Effectiveness of Thiocyanate Analog of Vitamin B_{12} in Pernicious Anemia.* (18798)

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Since the announcement of crystalline vit. B_{12}(1) two hemopoietically active modifications, B_{12a} and B_{12b}, have been described. Vit. B_{12} has been shown to be a cyano-cobalt coordination complex(2) and has been designated cyano-cobalamin(3). Vit. B_{12a} does not contain the cyano group(2). Comparative data now indicate vit. B_{12a} and B_{12b} are identical(4) and represent the replacement of the cyano group by an hydroxo group in the vit. B_{12} molecule(3). This substance therefore has been designated hydroxo-cobalamin(3). Other analogs of vit. B_{12} in which the cyano group of cyano-cobalamin has been replaced by other groups such as chloro, cyanato and sulfato have been prepared and tested for their microbiological activities(3). Vit. B_{12a}(4,5) and B_{12b}(6) had previously been reported to have a therapeutic potency in pernicious anemia comparable to that of vit. B_{12}. Studies on the effectiveness in pernicious anemia of other analogs of vit. B_{12} have not been reported. Buhs, Newstead, and Trenner(7) have prepared the thiocyanate analog of vit. B_{12} in which the cyanide ion of vit. B_{12} was replaced by the thiocyanate ion. Like vit. B_{12a} (hydroxo-cobalamin) it was found to be microbiologically equivalent to vit. B_{12} in the *L. lactis* cup assay but differed from vit. B_{12} in titrimetric assay methods(7). In the present paper we report studies showing the thiocyanate analog of vit. B_{12} to be fully active hemopoietically in pernicious anemia.

We reported previously(8) that certain biochemical changes can be observed preceding and during the hematologic response to crystalline vit. B_{12} in pernicious anemia. These include an early decrease in urinary excretion of phosphate, followed by increased uric acid excretion with beginning reticulocytosis, and increased phosphate excretion during the period of maximum reticulocytosis. In the present investigation similar biochemical studies were correlated with the hematologic and clinical responses to the thiocyanate analog of vit. B_{12}.

Two previously untreated white male patients with pernicious anemia in relapse were placed on a general hospital diet which furnished a constant intake of phosphorus (about 1.6 to 1.7 g phosphorus daily). Clinical diagnosis was substantiated by presence of a macrocytic anemia, megaloblastic bone marrow and histamine refractory achlorhydria. Crystalline thiocyanato-cobalamin, in saline solution, 15 μg per cc, was administered parenterally according to the schedule used with vit. B_{12}: 15 μg initially, then 5 μg daily

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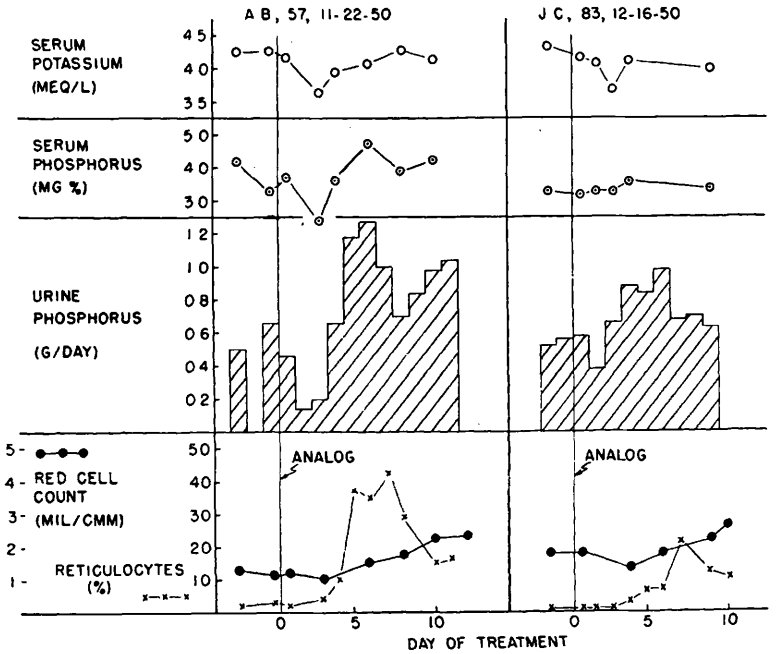

FIG. 1. Effects on Urinary Phosphorus, and on Serum Potassium and Phosphorus, with Relation to the Reticulocyte and Total Red Cell Response in 2 Cases of Pernicious Anemia Treated with the Thiocyanate Analog of Vit. B_{12}.

until the peak of the reticulocyte response after which 5 μg was administered every other day. The clinical response to treatment was similar to that observed by us with crystalline vit. B_{12} except for the neurological manifestations in the older patient. Striking in each patient was the remarkable effect on appetite, general strength and feeling of well-being. Patient A.B. (57 years) was discharged after 12 days treatment with the analog. He was then given vit. B_{12} since it was necessary for him to leave the city. During the period of hospitalization he received a total of 60 μg of thiocyanato-cobalamin in 10 days, an average of 6 μg per day. The only neurological finding was minor subjective tingling in the fingers, present for a week prior to admission, which he said improved following treatment.

