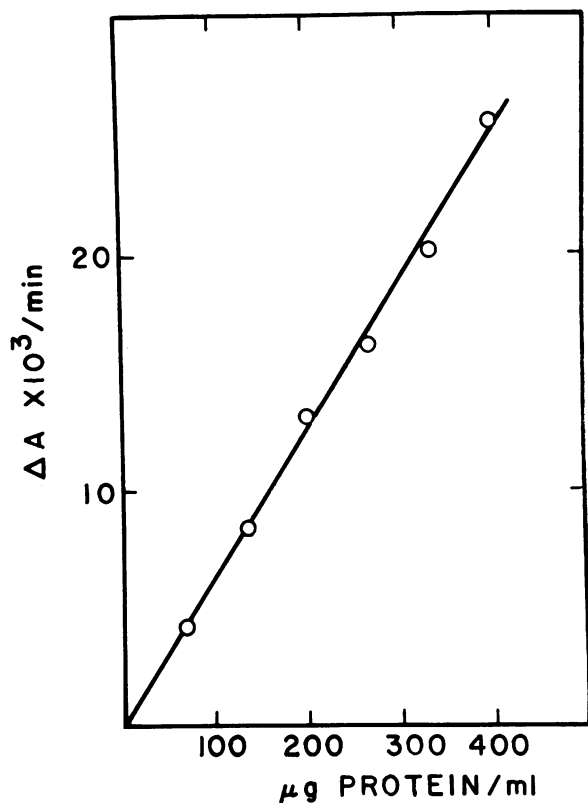


FIG. 1. The relation of reaction rate to enzyme concentration.

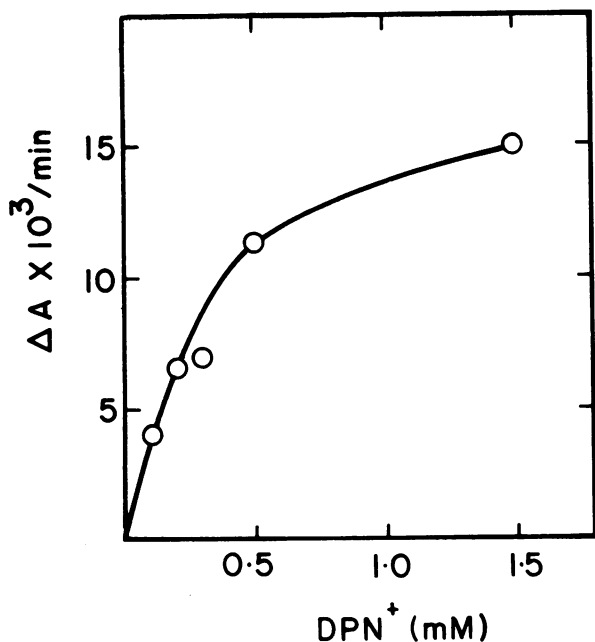


FIG. 2. Reaction rate as a function of DPN<sup>+</sup> concentration.

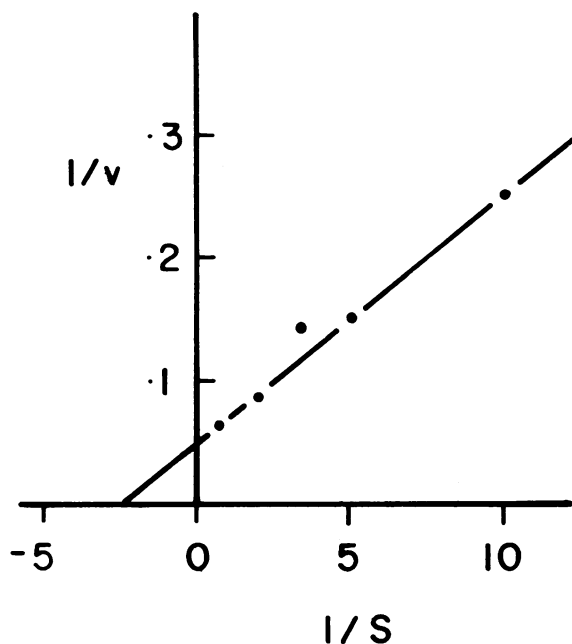


FIG. 3. Lineweaver-Burk plot of the effect of DPN on the reaction rate.

