I wish to thank Professor E. P. Kohler for suggestions offered during this investigation.

Experimental

Phenylmercuric Fluoride.—A solution of 40.6 g. (0.17 mole) of freshly precipitated silver oxide and 14 g. (0.35 mole) of 50% hydrofluoric acid in 400 cc. of water was shaken for five hours with 78 g. (0.25 mole) of phenylmercuric chloride, previously moistened with ethanol. The solid was filtered off and extracted with 400 cc. of boiling ethanol. After a small amount of precipitation by cooling, the solution was decanted and concentrated *in vacuo*. The residue was extracted with boiling ethanol and filtered. The cooled solution yielded 32 g. of phenylmercuric fluoride, m. p. 170°, or 43% of the theoretical. The chlorine-free compound was soluble in hot alcohol, hot xylene and hot chloroform and was crystallized from the latter solvent for analysis (m. p. 171°). It was insoluble in carbon tetrachloride, ethyl acetate, ether and hot acetone. The extracted silver chloride from the reaction mixture contained much unreacted phenylmercuric chloride; no doubt the yield can be increased by a longer period of reaction.

Anal. Calcd. for C_6H_5HgF: C, 24.27; H, 1.70. Found: C, 24.25; H, 1.99.

Reaction with Acetyl Chloride.—When 2.96 g. (0.01 mole) of phenylmercuric fluoride was refluxed with 1.57 g. (0.02 mole) of acetyl chloride, the acetyl fluoride was evolved immediately through the reflux condenser. After five hours the reaction was poured into iced sodium carbonate solution. No mercuric oxide precipitated. The solid was filtered off and crystallized from xylene to weigh 2.80 g. and melt at 257°. This 90% yield of phenylmercuric chloride was substantiated by mixed melting point.

Diphenylmercury.—When phenylmercuric fluoride was slowly destructively distilled at 200° under 10 mm. only diphenylmercury, and no diphenyl, could be found in the distillate.

CONVERSE MEMORIAL LABORATORY
HARVARD UNIVERSITY
CAMBRIDGE, MASS. RECEIVED SEPTEMBER 14, 1936

COMMUNICATIONS TO THE EDITOR

METAL ION ACTIVATION IN ENZYMATIC CATALYSIS. ARGINASE

Sir:

Recent investigations [Hellerman, Perkins, and Clark, *Proc. Nat. Acad. Sci.*, **19**, 855 (1933); Hellerman and Perkins, *J. Biol. Chem.*, **107**, 241 (1934); Bersin, *Z. physiol. Chem.*, **220**, 209 (1933)] support the idea that reversible chemical actions upon substituent thiol groups of certain enzymes (urease, papain, etc.) may account largely for their reversible inactivations by oxidation and by silver ion, phenylmercuric hydroxide, etc. However, the enzyme, arginase [Hellerman and Perkins, *J. Biol. Chem.*, **112**, 175 (1935)], was found to be little sensitive to phenylmercuric hydroxide, and most readily activated, not by reduction in the usual sense but rather by the use of reduced ions of the transition elements, manganese, cobalt or nickel, as well as ferrous ion, which had been associated previously with arginase activation. This, and other evidence cited, was considered to point strongly to metal coördination as a factor not merely in arginase activation but actually in the functioning of the enzyme itself. For example, dissociable, labile enzyme-substrate intermediates might be constructed by the "binding" to a metal ion of both enzyme and substrate through donor groupings of each. If so, an effective metal ion should alter the activity–*p*H curve of arginase in a characteristic way.

