Effect of an Elixir on the Absorption of Vitamin B_{12} by Healthy Young and Old Subjects*

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The absorption of orally administered vitamin B_{12}, even by healthy people, is poor.[1] In patients suffering from pernicious anemia,[2,3] or in those with complete gastrectomy,[4] vitamin B_{12} absorption is negligible. The oral coadministration of normal gastric juice or intrinsic factor preparations can increase the vitamin B_{12} absorption in the latter groups; however, whether such agents can also increase absorption in clinically healthy subjects has not yet been demonstrated by any systematic study. On the contrary, it has been found that an excessive dose of the usual intrinsic factor preparations may inhibit the absorption of vitamin B_{12} by normal rats,[5] by young, healthy humans,[6] and by gastrectomized subjects.[4] However, such data need not support the hypothesis that intrinsic factor, *per se*, is inhibitory. It has been demonstrated that highly purified preparations, or those prepared from sources without inhibitory substances, may actually enhance absorption.[6] This paper reports on several experiments designed to evaluate the ability of an elixir containing vitamin B_{12}, other vitamins, and lipotropic substances† (hereinafter referred to as the "elixir" with vitamin B_{12}) to enhance vitamin B_{12} absorption in both normal subjects and those suffering from pernicious anemia.

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METHOD

Medications Used

The following medications were used in these studies: commercial vitamin capsules containing all known vitamins and minerals except vitamin B_{12},‡ capsules containing 100 μg of vitamin B_{12}; solutions containing either 50 μg of radioactive (Co^{60}) vitamin B_{12} or 1000 μg of regular vitamin B_{12}; and the "elixir" containing vitamin B_{12}. One teaspoonful (5 ml) of the "elixir" with vitamin B_{12} contains the following ingredients: vitamin B_{12} (crystalline) 8.34 μg; riboflavin, 0.6 mg; niacinamide, 7.0 mg; pyridoxine, 2.0 mg; betaine, anhydrous, 700.0 mg; choline dihydrogen citrate, 150.0 mg; inositol, 150.0 mg; ferric pyrophosphate, 35.0 mg; caffeine citrate, 1 grain (65 mg); and alcohol, 15 per cent.

I. Studies of Vitamin B_{12} Serum Levels

A preliminary study was conducted in an old people's home by assaying the serum vitamin B_{12} levels of 21 elderly patients (65 years or older) who had been receiving the "elixir" with vitamin B_{12} (one teaspoon three times a day) for three months, and comparing the vitamin B_{12} serum levels of these patients with those of 139 patients who had not received this treatment (Fig. 1).

The results of the preliminary study prompted us to conduct a second one on 60 healthy patients at a different home for the

‡ Gevral, Lederle Laboratories, Pearl River, N. Y.

aged. All of the patients chosen had vitamin B_{12} serum levels of less than 175 $\mu\mu$g per ml. They were randomly divided into two groups of 30 patients each. Group A received a daily capsule containing all known vitamins and minerals *except* vitamin B_{12}; Group B received a capsule containing 100 μg of vitamin B_{12}, in addition to the capsule received by Group A. Serum levels[7] were determined at frequent intervals for seven months. At the end of this period all but 16 patients (8 from Group A and 8 from Group B) were dropped from the experiment because they had either failed to follow the regimen faithfully, or because they were unwilling to submit to the laboratory procedures. At the beginning of the eighth month, four of the eight patients remaining in Group B were given 15 ml of the "elixir" with vitamin B_{12} once a day (equal to a daily vitamin B_{12} dose; 25 μg). The remaining four patients continued to receive 100 μg of vitamin B_{12} a day. The blood serum levels of all groups were compared (Fig. 2).

A third study was then conducted on another 40 elderly patients with vitamin B_{12}

