Maintenance of Elevated Blood Levels of Vitamin B_{12} in Human Subjects

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THERE is increasing evidence that the administration of vitamin B_{12} via the oral route may occassionally give unreliable results in the treatment of Addisonian pernicious anemia and the neurologic disorders frequently associated with this disease.[1] Interest is thus referred back to the parenteral route and with it arises increasing need for a prolonged release form of the vitamin. This need prompted extension of our earlier findings[2] which had demonstrated prolonged urinary excretion following intramuscular injection of the vitamin in an oil-with-aluminum monostearate depot vehicle. Subsequently, we observed[3] that the depot effect of the preparation when tested in dogs was reflected in prolonged elevated blood levels of the vitamin. Administered in depot form, the vitamin remained in the blood twice as long as when it was given in an aqueous medium.

In this communication evidence from two studies with human subjects is presented to show that vitamin B_{12} may be expected to remain in the blood three to four times longer when it is given as a depot preparation than it does when it is given in water. The elevated levels, moreover, may persist for three to four weeks.

METHODS

Two studies were conducted. There were ten healthy subjects in each of two groups in the first study and five subjects in each of two groups in the second. In both studies each subject received 1,000 μg. of the vitamin intramuscularly in an aqueous (Ducobee® "1,000," 1,000 μg. vitamin B_{12} per ml.) or depot vehicle (Ducobee Depot, 1,000 μg. vitamin B_{12} per ml.). In the first study blood samples were taken at one, two, three, five, seven, nine, eleven, thirteen and fifteen days after administration. In the second study, samples were taken daily the first three days, three times during the following week, and then twice weekly for three weeks. Clotted blood samples were centrifuged and the serum collected. Serum was then diluted with pH 4.6 acetate buffer 1:5, steamed for thirty minutes, cooled to room temperature and centrifuged following the procedure of Gaffney et al.[4] Supernatant solutions were neutralized with 0.33 M Na_2HPO_4 and diluted further with water as needed.

The vitamin B_{12} contents of the suitably diluted aliquots were assayed microbiologically with L. leichmannii (A. T. C. C. No. 7830) following the U.S.P. titrimetric procedure.

RESULTS

The data of the two studies are summarized in Table 1. A sharp increase in the serum level of vitamin B_{12} followed the administration of 1,000 μg. of the vitamin in water. This level rapidly subsided so that in test No. 1, three and five days following medication, the average level was only moderately above the level before the test ($P = 0.02$ to 0.05). At seven days and thereafter the serum level was no longer significantly above the level before the test.

The serum of the subjects given the depot preparation, however, exhibited a sharp rise in the vitamin level following injection, and the level declined more slowly than had been anticipated in setting up the study. In contrast to the earlier study with dogs,[3] even at fifteen days the serum level was significantly above

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This study was presented at the Fifth International Congress on Nutrition, September 1–7, 1960, Washington, D. C.

TABLE I
Serum Vitamin B_{12} in Human Subjects Following the Intramuscular Injection of the Vitamin in Water or as a Depot Preparation in Oil with Aluminum Monostearate

Vitamin B_{12} Preparation	Post Medication Time (days)	Serum Vitamin B_{12} Av. ± S.E. ($\mu\mu$g./ml.)	P Compared to Initial
Test No. 1 (Ten Subjects in Each Group)			
Aqueous	Before test	384 ± 44	...
	1	1,819 ± 220	<0.001
	2	994 ± 132	<0.001
	3	652 ± 120	0.05
	5	641 ± 96	0.02
	7	562 ± 81	0.07
	9	531 ± 61	0.09
	11	524 ± 53	0.06
	13	487 ± 20	0.20
	15	449 ± 62	0.43
Depot	Before test	358 ± 39	...
	1	3,067 ± 262	<0.001
	2	2,806 ± 271	<0.001
	3	2,245 ± 256	<0.001
	5	2,066 ± 230	<0.001
	7	1,418 ± 198	<0.001
	9	1,162 ± 137	<0.001
	11	971 ± 100	<0.001
	13	911 ± 31	<0.001
	15	755 ± 66	<0.001
Test No. 2 (Five Subjects in Each Group)			
Aqueous	Before test	272 ± 11	...
	1	1,338 ± 160	<0.001
	2	881 ± 101	<0.001
	3	665 ± 62	<0.001
	6	485 ± 55	0.01
	8	408 ± 41	0.013
	10	403 ± 34	0.01
	13	378 ± 39	0.04
Depot	Before test	245 ± 27	...
	1	2,170 ± 224	<0.001
	2	1,810 ± 166	<0.001
	3	1,488 ± 146	<0.001
	6	1,189 ± 191	<0.001
	8	905 ± 115	<0.001
	10	808 ± 81	<0.001
	13	661 ± 78	0.001
	15	663 ± 75	0.001
	20	490 ± 55	0.004
	23	477 ± 53	0.005
	27	440 ± 43	0.005
	30	383 ± 36	0.015
	34	358 ± 32	0.03

the level before the test (P = <0.001) so that there was no basis for estimating the time required for the vitamin level to return to base values.

