Parenteral Studies with Vitamin B_{12} Complexes

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MUCH work and attention has recently been directed towards coenzyme vitamin B_{12} preparations. Barker and his associates[1,2] have demonstrated the properties of 5,6-dimethylbenzimidazole-B_{12} coenzyme and suggested that this may represent the metabolically active form of the vitamin. Simultaneously, hydroxocobalamin* has been proposed as a more physiologic form of vitamin B_{12} than cyanocobalamin in dogs[3] and has been shown to be better retained than cyanocobalamin after parenteral administration to man.[4]

This study was undertaken to compare the effects of parenteral administration of large single doses of cyanocobalamin, hydroxocobalamin and coenzyme vitamin B_{12} on the serum levels and urinary excretion in normal human subjects over an extended period of time.

PROCEDURE

Thirty healthy volunteer subjects between the ages of twenty-one and fifty-five years were studied. There were nine males and twenty-one females, mainly employees of the laboratory. They were ambulatory and lived outside of the institution and were considered reliable and competent to collect their urine samples properly.

The subjects were divided into three groups of ten. One group received cyanocobalamin, the second hydroxocobalamin, and the other coenzyme vitamin B_{12}.† The coenzyme vitamin B_{12} preparation was handled and administered under the strictest dark room conditions in view of its extreme photosensitivity.

Each patient received 1 mg. of the vitamin B_{12} preparation being tested by injection deep into the deltoid muscle. Control blood samples were drawn and a urine sample collected prior to administration of the drugs. Blood was collected subsequent to drug administration at five, twenty-four, forty-eight, ninety-six hours and one, two, three and four weeks. Quantitative urine samples were collected from zero to six, six to twelve, twelve to twenty-four, twenty-four to forty-eight and forty-eight to seventy-two hours. A random morning specimen of urine was collected after one, two, three and four weeks to determine whether or not vitamin B_{12} continued to be excreted in significant amounts. Urine samples were collected under toluene in scrupulously cleaned Mason jars. Blood was drawn with sterile disposable plastic syringes, transferred and centrifuged in sterile disposable plastic tubes. Serum and urine were frozen until assayed in sterile plastic tubes. Assays were run with Lactobacillus leichmannii by the method of Skeggs et al.[5] with the modifications described by Boger et al.[6]

RESULTS

The results of the serum study are depicted graphically in Figure 1. The geometric mean of each group, as given in Table 1, is plotted against the time interval. Surprisingly, the coenzyme vitamin B_{12} levels closely parallel those of cyanocobalamin, whereas the hydroxocobalamin group maintained statistically significant higher levels throughout the study than the other two groups.

The urinary recovery of the three complexes is shown in Figure 2. We approached the study of urinary excretion with some reservations since the normal urinary level is extremely low and represents a real challenge to the

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* Solutions of hydroxocobalamin are frequently referred to as aquocobalamin.

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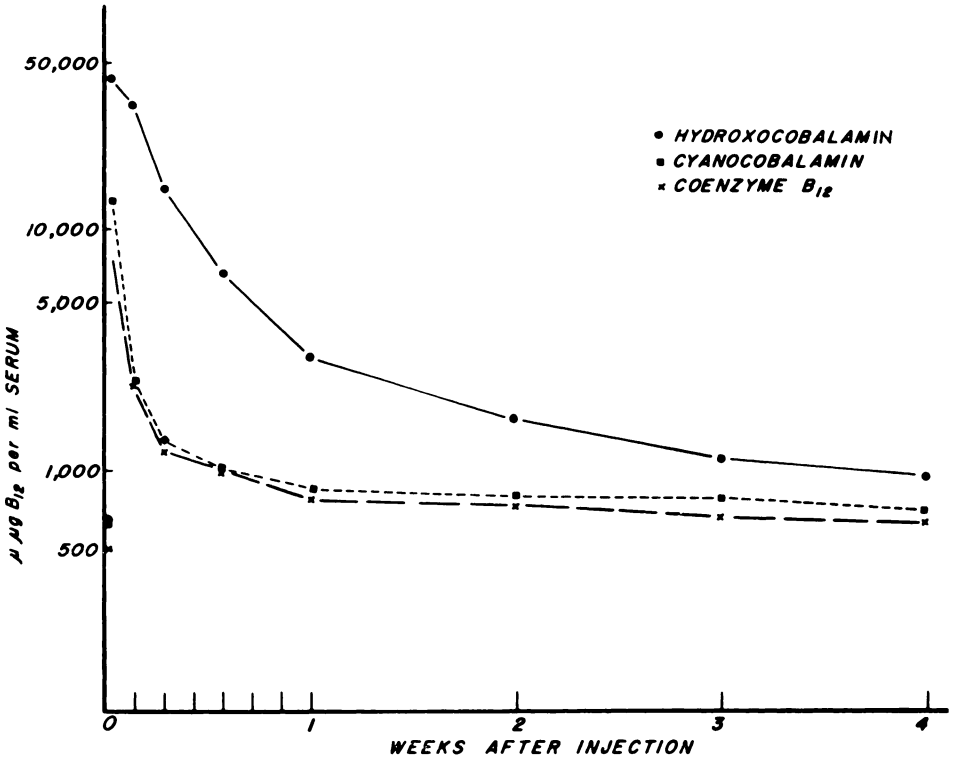