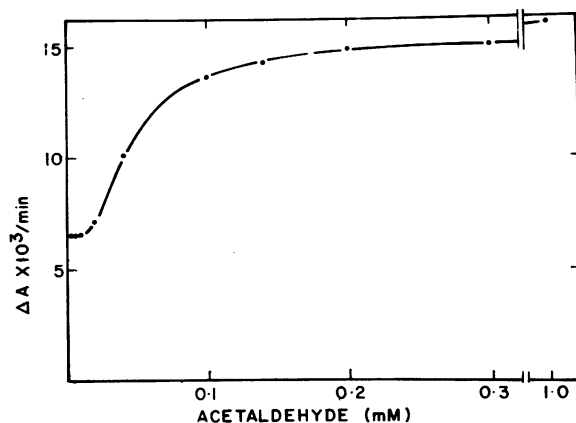


FIG. 4. Reaction rate as a function of acetaldehyde concentration. Enzyme, buffer and DPN, were added first to the reaction vessel, then the reaction was started with acetaldehyde. Note that the lowest concentration of acetaldehyde studied was 1  $\mu$ M, and the initial velocity of the reaction at this concentration was 40% of  $V_{max}$ . There was no increase in absorbance when no acetaldehyde was added.

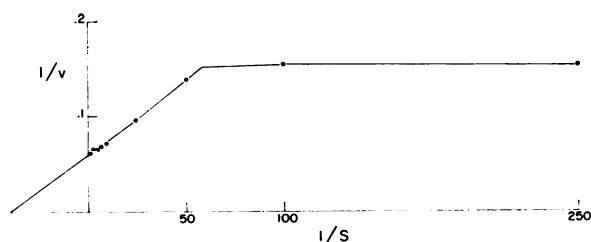


FIG. 5. Lineweaver-Burk plot of the reaction rate as a function of acetaldehyde concentration.

of 0.42 mM was calculated. On the other hand, the plot of reaction velocity versus acetaldehyde concentration was anomalous (fig 4, 5), the most striking feature being a fairly high velocity (about 40%  $V_{max}$ ) at very low acetaldehyde concentrations. In these studies enzyme, buffer and DPN were added first to the reaction vessel and the reaction was started with acetaldehyde. Under these conditions there is no increase in absorbance when no acetaldehyde is added, while the initial velocity at 1  $\mu M$  acetaldehyde is 40% of  $V_{max}$ . Initial velocities at acetaldehyde concentrations below 1  $\mu M$  were not studied. In contrast to acetaldehyde dehydrogenases from several other sources, no substrate inhibition was observed up to 2 mM substrate concentration. A Lineweaver-Burk plot of reaction rate as a function of acetaldehyde concentration (fig 5) failed to give the linear relationship which is expected according to Michaelis-Menten kinetics. The experimental points are distributed along 2 straight lines which intersect each other at an acetaldehyde concentration approximately 0.02 mM. Two Michaelis constants are found for acetaldehyde depending upon the range of acetaldehyde concentration. At the higher concentration the  $K_m$  is 0.026 mM. It has been estimated from experiments carried out with the Cary recording spectrophotometer (17), and not reported here in detail, that the  $K_m$  at the lower concentration is approximately 0.2  $\mu M$ .

A curve of this type for the interaction between reaction velocity and substrate concentration suggests either substrate activation or the presence of 2 acetaldehyde dehydrogenases. The experimental results obtained so far do not permit us to choose between these 2 hypotheses. Recently Erwin and Deitrich (5) have found the same kinetic behavior toward acetaldehyde with an aldehyde dehydrogenase from brain tissue.

**pH Effect.** The pH profile of the initial reaction velocity is shown in figure 6. The highest velocity is obtained in the pH range from 8.6 to 9.0. The enzyme is rapidly inactivated when the

Table II. *Substrate Specificity*

The reaction was followed spectrophotometrically. In the absence of enzyme, no upward drift in absorbance at 340  $m\mu$  was observed. The reaction rates are recorded relative to 1.7 mM acetaldehyde.

