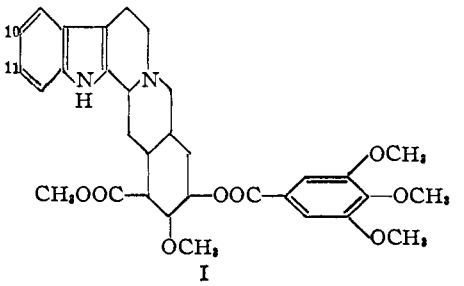
in thick pentagonal and hexagonal plates which melt in the capillary tube at 230–234° (cor.) with decomposition, $[\alpha]_D$ −163 ± 2° (*c* 0.5 in pyridine). The values obtained on analysis indicate the empirical formula $C_{32}H_{38}O_8N_2$ (578.6) (calcd. C, 66.42; H, 6.62; O, 22.12; N, 4.84. Found: C, 66.43; H, 6.51; O, 22.14; N, 4.91). The molecular weight determined by potentiometric titration with 0.1 *N* HCl was 579. The hydrochloride crystallizes from watery acetone in thin, rectangular plates, m.p. 247–253° (cor.) with decomposition; $C_{32}H_{38}O_8N_2 \cdot HCl$ (calcd. C, 62.48; H, 6.39; O, 20.81; Cl, 5.76. Found: C, 62.09; H, 6.15; O, 20.93; Cl, 5.86). In Keller's color reaction with glacial acetic acid containing ferric chloride, and concentrated sulfuric acid, canescine yields the same brownish-violet stain as yohimbine.

On alkaline hydrolysis, canescine yields an equivalent of trimethoxybenzoic acid, $C_{10}H_{12}O_5$ (212.2) (calcd.: C, 56.60; H, 5.70; O, 37.70. Found: C, 57.13; H, 5.90; O, 37.03), m.p. and m.p. when mixed with a genuine sample of trimethoxybenzoic acid were 170° (cor.)

If canescine is boiled with sodium methylate in methanol, canescinic acid methyl ester and trimethoxybenzoic acid methyl ester are obtained. Canescinic acid methyl ester has not yet been obtained in crystalline form; $C_{22}H_{28}O_4N_2$ (384.5) (calcd. C, 68.72; H, 7.34; O, 16.65. Found: C, 68.02; H, 7.41; O, 16.93), $[\alpha]^{20}_D$ −80 ± 2° (*c* 0.5 in pyridine).

The ultraviolet absorption spectrum of canescine in ethanol is composed of the chromophores of the unsubstituted indole system and of trimethoxybenzoic acid; λ_{max} 218 (log ε 4.79), λ_{max} 272 (log ε 4.26), λ_{max} 290 (log ε 4.07).

From these data and from biogenetic considerations it may be concluded, with a high degree of probability, that canescine has the structure of a 11-desmethoxyreserpine (formula I).



I

Investigations have shown that canescine possesses pharmacological properties similar to those of reserpine; above all, it produces a marked and prolonged fall in blood pressure.[8] The methoxy group in position 11 of the reserpine molecule, which is absent in canescine, therefore, does not seem to be necessary for the pharmacodynamic actions typical of these substances.

RESEARCH LABORATORIES
SANDOZ, INC. A. STOLL
BASLE, SWITZERLAND A. HOFMANN

RECEIVED JANUARY 5, 1955

(8) We are grateful to Prof. E. Rothlin, Director of the Pharmacological Laboratory, Sandoz, Inc., Basle, for supplying us with the preliminary pharmacological findings.

YEAST ALCOHOL DEHYDROGENASE, A ZINC METALLOENZYME

Sir:

Crystalline alcohol dehydrogenase (ADH) of yeast[1] is a zinc metalloenzyme; zinc is an integral and enzymatically functional component of the apoenzyme molecule.

Qualitative and quantitative emission spectrography was performed in duplicate,[2,3] as were microchemical determinations of zinc.[4,5] Protein weights were determined by trichloroacetic acid precipitation[6] and separately by measurement of absorbance at λ 280 mμ.

Crystalline preparations from our own and commercial sources uniformly contained large quantities of zinc, lesser and variable quantities of magnesium and insignificant amounts of all other elements.

Table I gives typical, quantitative spectrochemical data on twice crystallized preparations of yeast ADH, having high activity. Preparation 1 contained 1660 μg. of zinc per gram of protein, preparation 2 contained 1440 μg. per gram. Fractionation of ADH demonstrated an increase in the zinc:protein ratios in fractions in which the activity:protein ratio was increased. The metal:

TABLE I

EMISSION SPECTROGRAPHIC ANALYSIS OF TWICE CRYSTALLIZED YEAST ALCOHOL DEHYDROGENASE

Element	Preparation no. 1 Line/internal standard	Preparation no. 1 μg./g. yeast ADH	Preparation no. 2 Line/internal standard	Preparation no. 2 μg./g. yeast ADH
Zinc	Zn 3345 / Bi 2897	1660	Zn 3345 / V 3185	1440
Copper	By Na Diethyldithiocarbamate	165		[a]
Iron	Fe 3020 / V 3185	80	Fe 4283 / V 4395	81
Aluminum	Al 3961 / V 3185	79	Al 3961 / V 3185	48
Magnesium	Mg 2776 / Bi 2897	1180	Mg 2798 / V 3185	296
Calcium	Ca 4318 / Bi 2897	39	Ca 4302 / V 4395	105
Strontium	Sr 4077 / V 4111	4	Sr 4077 / V 4395	2
Barium	Ba 4554 / V 3185	11	Ba 4554 / V 4395	20
Manganese	Mn 2576 / V 4111	2	Mn 4030 / V 4395	π
Lead	Pb 4057 / V 4111	45	Pb 4057 / V 4395	π
Cadmium	Cd 2268 / V 3185	13	...	π
Chromium	Cr 4254 / V 4111	8	Cr 4254 / V 4395	π

Not detected: π, and also beryllium, cobalt, lithium, molybdenum, nickel, potassium, silver, tin.

[a] Lost.

(1) E. Racker, *J. Biol. Chem.*, **184**, 313 (1950).
(2) B. L. Vallee, in preparation for publication.
(3) B. L. Vallee and H. Neurath, THIS JOURNAL, **76**, 5006 (1954).
(4) B. L. Vallee and J. G. Gibson, 2nd, *J. Biol. Chem.*, **176**, 435 (1948).
(5) F. L. Hoch and B. L. Vallee, *ibid.*, **181**, 295 (1949).
(6) F. L. Hoch and B. L. Vallee, *Anal. Chem.*, **25**, 317 (1953).

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.

These studies were aided in part by a contract between the Office of Naval Research, Department of the Navy, and Harvard University Contract No. Nr5ori-07660, and grants-in-aid from the National Institute of Health of the Department of Health, Education and Welfare, the Rockefeller Foundation, the Research Corporation, and the Howard Hughes Foundation for Medical Research.

BIOPHYSICS RESEARCH LABORATORY
OF THE DEPARTMENT OF MEDICINE
HARVARD MEDICAL SCHOOL AND PETER BENT BRIGHAM HOSPITAL
BOSTON, MASSACHUSETTS
BERT L. VALLEE
FREDERIC L. HOCH

RECEIVED DECEMBER 14, 1954

(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, is unstable and tends to stretch

the O–O bond until the second activated complex (TETA)—Fe^{III}—OH 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)–