Absorption of Vitamin B_{12} Enhanced by D-Sorbitol

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INTRINSIC factor concentrate has long been the only substance known to increase absorption of vitamin B_{12} from the gastrointestinal tract. In recent papers,[1-3] however, we described our findings that some substance or substances in an experimental lipotropic elixir enhanced vitamin B_{12} absorption in man and rats[2] and produced serum vitamin B_{12} levels significantly higher than could be expected from an oral preparation containing no intrinsic factor concentrates. This paper describes the series of studies by which it was established that vitamin B_{12} absorption is increased by D-sorbitol, a crystalline, hexahydric alcohol, $CH_2OH.(CHOH)_4CH_2OH$, used as a moisture stabilizer.

METHOD

The elixir under study contained, in each 5 cc:‡

Vitamin B_{12}, crystalline	8.34 μg
Riboflavin	0.6 mg
Pyridoxine hydrochloride	2.0 mg
Niacinamide	7.0 mg
Betaine, anhydrous	700.0 mg
Choline dihydrogen citrate	150.0 mg
Inositol	150.0 mg
Ferric pyrophosphate (soluble)	35.0 mg
Caffeine citrate	65.0 mg (1 gr)
Alcohol	15%
D-sorbitol	q.s.

In addition, the elixir contained coloring and flavoring. Since the elixir contained a great number of components, we had to consider that the enhancing effect might be due to the interaction of a number of chemicals with possible synergistic effects. We decided, however, to search first for one or two factors. If more than two factors appeared to be involved, the problem would be considered too complex for our present test methods.

The "factors" considered were themselves combinations of components, as follows: (a) alcohol and caffeine; (b) D-sorbitol; (c) vitamins-riboflavin, niacinamide, and pyridoxine hydrochloride; (d) betaine, choline dihydrogen citrate, and inositol. Other ingredients, color, flavor, and ferric pyrophosphate, were considered to be a part of the base and were not investigated separately but were included in all experimental preparations.

Since we were looking for no more than two factors, we studied all possible combinations of factors taken two at a time, plus a control and the full formula in an exploratory study. As shown in Table I, this required an experiment using eight different formulae.*

Absorption of vitamin B_{12} was measured in male volunteers among inmates of a state prison. The men were clinically healthy and free from acute infectious diseases or metabolic disturbances at the time of the study. All volunteers worked in the prison shops and obtained their food from the same kitchen. They had a well balanced diet with adequate nutritional sources of vitamin B_{12}. Their daily ration contained at least 0.18 kg of meat and an average of 0.8 of an egg and 100 ml of milk each day in addition to the amounts of eggs and milk used in making breads and desserts.

Vitamin B_{12} absorption was estimated using

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* This experimental design constitutes a $1/2$ replication of a 2^4 factorial design.[4]

the technic of Schilling.[5] In all tests,* isotopic dilutions of vitamin B_{12} were made so that the specific activity of the final dilution was equal to 50 μc/mg. Each volunteer was given a solution of radioactive vitamin B_{12} containing 50 μg of the vitamin in 10 ml of a test solution. The beaker containing the radioactive vitamin B_{12} was rinsed two times with 10 ml of the test solution and three times with 10 ml of distilled water. The total fluid intake for each man was thus equal to 60 ml. Two hours after the volunteer ingested the test solution, he was given an intramuscular dose of 1 mg of unlabeled vitamin B_{12} to "flush" out the radioactive vitamin B_{12}.

A 24-hour urine collection was then made with the precautions described by Chow *et al.*[6] During the first 12 hours in which urine was collected, the volunteers were kept in one room and were supervised carefully. They were permitted to sleep in their dormitory during the night, but in the morning the men were again required to stay in one room in order that the 24-hour urine collection could be completed under close supervision.