Substrate	Relative rate of the reaction		
	1.7 mM	0.17 mM	0.017 mM
Formaldehyde	38	9	
Acetaldehyde	100	95	50
Butyraldehyde	45	42	
Caproic aldehyde	19	9	
Benzaldehyde	24	35	35
Glycolaldehyde	48	62	12
Glyoxylic acid	0	0	
Aldol	10	15	10

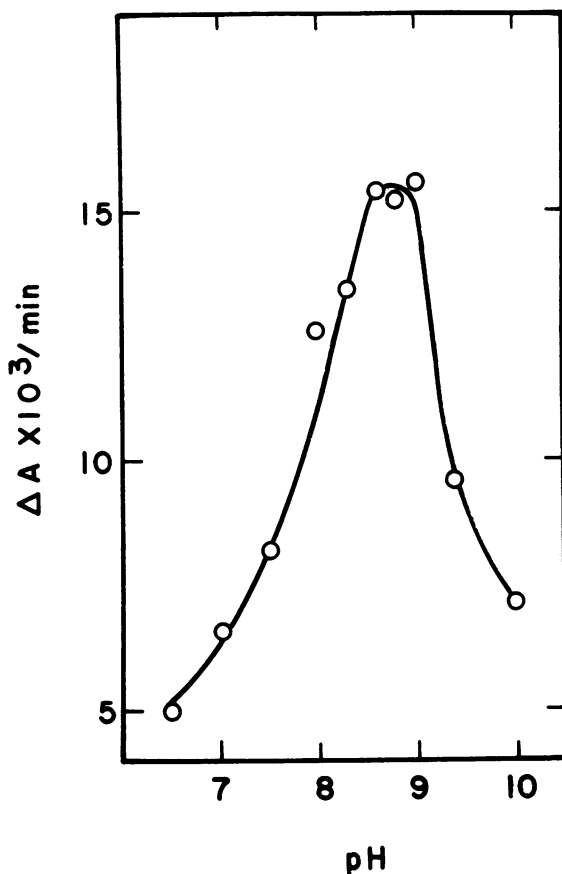


FIG. 6. Reaction velocity as a function of pH. Phosphate buffer at varying pH was used. Other conditions as described in Materials and Methods.

pH is in the neighborhood of 9, therefore it was found convenient to carry out the routine assay at pH 8.6.

**Specificity.** Table II shows the relative activity of several aldehydes at 3 concentrations taking 0.17 mM acetaldehyde as the standard. The enzyme is capable of oxidizing a wide variety of aldehydes. However it is more active toward acetaldehyde than any other of the substrates tested. Benzaldehyde and glycolaldehyde are inhibitory at the highest levels. Aldol inhibits the oxidation of acetaldehyde even at low concentration (fig 10). Glyoxylic acid is not oxidized. Myristic aldehyde, which is only sparingly soluble in water did not appear to be oxidized at all.

**Effect of Inorganic Ions.** The 2 aldehyde dehydrogenases found in yeast have been shown to require respectively  $K^+$  or  $Mg^{++}$  for activity (1, 19). When the assay mixture was buffered with 0.01 M K phosphate buffer, K, Na or Li chlorides were all slightly inhibitory (35% inhibition at 0.17 M concentration); 1 mM  $MgCl_2$  had no effect, but at higher concentrations it caused the formation of a heavy precipitate and a large inhibition. On the

Table III. *Activation by Phosphate in Comparison to Other Anions*

The enzyme preparation added to each incubation mixture (3 ml) contained 0.5  $\mu$ mole phosphate.

Buffer, pH 8.6	$\Delta A \times 10^3/\text{min}$
0.01 M potassium phosphate	10.0
0.10 M potassium phosphate	16.4
0.10 M sodium phosphate	17.4
0.10 M sodium arsenate	15.4
0.05 M sodium pyrophosphate	6.8
0.10 M sodium pyrophosphate	6.4

other hand, phosphate ion had an activating effect on this enzyme (table III, fig 7). Phosphate could be replaced by arsenate but not by pyrophosphate. No difference could be detected in this respect between Na and K phosphate buffers. In order to keep the ionic strength constant, various mixtures of K phosphate buffer and  $\text{Na}_2\text{SO}_4$  were added to the enzyme (table IV). The presence of  $\text{Na}_2\text{SO}_4$  did not appear to inhibit or stimulate the reaction.