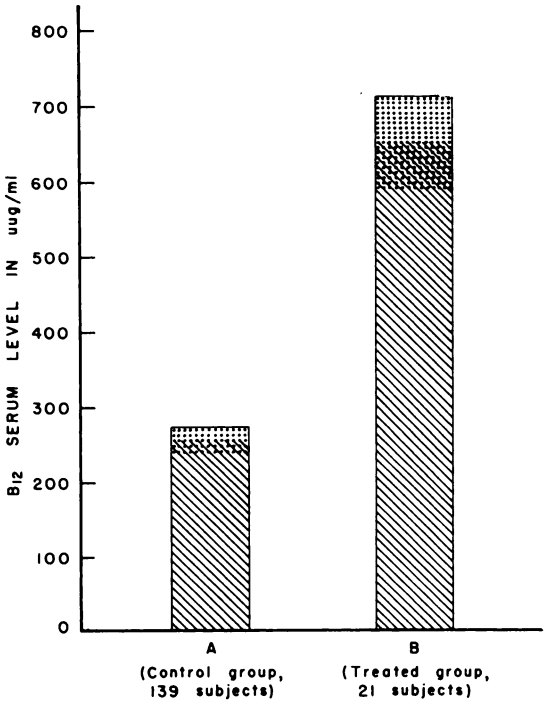

Fig. 1. Results of first serum level study. The cross-hatching in the columns denotes the magnitude of two standard deviations. The dividing line between the cross-hatching and the dotted portion immediately above it is the average value of the vitamin B_{12} serum level.

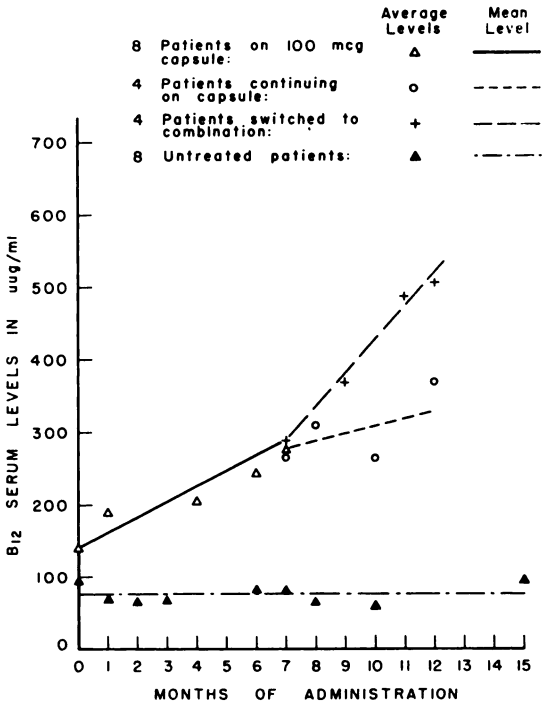

Fig. 2. Results of second serum level study.

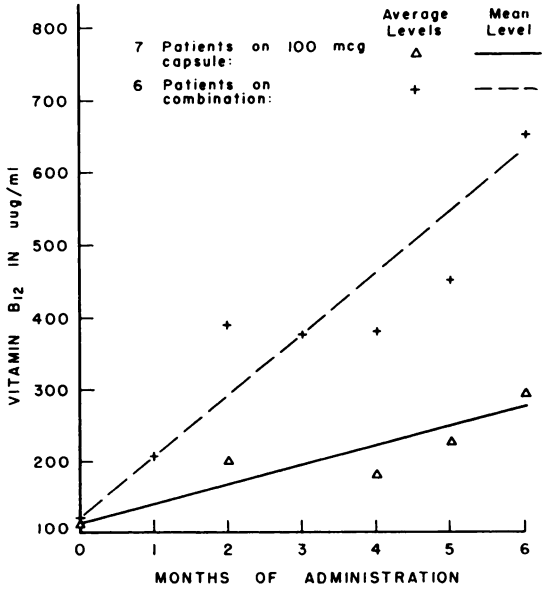

Fig. 3. Results of third serum level study.

serum levels of less than 175 $\mu\mu$g per ml. Once daily, 20 of them (Group A) received a capsule containing 100 μg of vitamin B_{12}. The other 20 (Group B) received 15 ml of the "elixir" with vitamin B_{12} (daily B_{12} dose; 25 μg). Blood specimens were taken at frequent intervals. After four months of treatment all but 13 patients (7 from Group A; 6 from Group B) had dropped from the experiment. A comparison of results in these 13 patients is given in Figure 3.

II. Urinary Excretion Studies

Method of Assay: The complete details of the urinary excretion test are published elsewhere.[8] Briefly, the method was as follows: 10 ml of an aqueous solution of 50 μg of radioactive vitamin B_{12} (specific activity, 14.4 μc/mg) were mixed with either 10 ml of water or 10 ml of the "elixir"; this "elixir" contained all the components of the "elixir" with vitamin B_{12} except vitamin B_{12}. Precisely two hours after taking one or the other solution, each patient received 1 ml of a vitamin B_{12} solution (1000 μg/ml) intramuscularly. A 24-hour urine specimen was collected. (At first, both 24- and 48-hour specimens were collected, but it was found that the 48-hour specimen yielded less than 5 per cent of the vitamin B_{12} excreted during the first 24 hours.)

