Enhancement of Serum Vitamin B_{12} by D-Sorbitol

WILLIAM P. BOGER, M.D.,* DAVID S. BRASHEAR, M.D.,† AND JOHN J. GAVIN, M.S.‡

IT HAS BEEN DEMONSTRATED in animals[1] and man[2] that D-sorbitol enhances the serum concentrations that result from the oral administration of vitamin B_{12}. In a previous publication[3] it was shown that patients with subnormal serum concentrations showed prompt elevations following the ingestion of a preparation containing D-sorbitol and vitamin B_{12}. It has been postulated that D-sorbitol forms a carbohydrate complex with the cyanocobalamin, resulting in the better absorption from the gut.[1] It has also been suggested that D-sorbitol serves as a nutrient for bacteria in the gastrointestinal tract with a resultant elaboration of increased quantities of vitamin B_{12} and, consequently, a greater absorption and elevation of the serum vitamin concentrations.[4] The study here reported was undertaken to test the hypothesis that the increases of serum value might have been due to the influence of the alcohol sugar, D-sorbitol, upon the gastrointestinal flora of man.

METHODS

A group of 30 patients was selected for study, all of whom had been institutionalized for a period of at least one year and whose weight had not fluctuated by more than five pounds during this period. All the patients studied were males and varied in age from 24 to 77 years; the body weights ranged between 108 and 183 pounds. The 30 patients were divided into three groups of 10 each, and the individuals served as their own controls. The groups ran in parallel and differed in treatment only during phase C of the study. Results were determined upon the basis of differences in the average serum vitamin B_{12} concentrations observed after the daily administration of the several preparations. The medication for all three groups was personally supervised by a member of the investigational team.

The pattern of study called for four phases of observation for each patient: (A) a pretreatment control period; (B) treatment with D-sorbitol alone; (C) administration of one of three preparations; and (D) a posttreatment control period.

During phase C, group I received 1 fluid ounce (20 g) of D-sorbitol administered in divided doses at 10 A.M. and 3 P.M. each day; group II received a similar dose of D-sorbitol, combined with a solution of crystalline cyanocobalamin, so that 50 μg were administered daily; and group III received 20 g of D-sorbitol per day and 50 μg of vitamin B_{12} in the form of a prepared dosage form, Vi-Sorbin.§

Blood samples were drawn from the antecubital vein at weekly intervals from all patients in the series. Blood was allowed to clot in the refrigerator overnight; the serum was then separated, pipetted into sterile vials, and held at -4°C until assayed. The *Lactobacillus leichmannii* method[5] was employed and all specimens were assayed at least twice. The specimens drawn each week were assayed as a group; then, as a check on the accuracy of method, the week-to-week results were corre-

From the Department of Research Therapeutics, Norristown State Hospital, Norristown, Pennsylvania.

* Director of Research, † Resident in Psychiatry, ‡ Fellow in Research, Norristown State Hospital.

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§ Vi-Sorbin is the trademark of Smith Kline & French Laboratories, Philadelphia, Pennsylvania, for a preparation containing crystalline vitamin B_{12} 25 μg, pyridoxine hydrochloride 6 mg, ferric pyrophosphate (soluble) 300 mg, folic acid 1.5 mg and D-sorbitol q.s., per 15 ml.

TABLE I

Plasma Concentrations ($\mu\mu$g) of Vitamin B_{12} Following Administration of Sorbitol

Patient	Wt (lb)	Age	Premedication control			$\mu\mu$g Vitamin B_{12} per ml serum							Postmedication control		
			1/30	2/6	2/13	2/20	2/27	3/6	3/13	4/3	4/10	4/17	4/24	5/1	5/15
N.C.	146	52	500	520	390	460	440	280	360	700	500	600	260	440	280
J.S.	125	68	290	260	370	180	220	160	200	300	250	200	100	220	100
C.M.	178	56	480	660	540	380	320	280	440	900	500	550	260	260	260
C.O.	169	69	120	200	200	260	160	N.S.	220	200	200	100	120	100	50
J.M.	132	73	20	160	100	120	100	124	100	100	100	120	60	60	130
E.G.	142	30	280	320	260	340	300	175	220	450	250	300	180	160	370
AVERAGE			278	353	310	290	257	204	257	442	300	328	163	207	198

