The Effect of Intrinsic Factor on the Absorption of Vitamin B_{12} in Older People

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VITAMIN B_{12} deficiency is known to exist in the absence of recognized clinical signs of primary pernicious anemia.[1] It has also been demonstrated that it may be associated with a lack of intrinsic factor. The simultaneous administration of vitamin B_{12} with intrinsic factor material has been advocated as a means of assuring absorption of vitamin B_{12} from the gastrointestinal tract, and of course has been used in the oral treatment of pernicious anemia.

This study was undertaken to determine (1) whether elderly people need added vitamin B_{12} in their diets and (2) the effect of exogenous intrinsic factor on the absorption of vitamin B_{12}. Two criteria were selected for estimating the absorption of this vitamin: urinary excretion measured by the Schilling test[2] and the plasma vitamin B_{12} level.[3,4]

METHODS AND MATERIALS

Choice of Subjects

Two hundred and one persons (76 females and 125 males), residents of the Marion County Home near Indianapolis, were selected for study. They had been admitted to this institution because of physical or mental illness or because they were unable to earn a living in the community. Their ages ranged from 37 to 92 years, the majority being over 50; the mean age was 69.3 years.

The diets at the Marion County Home were planned and prepared under the guidance of a trained dietitian. All subjects, prior to and during the test period, were offered a balanced diet daily.

Individuals were selected for absorption studies on the basis of the concentration of vitamin B_{12} in their plasma.[3,4] Of the total group of 201 people, 117 had plasma vitamin B_{12} levels of 0.5 mμg per ml or less and were used for further tests. Of these, 46 people had plasma vitamin B_{12} levels of 0.3 mμg per ml or below. Only 20 of the 46 subjects could cooperate enough to undergo tests for the estimation of intestinal absorption of vitamin $B_{12}Co^{60}$, with and without intrinsic factor, by the Schilling urinary excretion test.[2]

There were 71 people with plasma vitamin B_{12} levels of 0.3 to 0.5 mμg per ml and of these 40 were selected for studies in which plasma vitamin B_{12} levels were used as a criterion of absorption. They were divided into two groups with comparable individual and average plasma vitamin B_{12} values. One group of 20 then received oral vitamin B_{12} only and the other received intrinsic factor with vitamin B_{12}.

INTRINSIC FACTOR PREPARATION*

The intrinsic factor preparations used in these studies had been adequately standardized in patients with pernicious anemia in relapse and had been approved by the U.S.P. Antianemia Advisory Board. In the assay each patient received the intrinsic factor preparation with 15 μg of vitamin B_{12} orally for 36 consecutive days. All the anemic patients had an

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adequate reticulocyte response and obtained complete remission. A 165 mg dose of preparation A represented 75 per cent of the amount of intrinsic factor which when combined with 15 μg of vitamin B_{12} had been shown to equal one oral U.S.P. unit of activity. The 50 mg dose of preparation B used in the plasma study was the amount of intrinsic factor which when combined with 15 μg of vitamin B_{12} had been shown to equal one oral U.S.P. unit of activity.

PLASMA DETERMINATION OF VITAMIN B_{12}

Since the same results in the assay for vitamin B_{12} were obtained with plasma as with serum, the greater yield of plasma per unit of blood made its use preferable. The vitamin B_{12}-plasma values were determined from a 20 ml sample of heparinized blood. All samples were centrifuged within two hours after being drawn. The plasma was refrigerated overnight and analyses begun the following morning. All the specimens were analyzed by an individual who had no knowledge of the age or health of the patient from whom the specimen was obtained. Plasma vitamin B_{12} concentrations were estimated by a microbiologic technic using *L. leichmannii* according to the U.S.P. procedure.[5] A modification was developed whereby the plasma samples could be assayed without clarification. Each individual's plasma value was based on an average of six separate determinations. Crystalline vitamin B_{12} at specific concentrations was used as the standard and analyzed each time the unknowns were assayed.

