implies that different membranes possess unique enzyme activities which must reflect the functional activity of particular membrane systems.

Because of the vastly different experimental procedures used, it is difficult to compare the present study with those of OVTRACHT et al.[18] or BERGERON et al.[19]. In the work of OVTRACHT et al.[18], a crude enzyme mixture, takadiastase, was used which contains many different activities including proteases, glycosidases, phosphatases, RNases, etc.[20], it is difficult to separate enzyme activities which may be a part of the Golgi system as compared to those which may have become associated with the membranes after addition of takadiastase. A similar objection can be made to the use of lysosomal extracts to unstack Golgi. The use of large doses of ethanol by BERGERON et al.[20] results in toxic effects, which would be reflected in fluctuations of the enzymic activities. A recent report describes a nearly 100% increase of glycosyltransferase activities in rat liver Golgi after a single dose of ethanol[21]. An increase of ^{35}S incorporation into liver mucopolysaccharides after alcohol has also been reported[22]. We feel that the gentle homogenization procedure used in our laboratory represents a fair assessment of associated enzyme activities[23]

Zusammenfassung. Aus Rattenleber isolierte Golgi Apparate wurden unter milden Bedingungen homogenisiert und auf diskontinuierlichen Sucrose-Gradienten aufgetragen. Beim Zentrifugieren wurden 3 Banden mit jeweils Anreicherungen mehrerer Enzyme erhalten, welche einer partiellen Verteilung der Enzymaktivitäten entsprechen.

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Inhibition of Liver Aldehyde Dehydrogenase by Pyrogallol and Related Compounds

The observation that in vivo blood levels of acetaldehyde (AcH), the initial and probably most reactive intermediate in the metabolism of ethanol (EtOH), are significantly elevated during alcohol intoxication in rats if pyrogallol (PG; 1,2,3-trihydroxybenzene) is pre-administered[1,2] has prompted us to examine the effect of PG and several related derivatives on liver aldehyde dehydrogenase (AldDH; EC 1.2.1.3). It has been previously shown[2] that the increase in blood AcH levels in vivo following PG and EtOH is not contingent upon PG's known inhibition of catechol-*O*-methyltransferase (COMT) and probably is not mediated by activation of catalase-dependent EtOH metabolism. The effect of PG, PG metabolites, or related catechol compounds on the activity of AldDH has not been investigated.

Materials and methods. All chemicals were of the highest commercial quality. Mitochondrial AldDH was prepared from livers of male Sprague-Dawley rats essentially as described by TABAKOFF et al.[3], except that the disruption of isolated mitochondria was accomplished by 4 one-min sonication periods using a Bramson Sonifier at a power setting of 4. The final solution of AldDH was shown to be essentially free of monoamine oxidase (MAO) activity. MAO activity was assayed as described by TABAKOFF and ALIVISATOS[4] and was found to be $< 5\%$ of the activity found in the mitochondrial pellet.

Table I. Effect of pyrogallol and related compounds on in vitro activity of rat liver mitochondrial aldehyde dehydrogenase

Compound	Inhibition (%) Concentration (m*M*) 0.05	0.5	1.0
PG (1,2,3-trihydroxybenzene)	6.4	27.0	49.5
1,2,4-Trihydroxybenzene	38.5	49.5	60.0
Hydroquinone (1,4-dihydroxybenzene)	26.2	41.9	54.1
Chloral hydrate	—	—	56.2
DDC (diethyldithiocarbamate)	6.8	26.7	33.3
3-Methoxy-*O*-catechol	0	17.2	31.4
2,3-Dimethoxyphenol	0	4.3	6.9
Gallic acid	0	0	15.6
n-Propyl gallate	0	0	6.2
D,L-Shikimic acid	0	0	3.4
Tetrahydroxy-*p*-quinone	0	0	0

Aldehyde dehydrogenase activity was assayed at 25 °C in incubation mixtures containing 0.05 *M* Na_3PO_4, pH 7.0, 1 m*M* NAD^+, and enzyme (1–2 mg protein). The reactions were initiated by the addition of substrate, propionaldehyde, (2 m*M*). Measurements of AldDH activity were performed at pH 7, due to rapid oxidation of PG at higher pH's. For kinetic studies, the concentration of either NAD^+ or propionaldehyde was varied while the other components of the incubation mixture were kept constant. Inhibitors, when present, were preincubated with enzyme for 2 min before the addition of substrate. Incubations without inhibitor were assayed simultaneously with each incubation containing inhibitor. Formation of NADH was monitored spectrophotometrically using a Beckman Acta III recording spectrophotometer with a 0.1 absorbance unit, full scale expansion. Kinetic data, plotted as described by LINEWEAVER and BURK[5], were fitted by linear regression analysis using an Olivetti Programma 101. Each point represents the mean of 3 determinations, S.D. $< 7\%$.