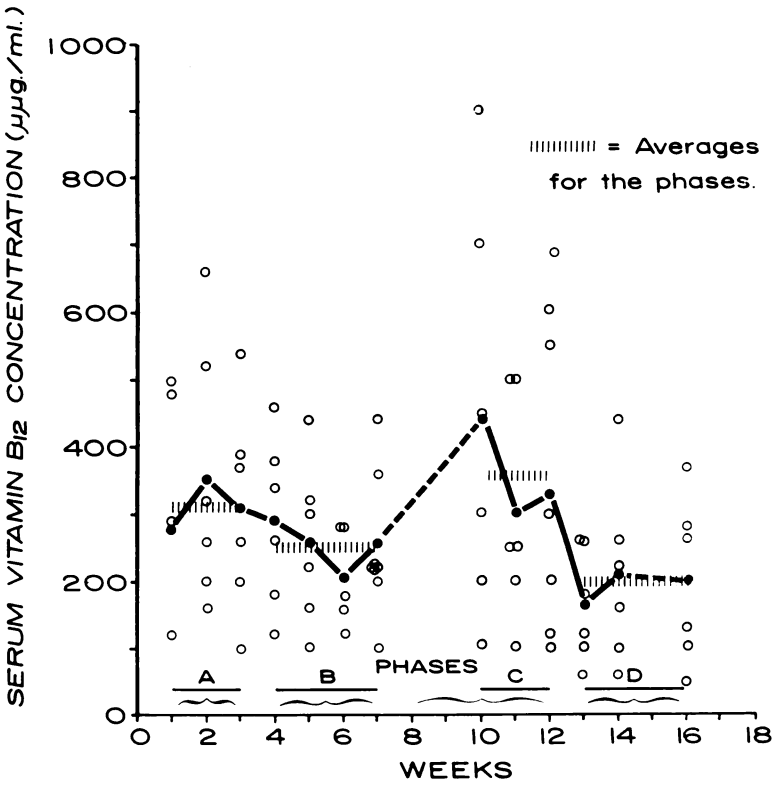

Fig. 1. Plasma concentrations ($\mu\mu$g) of vitamin B_{12} following administration of D-sorbitol alone (20 g/day).

lated by repeating a certain number of assays from the prior week. When the estimations failed to agree within plus or minus 20 per cent, the entire group of assays was repeated. Not only were weekly assays done, but upon the completion of the study all specimens from an individual patient in the series were assayed as a group on the same day. Thus every effort was made to control assay variability and to minimize its influence upon finding of difference between the treatments.

A "longitudinal" rather than a "cross-over" pattern of study was elected. This choice was dictated by previous observations that the serum vitamin B_{12} concentrations elevated by the administration of Vi-Sorbin were maintained long after discontinuance of the medication.[3] The patients in whom these observations were made had "subnormal" serum vitamin B_{12} concentrations and in this regard differed from the patients selected for the investigation reported here. Nevertheless, the

TABLE II

Plasma Concentrations ($\mu\mu$g) of Vitamin B_{12} Following Administration of Sorbitol Plus Crystalline Vitamin B_{12}

Patient	Wt (lb)	Age	Premedication control			$\mu\mu$g Vitamin B_{12} per ml serum: Sorbitol				Sorbitol plus B_{12}			Postmedication control		
			1/30	2/6	2/13	2/20	2/27	3/6	3/13	4/3	4/10	4/17	4/24	5/1	5/15
W.L.	183	43	300	420	520	310	280	200	460	700	450	750	450	280	N.S.
J.W.	118	59	460	200	200	100	180	100	220	500	300	400	680	320	120
W.S.	121	81	280	120	240	80	120	70	160	200	100	250	280	190	100
B.F.	125	24	220	240	200	110	140	120	220	550	350	500	180	160	150
P.Z.	113	77	520	380	860	300	460	500	J[a]	—	—	—	—	—	—
J.M.	122	53	240	480	640	380	340	280	360	650	450	650	240	240	140
AVERAGE			337	307	443	213	253	211	284	520	330	510	366	238	127

[a] Became jaundiced and was eliminated from the study.

