COMMUNICATIONS TO THE EDITOR

PERCHLORIC ACID SALT OF VITAMIN B_{12}

Sir:

During the course of an investigation of the titration of vitamin B_{12} in glacial acetic acid with perchloric acid, it was observed that the addition of an excess of the reagent resulted in the formation of an amorphous orange-colored precipitate. Vitamin B_{12a} responded in a like manner, except that it forms a red-colored product.

A well-defined product of constant composition could be obtained only when more than 8 equivalents of perchloric acid were added. In a typical experiment, 20 mg. of B_{12} was dissolved in 5 ml. of glacial acetic acid and 20 ml. of 0.01 *N* perchloric acid in glacial acetic acid added. The orange precipitate was centrifuged after standing for ten minutes, and washed with 5 ml. of glacial acetic acid and then successively with four 10-ml. portions of anhydrous ether. After preliminary drying in a vacuum desiccator the material was dried to constant weight at 56° over phosphorus pentoxide at 2 mm.

Anal. Calcd. for $C_{63}H_{84}N_{14}O_{14}PCo \cdot 6HClO_4$ (mol. wt. 1953): C, 38.7; H, 4.6; N, 10.0; P, 1.5; Co, 3.0; Cl, 10.9; CN, 1.4. Found: C, 38.4; H, 4.5; N, 10.1; P, 1.6; Co, 3.2; Cl, 10.6; CN,[1] 1.4.

That this product represents a simple salt of the unchanged vitamin with six moles of perchloric acid is furthermore evidenced by the following facts: (1) back titration with 0.01 *N* potassium acid phthalate[2] of the perchloric acid remaining in the supernatant showed that an amount corresponding to 5.93 and 6.02 equivalents had entered the precipitate; (2) in an experiment in which anhydrous ether was added for quantitative precipitation, 5.94 mg. of vitamin yielded 8.60 mg. of the perchloric acid salt (calcd. 8.59 mg.); (3) after decomposition of the salt with water (reappearance of pink color) the aqueous solution consumed alkali corresponding to 6 equivalents of the amount of vitamin used; (4) while qualitatively the ultraviolet absorption spectrum of the aqueous solution of the salt was identical with that of the vitamin, $E^{1\%}_{1\,cm.}$ at 360 mμ was 68% of the value given by the latter (calcd. 69.2). Similarly, in the microbiological assay the salt showed 66–69% of the potency of the crystalline B_{12} standard; (5) the infrared spectrum of the salt (Nujol) exhibited a band at 4.70 μ, characteristic for the cyano group.

The perchloric acid salt described above represents to our knowledge the first well-defined derivative of vitamin B_{12} having an intact cobalti-cyano linkage,[3,4,5] and is of structural interest insofar as its formation reveals the presence of at least six weakly basic groups in the vitamin molecule.

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(1) E. A. Schulek, *Anal. Chem.*, **62**, 337 (1923).

(2) P. C. Markunas and J. A. Riddick, *ibid.*, **23**, 337 (1951).

(3) N. G. Brink, F. A. Kuehl, Jr., and K. Folkers, *Science*, **112**, 354 (1950).

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(5) R. P. Buhs, E. G. Newstead and N. R. Trenner, *ibid.*, **113**, 625 (1951).

THE PREPARATION OF RADIOACTIVE VITAMIN B_{12} BY DIRECT NEUTRON IRRADIATION[1]

Sir:

It has been found possible to activate the cobalt atom in crystalline vitamin B_{12} by the $Co^{59}(n, \gamma)Co^{60}$ reaction with high Co^{60} retention and small over-all loss of biological activity. The one other preparation of vitamin B_{12}-Co^{60} has been accomplished by biosynthesis.[2] That the cobalt atom is firmly bound in the vitamin is evidenced by its complete lack of exchange with inorganic cobalt compounds, in agreement with the diamagnetic behavior of vitamin B_{12}.[3] It seemed reasonable, therefore, to expect that some radio- and bioactive B_{12} molecules would result from the neutron activation of this crystalline solid.[4] As will be shown, purification of irradiated B_{12} yields a fraction with ~80% of the original specific radioactivity and 100 ± 15% of the specific biological activity based on L.L.D. assay.

A sample of crystalline vitamin B_{12},[5] weighing 8.8 mg., sealed in a quartz ampoule *in vacuo*, was subjected to a thermal neutron flux of 1×10^{13} neutrons/sq. cm. for seven days at ~80°.[6] After irradiation, the sample was let stand for two months to allow short-lived activities to decay. The crystals were not visibly altered and were completely soluble in water (Solution I). To determine the purity of this aqueous solution and the subsequent fractions mentioned below, small aliquots were applied to sheets of Whatman No. 1 filter paper. These prepared sheets were then developed with ethyl acetate–acetic acid–water[7] in a descending chromatographic system. The developed and dried paper sheets were placed in contact with X-ray film for several days to locate the areas having radioactivity and then the *identical* sheets were placed on solid agar plates prepared for the L.L.D. assay[8] to locate areas having bioactivity. Solution I gave a chromatogram exhibiting both radio- and bioactivity at the origin and in an elongated area extending

(1) Work carried out under the auspices of the Atomic Energy Commission.

(2) L. Chaiet, C. Rosenblum and D. T. Woodbury, *Science*, 111, 601 (1950).

(3) (a) J. C. Wallmann, B. B. Cunningham and M. Calvin, *ibid.* 113, 55 (1951); (b) for a discussion of exchange in cobalt complexes as related to their structure, see B. West, *Nature*, **165**, 122 (1950).

(4) For data concerning retention in *liquids*, see J. M. Miller, J. W. Gryder and R. W. Dodson, *J. Chem. Phys.*, 18, 579 (1950).

(5) We wish to thank the following members of the Chas. Pfizer and Co., Inc. staff, J. H. Kane and J. Snell, for providing several samples of B_{12}; and T. Lees, for data on the L.L.D. plate assay.

(6) We wish to thank the personnel at the National Research Council for the irradiation performed at Chalk River, Ontario, Canada.

(7) M. A. Jermyn and F. A. Isherwood, *Biochem. J.*, **44**, 402 (1949).

(8) See note 5. Similar to the method described by W. F. J. Cuthbertson, H. F. Pegler and J. T. Lloyd, *The Analyst*, **76**, 133 (19 51).