protein ratio of all other elements studied *decreased* with increasing enzyme purification. Analytical and enzymological data thus far do not indicate that magnesium is an integral functional part of the apoenzyme. Preparations which had as little as 5 μg. of zinc per gram of protein have full activity.

Microchemical analyses for zinc[4,5] in many crystalline preparations have corroborated the spectrochemical data indicating the presence of about 0.20% of zinc, the exact value being a function of the state of purity of the preparation.

The zinc content of crystalline ADH preparations is not reduced below about 0.17% by dialyses under varying conditions, the choice of which indicated the successful maintenance of the zinc–protein bond against competitive agents.

Both the rate of appearance of DPNH at *p*H 8.8 in the presence of ethanol, and the disappearance of DPNH at *p*H 6.5 in the presence of acetaldehyde are inhibited strongly, *e.g.*, when the enzyme is preincubated with 1,10-phenanthroline. This inhibition is prevented by zinc ions but not by magnesium ions. The inhibition by 1,10-phenanthroline seems to be competitive with the coenzyme.

Based upon a molecular weight of yeast ADH of 150,000[7] the data in Table I give a ratio of 3.3 and 3.8 moles of zinc to 1 mole of ADH apoenzyme. One of our further purified and dialyzed preparations contained 1720 μg. of zinc per gram of protein, corresponding to 3.9 moles of zinc per mole of ADH. A ratio of four moles of zinc to one mole of ADH apoenzyme seems to be the correct value. These data establish yeast ADH as a zinc metalloenzyme as previously defined.[8,9]

These findings throw new light upon the interaction of DPN with ADH. The zinc content of yeast ADH, the participation of zinc in enzyme activity, the kinetics of competitive inhibition and the strong zinc–protein bonding suggest that the four molecules of DPN or DPNH involved in yeast ADH activity are bound to the apoenzyme through zinc.

The hypothesis that other dehydrogenases linked with pyridine nucleotides may similarly prove to be metalloenzymes is being tested experimentally. In this regard it may be suggested that liver ADH, with a molecular weight of 73,000,[10] which binds two molecules of DPN, does so in similar fashion. Also, the function of the hitherto unexplained large amounts of zinc in retina[11,12] may now be attributed to a high content of ADH, active in the dehydrogenation or formation of Vitamin A_1.

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(7) J. E. Hayes, Jr., and S. F. Velick, *J. Biol. Chem.*, **207**, 225 (1954).

(8) B. L. Vallee, *Scientific Monthly*, **62**, 368 (1951).

(9) B. L. Vallee, F. L. Hoch, and W. L. Hughes, Jr., *Arch. Biochem. Biophys.*, **48**, 347 (1954).

(10) H. Theorell and R. Bonnichsen, *Acta Chem. Scand.*, **5**, 329 (1951).

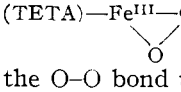
(11) M. Leiner and G. Leiner, *Biol. Zentr.*, **64**, 293 (1944).

(12) J. M. Bowness, R. A. Morton, M. H. Shakir and A. L. Stubbs, *Biochem. J.*, **51**, 521 (1952).

A SYNTHETIC COMPOUND WITH CATALASE-LIKE ACTIVITY

Sir:

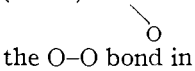
Triethylenetetramine (TETA), $H_2NCH_2CH_2NH$-$CH_2CH_2NHCH_2CH_2NH_2$, forms a quadridentate chelate compound with ferric ion. Steric considerations show that it is energetically improbable to have the four N atoms and the Fe^{III} ion in (TETA)—Fe^{III} located in one plane, but that the structure with one primary amine N atom above and the other below the plane determined by the two secondary amine N atoms and the Fe^{III} ion is stable. In this chelate ion the four N atoms form four coördination bonds with Fe^{III} along four of the six octahedral d^2sp^3 hybrid atomic orbitals of the latter, the two unoccupied hybrid orbitals are directed along the two remaining adjacent octahedral directions. In aqueous solutions these last two orbitals are presumably used to form two additional coördination links between Fe^{III} and two hydroxide ions or a hydroxide ion and a water molecule. In the presence of hydrogen peroxide, either or both of these unidentate ligands may be displaced by the OOH^- ions. Each OOH^- ion is potentially capable of forming two coördination links with Fe^{III}, with one electron pair donated by each O atom. But since the O–O bond length is only 1.3 Å., the resulting chelate,