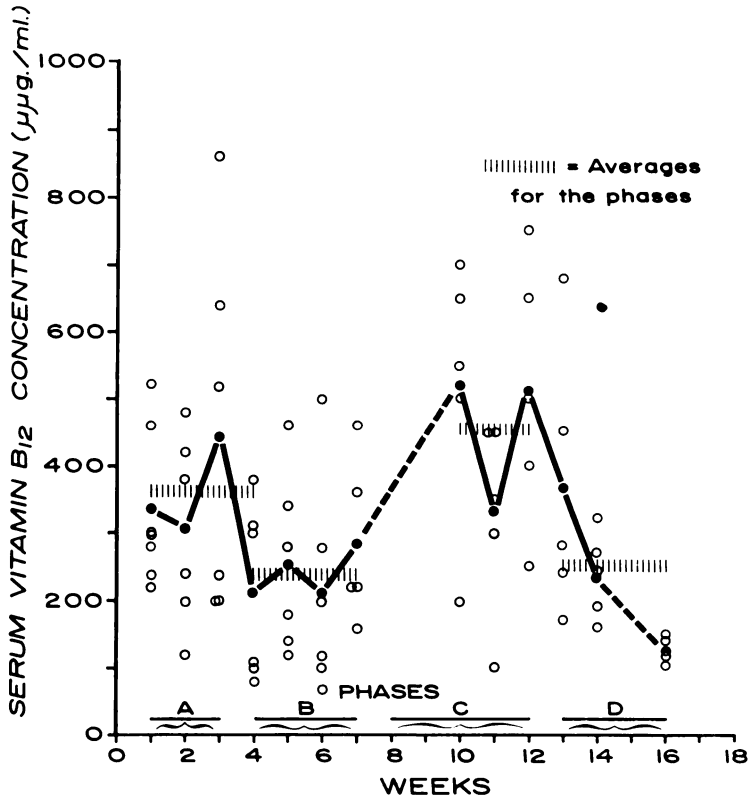

Fig. 2. Plasma concentrations of vitamin B_{12} ($\mu\mu$g) following administration of D-sorbitol (20 g/day) plus crystalline vitamin B_{12} (50 mg/day).

"longitudinal" pattern of study was selected.

RESULTS

The data are presented in Tables I, II, III, and Figures 1, 2, and 3. Of the original 30 patients selected for study only 18 could be included in the final tabulations as having fulfilled all of the criteria of full cooperation, complete blood sampling, and assay reproducibility.

DISCUSSION

In a previous study it was pointed out that for any particular individual, there appears to be a reasonably constant serum vitamin B_{12} concentration.[3,6] It has been postulated that

TABLE III

Plasma Concentrations ($\mu\mu$g) of Vitamin B_{12} Following Administration of Vi-Sorbin[a]

Patient	Wt (lb)	Age	Premedication control			$\mu\mu$g Vitamin B_{12} per ml serum: Sorbitol				Vi-Sorbin			Postmedication control		
			1/30	2/6	2/13	2/20	2/27	3/6	3/13	4/3	4/10	4/17	4/24	5/1	5/15
J.B.	140	48	500	300	640	180	360	180	360	750	650	700	260	320	200
T.R.	108	52	640	640	620	400	460	350	400	—	—	—	—	—	—
E.H.	142	75	300	260	320	140	200	180	220	750	300	650	240	160	110
J.M.	113	47	300	300	280	180	260	240	340	750	450	N.S.	280	280	380
R.U.	134	54	140	100	100	70	80	60	220	300	450	200	120	180	80
J.C.	155	28	440	320	320	280	200	180	240	650	250	360	240	480	200
AVERAGE			387	320	380	208	260	168	267	640	420	477	228	284	194

[a] Crystalline vitamin B_{12} 25 μg, pyridoxine hydrochloride 6 mg, ferric pyrophosphate (soluble) 300 mg, folic acid 1.5 mg, and D-sorbitol q.s., per 15 ml.

