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.

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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.

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