The Absorption and Utilization of Vitamin B_{12}

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THE HEMOPOIETIC activity of orally administered vitamin B_{12} is enhanced by the simultaneous oral administration of intrinsic factor. The mechanism of this enhanced hemopoietic effect has not been proved, but enhanced absorption of the vitamin has been demonstrated beyond reasonable doubt to occur as one action of intrinsic factor. Another possible explanation of the action of intrinsic factor is the formation with vitamin B_{12} of a complex which is a more potent hemopoietic agent than vitamin B_{12} alone. Intrinsic factor might also retard bacterial utilization of intestinal vitamin B_{12} and thus allow the host to absorb more of the vitamin.

When it was established that vitamin B_{12} was an extrinsic (food) factor as well as an anti-pernicious anemia factor, a logical function to assign to intrinsic factor was the promotion of intestional absorption of the vitamin.[1] Heinle, Welch, Scharf, Meacham and Prusoff[2] demonstrated that intrinsic factor decreased fecal radioactivity in patients with pernicious anemia given radioactive vitamin B_{12} ($B_{12}Co^{60}$) by mouth. A reasonable conclusion was that intrinsic factor enhanced the absorption of the vitamin. Table I is a compilation of data from the literature in which the fecal radioactivity test was used to study pernicious anemia patients for their ability to absorb vitamin B_{12} from the gastrointestinal tract.

The liver has long been known to be a good source of the anti-pernicious anemia factor. The vitamin B_{12} normally in the liver must have been absorbed from the intestine. Glass and co-workers[3] demonstrated that the liver of normal persons who ingest radioactive vitamin B_{12} will contain radioactivity detectable by external scintillation counting, but the patient with pernicious anemia does not demonstrate such radioactivity unless intrinsic factor is given with the ingested radioactive vitamin. These workers have clearly demonstrated an inverse relationship between the oral dose of vitamin B_{12} and the percentage of the dose deposited in the liver, i.e. absorbed.[4] Swenseid and her colleagues[5] have demonstrated a similar limit of absorption of vitamin B_{12} as estimated by the fecal radioactivity technic.

TABLE I

Summary of Fecal Radioactivity Excretion Data in Pernicious Anemia

No. patients	Observations	Intrinsic factor	Per cent radioactivity excreted
34	50	—	72–100
23*	27	added	25–66

* Of the 34 patients above.

Normal subjects who take 1 or 2 μg. of radioactive vitamin B_{12} by mouth will excrete none of the radioactivity in the urine in the following 24 hours unless a large (1000 μg.) "flushing" dose of nonradioactive vitamin B_{12} is injected about the time of taking the oral labeled vitamin.[6] Using this "*in vivo* carrier" technique, 9 normal subjects excreted 7–21 per cent of the orally administered radioactivity in the 24-hour urine, whereas 23 patients with pernicious anemia excreted from 0–2.3 per cent of the radioactivity in the 24-hour urine (Table II).

It is to be noted that 26 subjects with histamine-fast achlorhydria but no other history or signs of pernicious anemia excreted from 3.2–29.6 per cent of the radioactivity in the urine. The radioactivity appearing in the

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TABLE II

Urinary Radioactivity after Oral Vitamin $B_{12}CO^{60}$

No. patients	Status	Gastric acid	Added intrinsic factor	Per cent Minimum	Per cent Maximum	Per cent Average
9	control	present	—	7.0	21.0	14.7
26	no P.A.*	achlorhydria	—	3.2	29.6	13.0
23	P.A.	—	—	0	2.3	0.6
19	P.A.	—	+	3.4	15.0	9.5
8	Total gastrectomy	—	—	0	1.0	0.2

* P.A. = pernicious anemia.

The figures represent the per cent of orally administered radioactivity which was excreted in the urine collected for 24 hours after ingestion of the radioactive vitamin.

urine in this test has been shown by MacLean and Bloch[7] to have a chromatographic mobility identical with vitamin B_{12}. They also report that 3 normal individuals excreted 20–29 per cent of the orally administered radioactivity in the urine within the first 24 hours, corresponding to 34–39 per cent of the absorbed dose.