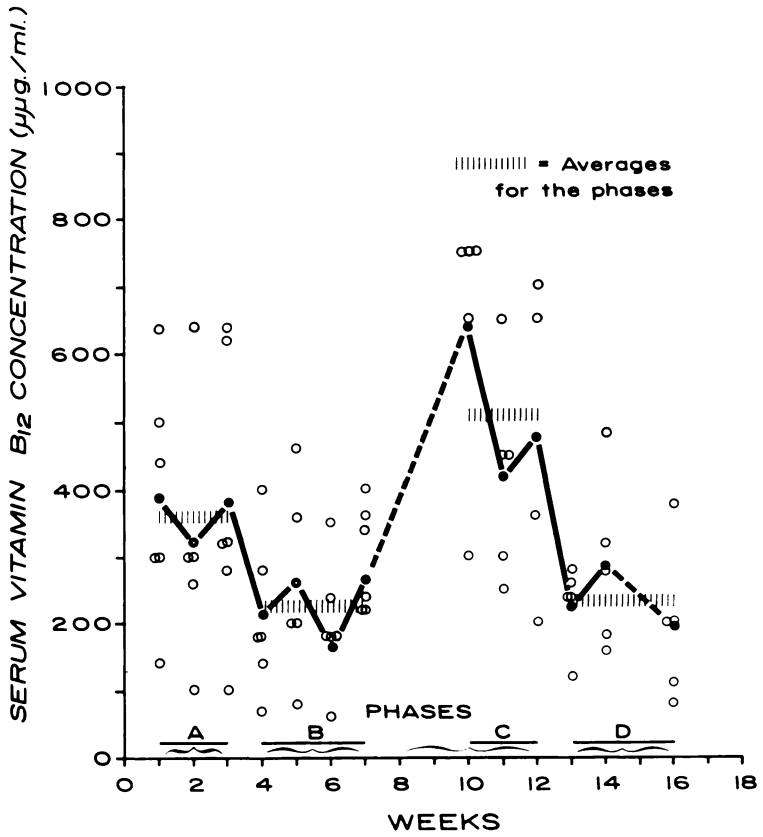

Fig. 3. Plasma concentrations ($\mu\mu$g) of vitamin B_{12} following administration of D-sorbitol (20 g/day) plus Vi-Sorbin (50 μg/day).

this relatively stable situation is a reflection of a balance between the bodily reservoirs of vitamin B_{12} and the ability of the patient to bind and transport the vitamin. In order to establish the "base-line" (phase A) against which the results of ingesting sorbitol alone or sorbitol plus vitamin B_{12} might be judged, three samples of blood were obtained from each patient and assayed for vitamin B_{12} content. In Tables I, II, and III it can be noted that a relative constancy tends to be confirmed, but there are some sharp discrepancies (J. M.,

Table I; J. B., Table II; P. Z. and J. H., Table III). Either day-to-day physiologic differences or the methodologic shortcoming inherent in a microbiologic assay can be invoked; the second alternative seems the more likely. With the exception of patients J. M. (see Table I) and R. U. (see Table III), all patients selected for this study had serum values for vitamin B_{12} that fell well within the previously defined normal range.[7] There was no explanation for the low values in these two patients. There were no evidences of hematologic, neurologic, or gastrointestinal dysfunction.

All patients in the series received sorbitol alone in the daily dose of 20 g for a period of four weeks (phase B). Powerful antibacterial substances produce alterations of gastrointestinal flora within a matter of days, but there was no anticipation that an alcohol-sugar such as D-sorbitol would produce either a prompt or profound effect. It was assumed, however, that a four-week period would be sufficient to test the hypothesis that D-sorbitol as a carbohydrate might alter the bacterial content of the gut.

Larger daily doses, based on body weight, of sorbitol have sometimes caused animals to show diarrhea and looseness of stools.[4] Similar observations have been made in man with 30 or 40 g daily doses of sorbitol. Soft stools were observed in three of our patients, but otherwise there was no indication of disturbed gastrointestinal function produced by the daily administration of sorbitol.

Tables I, II, and III show that neither the individual nor the average data of 18 patients suggest any elevation of serum vitamin B_{12} content during phase B. The serum concentrations seemed to be somewhat lower during the four-week period of sorbitol administration than in the prior control period.

During phase C, each group received a different preparation. The sorbitol group (see Table I) showed little change during an *additional* five weeks of treatment. Yet as compared with the previous four weeks on sorbitol the values tended to be higher. The group that received a supplementation of crystalline vitamin B_{12} along with sorbitol (see Table II) showed an elevation of serum vitamin B_{12} concentration above that which had been observed in the previous period on sorbitol alone (phase B) and above the original control level (phase A). The patients who received the special formulation, Vi-Sorbin, showed a greater rise of serum vitamin B_{12} concentrations (see Table III) above the control and D-sorbitol-alone periods than did either of the other two groups.

The differences between the treatments must be interpreted within the framework of the inherent error of the microbiologic assay for cyanocobalamin and the little-known factors of individual patient difference. Our assay technique has been repeatedly cross-checked with other laboratories by splitting samples and running a "blind" series of unknowns, and there is every assurance that the reliability of assay compares favorably with that reported in the literature. For this particular study, every precaution was taken to minimize assay variability as a factor in producing difference between treatments. Nevertheless, the over-all results clearly indicate that a 22 per cent variance in vitamin B_{12} assay existed. This figure exactly matches that which has been recently cited in critical reviews[9,10] as the limitation of the assay methods for cyanocobalamin.*

The assay variability as observed is regarded as unavoidable, and hence any difference between treatments must exceed this methodologic limit. Although some change in the average serum concentrations of group I (see Table I) were observed in the various phases of study, none of the changes exceeded the limits of assay variability and, accordingly, the findings of this investigation are interpreted as meaning that D-sorbitol alone produced no change in serum concentration of vitamin B_{12}.