FIG. 1. Serum vitamin B_{12} level following single parenteral dose (1 mg.) of indicated cobalamin.

sensitivity of the assay in the presence of the variable urinary constituents. We were pleasantly surprised to find the normal values fell within a narrow range. However, the stability of hydroxocobalamin and the coenzyme preparation in urine, especially when the collection period was for twenty-four hours, represents an unknown quantity. Our total recovery of hydroxocobalamin is lower than that obtained in the studies of Glass[4] and Killander and Schilling[7] in which radiolabeled hydroxocobalamin was used. The range of recovery of hydroxocobalamin was from 21 to 45 per cent with a geometric mean of 30.9 per cent. Coenzyme vitamin B_{12} represents even more of an unknown quantity with respect to stability in urine. The mean recovery was 42 per cent but the individual variation was from 0.2 to 87.5 per cent. Since the subject from whom we recovered 0.2 per cent exhibited essentially a homeostatic serum level throughout the study, we are inclined to believe she represents a dark room casualty and that perhaps a substantial portion of the dose leaked from the site of injection or from the syringe. If this person is omitted from the group, the statistical findings do not change from those to be reported, but the mean increases to 46 per cent and the lowest recovery in the group was 23 per cent.

According to the statistical analysis of the data, at six hours, all excretion percentages

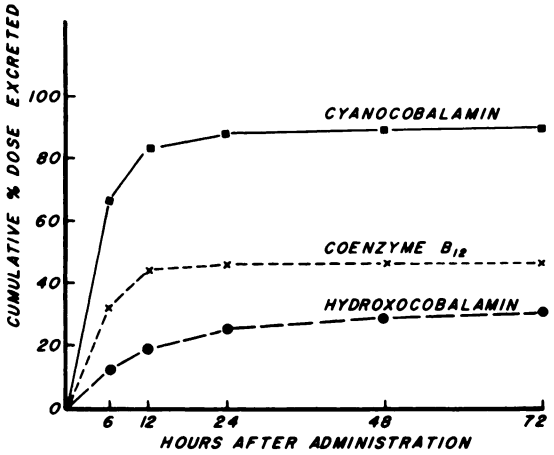

FIG. 2. Urinary recovery of cobalamin. Results are expressed in terms of geometric means.

TABLE I
Serum Vitamin B_{12} Levels after Single Intramuscular Dose of Cobalamins*
(mμg./ml. serum)

Time After Administration	Cyanocobalamin	Hydroxocobalamin	Coenzyme Vitamin B_{12}
0 hour	0.61	0.63	0.48
5 hours	13.0	42.5	7.9
24 hours	2.3	33.7	2.2
48 hours	1.3	14.3	1.2
96 hours	1.0	6.5	1.0
1 week	0.83	2.9	0.75
2 weeks	0.77	1.6	0.71
3 weeks	0.76	1.1	0.63
4 weeks	0.67	0.92	0.59

* Results are expressed as geometric mean of ten patients in the groups receiving hydroxocobalamin and coenzyme vitamin B_{12} and of eight patients in the group receiving cyanocobalamin. Two patients in the cyanocobalamin group were omitted because they were found to be on supplemental vitamin B_{12} therapy. One milligram doses were administered.

were different with cyanocobalamin yielding a greater percentage than either hydroxocobalamin ($P < 0.001$) or coenzyme vitamin B_{12} ($P < 0.05$). The rate of decrease over the seventy-two hour period was essentially the same for both cyanocobalamin and coenzyme vitamin B_{12}, both decreasing at a steeper rate than hydroxocobalamin ($P < 0.001$). As compared with the recovery figures cited, the mean excretion of cyanocobalamin was 90.8 per cent, higher than that obtained with coenzyme vitamin B_{12} ($P < 0.5$) or hydroxocobalamin ($P < 0.001$). As compared with the recovery figures cited, the mean excretion of cyanocobalamin was 90.8 per cent, higher than that obtained with coenzyme vitamin B_{12} ($P < 0.5$) or hydroxocobalamin ($P < 0.01$). Excretions of the latter two preparations were not significantly different.