 Table IV. *Activation by Phosphate in Relation to Total Ionic Strength*

Reactions were carried out in 3.0 ml total volume. Phosphate was added as potassium phosphate buffer, pH 8.6. Sulfate was added as sodium salt.

Added to the reaction mixture		$\Delta A \times 10^3/\text{min}$
Phosphate $\mu$ mole	Sulfate $\mu$ mole	
300	0	15.2
100	0	12.2
100	200	12.0
50	0	9.8
50	250	10.2

In the normal purification procedure, after the isoelectric precipitation, the enzyme was dissolved in 0.05 M K phosphate buffer pH 7.5. Substitution of Tris HCl buffer for phosphate resulted in rapid and irreversible inactivation. However the enzyme could be prepared in a phosphate-free medium as follows: the isoelectric precipitate was suspended in  $\text{H}_2\text{O}$  and 1 M  $\text{KHCO}_3$  was added dropwise at  $0^\circ$  until the enzyme dissolved; the resulting solution was approximately pH 7.0 and was as active as the enzyme prepared in phosphate buffer.

The bicarbonate enzyme retained its activity until it was frozen. Using enzyme which had been prepared in bicarbonate and 0.02 M K-glycine buffer pH 8.6 in the reaction mixture, it was possible to obtain a curve for the phosphate activation effect (fig 7). This enzyme still contained 0.097  $\mu$ mole Pi per assay tube. The inorganic anion was presumably adsorbed to the protein.

*Inhibitor Studies.* Like other known aldehyde dehydrogenases, the peanut enzyme is sensitive to thiol reagents (table V); however low molecular

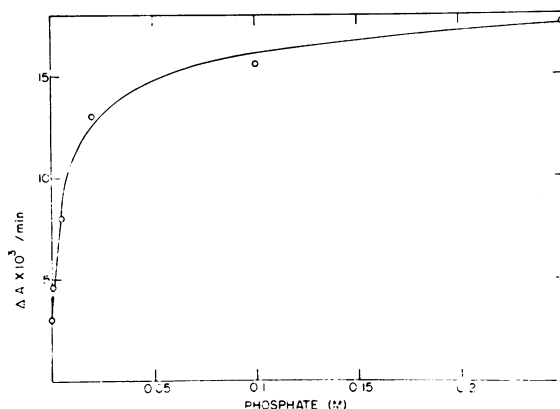


FIG. 7. Effect of phosphate concentration on reaction velocity. The enzyme was prepared in  $\text{KHCO}_3$  as described in the text and contained 0.097  $\mu$ mole Pi per 3 ml reaction mixture. The reaction mixtures contained 0.02 M K glycine buffer pH 8.6 plus varying amounts of K phosphate buffer at the same pH and 1.7 mM acetaldehyde.

weight thiols had no stimulating effect: mercaptoethanol, cysteine and CoA were inhibitory while GSH had a slight protective effect that became apparent on prolonged incubation (fig 8). Acetyl-CoA was decidedly inhibitory and this effect was more pronounced with the higher acyl-CoA derivatives (fig 9, table VI). Another inhibitor was the substrate analogue aldol (fig 10), itself a poor substrate for the enzyme (table II). Because of the kinetic peculiarities of the uninhibited reaction it was not possible to submit this inhibition to a classical kinetic analysis.

*Other Studies.* Attempts to purify the enzyme further were unsuccessful. Although the enzyme was stable for several weeks upon storage at  $-15^\circ$ , it was destroyed rapidly under conditions that involved either high ionic strength or high dilution of the protein. Ammonium sulfate precipitation,

 Table V. *Inhibition by Thiol Reagents*

Inhibitor	Final concentration mM	$\Delta A \times 10^3/\text{min}$	% inhibition
None	0.1	18.8	...
Arsenite	0.1	16.8	10
Iodoacetamide	0.1	8.6*	55
$\text{HgCl}_2$	0.1	10.0*	47
$\text{CuCl}_2$	0.1	9.0*	52
<i>p</i> -Chloromercuribenzoate	0.001	15.0	20
<i>p</i> -Chloromercuribenzoate	0.005	11.4	39
<i>p</i> -Chloromercuribenzoate	0.01	0	100

\* The inhibition was progressive during the 6 min that the reaction was followed. The initial rates of the reaction are recorded.