URINARY EXCRETION OF VITAMIN B_{12}

The method described by Schilling[2] was used to determine absorption of vitamin B_{12}, first, without exogenous intrinsic factor, and then with 165 mg ($^3/_4$ U.S.P. unit) of intrinsic factor preparation A. The amount of vitamin B_{12}-Co^{60} given was 3.36 mg (equivalent to 0.15 μc of Co^{60}) for each test. The radioactive vitamin B_{12} was administered in a capsule, as was the intrinsic factor. The patients were hospitalized in a special metabolic ward where administration of material and collection of urine was done by graduate nurses trained in metabolic procedures. Great care was taken to hydrate each patient in order to insure a good 24-hour urine collection. Unless this was done, reproducible results could not always be obtained.

After collecting the urine for 24 hours, the total volume was recorded and a 600 ml sample was removed. The urine was stirred for five min with a mixture of 1 g of Hy-Flo Supercel and 3 g of Magnesol. This slurry was then transferred to a sintered glass funnel and filtered using vacuum. This procedure was repeated on each urine filtrate to insure the removal of all the radioactive vitamin B_{12} present. The bottom of the funnel had been removed, and the underside of the sintered plate ground smooth. The funnels were placed on a $1^3/_4$" x 2" solid thallium-activated, sodium iodide crystal of a Baird-Atomic scintillation spectrometer instrument, and the recovered radioactivity was determined over the entire Co^{60} spectrum. A sample from each lot of the vitamin $B_{12}Co^{60}$ solution was used as a standard. The standard was prepared by adding 0.1 microcurie of vitamin $B_{12}Co^{60}$ to 600 ml of water and the mixture was taken through the above procedure. The counts obtained from this standard were increased by a factor of 1.5 for calculation. This standardization was done in order to correct for difference in geometric configuration of samples counted in capsule and funnel. The results were expressed in terms of the percentage of the dose of vitamin $B_{12}Co^{60}$ recovered in the urine. Each Schilling assay was performed twice, the second test being done after three to eight weeks of elapsed time. Before each procedure urine was collected overnight to check for the absence of vitamin $B_{12}Co^{60}$.

Patients on whom Schilling assays were done were subjected to gastric analysis. A fasting sample was obtained and the subject was given 50 mg of Histalog* subcutaneously.[6] Samples were then collected at 20-minute intervals for

TABLE I

The Range and Average Level of Vitamin B_{12} in Plasma of 201 People According to Age in Increments of 10 Years

Ages (yrs)	Number of subjects	Vitamin B_{12} mμg per ml of plasma	
		Range	Average
31–40	2	0.35–0.94	0.65
41–50	8	0.28–0.93	0.54
51–60	39	0.23–1.60	0.61
61–70	57	0.08–0.98	0.48
71–80	54	<0.06–0.93	0.45
81–90	40	<0.01–1.25	0.439
91–100	1	0.36–0.36	0.36

* "Histalog" (Betazole, Lilly).

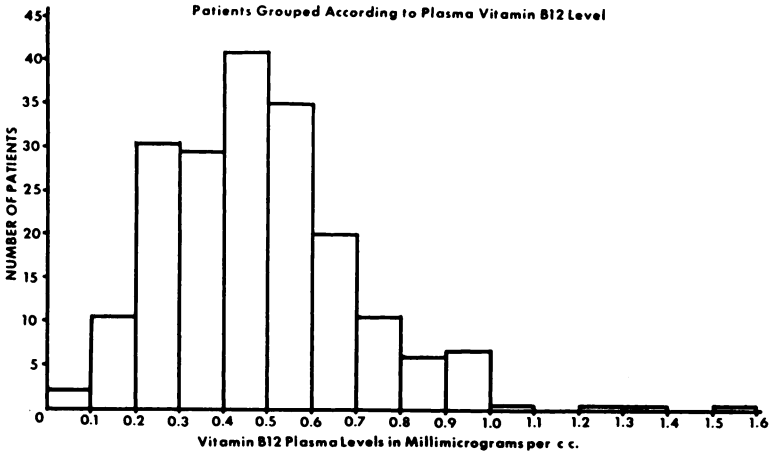

Fig. 1. Concentration of vitamin B_{12} in the plasma of 201 subjects.

one hour. The pH of the gastric juice was determined by means of a Beckman model H2 glass electrode pH meter. The patient was considered to have free acid in his gastric juice if any sample had a pH of less than 7.0.[7]