Each specimen was measured by pouring one half of the urine into a beaker containing 50 μg of unlabeled vitamin B_{12} as carrier. After evaporation on a steam bath, the contents of the beaker were transferred into a bottle graduated to 50 ml and made up to volume. The bottle was then inserted into the well of a scintillation counter for radioactivity measurement.

Study Procedure: The study was conducted over an eight-month period on 60 young, healthy volunteers (ages 22–42) from a penal institution. Four separate trials were made in order to compare the urinary excretion of radioactivity in a number of patients who received radioactive vitamin B_{12} plus water, with an equal number who received radioactive vitamin B_{12} with the "elixir" (Table I).

In addition, 15 volunteers received both regimens (Table II); 7 of these (Group A) began on radioactive vitamin B_{12} plus water and were switched to radioactive vitamin B_{12} plus the "elixir"; 8 (Group B) began on radioactive vitamin B_{12} plus the "elixir" and were then switched to the radioactive vitamin plus water.

Urinary excretion tests were also performed in six subjects who had pernicious anemia in remission (Table III). They were given oral doses of 2 μg of radioactive vitamin B_{12} in

TABLE I

The Effect of Administration of an Elixir on the Urinary Excretion of Orally Administered Radioactive Vitamin B_{12} in Young, Healthy Adults

Study	Average radioactive vitamin B_{12} in 24-hr urine samples (mμg)		Statistical significance of the differences
	Controls (25)	Treated group (35)	
I	450 (7)	878 (6)	$P < 0.05$
II	470 (7)	951 (5)	$P < 0.05$
III	529 (7)	622 (6)	$P < 0.05$
		753 (6)	
IV	546 (4)	765 (6)	$P < 0.05$
		670 (6)	

( ) indicates number of subjects studied.

TABLE II

The Urinary Excretion Test on Radioactive Vitamin B_{12} with or without an Elixir by Switchback Experiment

Group	Therapy	Urinary excretion	Difference between (1) and (2)
		mμg in 24 hrs	
A (7)	1. 50 μg + water	706 ± 168	
	2. 50 μg + elixir	1680 ± 110	$P \ll 0.05$
B (8)	1. 50 μg + elixir	778 ± 49	
	2. 50 μg + water	575 ± 90	$P < 0.05$

( ) indicates number of subjects studied.

TABLE III

The Effect of an Elixir on Urinary Excretion of Orally Administered Radioactive Vitamin B_{12} in Patients with Pernicious Anemia in Remission

Subject	Treatment*	
	2 μg vitamin B_{12}	2 μg vitamin B_{12} +30 ml Elixir
A	0.80	1.4
B	0.60	3.0
C	—	0.97
D	0.92	4.1
E	0.8	2.6
F	—	0.9

* Urinary excretion expressed as per cent of the orally administered radioactive vitamin B_{12}.

30 ml of the "elixir," and then received 1.0 mg of unlabeled vitamin B_{12} two hours later by injection.

RESULTS

Serum Level Studies: The results of the first study (Fig. 1) show that the mean vitamin B_{12} serum level in the 21 patients receiving the "elixir" with vitamin B_{12} was 652 ± 61.7 $\mu\mu$g/ml. In 139 patients who did not receive the "elixir" with vitamin B_{12}, the mean serum level was 255 ± 15.8 $\mu\mu$g/ml. This difference is statistically significant.

It should be pointed out that several patients in the control group took the "elixir" with vitamin B_{12} at irregular intervals.* This fact, however, would only tend to increase the vitamin B_{12} serum level of the control group. Undoubtedly, then, the effect of the "elixir" on the vitamin B_{12} serum level was marked.

The results of the second study (Fig. 2) demonstrate that it required from six to eight months to replete tissue reserves by the daily oral administration of 100 μg of vitamin B_{12}. The mean serum level climbed from 100 to 250 $\mu\mu$g/ml. The four subjects (circles) who were continued on a 100-μg vitamin B_{12} capsule for two more months experienced only a slight elevation (from 250 to 369 $\mu\mu$g/ml). On the other hand, the four patients (crosses) who were switched from 100 μg of vitamin B_{12} alone to 25 μg of vitamin B_{12} in the "elixir" experienced a marked increase in serum levels (from 250 to 505 $\mu\mu$g/ml).

