two pieces of information in addition to the geometrical array in the triangular faces. First, adjoining planes are frequently visible in which the molecular arrangement is like that of an orthographic projection of (200) faces. Secondly, where molecules are lying on the triangular surfaces or where steps occur, the shadow lengths indicate that the molecules are approximately as thick as their diameter in the triangular faces. Apparently the molecules are approximately spherical, about 80 Å. diameter in the dry crystal.

With an assumed density of 1.30 for the crystal,[1] the calculated molecular weight is 290,000 agreeing well with values deduced by other methods.[1] Although the unit cell of edestin has not been measured by X-ray methods, the structure of a similar globulin from tobacco seed has been reported as face-centered cubic with $a = 123$ Å. in the dry crystal.[2] Although this value is a little larger than that obtained for edestin, the agreement appears satisfactory insofar as there may be actual differences between the two proteins or differences in hydration as examined.

(1) See E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids, and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943.
(2) D. Crowfoot and I. Fankuchen, *Nature*, **141**, 522 (1938).

DEPARTMENT OF BIOLOGY C. E. HALL
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE, MASSACHUSETTS

RECEIVED JUNE 16, 1949

CRYSTALLIZATION OF VITAMIN B_{12b}

Sir:

The existence of more than one pink clinically-active pigment in liver extract was noted by Smith.[1] In the present study, a crystalline fraction was separated by chromatography having absorption spectrum maxima different from those at 278, 361 and 550 mμ which have been reported for vitamin B_{12}.[2,3] The biological activity of an impure preparation of this new fraction, obtained from liver extract, for chicks and in the microbiological assay was described elsewhere.[4] A similar fraction was obtained from cultures of *Streptomyces aureofaciens*[5] by adsorption with charcoal followed by elution and chromatography upon silicic acid columns.[6] Two characteristic pink bands were thus separated and were eluted. Fractional precipitation of the first of these with acetone yielded small rod-like red crystals which contained cobalt and phosphorus and which showed absorption spectrum maxima at 273, 351 and 525 mμ. The secondary "peaks" at 307 and 325 mμ, which are shown by vitamin B_{12}, were absent. The crystals were biologically active in the chick assay[4] and in the assay with *L. leichmannii* 313.[7] Since the term "vitamin B_{12a}" has recently been applied to a biologically active compound related to vitamin B_{12},[8] the term "vitamin B_{12b}" is suggested for the preparation described in the present investigation.

The second pink fraction had an absorption spectrum which was characteristic of vitamin B_{12}.[2,3] It was concentrated to yield needle-like crystals which appeared similar to those of vitamin B_{12}.

(1) Smith, *Nature*, **161**, 638 (1948).
(2) Ellis, Petrow and Snook, *J. Pharm. and Pharmacol.*, **1**, 60 (1949).
(3) Brink, Wolf, Kaczka, Rickes, Koniuszy, Wood and Folkers, THIS JOURNAL, **71**, 1854 (1949).
(4) Stokstad, Jukes, Pierce, Page and Franklin, *J. Biol. Chem.*, in the press (Sept. 1949).
(5) Duggar, *Annals N. Y. Acad. Sci.*, **51**, 175 (1948).
(6) Smith and Parker, *Biochem. J.*, **43**, viii (1948).
(7) Hoffman, Stokstad, Franklin and Jukes, *J. Biol. Chem.*, **176**, 1465 (1948).
(8) Kaczka, Wolf and Folkers, THIS JOURNAL, **71**, 1514 (1949).

LEDERLE LABORATORIES DIVISION J. V. PIERCE
AMERICAN CYANAMID COMPANY A. C. PAGE, JR.
PEARL RIVER, NEW YORK E. L. R. STOKSTAD
T. H. JUKES

RECEIVED JULY 22, 1949

PROTEIN SYNTHESIS BY CHYMOTRYPSIN

Sir:

Bergmann and Fruton[1] showed that chymotrypsin can synthesize anilides. It has now been found that an insoluble protein forms when very small quantities of chymotrypsin are added to Witte peptone. The clear peptone solution gradually changes into a solid gel. The reaction appears to have an optimum *p*H very close to 7.00. Crude lima bean trypsin inhibitor and crystalline lima bean trypsin inhibitor cause temporary inhibition.[2] The synthetic protein is soluble in hot water. It is precipitated by trichloroacetic acid and by a saturated ammonium sulfate solution which has been adjusted to *p*H 7.00. It gives the usual protein color reactions and a pink biuret test.

TABLE I

Sample	Chymotrypsin	After 16 hours	After 40 hours	Insoluble protein N, mg.
1	50 gamma	Viscous solution	Solid gel	3.60
2	1 mg.	Solid gel	Heavy precipitate	9.84
3	None	Clear solution	Clear solution	None

Each sample contained 0.66 g. of Witte peptone in 2 cc. of distilled water. The chymotrypsin was dissolved in 0.25 cc. of distilled water. Sample 3 contained 0.25 cc. of distilled water instead of the enzyme solution. The *p*H was 7.00. The temperature was 37°. A generous quantity of salt-free crystalline chymotrypsin was very kindly furnished by Dr. M. Kunitz of the Rockefeller Institute for Medical Research. Identical results were obtained with a commercial preparation of crystalline chymotrypsin Worthington Biochemical Laboratory—4 × crystallized, ap. 50% $MgSO_4$). Three different Witte peptone preparations gave identical results. A few commercial peptones (not Witte peptone) were unsuitable for the protein synthesis.

(1) N. Bergmann and J. S. Fruton, *Ann. N. Y. Acad. Sci.*, **45**, 409 (1944).
(2) H. Tauber, B. B. Kershaw and R. D. Wright, *J. Biol. Chem.*, in press.