A new method of comparing several treatments was developed for this study because the common procedure is too often unwieldy and quite variable. Under ordinary procedures, individual doses are pipetted from bulk supplies of each of the test solutions. Men are treated in order of their arrival and all doses of one solution are administered before the next solution is given. Consequently, whenever a large number of men are studied at one evaluation, the last man receives his dose of test solution long after the first man has been treated. Since doses are given on an empty stomach, one may question whether observed differences in response are caused by differences in the solutions or by differences in the time of administration. In addition, so much time may be required to administer the solutions that it is difficult to control the timing of the two-hour intramuscular "flushing" injection of vitamin B_{12}.

The method used in this study greatly reduces these problems. Test solutions were prepackaged in individual 30 ml doses. Each of the different test solutions was given once, in random order, before any one solution was given a second time. This procedure was repeated as often as necessary. Each evaluation was thus balanced for time effects and all doses were measured exactly and without haste. Each bottle of solution was assigned a code number which designated the order in which it was to be given, and which enabled us to keep the study "double-blind."

The code number identification was carried through the laboratory work, thus providing a randomized plan for the whole investigation. In particular, single regimens were not treated as one group so that any variation in laboratory work would not be credited to differences between the regimens. The code was not broken until all laboratory work was recorded for each code number, and the results were tabulated by specific treatments. The final results were balanced for time effects and included only random laboratory variation and biologic variation. It was found that variability between subjects was materially reduced by using this method of pre-packaging test solutions.

Additional work showed that when this technic was used, we needed only eight men for each test solution to detect meaningful differences in response to treatment. It was also found that an optimum size for any one day's experiment was 24 to 32 men. This limited size of the experiment helped to control the timing for the flushing, intramuscular dose of vitamin B_{12}.

RESULTS

The results of this study are shown in Tables I–IV. From the first series of studies (Table I) it appeared that none of the combinations reached the level of absorption given by the full formula; however formulas which contained vitamins were closer to the results of the full formula than those without vitamins. It was therefore decided to investigate vitamin B_{12} absorption from solutions containing riboflavin, niacinamide and pyridoxine. Since formula 7 which contained D-sorbitol produced

* The radioactive vitamin B_{12} labeled with Co^{60} and Co^{58} used in the Schilling tests was obtained from Merck & Company.

TABLE I

Schilling Test. Exploratory Study

Formula no.	Mixture tested*	Number of subjects	Average vitamin B_{12} μg (in 24 hr)
1	Control (water alone)	9	545
2	Lipotropics, alcohol and caffeine	8	549
3	D-sorbitol, alcohol and caffeine	9	586
4	Lipotropics, D-sorbitol	6	431
5	Vitamins, alcohol and caffeine	7	759
6	Vitamins, lipotropics	8	735
7	Vitamins, D-sorbitol	7	833
8	Full formula	8	900

* Basic formula: color, flavor, ferric pyrophosphate.
Standard deviation: 250.
Significant difference between treatment averages: 250.

TABLE II

Schilling Test. Individual Vitamin Study

Mixture tested*	Number of subjects	Average vitamin B_{12} $m\mu g$ (in 24 hr)
Control (water)		565 ± 50 ($P_{.05}$)
Riboflavin, Niacinamide	7	758
Riboflavin, Pyridoxine	8	582
Niacinamide, Pyridoxine	7	712
Riboflavin, Niacinamide, Pyridoxine	8	731

* Basic formula: 40% D-sorbitol.
Standard deviation: 159.
Significant difference between treatment averages: 159.

vitamin B_{12} levels nearest to those of the full formula, D-sorbitol was used in the base of all of these solutions.

The results of this study (Table II) showed that none of the preparations containing combinations of the vitamins (riboflavin, niacinamide and pyridoxine) enhanced absorption as much as the full formula did. However, the solution without niacin yielded somewhat lower vitamin B_{12} levels than did the others, although all vitamin solutions tested were higher than the control (50μcg of vitamin B_{12} in aqueous solution). These results seemed to indicate that either niacin was the active component or the enhancing substance was present in all four solutions tested.

TABLE III

Urinary Excretion. Effect of Niacinamide

Mixture tested*	Number of subjects	Average vitamin B_{12} $m\mu g$ (in 24 hr)
Control	8	579
Niacinamide	8	611
Niacinamide and D-sorbitol	7	764
Full formula	8	806

* Standard deviation: 154.
Significant difference between treatment averages: 154.