(TETA)—Fe^{III}—OH

O

the O–O bond until the second activated complex



(TETA)—Fe^{III}—OH

O

is formed. Direct splitting of the O–O bond in an isolated H_2O_2 molecule requires about 35 kcal. of energy per mole. But in the above complex ion the energy consumed in splitting the O–O bond is partially compensated by the energy gained through the formation of more stable Fe^{III}–O bonds, because the O atom and the OH^- ion are now separate ligands and can orient themselves for maximum overlapping with the two vacant octahedral atomic orbitals of the Fe^{III}. The second activated complex can then readily react with a second OOH^- ion to yield O_2 and OH^-.

This reasoning was subsequently substantiated by measurements of the rate of catalytic decomposition of H_2O_2 by (TETA)-Fe^{III}. In each experiment, a measured volume of about 0.15 *M* H_2O_2 solution was mixed with a known amount of TETA + (TETA)–Fe^{III} solution at zero time. Aliquot parts of the reaction mixture were taken at short time intervals, mixed with equal volumes of 2.5 *N* H_2SO_4 and titrated with standard ceric sulfate solution with *o*-phenanthroline–Fe^{II} as indicator. The turnover numbers of (TETA)–

Fe^{III}, expressed in moles of H_2O_2 decomposed per min. per mole of (TETA)–Fe^{III}, were computed from the initial rates of decompositions of H_2O_2, and are summarized in Table I. In order to correct for traces of Fe^{III} present in the system as an impurity (from reagents and glassware), a blank measurement with TETA and H_2O_2 but without added Fe^{III} was made at each temperature. These blank rates were subtracted from the corresponding measured rates before the computations for turnover numbers were made. The activation energy for the reaction computed from Table I is 6.6 kcal./mole. Results of a detailed study on the subject will be reported in a later publication.

TABLE I

CATALYTIC DECOMPOSITION OF H_2O_2 BY (TETA)–Fe^{III}

Concn. of Total TETA, mole/l.	Concn. of Total Fe^{III}, mole/l.	pH	Temp., °C.	Turnover number $min.^{-1}$
2.9×10^{-3}	3.8×10^{-7}	9.5	25.2	11000
2.9×10^{-3}	5.7×10^{-7}	9.6	25.2	11000
2.9×10^{-3}	7.6×10^{-7}	9.6	25.3	11000
2.9×10^{-3}	3.8×10^{-7}	9.5	1.8	4400
2.9×10^{-3}	3.8×10^{-7}	9.5	13	6700
2.9×10^{-3}	3.8×10^{-7}	9.5	41.5	21000

Although (TETA)–Fe^{III} is not as efficient as natural catalases, these data show that it is possible to construct small molecules with turnover numbers in the enzymic range.

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BOOK REVIEWS

Einschlussverbindungen. By FRIEDRICH CRAMER, Dozent am Chemischen Institute der Universitat Heidelberg. Springer-Verlag, Reichpietschufer 20, Berlin W 35, West Berlin, Germany. 1954. iv + 115 pp. 14 × 21 cm. Price, DM 14.80.

