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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RECEIVED JUNE 28, 1951

(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).

(4) E. A. Kaczka, D. E. Wolf, F. A. Kuehl, Jr., and K. Folkers, *ibid.*, **112**, 354 (1950).

(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 (1951).

to the limit of the solvent migration. Consequently, an elution chromatogram of a portion of Solution I was made by diluting with acetone and running on a silica gel column (0.9 × 30 cm.) using acetone–water mixtures. The radioactivity of the fractions was measured by counting evaporated drops of the eluate on aluminum discs with a G.M. counter. One fraction (Solution IX) behaving in the column in a manner similar to authentic B_{12}, was then subjected to further study. This solution exhibited absorption maxima at 2780 and 3610 Å. No radioactivity could be detected in the organic phase when IX was extracted with dithizone in chloroform. A paper chromatogram of IX revealed a concentrated spot with little tailing, coincident for both the radio- and bioactivity. The R_f of this spot (0.79–0.80) was in agreement with a B_{12} standard run simultaneously.

The radioactivity of the original and purified samples was shown to be Co^{60} (5.3 y) by measurement of the gamma ray spectrum in a scintillation spectrometer. On a G.M. counter (geometry ~9%), Solution I had 689 ± 16 cpm./γ, solution IX 567 ± 30 cpm./γ, giving a retention value of ~80%. By employing serial dilutions of I and IX against standard B_{12}, it was determined that the *bio*-activity in Solution I was 80 ± 20% and in Solution IX was 100 ± 15% of the standard by weight.

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ENZYMATIC DISINTEGRATION OF WHEAT GERM DESOXYPENTOSE NUCLEIC ACID

Sir:

Previous work in this Laboratory[1] has shown that the degradation of calf thymus desoxypentose nucleic acid (DNA) by crystalline pancreatic desoxyribonuclease proceeded according to a complex pattern, resulting in the formation of dialyzable fragments and of a non-diffusible core which was characterized by greatly increased ratios of adenine to guanine, thymine to cytosine, and purines to pyrimidines.

A study of the generality of this phenomenon appeared important, since it offers an additional tool for the differentiation between DNA preparations of different origin and for the understanding of the relationship between nucleotide sequence and enzyme action. Wheat germ DNA, highly polymerized and entirely free of pentose nucleic acid, was employed. This DNA contains an appreciable quantity of a third pyrimidine, 5-methylcytosine,[2] and provides thereby one more marker, useful for the study of the enzymatic attack. The results summarized in Table I indicate the trend of degradation; "19% core" and "8% core" refer to the dialysis residues recovered after 81 and 92% of the DNA, respectively, had been converted to dialyzable products.

TABLE I

Wheat Germ DNA; Intact Preparation and Enzymatically Produced Cores (as moles per 100 moles P)

Constituent	Intact DNA	19% Core	8% Core
Adenine	26.3	33.2	35.4
Guanine	21.8	20.0	19.8
Cytosine	16.2	11.8	10.3
5-Methylcytosine	5.8	4.3	3.6
Thymine	26.1	26.2	23.4
Total	96.2	95.5	92.5
Purine to pyrimidine ratio	1.00	1.26	1.48

TABLE II

Liberation of Adenine (as moles per 100 moles P)

Agent	Intact DNA	19% Core	8% Core
1 *N* H_2SO_4, 100°, 1 hr.	26.5	31.2	31.6
98% HCOOH, 175°, 2 hr.	26.3	33.3	35.4
7.5 *N* $HClO_4$, 100°, 1 hr.		33.2	

Several points appear of interest. The ratio of cytosine to 5-methylcytosine remained constant in all stages, *viz.*, 2.8. In the intact DNA, the sum of these two pyrimidines equalled the molar concentration of guanine, a relationship observed with respect to the ratio of guanine to cytosine in almost all DNA specimens studied.[3] As judged from the extent of its liberation by various hydrolyzing agents, adenine seems to occur in two types of linkage, one of which is enriched in the cores (Table II). The procedures employed for the isolation and purification of the DNA will soon be discussed in detail. The enzyme used was supplied by the Worthington Biochemical Laboratory, Freehold, N. J. The analytical methods have been described before.[4]

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(3) E. Chargaff, *Experientia*, **6**, 201 (1950); *J. Cell. Comp. Physiol.*, in press; *Federation Proc.*, **10**, in press.

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ON THE SYNTHESIS OF CORTISONE ACETATE

Sir:

We wish to report the synthesis of cortisone acetate from *allo*pregnane-3β-ol-11,20-dione acetate, I, made available recently from $\Delta^{5,6}$ steroids, devoid of functional groups in ring C.[1,2]

Hydroxylation of the *allo*pregnane I at the 17-position by conversion into its enol acetate and treatment with perbenzoic acid followed by caustic saponification[3] yielded *allo*pregnane-3β,17α-diol-

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