The urinary excretion of cyanocobalamin and coenzyme vitamin B_{12} was virtually complete at twenty-four hours whereas considerable hydroxocobalamin was still being excreted in the period from forty-eight to seventy-two hours. The data in Table II show that the urinary level of hydroxocobalamin remained higher than for the other complexes for the duration of the experimental period, although beyond the first week the differences probably are not statistically significant.

COMMENTS

From these studies, it appears that the serum vitamin B_{12} activity is maintained at ele-

TABLE II
Urinary Levels of Vitamin B_{12} after Single 1 mg. Dose of Cobalamins Given Intramuscularly

Time After Administration	Vitamin B_{12} (μμg./ml. urine*)		
	Cyanocobalamin	Hydroxocobalamin	Coenzyme Vitamin B_{12}
0 hour	51 (27–94)	33 (17–104)	38 (0–137)
24 hours	939,000 (764,000–1,142,000)	286,000 (173,000–577,000)	657,000 (342,000–1,845,000)
48 hours	824 (129–3160)	54,500 (20,500–125,000)	475 (130–4,480)
72 hours	145 (39–354)	7,450 (2,610–25,900)	144 (87–486)
1 week	74 (13–242)	485 (198–5,160)	59 (5–199)
2 weeks	46 (0–77)	113 (24–1,340)	58 (0–132)
3 weeks	40 (0–47)	90 (11–300)	44 (0–123)
4 weeks	44 (21–160)	82 (28–199)	29 (11–105)

* Figures in parentheses indicate range of values in obtaining geometric mean.

vated levels for a longer period of time after the administration of hydroxocobalamin as contrasted with a rapid decline in serum activity following the parenteral administration of equal doses of either cyanocobalamin or coenzyme vitamin B_{12}. Urinary excretion studies support the findings in serum in that the rate of excretion of hydroxocobalamin is considerably slower than that of the other two complexes. Paradoxically, the total excretion of coenzyme vitamin B_{12} resembles the total excretion of hydroxocobalamin and both are much lower than the total excretion of cyanocobalamin. The questionable stability of both hydroxocobalamin and coenzyme vitamin B_{12} in urine is undoubtedly a factor to be considered in the interpretation of these data, which were obtained by microbiological assay. However, taking the findings at face value, the possibility that coenzyme vitamin B_{12} is more rapidly cleared from the serum and stored more tenaciously in tissues than are the free cobalamins cannot be ruled out, although such reasoning would be at variance with the concept that the serum vitamin B_{12} level is indicative of the total body stores of the vitamin. In normal healthy persons such as these, one would expect the picture to be similar to that observed with hydroxocobalamin when an excess of an essential metabolite is presented to the body; a gradual decline in serum levels and evidence of sustained elimination in the urine. It has always been a puzzle as to why cyanocobalamin, albeit so remarkable in its therapeutic effectiveness, is excreted, as Gräsbeck[8] expresses it, in a pharmacologic manner, rather than in a physiologic pattern.

SUMMARY

Thirty healthy, noninstitutionalized volunteer subjects were divided into three groups and given an intramuscular dose of 1 mg. of either cyanocobalamin, hydroxocobalamin or coenzyme vitamin B_{12}. Serum vitamin B_{12} was measured over a four week period and urinary excretion was followed quantitatively for seventy-two hours and qualitatively for the duration of the study period.

The group receiving hydroxocobalamin maintained statistically significant higher serum levels over the period of the study, with a slower rate of urinary excretion than those receiving either cyanocobalamin or coenzyme vitamin B_{12}.

The total urinary excretion over a seventy-two hour period was much greater in the group receiving cyanocobalamin than in either of the other two groups. The low total recovery of coenzyme vitamin B_{12} in the absence of sustained elevation of serum levels is paradoxical and may be due to the instability of the compound or to tissue retention, but, at the moment, it is unexplained.

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