Table VI. *Inhibition of Acetaldehyde Dehydrogenase by Acyl-CoA Derivatives*

Compound added	Final concentration mM	% inhibition
Malonyl-CoA	0.33	0
Acetyl-CoA	0.33	14
Butyryl-CoA	0.33	34
Palmityl-CoA	0.33	80
Palmityl-CoA	0.11	43

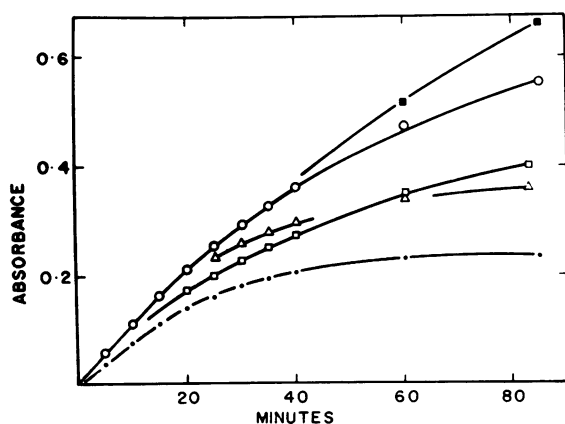


FIG. 8. Effect of mercaptans on the activity of acetaldehyde dehydrogenase. Incubations at 35° in the presence of 1.7 mM acetaldehyde. ○ — ○, control; ■ — ■, 1.7 mM GSH; □ — □, 1.7 mM mercaptoethanol; △ — △, 1.7 mM cysteine; ● — ●, 0.33 mM coenzyme A.

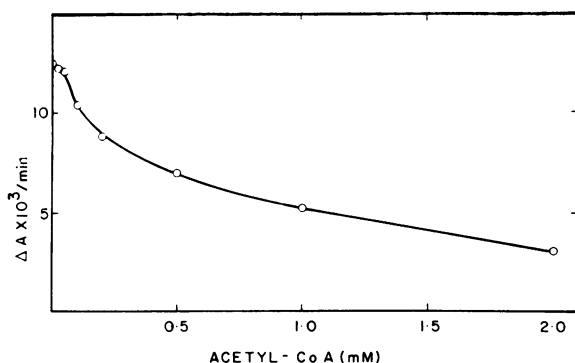


FIG. 9. Effect of acetyl-CoA concentration on the reaction rate. The substrate was 0.1 mM acetaldehyde.

adsorption by Alumina C $\gamma$ , chromatography on cellulose ion exchangers and on Sephadex columns, and fractionation by prolonged high-speed centrifugation in sucrose gradient resulted in total loss of activity. Refinement of the precipitation techniques by addition of acetone or acid did not inactivate the enzyme, but failed to yield further purification.

Study of the molecular weight of the enzyme was also attempted. Enzyme preparations containing 80 units acetaldehyde dehydrogenase and 6 mg protein in a volume of 0.2 ml were applied to short Sephadex G-50, G-100, and G-200 columns. Acetaldehyde dehydrogenase was completely excluded from Sephadex G-50, G-100, and G-200. Under the same conditions yeast alcohol dehydrogenase (molecular weight 150,000) was partially retained by the G-200 column. Unfortunately, since acetaldehyde dehydrogenase is unstable under these conditions, only very short columns (5.4 cm) could be employed as compared with columns normally used for this type of study. No significant purification was obtained, but the result of this experiment provided suggestive evidence that the molecular weight of this enzyme is greater than 200,000.

Acetate was the only radioactive product that could be detected when the reaction was allowed to proceed using acetaldehyde 1,2- $^{14}$ C as the substrate. However because of the stimulating effect of Pi and the inhibitory effect of acetyl-CoA, it was important to consider the possibility that an active acetyl intermediate (acetyl-P or acetyl-CoA), might be the real product of the reaction, which is subsequently hydrolysed to yield acetate. Furthermore, a DPN dependent dehydrogenase which oxidizes acetaldehyde to acetyl-CoA in the presence of CoA is known to exist in *Clostridium kluyveri* (2, 22), and 3-phosphoglycerdehyde dehydrogenase has been shown to oxidize acetaldehyde to acetyl phosphate in the presence of Pi (7). With the peanut enzyme no evidence for an energy-rich intermediate could be found.