PLASMA VITAMIN B_{12} STUDY

Of the group of forty subjects with plasma vitamin B_{12} levels between 0.3 and 0.5 mμg per ml 18 individuals were given 5 μg of vitamin B_{12}, and 20 received 5 μg of vitamin B_{12} plus 25

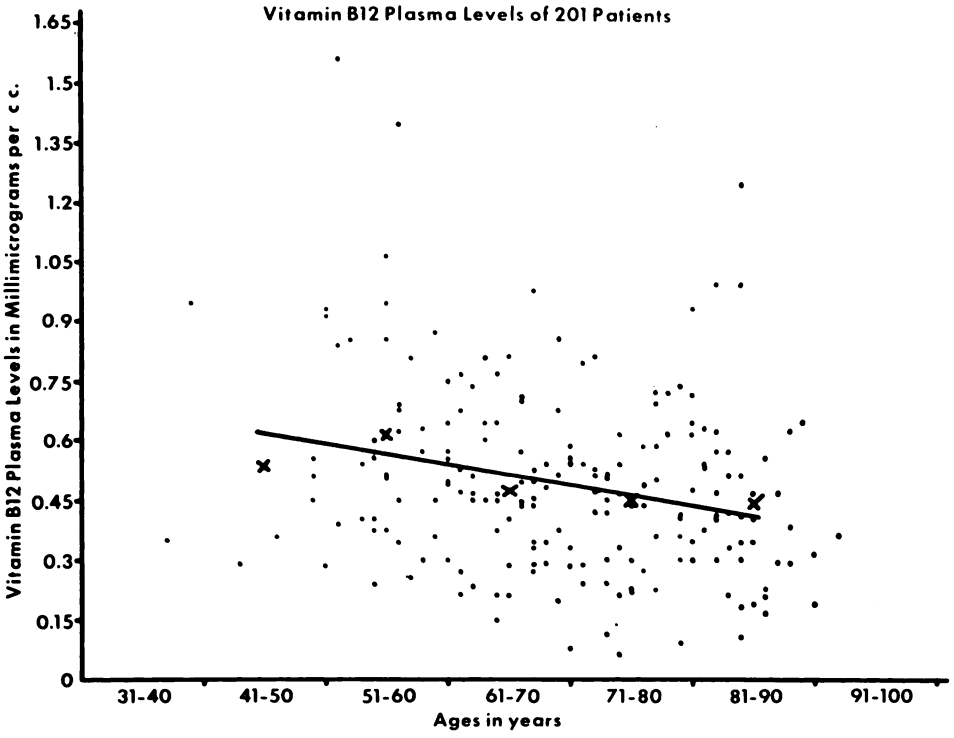

Fig. 2. Distribution of vitamin B_{12}, in mμg per ml, in the plasma of 201 subjects grouped according to age.

mg ($^1/_2$ U.S.P. unit) of intrinsic factor preparation B, twice daily for five months. Two subjects did not complete the study.

RESULTS

The plasma vitamin B_{12} level was determined on 201 subjects. Distribution of the plasma levels in relation to 0.1 mμg increments of plasma vitamins B_{12} is shown in Figure 1. The pattern was slightly skewed to the right. The general appearance of the curve, however, was compatible with the frequency distribution pattern of a random effect. The mean plasma vitamin B_{12} level in this series was 0.492 mμg per ml.

Figure 2 presents the distribution of plasma levels according to age. The range and mean plasma levels for age groups in 10 year intervals are shown in Table I. All subjects who had low plasma vitamin B_{12} concentrations (0.2 mμg per cc or less) were 64 years of age or older (Fig. 2). There were 45 patients with a plasma vitamin B_{12} level of 0.3 mμg per cc or less. Thirty-nine of these 45 subjects were 61 years of age or older. These data suggest a gradual decline in the plasma vitamin B_{12} levels with increasing age. This decline, calculated by the method of least squares, was 0.00521 mμg per ml of plasma per year of age. Statistical analysis indicates that the relationship shown above would be expected to occur by chance less than one time in 100.