Figure 1 is a scattergraph of urine volume versus urine radioactivity, and it is apparent

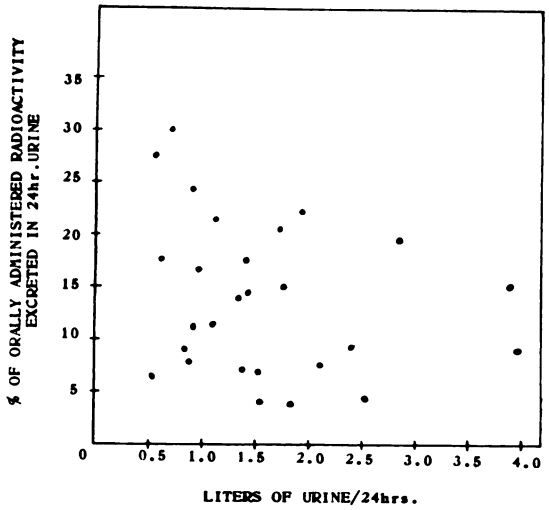

Fig. 1 Scattergraph of urine radioactivity versus urine volume in 27 subjects with histamine-fast achlorhydria but no other evidence of pernicious anemia. Details of technique in text.

that the two functions are not directly dependent.

Chow[8] has reported data suggesting that elderly subjects might absorb vitamin B_{12} less efficiently than young persons. Figure 2 is a scattergraph plotting age of subject against urine radioactivity after oral radioactive vitamin B_{12}. These data offer no support for the theory that older persons absorb vitamin B_{12} less efficiently than do younger subjects. The data in Figure 2 are evidence that most achlorhydric subjects without other signs of pernicious anemia function as do the control subjects in this test of vitamin B_{12} absorption and excretion.

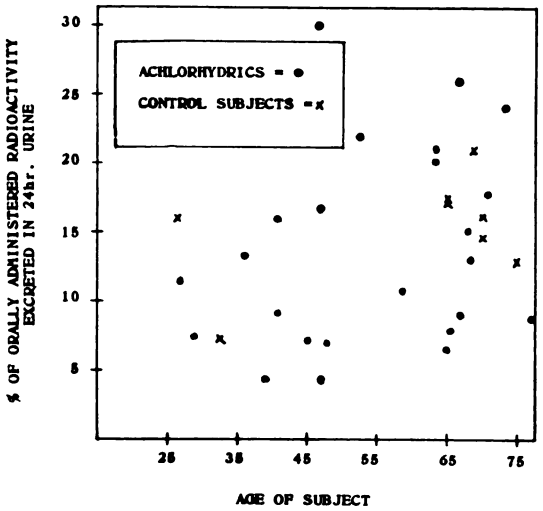

Fig. 2 Scattergraph of urine radioactivity versus age of patient. See text for technique.

As estimated by the urinary excretion of radioactivity, the absorption of vitamin B_{12} by a patient with pernicious anemia was not increased when the intestinal bacterial population was reduced by oxytetracyclene therapy.[6] Thus, as in Ungley's earlier study[9]

using hemopoiesis as an index of vitamin absorption, no evidence was found to suggest that intestinal bacteria were preventing vitamin B_{12} absorption. The observed hemopoietic effects of antibiotics in some megaloblastic anemias[10,11] might be due to increased bacterial production of folic acid or citrovorum factor. As would be anticipated from the earlier work of Castle,[12] Paulson, Conley, and Gladsden,[13] McDonald, Inglefinger, and Belding,[14] studies with radioactive vitamin B_{12} have demonstrated that totally gastrectomized patients are quantitatively similar to patients with pernicious anemia in their inability to absorb vitamin B_{12}[15,16] (Table II). Two of the patients in our series had had end-to-end esophago-duodenostomies, and their vitamin B_{12} absorption was as low as in those patients having an esophago-jejunostomy with a blind loop of duodenum. The data from observations on totally gastrectomized persons also indicate that the stomach is not necessary for vitamin B_{12} absorption if intrinsic factor is supplied. The small intestine may be considered the site of absorption of vitamin B_{12}.