The physiologic differences between individuals are extremely difficult to evaluate. An attempt was made in this study to select patients who had "normal" serum vitamin B_{12} values and this level was established in the pretreatment control (phase A). It can be noted that in the posttreatment control (phase

* It is worthy of emphasis that this large assay variability applies to *Euglena gracilis* as well as to *Lactobacillus leichmannii* methods.[10]

D) the values tend to be the same. In groups II and III the posttreatment levels represent a prompt decline from elevated values attained during the treatment periods with combinations of D-sorbitol and cyanocobalamin. This prompt return to pretreatment values in this study stands in contrast to the maintained elevations that were observed in our previous study of patients who had originally "subnormal" serum concentrations of vitamin B_{12}.[3]

In explanation of the difference noted between patients who had initially "normal" values and those who had "subnormal" serum vitamin B_{12} concentrations, it is suggested that reduced body reservoirs of cyanocobalamin can be replenished by the administration of a sorbitol-vitamin B_{12} combination (at least in part during a short-term study), and thereafter the serum concentrations will continue for a period of time at a more nearly normal level, even after discontinuance of medication. Such an explanation is compatible with observations that have been made of widely different rates at which serum vitamin B_{12} concentrations fall after various parenteral treatment schedules of patients with true vitamin B_{12} deficiency.

The present study, carried out in individuals who had initial serum concentrations that were declared "normal," showed that following discontinuance of the sorbitol-vitamin B_{12} preparations, the elevations of the vitamin were not maintained and the serum values returned promptly to pretreatment level. These findings parallel those made in young, healthy adults who had "normal" serum vitamin B_{12} concentrations.[8]

A low serum vitamin B_{12} concentration may reflect a modest deficit of body reservoirs which results from long-standing limited absorption of vitamin B_{12}, whether due to dietary intake of vitamin B_{12} and/or coupled with declining production of intrinsic factor of an aging gastric mucosa. In such patients, the administration of adequate parenteral vitamin B_{12} or the long-term administration of large amounts of oral vitamin B_{12} either alone or in combination with intrinsic factor or sorbitol might result in improvement of the body reservoirs and a maintenance of serum vitamin B_{12} concentrations for long periods after discontinuance of vitamin B_{12} supplementation. If one were to fully restore body depots to "normal" by any treatment, it would be anticipated that 3 to 5 years would be required for vitamin B_{12} levels to again decline to "subnormal" or "deficient" concentrations.

SUMMARY AND CONCLUSIONS

This study, undertaken to test the hypothesis that D-sorbitol might, by itself, exert a favoring effect upon serum vitamin B_{12} concentrations by an influence upon the flora of the gastrointestinal tract, has not shown such an effect. A group of 18 patients, serving as their own controls, were given 20 g of sorbitol per day for a period of four weeks; there was no observable influence upon the serum vitamin B_{12} concentration. Six patients received 20 g of sorbitol per day for an additional five weeks (total of nine weeks); no effect on vitamin B_{12} concentration was noted.

Five patients who were given daily 20 g doses of sorbitol plus 50 μg of crystalline vitamin B_{12} for a period of five weeks immediately following a four-week period during which sorbitol alone was administered showed a modest elevation of serum vitamin B_{12} concentrations.

Six patients who received treatment with Vi-Sorbin for five weeks showed even greater elevation of serum vitamin B_{12} concentrations. All 18 patients showed a prompt return to pretreatment levels of serum vitamin B_{12} concentrations when sorbitol vitamin B_{12} preparations were discontinued. This prompt return to pretreatment levels strengthens the interpretation that the changes observed during phase C of the study were due to treatment difference. It is emphasized that the microbiologic assay for vitamin B_{12}, in the best laboratories, has a variability of approximately 20 per cent.

One of the important features of the present study was the demonstration of the real difficulty of showing differences between treatments when serum vitamin B_{12} is used as a criterion. However, the findings of this study suggest that (a) sorbitol alone, in a daily dose

of 20 g, does not influence serum vitamin B_{12} concentrations; (b) D-sorbitol does appear to enhance the absorption of administered vitamin B_{12}; and (c) Vi-Sorbin seemed to elevate serum vitamin B_{12} concentrations to a slightly greater degree than did D-sorbitol supplemented with crystalline cyanocobalamin. The reason for and significance of this are not clear.

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