Ethanol-1- $^{14}$ C was oxidized by a crude enzyme preparation (containing alcohol dehydrogenase) in phosphate buffer, in the presence of DPN; non radioactive acetyl-CoA was added as a trap. Since radioactive acetate is formed under these conditions the incorporation of radioactivity from acetate to

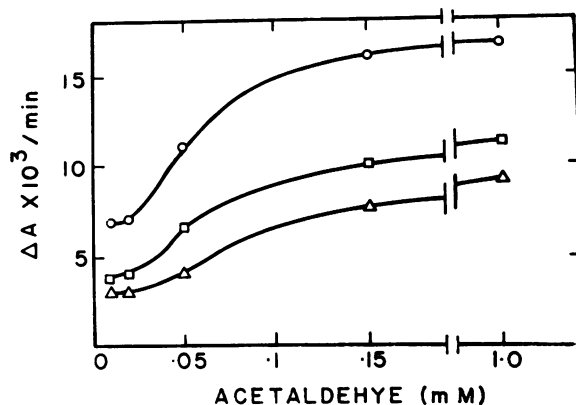


FIG. 10. Inhibition by aldol. ○ — ○, control; □ — □, 0.06 mM aldol; △ — △, 0.30 mM aldol.

acetyl-CoA was studied in a parallel incubation. At the end of the reaction, the radioactivity in the acetate was determined by the butanol extraction method (14) and the radioactivity trapped as active acetate was determined by the acetyl-hydroxamic acid method (18). The radioactivity incorporated into active acetate compounds under these conditions was very low. This incorporation could be accounted for by oxidation of ethanol- $^{14}\text{C}$  to acetate- $^{14}\text{C}$  and subsequent conversion of acetate- $^{14}\text{C}$  to acetyl- $^{14}\text{C}$ -CoA; it was far too low to suggest the oxidation of ethanol via acetaldehyde to acetyl-CoA.

Attempts to trap an active acetyl intermediate by adding  $\text{NH}_2\text{OH}$  to a reaction mixture in which acetaldehyde 1,2- $^{14}\text{C}$  was oxidized enzymatically gave only negative results, but it was difficult to select the incubation conditions so that the acetaldoxime formation could be minimized. When the enzymatic oxidation of radioactive acetaldehyde was allowed to proceed in the presence of CoA, oxalacetate and citrate synthetase, no radioactive citrate was detected.

Acetyl-CoA and acetyl-P did not oxidize DPNH when these compounds were incubated with the purified acetaldehyde dehydrogenase. The oxidation of DPNH by these substrates is thermodynamically possible and was studied by Burton and Stadtman with the *Clostridium kluuyveri* enzymes (2), and by Harting and Velick (7), with crystalline 3-phosphoglyceraldehyde dehydrogenase. The purified acetaldehyde dehydrogenase from peanuts contained at the most, only a trace of phosphatase and deacylase activities, which could not possibly interfere with the demonstration of the reversible oxidation of DPNH by these high energy acetyl compounds. Finally, the very fact that the purified enzyme does not require CoA argues against an acetyl-CoA intermediate.

## Discussion

DPN and TPN enzymes which catalyze the oxidation of acetaldehyde to acetate have been described from a variety of sources (9). All of these enzymes contain essential sulphydryls. The peanut enzyme shares with a DPN specific aldehyde dehydrogenase from *Pseudomonas fluorescens* the requirement for inorganic phosphate (8). In neither case is a high energy acetate compound formed in the reaction.

It has already been mentioned that crystalline 3-phosphoglyceraldehyde dehydrogenase catalyzes the reversible formation of acetylphosphate from acetaldehyde and inorganic phosphate (7). However the affinity of this enzyme for acetaldehyde is low, the  $K_m$  being of the order of 0.1 M.

Nygaard and Sumner (16) described a crude 3-phosphoglyceraldehyde dehydrogenase preparation which showed a relationship between velocity and

substrate concentration qualitatively similar to the one found by us with the peanut enzyme (fig 4, 5) and by Erwin and Dietrich (5) with the brain enzyme. Upon further purification Nygaard and Sumner obtained a decrease in the affinity of their preparation toward acetaldehyde and a more normal Lineweaver and Burk plot. These results suggest that they were dealing with a mixture of aldehyde dehydrogenases of different relative affinities for various substrates; and the same thing could be true of the peanut and brain enzyme preparations.