The vitamin B_{12} content of the feces from a patient with pernicious anemia in relapse is more than adequate to treat him by injection.[17] Patients with pernicious anemia in relapse have been treated by intramuscular injections of vitamin B_{12} extracted from their own feces.[18] If intrinsic factor is given 12 hours before the oral vitamin B_{12}, the hemopoietic activity of the vitamin will not be increased.[1] From this one might conclude that the intrinsic factor is inactivated during this period, or, possibly, when the intrinsic factor has reached the colon, it can no longer effect the absorption of vitamin B_{12}. Evidence for the latter postulate is the observation by Best and colleagues,[19] who found that an enema of radioactive vitamin B_{12} and intrinsic factor did not lead to urinary excretion of radioactivity after a "flushing" injection of nonradioactive vitamin B_{12}.

The poor absorption of vitamin B_{12} observed frequently in sprue is not corrected by adding intrinsic factor.[3]

If massive oral doses of vitamin B_{12} (1000–5000 μg./day) are given to patients with pernicious anemia in relapse, excellent hemopoietic responses will be observed in the absence of added intrinsic factor.[9] Nasal instillation and aerosol inhalation of vitamin B_{12} have been reported as effective modes of therapy for pernicious anemia.[20,21] Intramuscular injections of vitamin B_{12} at three- to four-week intervals in amounts equal to 1 μg./day have been found to be satisfactory for maintenance therapy.[22]

Latner and his colleagues[23] have reported the "isolation of the intrinsic factor." At the oral dose level of 1 mg., their material decreased the fecal radioactivity in patients with pernicious anemia given radioactive vitamin B_{12}. By electrophoretic and ultracentrifugal study this preparation was considered to be pure. In unpublished observations by the author a hog mucosal concentrate* was found to be active at 1 and 2 mg. levels in the urinary radioactivity assay for intrinsic factor.

Several chemical forms of vitamin B_{12} in addition to cyano-cobalamin are known.[24,25] Hydroxo-cobalamin[25,26] and nitro-cobalamin[25] (vitamin B_{12c}) possess anti-pernicious anemia activity when injected. Hydroxo-cobalamin will also serve as an extrinsic factor;[26] hence any postulated chemical bond between extrinsic and intrinsic factors cannot be dependent upon the specific cyanocobalamin configuration. Pseudo-vitamin B_{12} contains an adenine moiety instead of the 5,6-dimethyl benzimidazole of the usual cobalamins, and it is reported to lack anti-pernicious anemia activity.[27]

Vitamin B_{12} which is absorbed has a slow turnover rate. The prolonged remissions[28] which sometimes persist in patients with pernicious anemia after therapy with B_{12} is discontinued suggest this. Direct observations of hepatic radioactivity after therapy with radioactive vitamin B_{12} show that much of the activity remains in the liver for more than one year. That excessive metabolic activity may increase the need for vitamin B_{12} is suggested by the fact that there are in the literature at least 75 instances of the co-

* Supplied to the author by W. F. White of Armour Laboratories.

existence of hyperthyroidism and pernicious anemia.[29] The author feels that this figure is higher than would be anticipated from chance alone: statistical proof, however is not available. The hypermetabolic rat has an increased vitamin B_{12} requirement.[30]

The classical human example of vitamin B_{12} deficiency is that caused by an unexplained lack of intrinsic factor activity, pernicious anemia. The parenteral injection of vitamin B_{12} will completely correct the hematologic defects of this disease, and the neurologic defects will be halted or reversed. The sore tongue will disappear. Lajtha[31] and Thompson[32] have reported independently that vitamin B_{12} added *in vitro* to cultures of marrows from pernicious anemia patients did not cause a maturation of the megaloblasts. Folic acid, however, did cause such a maturation *in vitro*. When vitamin B_{12} was added with intrinsic factor, maturation occurred. Horrigan, Jarrold, and Vilter[33] demonstrated that local *in vivo* instillation of vitamin B_{12} into one iliac marrow caused local maturation of that marrow in 24 hours, but not of the marrow in the opposite ilium of the patient with pernicious anemia. Wallerstein and colleagues[34] found no consistent evidence that intravenous B_{12} and gastric juice was a more effective hemopoietic combination than intravenous vitamin B_{12} alone.