A second test was performed to gain definitive information on the relative release rates of the vitamin from the injection site as reflected by elevated blood levels. From the results summarized in the table it may be seen that following administration of the vitamin as the aqueous preparation the serum levels fell after the initial rise to about 400 $\mu\mu$g. per ml. and then leveled off so that successive readings were 408, 403 and 378 $\mu\mu$g. per ml. Stabilization occurred at the eighth day after medication and fell within the range of the seven-day 562 $\mu\mu$g. per ml. endpoint of the first study. We consider these values to have returned to the range of serum vitamin B_{12} levels before the test. The vitamin level in the serum of the subjects given the depot preparation returned to the level existing before the injection thirty days after medication.

Judging by the maintenance of elevated blood levels, a three- to fourfold depot effect occurred. It can also be noted that the vitamin given in oil with aluminum monostearate remained for four to five days above the twenty-four-hour peak of the vitamin level of the patients given the aqueous preparation.

COMMENTS

In an earlier study[2] with human subjects evidence was presented to show that the urinary vitamin level rapidly declined following administration of the vitamin in water. When the vitamin was given in a depot vehicle (oil and aluminum monostearate), significant amounts of the vitamin were still being excreted four days following medication.

When vitamin B_{12} was administered in an aqueous menstruum to the human subject, the serum vitamin level remained elevated for eight or nine days, but when the depot form was administered, its effect was exerted over a period of twenty-seven days with return to levels existing before the test at thirty and thirty-four days.

Both studies showed sustained higher serum levels twenty-four hours after administration of the depot vitamin than after injection of the aqueous one. The elevated serum level following the administration of the depot form was as pronounced at the end of four or five

days as it had been at the twenty-four hour level following the injection of the aqueous preparation. Thompson and Hecht[5] in a similar study with human subjects observed that a comparable depot effect was achieved following administration of the vitamin as a cyanocobalamin zinc tannate complex. Preliminary reports[6,7] also suggest that the vitamin is released more slowly when administered as hydroxocobalamin than as cyanocobalamin.

When high serum levels of vitamin B_{12} are indicated, the depot preparation offers definite advantages over the aqueous one.[8] It remains to be proved, however, that the prolonged high serum levels are associated with increased utilization and hence of benefit to the patient.

SUMMARY

Serum levels of vitamin B_{12} were followed in two groups of healthy subjects given the vitamin intramuscularly either in the usual aqueous medium or as an oil-aluminum monostearate depot preparation.

Following the administration of the vitamin in water, the blood levels rose and then subsided to levels existing before the test in eight to nine days.

In both groups of subjects given the vitamin in the depot vehicle, the serum level of the vitamin at fifteen days was clearly above the levels before the test. One group, followed beyond this time, exhibited elevated levels through twenty-seven days with return to levels existing before the test at the thirtieth and thirty-fourth days. The groups given the vitamin in depot form exhibited serum levels elevated for four to five days above the twenty-four-hour level of the group given the aqueous preparation.

REFERENCES

1. Bethell, F. H., Castle, W. B., Conley, C. L. and London, I. M. Present status of treatment of pernicious anemia, ninth announcement of the U. S. P. anti-anemia preparations advisory board. *J.A.M.A.*, 171:2092, 1959.
2. Arnold, A., Berberian, D. A. and Sobell, S. D. Prolonged excretion of vitamin B_{12} following its intramuscular injection as a depot preparation. *Am. Pract. & Digest. Treat.*, 9:1835, 1958.
3. Sobell, S. D., Arnold, A. and Coulston, F. Prolonged elevated serum levels of vitamin B_{12} following its intramuscular injection as a well tolerated depot preparation. *Am. J. Clin. Nutrition*, 8:412, 1960.
4. Gaffney, G. W., Horonick, A., Okuda, K., Meier, P., Chow, B. F. and Shock, N. Vitamin B_{12} serum concentration in 528 apparently healthy human subjects of ages 12 to 94. *J. Gerontol.*, 12:32, 1957.
5. Thompson, R. E. and Hecht, R. A. Studies on a long-acting vitamin B_{12} preparation. *Am. J. Clin. Nutrition*, 7:311, 1959.
6. Boger, W. P. and Gavin, J. J. Hydroxocobalamin: comparison with coenzyme B_{12}, cyanocobalamin and cyanocobalamin zinc tannate. Presented at the Fifth International Congress on Nutrition, September 1–7, 1960, Washington, D. C.
7. Killander, A. and Schilling, R. F. Preferential retention of hydroxocobalamin over cyanocobalamin in humans. *J. Lab. & Clin. Med.*, 56:917, 1960.
8. Woodrow, M. The use of long-acting vitamin B_{12} (Ducobee Depot) in 420 patients in a general medical practice. *Current Therap. Res.*, 2:305, 1960.