TABLE IV

Urinary Excretion. Confirming Effects of D-Sorbitol

Mixture tested*	Number of subjects	Average vitamin B_{12} $m\mu g$ (in 24 hr)
Control	9	581
D-sorbitol	8	852

* Standard deviation: 201.
Significant difference between treatment averages: 195.

It was decided to test the effect of niacin first. The scheme of the study and the results are given in Table III. The results indicate that niacin without D-sorbitol was not superior to the control, but when D-sorbitol was present, vitamin B_{12} absorption was close to that of the full formula. This study led us to question our tentative assumption that the active agent was a vitamin and suggested that D-sorbitol may have been the substance which enhanced the absorption of vitamin B_{12} in the elixir.

A fourth study was conducted to test whether D-sorbitol was the active agent. To this end, nine subjects were given 50 μg of radioactive vitamin B_{12} in an aqueous solution; whereas, the second group of eight subjects received 50 μg of radioactive vitamin B_{12} mixed with D-sorbitol. The results of this study are given in Table IV. They clearly indicate that D-sorbitol can enhance the absorption of vitamin B_{12} from the gastrointestinal tract (this effect of D-sorbitol has been noted also in similar studies in rats).*

* Greenberg, S. *et al.*, Smith, Kline & French Laboratories. Personal communication.

DISCUSSION

In spite of the limitations of the Schilling urinary excretion test as a measure of the absorption of vitamin B_{12}, it provided a convenient means for comparing the activity of various test substances. The precision of the measurements in this study was increased by testing the substances on an unusually homogeneous group of subjects: prisoner volunteers who received the same food and who could be carefully controlled. Moreover, by pre-packaging and randomizing test solutions, individual responses within each group were made comparable. These procedures not only saved time, but avoided much of the confusion which ordinarily arises when a large group of individuals is involved in a volunteer test. Furthermore, by administering the test solutions within a short period of time, the differences in physiologic reactions such as secretion of gastric juice or psychologic factors which may influence the absorption were minimized.

TABLE V

Control Observations

Number of subjects	Average vitamin B_{12} $m\mu g$ (in 24 hr)
9	546
9	545
8	579
9	581
8	575
8	577
5	580
5	607

The advantages of this procedure are best indicated by the constancy of excretion of radioactivity in the urine of the control subjects who received the solution at different times over a period of more than one year. The results are presented in Table V. The constancy of our control values enables us to interpret with fair confidence the small differences in the urinary excretion among the groups.

At the present time, we are not certain how D-sorbitol aids the absorption of vitamin B_{12} from the gastrointestinal tract. The enhancing effect may be physiologic or it may be the result of a chemical reaction between vitamin B_{12} and D-sorbitol. It may even be caused by a contaminant in the D-sorbitol.

Regardless of the mechanism, the finding that a simple chemical substance, not derived from the usual animal sources of intrinsic factor, can increase vitamin B_{12} absorption is of academic interest and practical importance. The observation suggests a new line of research into the mechanisms of vitamin B_{12} absorption and deficiency disorders. It also suggests further study which may lead to a means for improving vitamin B_{12}-therapy for pregnant, convalescent, geriatric, and other patients who may have a mild state of vitamin B_{12} deficiencies.

SUMMARY AND CONCLUSIONS

A multiple-component lipotropic elixir which enhanced absorption of vitamin B_{12} was analyzed to determine which of its many ingredients caused the increased absorption.

In order to analyze the elixir, the usual methods of studying the effects of nutritional substances upon human volunteers had to be improved. A new method was developed which has a number of statistical advantages in addition to improving the precision and reducing the time required to conduct such studies.

Study results showed that D-sorbitol, a widely used moisture stabilizer, substantially enhanced the absorption of orally administered vitamin B_{12}. This is the first substance other than intrinsic factor concentrate shown to have the ability to increase vitamin B_{12} absorption from the gastrointestinal tract.

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