

Comments and Communications

Vitamin B₁₂, A Cobalt Complex

Vitamin B₁₂ was described by the undersigned (*Science*, April 16, p. 396) as a crystalline red compound which is highly active for producing hematological responses in patients with pernicious and other anemias (R. West. *Science*, April 16, p. 398; T. D. Spies, *et al.* *S. med. J.*, 1948, 41, 522, 523) and for the growth of *Lactobacillus lactis* (E. L. Rickes, *et al.* and M. S. Shorb. *Science*, April 16, pp. 396, 397), chicks (W. H. Ott, E. L. Rickes, and T. R. Wood. *J. biol. Chem.*, 1948, 174, 1047), and rats (G. A. Emerson, M. E. Zanetti, and T. R. Wood. To be published.) Independent isolation of an anti-pernicious anemia factor, presumably identical with B₁₂, has also been reported (E. L. Smith. *Nature, Lond.*, 1948, 161, 638).

Information on the chemical nature of B₁₂ is of primary interest. The following observations on B₁₂ have been made which appear useful for other chemical and biological studies.

Emission spectrographic analysis¹ of B₁₂ has shown the presence of cobalt.² Vitamin B₁₂ appears to be a cobalt coordination complex which, having 6 groups about the cobalt atom, could involve one or more organic moieties. The red color of B₁₂ appears to be at least in part associated with its cobalt-complex character.

The presence of cobalt in vitamin B₁₂ reflects significantly upon many biological studies which have shown that cobalt is an essential trace element in nutrition, and perhaps upon suggestions concerning cobalt as a trace contaminant in iron therapy of anemias (E. J. Underwood. *Proc. Soc. exp. Biol. Med.*, 1937, 36, 296). The nutritional significance of cobalt must be re-evaluated as the biological function of B₁₂ is developed.

Cobaltous ion (1 µg/ml) was without activity for *L. lactis* as contrasted with the high potency of B₁₂ (0.000013 µg/ml, half-maximal growth).

Randolph West has tested cobalt ion in two cases of pernicious anemia with negative results.³ The average adult daily dietary intake of cobalt has been estimated at 100 µg (B. Ahmad and E. V. McCollum. *Amer. J. Hyg.*, 1939, 29A, 24).

Spectrographic examination of B₁₂ also showed the presence of phosphorus. Although nitrogen was found to be present, tests for sulfur were negative.

¹We wish to thank Dr. Charles Rosenblum and Luise Anderson for this determination.

²Private communication from Dr. H. M. Walker, of Glaxo Laboratories, Ltd., discloses that Glaxo's factor also contains cobalt. E. L. Smith and L. F. J. Parker. *Proc. biochem. Soc.*, in press; E. L. Smith. *Nature, Lond.*, in press.

³Personal communication; levels of 500 and 150 µg of cobaltous ion administered subcutaneously as acetate and chloride, respectively.

Microbiological assay of an aqueous solution of B₁₂ (74 µg/0.5 ml) showed that autoclaving for 15 min at 121° C did not change the activity within the experimental error of $11.4 \times 10^6 \pm 0.6 \times 10^6$ u/mg.

Vitamin B₁₂ in 0.015 N sodium hydroxide solution (0.2 µg/ml) was inactivated (microbiological assay) at room temperature as follows: 20% (0.67 hr), 45% (6 hrs), 90% (23 hrs), 95% (95 hrs); it was inactivated in 0.01 N hydrochloric acid solution (10 µg/ml) as follows: 18% (3 hrs), 75% (23 hrs), 89% (95 hrs).

The cobalt-complex nature of vitamin B₁₂ is an outstanding property.

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A Laboratory Test for the Virus of Poliomyelitis

The need for a reliable test for the presence of the virus of poliomyelitis other than expensive monkey inoculations requires no emphasis.

Preliminary experiments indicate that such a test might be satisfactorily obtained through an interference phenomenon resulting in the protection of mice inoculated with material suspected of containing the virus of poliomyelitis against a subsequent inoculation with the Lansing strain of virus.

Virus is extracted and concentrated from the supposedly infected material in the usual manner (P. Lépine. *C. R. Soc. Biol., Paris*, 1939, 131, 573). Five mice, 4-6 weeks of age, are injected intracerebrally with the extracted materials. Two days later the mice are given another intracerebral injection with active Lansing mouse-adapted virus. Five control mice are similarly treated, each receiving about 50 LD₅₀.

The answer is drawn from the results observed on the 10th-11th day, when 80% (4 out of 5), at least, of the controls should be paralyzed or dead, and at least 3 out of the 5 mice inoculated with the suspected material should show a significant protection. It should be mentioned that this significant difference between the two groups of animals may be the result either of permanent protection or of merely delayed onset of symptoms in the protected group.

Our experiments have included (a) cord material from monkeys inoculated with one standard laboratory strain and (b) fecal material collected from patients during the summer of 1946 and preserved in the frozen stage since that time.

Extracts from normal human feces, or cord material inactivated after heating at 80° C for 15 min, showed no protective action.

Attempts to obtain interference protection by other means of inoculation (oral, nasal, intraperitoneal, intracutaneous, etc.) or with unconcentrated material, or through a longer interval between inoculations, have so far failed.