The results obtained for the twenty subjects with plasma vitamin B_{12} levels of 0.3 mμg per ml or less that were studied by Schilling assay are shown in Table II. The first four subjects (G. B., J. G., E. S., and L. R.) had radioactive vitamin B_{12} uptakes in the same range as patients with pernicious anemia.[8] Subject E. S. suddenly became a markedly agitated paranoid-schizophrenic during the evaluation and on transfer to another institution was lost to the study. The other three men (G. B., J. G., and L. R.) with plasma vitamin B_{12} levels in the range usually seen in pernicious anemia, did not have the typical physical or hematologic findings characteristic of pernicious anemia. An examination by the staff neurologist, however, revealed abnormalities in sensory and motor function.

Case G. B. (Table II) was known to have had diabetes for 28 years. He had a loss of vi-

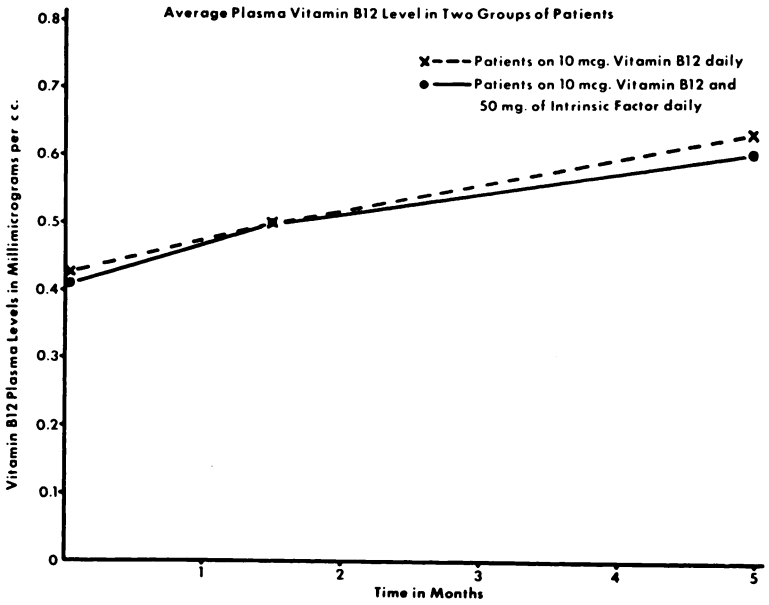

Fig. 3. Average vitamin B_{12} levels in the plasma of 18 people who received vitamin B_{12} alone and 20 subjects who received both vitamin B_{12} and intrinsic factor for five months.

bratory and position sense in his lower extremities. There was dysesthesia on the plantar surface of both feet. These findings were interpreted as peripheral neuritis rather than combined degeneration of the cord. No free hydrochloric acid was found in his gastric juice after Histalog stimulation. X-ray of the upper gastrointestinal tract revealed no abnormality. The hemoglobin was 88.6 per cent and the red blood cell count was 4.60 million per cu mm at the time the Schilling assay was performed.

J. G. (Table II)was known to have adenocarcinoma of the prostate and had been reported to have had a cerebral thrombosis two years previously. Neurologic examination revealed no evidence of motor or sensory changes that were typical of combined cord degeneration. No focal brain lesions could be demonstrated and the final diagnosis was "diffuse cerebrovascular disease and aging." The hemoglobin was 76.4%; red cell count was 4.60 million per cu mm. Roentgenogram of the stomach showed no abnormality. Acid phosphatase was 3 and alkaline phosphatase 10.1 King-Armstrong units.

L. R. (Table II) had been forced to give up his job and live in the Marion County Home because of difficulty in walking. A diagnosis of "multiple sclerosis" had been made in 1952, but examination by the neurologist during this study excluded this as a cause of present disability. At the time of the Schilling assay minimal evidence of cord involvement was observed. Changes in the left side were described as due to cerebral vascular disease. The red cell count was 4.68 million/cu mm; hemoblogin was 97.3 per cent. Gastric analysis after Histalog stimulation revealed no free acid in his gastric juice. X-ray of stomach showed no abnormality.