On the other hand, the unusual interaction between reaction velocity and substrate concentration could be attributed to specific configurational changes of a single enzyme. The sigmoid relationship between reaction velocity and substrate concentration (fig 4) and the cooperative effect in the inhibition of this enzyme by acetyl CoA (fig 9) suggest that the peanut aldehyde dehydrogenase is an allosteric enzyme. A formal analysis of these 2 possibilities, either a mixture of 2 or more enzymes, or a single enzyme undergoing conformational changes with substrate concentration, has been presented elsewhere (17). Unfortunately, the experimental evidence which is available thus far, does not enable us to choose between them.

Regardless of the actual enzymatic mechanisms involved, our data suggest that the oxidation of acetaldehyde proceeds with greater affinity at low substrate concentration, and with lesser affinity when the substrate concentration exceeds a certain critical value (ca. 0.02mM). Thus, if the rate of acetaldehyde production should become abnormally high, the rate of acetaldehyde removal by oxidative reactions would not become saturated until the substrate concentration exceeds 0.1 mM (fig 4). This situation could be physiologically advantageous, particularly if acetaldehyde is the branching point between 2 metabolic pathways (fig 11). Laites has observed that acetaldehyde is given off by activating potato tuber slices and that the addition of acetaldehyde to the medium inhibits the activation process (10). It is not unlikely that acetaldehyde is a metabolite of equally critical importance in other plant tissues. Acetaldehyde dehydrogenase,

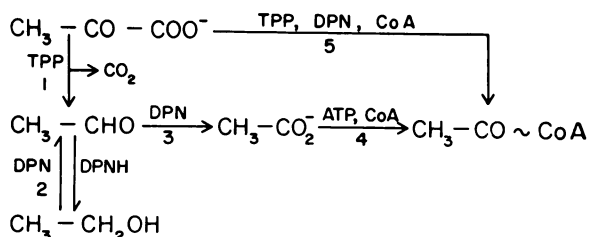


FIG. 11. Acetaldehyde dehydrogenase in relation to some other enzymes of pyruvate catabolism. 1) pyruvate decarboxylase; 2) alcohol dehydrogenase; 3) acetaldehyde dehydrogenase; 4) acetyl thiokinase; 5) pyruvate oxidase (mitochondrial).

the enzyme which mediates the oxidative metabolism of this intermediate, could play a critical role in tissues such as germinating peanut cotyledons and activating potato slices, in which the capacity to carry out aerobic respiration is rapidly increasing.

Acetaldehyde dehydrogenase appears to be involved in the oxidation of exogenous radioactive ethanol by peanut and pea cotyledon slices, castorbean endosperm slices, and activated potato tuber slices. In all these tissues aldol at the proper concentration will inhibit the liberation of  $^{14}\text{C}$  from ethanol- $^{14}\text{C}$  but not from acetate- $^{14}\text{C}$ . Aldol has no effect on the succinate and DPNH oxidation by isolated potato and castorbean mitochondria. It appears therefore that acetaldehyde dehydrogenase could be involved in the oxidation of endogenous alcohol to acetyl-CoA, a process which is probably physiologically significant. Acetaldehyde can also be formed by the decarboxylation of pyruvate. Consequently one can conceive of an alternate route for the conversion of pyruvate to acetyl-CoA involving pyruvate decarboxylase, acetaldehyde dehydrogenase and acetyl thiokinase, instead of the mitochondrial pyruvate oxidase complex (fig 11). Experiments are now under way which are aimed at elucidating the physiological role of acetaldehyde dehydrogenase.

Since fatty acid metabolism is very active in germinating peanut cotyledons, it is tempting to ascribe a regulatory function to the inhibition of acetaldehyde dehydrogenase by acyl-CoA derivatives. However Taketa and Pogell (23) have shown recently that many enzymes are inhibited by higher acyl-CoA derivatives, including some which are not related in any obvious way to fatty acid metabolism.

### Acknowledgment

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