Patient J.C. (83 years) was treated continuously with the analog for a period of 61 days and received a total of 225 μg, 75 of which were given during the 9 days of hospitalization.‡ Neurological symptoms were moderately severe and had been present for about 6 weeks. In general the neurological response was disappointing. After discharge he received a single dose of 15 μg per week for 2 weeks. The dose of the analog was then increased to 30 μg a week for 3 weeks without noticeable improvement. When our supply was exhausted (after 61 days) he was given vit. B_{12} and about a month later, after 90 μg, stated there was some subjective improvement.

Maximum reticulocytosis occurred in each subject on the 7th day of treatment, but response in the younger man, A.B., was more vigorous (Fig. 1). Increases in hemoglobin and hematocrit were consistent with improvement in the red cell count. Leucopenia (<3000) was present prior to treatment in both instances. In A.B. a normal leucocyte count was obtained on the 4th day of therapy, while in J.C. a moderate leucopenia (2350 to 4200) persisted until the 9th day of treatment after which normal counts were obtained.

Fig. 1 shows the response in urinary phosphorus excretion in both men. The effect

‡ This patient received 15 μg on 8th and 9th days.

was the same as that observed previously with crystalline vit. B_{12} administered in the same dosage. There was a prompt diminution in urinary phosphate excretion which occurred before any change in reticulocyte count. This was more marked in A.B. and was accompanied by a drop in serum phosphorus and serum potassium. Following the initial decrease there was a marked increase in urinary phosphorus parallel with the reticulocyte rise. Serum alkaline phosphatase in A.B. was 2.7 to 2.8 Bodansky units per 100 cc before and after treatment except for a value of 3.2 units on the day when serum and urine phosphorus were greatest. An increase in urinary uric acid-creatinine ratio accompanied reticulocytosis. Similar changes occurring after vit. B_{12} in pernicious anemia patients were interpreted by us as being due to increased utilization of phosphate accompanying a profound stimulation of nucleoprotein metabolism(8).

Summary. The thiocyanate analog of vit. B_{12} (thiocyanato-cobalamin) has been shown to be fully active hematologically in pernicious anemia. In two patients the early biochemical and hematologic responses were the same as observed with same dosages of vit. B_{12}. Further evaluation of the neurological response to this analog is necessary.

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Ergothioneine Depletion in Rabbit Erythrocytes and Its Effect on Methemoglobin Formation and Reversion. (18799)

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Recent studies on methemoglobin (MHb) formation and reduction in nitrite-treated rabbit erythrocytes revealed an acceleration in both the appearance and reversion of MHb in red cells of animals raised on a purified diet(1). Liver paste added to this diet retarded the MHb formation rate but various crude supplements such as liver, yeast, wheat germ, and kale failed to alter the MHb reversion rate. Supplements of ergothioneine, glutathione, and vit. B_{12} have since been tested and it has been found that the ergothioneine retards MHb formation or reduction to a rate characteristic of corpuscles of animals raised on a stock diet. Moreover, the blood ergothioneine level varies inversely with the MHb rates, being low in rabbits on the purified diet and high in those fed the stock diet or treated with ergothioneine.

Procedure. Fifty male New Zealand strain albino rabbits weighing 1600-1800 g were placed on the purified diet designated as No. 672, while 12 animals were put on the stock diet composed of Purina rabbit pellets(1). Diet 672 contained sucrose, casein, cellophane, Wesson oil, O.M. salt mixture and vitamins as described previously. After 5 months the test rabbits were separated into 5 equal groups. Equal blood samples of the individuals in each group were pooled, and the rate of MHb formation was determined in the washed substrate-free erythrocytes following addition of a standard dose of $NaNO_2$. This test and the determination of the rate of reversion of MHb after addition of a standard $NaNO_2$ dose to corpuscles suspended in a medium containing lactate as substrate were carried out according to the procedure already outlined(1). Supplements of vit. B_{12}, ergothioneine and glutathione, were then given three groups (Table I). Three months later the MHb tests were repeated, and in addition the erythrocyte ergothioneine and glutathione content was measured using the most recent Hunter method for ergothioneine(2). In or-

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