Since this communication was prepared, an important paper on the subject by R. W. Estabrook has appeared[6].

D. Boulter
E. Derbyshire
C. S. Semmens

Department of Botany,
University of London,
King's College,
London, W.C.2.
Jan. 17.

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Immunobiological Studies on Crystalline Alcohol Dehydrogenases from Closely Related Yeast Species

Hitherto, there have been few reports on comparative immunological studies on functionally homologous enzymes from closely related species of microorganisms. Detailed immunobiological and immunochemical studies of this kind have been carried out only upon lecithinases from *Clostridia*[1-3].

We have been studying the immunobiological and immunochemical properties of crystalline alcohol dehydrogenase isolated from two subspecies of *Saccharomyces cerevisiae* (bakers' yeast and brewers' yeast) by the methods of Racker[4] and Keleti[5]. The enzymes from both species acted as antigens on rabbits.

The rabbits were given 260 mgm. alcohol dehydrogenase from brewers' yeast or 130 mgm. alcohol dehydrogenase from bakers' yeast intravenously. The undiluted immune sera were found to have 0·484 mgm./ml. and 1·704 mgm./ml. of the respective antibodies. The enzyme from both species proved to be immunologically homogeneous, since the neutralization zones determined in our precipitation systems had sharp limits.

In our work we first determined the optimal proportions by the dilution method and then we studied the quantitative relationships following Heidelberger and Kendall[6], using the spectrophotometric method of Gittlin[7].

We found that both antigens mutually absorb completely the homologous as well as the heterologous antiserum. The quantities of antigen needed for absorption and the quantities of antibody precipitated by the different antigens are different for the alcohol dehydrogenases isolated from the two species.

The differences between enzymes found by immunological studies is supported by mutual dissolution studies following Landsteiner and Heidelberger[8].

We found that the anti-enzyme-containing serum inhibits the activity of alcohol dehydrogenase from both species. At maximal (100 per cent) inhibition, the ratio of enzyme-antigen to antibody agrees with the ratio of antigen to antibody found at the neutralization point determined in our precipitation systems.

Inhibition becomes total two minutes after the addition of immune serum. This period remains unchanged if the enzyme is pre-incubated with diphosphopyridine nucleotide, or with its substrate previously to the addition of immune serum. However, if the immune serum is added to a functioning enzyme (that is, to antigen which has been previously mixed with diphosphopyridine nucleotide and substrate) the inhibitory action of antibody is delayed. Total inhibition then develops after 20 min. instead of 2 min.

The inhibitory action of antibody does not compete with substrate.

A detailed account of these experiments will be published in *Acta Physiologica Hungarica*.

F. Antoni
T. Keleti

Biochemical Institute,
Hungarian Academy of Sciences,
Budapest.
Jan. 2.

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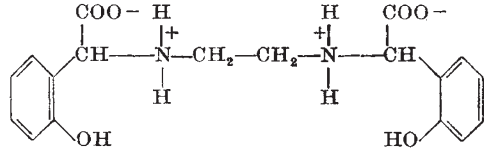
Chelating Tendencies of N,N′-Ethylene*bis*-[2-(o-Hydroxyphenyl)] Glycine

Recent investigations of a wide variety of chelate compounds as carriers of ferric ions in plant nutrition showed the ferric chelate of N,N′-ethylene*bis*-[2-(*o*-hydroxyphenyl)] glycine to be a very effective iron carrier in biological systems. Because of the remarkable properties of this new chelating agent, investigation of its affinity for ferric ions and other metal ions seemed desirable. The structure of this substance (I) differs from that of most of the common ferric ion sequestering agents in that the hydroxyl groups are phenolic rather than aliphatic in nature.



(I)

The acid dissociation constants and chelate stability constants, as determined from the potentiometric titration curves in Fig. 1, are listed in Table 1. The second, third and fourth overlapping dissociations of N,N′-ethylene*bis*-[2-(*o*-hydroxyphenyl)] glycine were calculated graphically by the method of Schwarzenbach and Ackermann[1]. The stability constants were calculated by a conventional algebraic method, except for those for calcium and magnesium

Table 1. Acid Dissociation Constants and Chelate Formation Constants of (I)

Metal	Equilibrium constants*			
H+	pK_1 6·32	pK_2 8·64	pK_3 10·24	pK_4 11·68
	log $K(MH_2A)$	pK_a	pK_b	
Cu(II)	>15	4·98	8·04	
Ni(II)	11·40	6·03	7·63	
Zn(II)	9·26	6·64	7·74	
Cd(II)	7·77	7·86	8·70	
	log $K(MH_2A)$	log $K(MHA)$	log $K(MA)$	
Ca(II)	1·6	3·5	6·1	
Mg(II)	2·3	4·6	7·6	

* pK's refer to dissociation constants; K_z values are formation constants for metal complex X.