In the three subjects described above and

TABLE II

Effect of Exogenous Intrinsic Factor on the Urinary Excretion of Vitamin B_{12}

Patient	Age	Sex	Schilling assay: Vitamin B_{12} — Per cent B_{12} recovery	Schilling assay: Vitamin B_{12} and intrinsic factor — Per cent B_{12} recovery	Initial vitamin B_{12} plasma levels *mμg per ml*	pH of gastric juice
G. B.	74	M	0.46	8.95	<0.06	7.15
J. G.	84	M	0.88	3.20	<0.01	8.20
E. S.	54	M	1.30	7.38	0.23	Unknown
L. R.	70	M	1.36	7.29	0.08	7.90
J. S.	73	M	3.27	12.15	0.11	8.20
C. H.	64	M	4.04	4.60	0.20	Unknown
C. F.	77	M	4.32	5.96	0.22	1.00
D. D.	61	F	4.92	6.74	0.27	1.20
J. D.	62	M	5.08	8.80	0.24	7.20
L. T.	65	M	5.51	5.95	0.20	1.50
J. S.	76	M	6.36	6.90	0.27	5.80
E. L.	72	M	6.58	7.61	0.21	Unknown
J. R.	88	M	8.14	11.55	0.29	1.20
S. B.	71	F	8.57	7.72	0.23	1.80
M. W.	65	F	8.92	7.75	0.29	8.00
E. B.	83	F	9.05	8.10	0.20	1.30
E. W.	70	F	9.84	8.58	0.28	1.40
F. B.	67	M	9.90	12.28	0.27	1.05
C. M.	50	M	12.40	9.70	0.28	1.40
B. S.	72	M	14.50	14.40	0.29	1.40

Each subject received 3.36 μg of vitamin $B_{12}Co^{60}$ containing 0.15 μc of radioactivity. One dose of intrinsic factor contained 165 mg (75 per cent of 220 mg) of a standardized intrinsic factor preparation.

the fourth individual, E. S., a marked increase in absorption of vitamin B_{12} was accomplished by the simultaneous administration of intrinsic factor (Table II). A paired *t*-test applied to differences between the two treatments in these four people indicated that the differences were significant in that the results would not occur by chance 95% of the time.

In another subject (J. S.) (Table II) the per cent recovery of radioactive material from the urine was increased from 3.27 when vitamin B_{12} was given alone to 12.15 per cent when intrinsic factor was administered with the labeled cyanocobalamine. The concentration of vitamin B_{12} in the plasma of this patient was low, 0.11 mμg. He had no neurologic or hematologic changes. Gastric analysis after Histalog stimulation revealed no free acid in his gastric juice.

One individual (M. W.) (Table II) who had gastric juice of pH 8 exhibited no impairment of ability to absorb vitamin B_{12}, and the simultaneous administration of intrinsic factor did not produce a significant change in the percent of recovery of vitamin B_{12} in urine during Schilling assay in this subject.

Excluding the first four patients with extremely impaired absorption of vitamin B_{12}, the average urinary excretion of vitamin B_{12} was 8.67 per cent of the amount administered when given with intrinsic factor. In the same individuals the average vitamin B_{12} urinary excretion during Schilling assay when cyanocobalamine alone was given, was 7.59 per cent, Table II. Application of the paired *t*-test to individual patient differences showed that these results were not significantly different at the 5 per cent level. In short, of the 20 patients with low plasma levels (under 0.3 mμg/ml) four showed impaired absorption of vitamin B_{12} by the Schilling assay. Administration of intrinsic factor greatly increased the absorption of the vitamin in the four but not in the others.

The results, in the two groups of people, each with plasma vitamin B_{12} levels between 0.3 and 0.5 mμg per ml and who received vitamin B_{12} with and without intrinsic factor, orally, for five months are shown in Figure 3. Two people did not complete the study. Statistical analysis of the data indicates that the increase in plasma vitamin B_{12} for all 38 people at one and one-half months and five months was very highly significant at the probability level of 99.95 per cent. The difference in the rise of the plasma vitamin B_{12} between groups was not significant at the 95 per cent level of probability. In other words, in this group of subjects with moderately low plasma levels, intrinsic factor did not have any significant effect on the plasma level of vitamin B_{12} during supplementation over a prolonged period. However, oral vitamin B_{12}, with or without intrinsic factor, produced a statistically significant rise in plasma level.