To ascertain whether the inhibition by PG was reversible, AldDH was pre-incubated with PG (10 m*M*) for

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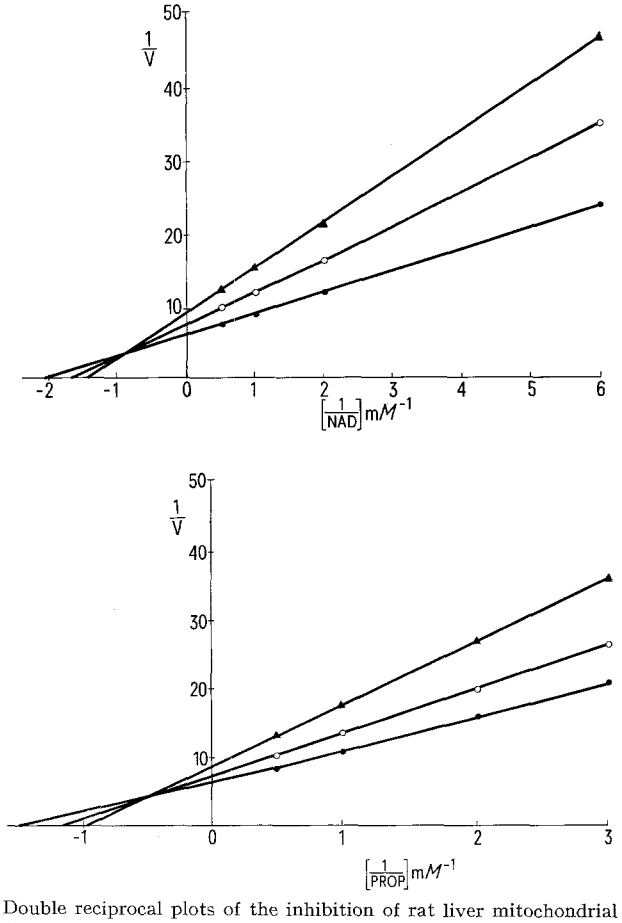
5 min. An aliquot of this incubation was then diluted into an assay incubation described above, such that the final PG concentration was 1 m*M*, and AldDH activity was determined.

Results and discussion. PG, 1,2,4-trihydroxybenzene, and hydroquinone were found to significantly inhibit mitochondrial AldDH activity (Table I), being approximately equipotent with chloral hydrate and more potent than DDC under the assay conditions utilized. A similar inhibition (78%) of rat liver mitochondrial AldDH by 2 m*M* chloral hydrate was previously reported by GRUNNET[6], and purified horse liver AldDH was also found to be inhibited approximately 50% by 1 m*M* chloral hydrate[7]. Both DDC and chloral hydrate have been used extensively as characterisitc inhibitors of AldDH[6–8]. Inhibition of AldDH activity by PG was found to be reversible by dilution (Table II), and the inhibition was not increased by pre-incubating enzyme in the presence of PG for longer periods (up to 15 min) before addition of substrate.

Table II. Reversibility of inhibition of aldehyde dehydrogenase by pyrogallol

Pyrogallol (m*M*)	Activity[b]	% Inhibition
0	12.5 ± 1.1	—
10.0	0.9 ± 0.2	92.1
1.0	6.3 ± 0.7	49.5
1.0[a]	6.3 ± 0.6	49.5

[a] Aldehyde dehydrogenase was pre-incubated with 10 m*M* pyrogallol and an aliquot of this mixture was diluted into a final assay mixture such that the final pyrogallol concentration was 1 m*M*. For further details see text. [b] Activity is expressed as moles $\times 10^{-11}$ NADH produced/min/mg protein.



Double reciprocal plots of the inhibition of rat liver mitochondrial AldDH by PG, indicating non-competitive inhibition with respect to the substrate, propionaldehyde (PROP) and to the cofactor, nicotinamide adenine dinucleotide (NAD). PG concentrations: ●, none; ○, 0.1 m*M*; ▲, 0.5 m*M*.

Kinetic experiments (Figure) indicated that PG was a non-competitive[9] inhibitor of AldDH. Similar inhibition patterns were obtained whether NAD^+ or propionaldehyde were the variable substrates.

Investigators have considered PG primarily an inhibitor of COMT. Our present studies demonstrate that PG and certain related compounds are inhibitors of liver AldDH as well, and suggest that the elevated AcH levels in vivo after PG and EtOH can be explained in part by this inhibition. In addition, mitochondrial NAD^+ levels have been shown to be reduced after administration of EtOH to rats[10]. The resultant low NAD^+ levels would contribute further to the inhibition of AldDH activity in the presence of PG.

If PG also inhibits AldDH in brain and nerve, increases in levels of the biogenic aldehydes derived from MAO action on amine neurotransmitters – in particular (because of concomitant COMT inhibition), the catechol aldehydes – may occur in PG-treated animals. The biogenic aldehydes could have effects[11] which contribute to the increased duration of response to catecholamines which is seen with PG[12]. The endogenous aldehydes presumably could condense with parent amines, forming tetrahydroisoquinolines or β-carbolines with β-sympathomimetic activity[13]. In support of this condensation possibility, PG has been found to promote tetrahydroisoquinoline formation from exogenous AcH and brain dopamine during EtOH metabolism in rats[14,15].

Zusammenfassung. Nachweis, dass Pyrogallol und ähnliche Substanzen eine der mitochondrialen Alkoholdehydrogenasen aus Rattenleber hemmen.

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