by melting point and rotation, in the effluent. The material from the lowest zone consisted of β-D-maltose octaacetate (m. p. 158–160°, unchanged on admixture with an authentic specimen; $[\alpha]^{25}D$ +62.5°, *c* 1.1, chloroform). The material from the next higher zone was rechromatographed in the same manner, and the eluent from the lower zone which crystallized from ethanol was identified as β-D-isomaltose octaacetate (m. p. 144–145°, unchanged on admixture with an authentic specimen; $[\alpha]^{26}D$ +98°, *c* 1.0, chloroform); yield 92 mg.

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## ERYTHEIN AND APOERYTHEIN AND THEIR RELATION TO THE ANTIPERNICIOUS ANEMIA PRINCIPLE

*Sir:*

Normal gastric juice has been found to contain a non-dialyzable, heat labile substance which combines, apparently stoichiometrically, with erythrotin,[1] (vitamin $B_{12}$)[2] to form a complex (erythein) which is non-dialyzable and not dissociated by dialysis. Erythrotin in this combination is not available to microörganisms (*Escherichia coli, Lactobacillus lactis Dorner, Lactobacillus leichmannii*), but is released by heat, much as biotin is released from avidin, whereupon it is again microbiologically active. Heated gastric juice contains no principle capable of combining with erythrotin.

Quantitative determination of heat labile principle (apoerythein) is readily performed by measuring in an erythrotin assay (*Escherichia coli*)[1] the inhibition of growth resulting when aliquots of the juice are added (unheated) to cultures containing just sufficient erythrotin to elicit a maximum response. The erythrotin combining capacities (millimicrograms of erythrotin per ml. of secretion) of samples of gastric juice from normal and anemic subjects were found to be respectively, 20, 60[3], 60, 15; and 5, $<5$,[3] $<1$, $<1$ $>15$.

Commercial preparations of hog gastric mucosa made for therapeutic use have been found to be rich in a principle which appears on the basis of chemical and biological properties to be analogous to the apoerythein in gastric juice. Other biological materials tested, including commercial pepsins, contain very little or none of the active substance. Less than 2000 parts by weight of a concentrate prepared from hog gastric mucosa completely counteracted consistently the microbiological growth stimulation of one part of erythrotin.

For preparative purposes hog gastric mucosa has been used, and the principle can be precipitated from an aqueous extract by alcohol, acetone or ammonium sulfate (80% saturation). The principle is highly selective in its action and inactivates erythrotin but does not diminish the biological action of the end-products of erythrotin-catalyzed processes which can substitute for this vitamin in microbiological assays—methionine (*Escherichia coli* test)[1] and desoxyribosides (*Lactobacilli* tests).[4]

The complex formed when erythrotin combines with apoerythein decomposes upon heating (120° fifteen minutes) into erythrotin (or a compound which cannot be distinguished from it biologically or chromatographically), and a residue no longer possessing the ability to bind erythrotin. In combined form erythrotin is not as susceptible to destruction by alkaline or oxidative treatments which inactivate the unbound vitamin, since heat liberation following such treatment of the complex yields the original erythrotin activity.

These experiments point to the probability that apoerythein is the intrinsic factor of Castle or an important component thereof. Clinical trials are now in progress to test this conclusion.

We are deeply indebted to Dr. William Shive for generous supplies of erythrotin before vitamin $B_{12}$ was available and for prepublication disclosures concerning erythrotin tests, and to Dr. Edward Campbell, Eli Lilly and Company, who furnished biological preparations and gastric samples.

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(1) W. Shive, *Ann. N. Y. Acad. Sci.*, in press.
(2) E. L. Rickes, *et al.*, *Science*, **107**, 396 (1948).
(3) Pooled samples from at least three subjects.
(4) W. Shive, J. M. Macow and R. E. Eakin, THIS JOURNAL, **70**, 2614 (1948).