We have now studied the effect of such ions upon the arginase–arginine reaction in buffers of widely varying *p*H values but having approximately constant ionic strength ($\mu = 1$). Activity–*p*H curves constructed from the data clearly show characteristic differences. Liver-arginase action is enhanced greatly by nickelous and especially cobaltous ion from *p*H 5 to 7.7; activation by manganous ion is not significant below *p*H 6.7. The optima for cobaltous, nickelous and manganous ion are, respectively, *p*H 7.5 to 7.7, 6.7 to 7.7 and 10 as compared with the optimum for our arginase, without added metal, 7.7 to 9.0. The variations may be considered in relation to the corresponding stabilities of the coordination complexes of these ions with substituted ammonias. For example, we ascertained (by a potentiometric titration method) that the dissociation constants of the complex ions derived from *d*-arginine with cobaltous, nickelous

and manganous ion, respectively, are approximately of the order, 2×10^{-10}, 4×10^{-11} and 1×10^{-6}. Manganous ion apparently cannot appreciably activate arginase in the more acid *p*H range where it coördinates only slightly.

A similar study has also been made with the new catalyst for *d*-arginine hydrolysis, which Hellerman and Perkins found was "elicited," in the presence of cobaltous, nickelous or manganous ion from some constituent of their preparations of jack bean urease. This "urease-metal" effect, in contrast to liver–arginase activity, is negligible below *p*H 7.0; "with Co^{++}" the curve relating activity (ordinate) with *p*H (abscissa) rises sharply from *p*H 7.1 to 7.7, the optimum. This practically proves the non-identity of the two catalysts. Nevertheless, the characteristics of the kinetics of arginine hydrolysis with either enzyme (plus Co^{++}) are similar (pseudounimolecular at *p*H 7.5). Moreover, the striking specificity of liver–arginase is retained in the new catalyst; this was tested with argininic acid, δ-guanidinovaleric acid, etc.

The results will be published in detail in another place.

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
THE JOHNS HOPKINS UNIVERSITY LESLIE HELLERMAN
BALTIMORE, MARYLAND C. CHESTER STOCK

RECEIVED OCTOBER 23, 1936

AN ANTIRACHITIC SUBSTANCE FROM TUNA LIVER OIL

Sir:

Drummond and Haslewood [*Chem. & Ind.*, **55**, 598 (1936)] report the isolation of an antirachitic substance from tuna liver oil and indicate that their substance may not be identical with that of Brockman [*Z. physiol. Chem.*, **241**, 104 (1936)]. Our own findings may be of interest, particularly since we arrive at essentially the same end product as Brockman by a somewhat different route. We have not used the chromatographic technique which he deems essential.

Starting with an alcohol-soluble fraction from the non-saponifiable of tuna liver oil, the sterols were esterified with phthalic acid, leaving the hydrocarbons behind. This was followed by distribution of the sterol mixture between solvents in the manner described for a number of similar mixtures by various investigators. Removal of cholesterol by digitonin, freezing, treatment with decolorizing carbon and finally formation of the 3,5-dinitrobenzoic ester gave a crystalline product which melted sharply at 128.5°. The alcohol from this showed a narrow absorption band with a peak at 2650 Å. and an extinction coefficient $E^{1\%}_{1cm.} = 350$ in Normalbenzin (Kahlbaum). The dinitrobenzoic ester showed 4.94% of nitrogen; calcd. 4.83%. The biological activity was 30×10^6 international units per gram. No evidence of any considerable amount of biological activity residing in substances with other absorption peaks was obtained, although in the stages of lesser purity low absorption peaks at both longer (Drummond) and shorter wave lengths were very evident.

We agree with Brockman that the material he identified essentially characterizes the Vitamin D of this type of liver oil. Our slightly higher biological activity as well as our slightly but distinctly narrower absorption band (calculated to the same units as Brockman uses), cause a little hesitation in concluding that either Brockman's D_3 or our material is pure. The difference in absorption is not likely to be a solvent effect (hexane *vs.* Normalbenzin), particularly since the position of the peak is identical. All these findings have as yet no bearing on the question of multiplicity of forms of naturally occurring Vitamin D.

DEPARTMENT OF PATHOLOGY E. J. H. SIMONS
COLLEGE OF PHYSICIANS AND SURGEONS T. F. ZUCKER
COLUMBIA UNIVERSITY

RECEIVED NOVEMBER 14, 1936