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This Week's Citation Classic

Theorell H & Bonnichsen R. Studies on liver alcohol dehydrogenase, I. Equilibria and initial reaction velocities. *Acta Chem. Scand.* **5**:1105-26, 1951.
[Medicinska Nobelinstitutets Biokemiska avdelning, Stockholm, Sweden]

This paper shows that liver alcohol dehydrogenase binds two molecules of NADH through sulfhydryl bonds with simultaneous shift of the absorption band at 340 to 325 nm. This discovery made possible closer studies of the mechanism of alcohol oxidation. NADH is bound more firmly than NAD and thus rate determining. [The *SCI*® indicates that this paper has been cited over 300 times since 1961.]

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"Alcohol in its various aspects has interested mankind as long as it has been available. In our laboratory at the Nobel Institute of Biochemistry, Britton Chance had just found that a catalase-peroxide complex oxidizes alcohol. It was known that an oxidizing enzyme in liver converted alcohol to acetaldehyde and the question ensued: how is alcohol combusted in man? At that time Einar Lundsgaard from the Rockefeller Institute in Copenhagen was visiting us and during an afternoon discussion we decided to isolate and identify the dehydrogenase from liver. This was a long time ago and the isolating methods were rather primitive.The various chromatographic methods were not yet invented. For the kinetic studies we needed hundreds of mgs of enzyme and as most preparations of this kind sooner or later end up in the sink, preparations were a limiting factor. Subsequently, we found a better way to prepare the enzyme with higher yield. NAD, then DPN, was not commercially available and we had to prepare it by rather laborious methods until Neilands and Åkeson at the laboratory prepared NAD using an ion exchange column. We prepared the reduced form enzymatically and then extracted the coenzyme. We did not at that time realize that the enzyme preparations consisted of different variants of the enzyme. We usually got three peaks in the electrophoresis pattern, thought it to be impurities, and purified and recrystallized the preparation until we got one fraction by electrophoresis. Until the end of World War II the only spectrophotometer available was a Warburg-Negelein instrument and kinetic studies with this instrument were not possible. Britton Chance, however, brought with him the first Beckman spectrophotometer we had seen and we invested in a couple of these instruments.

"Misuse of alcohol is one of the greatest problems in our civilization and more than 100,000 papers have been written about alcohol and its effect on man. The knowledge of the ADH enzyme, as we coined it, seemed to us one of the keystones to solve or minimize the damage of alcohol. The discovery of the spectral shift of NADH-absorption from 340 to 325 nm following its binding with sulfhydryl groups made possible the kinetic studies in this and following works. We believe this to be the main reason why this work has been so much cited, as it started an extensive study of this enzyme, various isoenzymes with different properties and specificities, as well as studies of structure and reaction mechanism.[1] Accumulation of reduced NAD has been proved to be responsible for many of the toxic effects of alcohol. Still 30 years later there are many controversial theories concerning alcohol turnover, e.g., the role of microsomal tissue, catalase, peroxidase, and the small but very active, recently found variants of ADH, as well as its physiological substrate."

1. Brändén C I, Jörnvall H, Eklund H & Furugren B. Alcohol dehydrogenases. (Boyer P, ed.) *The enzymes.* New York: Academic Press, 1975. Vol. 11, pt. A. p. 103-90.