DISCUSSION

These data indicate that at the age levels studied the plasma vitamin B_{12} concentration varies considerably from patient to patient. Similar variations have been observed by Boger and associates.[9] There is, however, a trend toward lower plasma concentrations of vitamin B_{12} with increasing age. This may mean that older people either eat a diet containing lesser amounts of vitamin B_{12}[10] or that they are unable to absorb adequate amounts of the ingested vitamin B_{12} because of a partial or complete lack of intrinsic factor. In Schilling assay studies Tauber and associates suggest the possibility of a decreased secretion of intrinsic factor.[11] Chow and associates have shown a lesser elevation of vitamin B_{12} blood level in older people than in young subjects following a standard oral dose of vitamin B_{12}. They concluded that older people cannot absorb vitamin B_{12} from the intestinal tract as well as the young. They also thought this was possibly due to lack of intrinsic factor.[12,13] Further evidence that old people have some decrease in the absorption of vitamin B_{12} has been obtained by Glass who states that, "The addition of a potent intrinsic factor preparation increased the hepatic uptake of radioactive vitamin B_{12} over 25 per cent in almost one-half of the cases in all age groups."[14,15]

In our studies those subjects who initially had a low vitamin B_{12} uptake (Table II) showed an increased absorption of radioactive vitamin B_{12} when intrinsic factor was given in addition to vitamin B_{12}.

In persons presumably having a defect in vi-

tamin B_{12} absorption associated with a lack of intrinsic factor, the concomitant use of a potent intrinsic factor preparation with vitamin B_{12} may avoid the development of neurologic changes that may occur in chronic vitamin B_{12} deficiencies.[16,17] This concept is particularly applicable to patients G. B., J. G., E. S., and L. R. They did not have the hematologic findings which ordinarily lead to a diagnosis of pernicious anemia.[8] Whether or not these neurologic changes are related to the vitamin B_{12} deficiency and could have been altered by the administration of vitamin B_{12} and intrinsic factor cannot be determined.

In the two groups of patients in which plasma vitamin B_{12} levels were used as an index of absorption, the parallel response obtained when vitamin B_{12} was given orally, with and without intrinsic factor, was expected. Both groups had vitamin B_{12} plasma levels in the low normal range at the beginning of the study and therefore presumably did not lack intrinsic factor. Similarly the subjects whose urinary excretion of vitamin $B_{12}Co^{60}$ was in the normal range were not expected to have a greater excretion of radioactive material when intrinsic factor was administered with the vitamin B_{12}.

The data also show definitely that the intrinsic factor preparations used in this study did not impair the absorption of vitamin B_{12}, after either acute or chronic administration.

SUMMARY

Determinations of the vitamin B_{12} level in the plasma were made on 201 persons between the ages of 37 and 92 years. A statistically significant decline in the plasma vitamin B_{12} level with age was found. This decline, calculated by the method of least squares, was 0.00521 mμg per ml of plasma per year of age.

Schilling assays were done on twenty elderly subjects. Administration of intrinsic factor increased the urinary excretion of radioactive vitamin B_{12} in those who had poor vitamin B_{12} absorption as well as low plasma levels. On the other hand, the subjects with a urinary excretion of vitamin $B_{12}Co^{60}$ in the normal range, but with low plasma levels, did not show a statistically significant change when intrinsic factor was given in addition to vitamin B_{12}.

Thirty-eight people with low normal plasma vitamin B_{12} levels (0.3 to 0.5 mμg per ml) were given vitamin B_{12} orally for five months. There was a statistically significant increase in plasma concentration of vitamin B_{12}. However, the increase in the amount of vitamin B_{12} in the plasma was just as great when intrinsic factor was given with vitamin B_{12} as when the subjects received vitamin B_{12} alone. No evidence for any inhibitory activity of the intrinsic preparations was found.

CONCLUSION

Low plasma levels of vitamin B_{12} were occasionally found among elderly people with no evidence of pernicious anemia. The oral administration of active intrinsic factor preparations enhanced the absorption of vitamin B_{12} in those subjects who showed impaired uptake by the Schilling test but had no significant effect if absorption was adequate. Prolonged administration of oral vitamin B_{12} raised plasma levels significantly.

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