The results of the third experiment (Fig. 3) again demonstrated the superiority of the "elixir" with vitamin B_{12} (crosses) to vitamin B_{12} alone (triangles), as determined by serum levels. The average serum level of those on the "elixir" was 630; of those on vitamin B_{12} alone, 275 $\mu\mu$g/ml.

The results of four separate trials on 60 young volunteers (Table I) demonstrate that each subject receiving the "elixir" excreted significantly greater amounts of vitamin B_{12} than those who received vitamin B_{12} alone. Every patient who received both regimens excreted more vitamin B_{12} when taking the "elixir" (Table II). There was, however, considerable variation in the amounts excreted from experiment to experiment.

The results in patients with pernicious anemia (Table III) demonstrate that the "elixir" with vitamin B_{12} was of questionable value in increasing their vitamin B_{12} absorption. Of the 6 patients in remission, 3 had a partial response only; 3 had a negligible response. Of 3 patients in relapse, 2 responded, but the third did not. However, the latter patient had also failed to respond to any other oral therapy.

DISCUSSION

Vitamin B_{12} is essential for human nutrition[9,10] and for the treatment of certain diseases. Its use may be broadened as its importance in treating many metabolic disorders becomes better understood; therefore, it is of the utmost importance to find a means of assuring absorption when this vitamin is given orally. Although it is logical to assume that the oral coadministration of intrinsic factor preparations will increase the absorption of vitamin B_{12} by healthy individuals, data to support this hypothesis are inconsistent and meager. According to some reports[4,5,6] the use of certain intrinsic factor preparations is contraindicated in individuals without achylia gastrica. In our laboratory we have been interested in obtaining simple chemical substances capable of enhancing the absorption of vitamin B_{12}.

It is of particular interest to note that the "elixir" reported on in this paper, which possesses a marked ability to increase vitamin B_{12} absorption, is not derived or extracted from the usual animal sources of intrinsic factor. While differences in excretion were demonstrable in each test, the variation among the test subjects was great. This variation is primarily a reflection of our lack of precise knowledge of the mode of action (be it physical or chemical). It is conceivable that the ability of the combination to enhance vitamin B_{12} absorption may be due to its physical characteristics; to

* In an unknown number of instances, subjects in this control group requested this mixture; hence, the average value of the vitamin B_{12} serum level in the control group is higher than the mean value of serum obtained from other populations.

some chemical ingredients; or even to a chemical reaction which alters the chemical structure of vitamin B_{12} to a form[11] more easily absorbed than is cyanocobalamin.

Intrinsic factor is defined by Castle and associates[12] as a substance present in gastric juice which, when given with vitamin B_{12} by mouth, will produce a therapeutic response in pernicious anemia patients. Subsequent study by Schilling[3] showed that it will increase the absorption of vitamin B_{12} in such patients, according to the urinary excretion test. Thus, the "elixir" used in our study cannot be classified as an intrinsic factor-like substance unless it can regularly enhance absorption in patients with pernicious anemia. Because of its failure to regularly or markedly increase absorption in patients with pernicious anemia, it is questionable whether this combination itself can be considered to possess intrinsic factor activity. Whether the addition of other substances, such as those which bind vitamin B_{12}—a property believed by some to be inherent and essential to intrinsic factor activity—will result in a mixture with true intrinsic factor properties, remains to be answered by further experiments.

SUMMARY AND CONCLUSIONS

In order to evaluate the ability of an elixir containing vitamin B_{12}, other vitamins, and lipotropic substances to enhance the absorption of vitamin B_{12}, several studies were performed. They compared the serum vitamin B_{12} levels of subjects receiving the elixir with those receiving vitamin B_{12} alone. These studies involved 258 patients (189 old people, 60 young normal volunteers, and 9 patients with pernicious anemia). In the elderly people, vitamin B_{12} absorption was evaluated by assays of blood serum; in the volunteers and patients with pernicious anemia, by urinary excretion studies of radioactive vitamin B_{12}.

The results of every study on healthy subjects revealed that the elixir was capable of producing significantly higher serum vitamin B_{12} levels than could be obtained with larger doses of vitamin B_{12} alone. The elixir was of questionable value in treating patients with pernicious anemia.

It is concluded that this elixir is a singularly effective agent for treating vitamin B_{12} deficiency not caused by deficiency of intrinsic factor; that it enhances the absorption of B_{12}; and that the preparation cannot as yet be considered to have intrinsic factor properties.

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