This ably written and documented monograph (167 references) brings together the present available information on a relatively new and remarkable type of compound to which Schlenk in 1949 gave the name "Einschlussverbindungen" or *inclusion compounds*. These are the compounds which involve purely a spatial combination between partners, involving no principal or secondary valences. One kind of molecule is simply trapped within open spaces created by the other and cannot escape. Inclusion compounds are classified as formed by crystal lattices, by molecules and by macromolecular materials. Examples of the first are the adducts of urea with *n*-paraffin derivatives (canal "hohlraum"), thiourea with cyclic hydrocarbons (canal), desoxycholic acid with paraffins, fatty acids and aromatics (canal), dinitrodiphenyl with diphenyl derivatives (canal), hydroquinone with HCl, SO_2, C_2H_2, rare gases (cage), gas hydrates (halogens, rare gases, CH_4, etc.) (cage), *o*-trithymotide with cyclohexane, benzol, chloroform, (cage), oxyflavane with organic bases (cage), dicyanoamminobenzolnickel with benzol, thiophene, furane, pyrrol, aniline (cage), and substances which form blue iodine adducts (canals). The only example of a fairly low molecular weight compound which provides an inclusion space in a single molecule is cyclodextrin. The space is a canal, or in solution a cage, enclosing hydrocarbons, iodine, alcohols, halogenated paraffins, aromatic compounds and dyestuffs. Macromolecular materials forming inclusion compounds are minerals such as zeolites and clay minerals, graphite, cellulose, starch and proteins (inclusion of dyestuffs, lipoids, etc.). For most of these cases X-ray crystal structure data are summarized, together with absorption spectra data directed to the question whether the included molecules are completely unchanged in the canals or cages. Trapping and trapped molecules cannot be separated, hence inclusion compounds are especially stable when the system possesses high electron density. Hence the "hohlraum" may serve as an electron donor and as a base in the Brönsted–Lewis sense. Also negative and positive catalysis by inclusion compounds might be expected, and examples are cited; and these also serve as models for ferment reactions (especially cyclodextrin). The criteria for the building of inclusion compounds are summarized as a means of prediction of new systems. The author has clearly demonstrated his mastery of the synthesis and theory and properties of these curious compounds in which one kind of molecule imprisons another tightly but is not chemically combined with it. Incidentally, the term "clathrate" for this type of compound is not mentioned.

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An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry. By A. E. GILLAM, D.Sc. (Liverpool), F.R.I.C., Late Senior Lecturer in Chemistry, and Special Lecturer in Chemical Spectroscopy, The University, Manchester and E. S. STERN, Ph.D. (London), A.R.C.S., Chief Research Chemist, J. F. Macfarlan and Co., Ltd., Edinburgh. St. Martin's Press, Inc., 103 Park Avenue, New York 17, N. Y. 1954. vii + 283 pp. 15 × 22.5 cm. Price, $8.00.

The organic student who nowadays must have a working knowledge of ultraviolet spectroscopy in relation to organic structural problems will find in this book a relatively easy and painless means of achieving this objective. And since the book was presumably intentionally limited to this specific objective we shall not carp about the rather inadequate theoretical discussion nor about the failure to review fully developments of the last three or four years.

Beyond a passing mention of valence-bond theory and molecular orbital theory, the treatment is descriptive, factual and frankly empirical. The index contains not a single entry under either "Selection Rules" or "Symmetry Characters." Absorption bands are K, R or B, each characterized by its own set of rules or generalizations as to the effects of solvents, substituents, conjugation, etc. This characterization serves the purpose of convenience, if not understanding, in cataloging the relationships between structure and absorption spectra for a considerable variety of organic compounds. The text is well-supported by tabular data, figures and literature references.

The style is free and easy, occasionally to the point of absurdity, as, to quote a particularly glaring example (p. 67), "The generalization that two chromophores separated by a carbon atom interact but little does not necessarily apply when the atom separating the two groups is not carbon: thus. . . ."

The authors are at their best in describing the applications of spectroscopic methods in specific situations as, for example, in the chapter (Chapter 12) dealing with the spectrophotometric determination of organic compounds, and again in the chapter (Chapter 14) containing the data and the arguments actually applied in representative struc-