Since the ADH reaction should never be rate-limiting, relatively high concentrations of ADH must be added to the assay mixture in view of the low affinity of ADH for propionaldehyde. Preliminary experiments have shown that the enzyme activity becomes independent from ADH concentrations only above 50–100 μg of ADH per sample. A safe amount of enzyme, in the assay mixture, was considered to be 500 μg.

Another pre-requisite for the appropriate use of a coupled system to assay the dioldehydrase activity is that no other reaction involving NADH oxidation occurs in the system. The specificity of the assay has therefore been checked, as reported in Table I. It may be seen that unspecific interference in our preparations accounts for at most 7%. Most probably such an interference may be completely excluded by the use of respiratory inhibitors. Under the experimental conditions assessed, a strict proportionality was found between rate measured and amount of enzyme protein added to the assay mixture. This is clearly illustrated by the graph reported in Figure 1.

A direct comparison of the method described with the conventional dinitrophenylhydrazone method is reported in Table II. Clearly the results obtained by the 2 methods are perfectly comparable but the extent of the experimental error is definitely lower with the method we propose than with the method of BOEHME and WINKLER[2]. The standard error found in the former case is 6–7 times less than in the latter.

Some kinetics parameters of dioldehydrase from *A. aerogenes* have been also reinvestigated by this technique and compared with those reported in literature. Data reported in Table III indicate a good agreement of our data with those already published by other authors[6].

Discussion. The method described has the advantage of being a continuous recording of the kinetics rather than a sampling method. This allows accurate measurements of initial rates and, in addition, leaves much less room for experimental error. The sensitivity of the method is rather high as compared with the classical dinitrophenylhydrazone method. The sensitivity may be further increased by recording at 340 nm instead of 366 nm or even further by recording the fluorescence change of the nucleotide.

The method has limitations, however, being appliable only to relatively purified systems. This limitation may be, to a certain extent, circumvented by the use of the respiratory inhibitors. A second limitation arises from the necessity of using high concentrations of purified ADH which should never be rate-limiting. Preliminary experiments are therefore necessary to ensure that this condition is fulfilled. This point makes the method far more expensive than the previous one. All things considered, however, we feel that the balance between advantages and disadvantages of the new method favours it and disfavours the sampling method of BOEHEME and WINKLER.

Table I. Specificity of ADH-coupled dioldehydrase activity

	Enzymic activity (%)
Complete system	100
DMBC	7
ADH	7
1,2-Propanediol	7
KCl	66
BSA	90
Apodioldehydrase	0

Experimental conditions as described under materials and methods.

Table II. Dioldehydrase activity determined by ADH-coupled reaction and according to BOEHME and WINKLER[2]

nmoles propionaldehyde · mg^{-1} protein · min^{-1}	
ADH-coupled system	Dinitrophenylhydrazone system
120.3 ± 0.91 (6)	119.6 ± 6.5 (6)

Data ± S.E.M. In parenthesis number of determination.

Table III. Comparison of some kinetics parameters of dioldehydrase from *A. aerogenes* determined by the new assay method with those reported in literature

	New technique	Literature
Km of 1,2-propanediol	$1.20 \times 10^{-4} M$	$0.81 \times 10^{-4} M$ (ref.[6])
Ki of styrene glykol	$3.56 \times 10^{-2} M$	$3.84 \times 10^{-2} M$ (ref.[6])

Zusammenfassung. Es wird eine raschere und empfindlichere Methode zur Bestimmung der Aktivität von Dioldehydrase beschrieben.

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[6] T. TORAYA and S. FUKUI, Biochem. Biophys. Res. Commun. *36*, 469 (1969).

CONGRESSUS

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