The fact that the cells from the vaginal and gastric mucosa in pernicious anemia in relapse are macrocytic and often multinucleated[35] is evidence that vitamin B_{12} is required for normal development of cells from several systems in addition to the hemopoietic and neurologic.

Summary

The absorption of vitamin B_{12} in the small intestine from the dietary intake requires an adequate supply of intrinsic factor. In man, the only apparent gastrointestinal source of intrinsic factor is the gastric mucosa. Less than 1 μg./day is the normal requirement of absorbed vitamin B_{12}. The total daily dietary requirement is not known. There is evidence that the biologic rate of decay of this vitamin is relatively slow.

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DISCUSSION

DR. J. R. KREVANS (Baltimore, Md.): I'd like to make just a few very brief comments about Dr. Schilling's excellent paper, and ask some questions.

We got very much the same results that Dr. Schilling did with respect to age, using the technique of feeding a patient a certain amount of labeled vitamin B_{12} and recovering radioactivity in the feces. We feel that this adds strength to the argument. At least, by these two techniques, age does not seem to be an important factor in the percentage of B_{12} absorbed, and I think our method of study helps to strengthen that argument because it bypasses the question of whether or not the elderly individual's kidneys are working as well as the young adult's.

Second, we were able to confirm the observations about the inability of terramycin to improve the absorption of vitamin B_{12} in patients with pernicious anemia.

We had an opportunity to study an indivi-

dual who had a vitamin B_{12} deficiency on another basis, multiple small intestinal diverticulae, and who had neurologic changes. Now, this individual's B_{12} absorption was very definitely improved by the prior administration of terramycin, suggesting that at least in this situation bacterial competition played an important role.

This observation is confirmed by a very interesting case report by Scandinavian workers, who reported a patient with multiple intestinal strictures and fistula who had pernicious anemia, and who had a clinical and hematological response to the administration of terramycin rather than vitamin B_{12}. I think it is worth pointing out that not all patients with sprue have an absorption defect for vitamin B_{12}, and I think both Dr. Glass and our group have found some individuals with a full-blown picture of sprue who are able to absorb vitamin B_{12} perfectly normally.

Finally, we have been able to follow some 50 patients now, with pernicious anemia, who have been treated with orally administered vitamin B_{12}. Ten of these patients have been started and maintained on orally administered B_{12}, and some 20 patients have been started on oral B_{12} but not maintained on this therapy. Others have been maintained on oral B_{12}, although they had been started on liver extract or parenteral vitamin B_{12}. The longest any of these patients has been maintained on oral B_{12} has been since the winter of 1950. The patients received initially five milligrams of vitamin B_{12} orally, without any gastric juice or intrinsic factor, and are maintained on one milligram per week. To date, we have had no clinical or hematological relapses on this schedule.

I would like to ask Dr. Mueller this question: Do those patients with pernicious anemia who were maintained on folic acid for long periods of time, and who eventually relapsed, show a picture of hypoplasia in the bone marrow, and no megaloblastic changes? I'd like to hear this discussed further. It is so convenient to think of the megaloblastic picture as part of the same general B_{12} deficiency state that causes immature cells to be found in the gastric mucosa, and I wonder why these individuals do not have megaloblastic marrows when all their B_{12} has been consumed?

Dr. J. F. Mueller (Cincinnati, Ohio): The observation has been repeatedly noted that a hypocellular nonmegaloblastic bone marrow is present in patients who are treated for months or years with folic acid. We interpret this finding as showing a severe B_{12} deficiency, certainly with no folic acid deficiency. It fits in well with the published reports of experiments with swine. In these animals pure dietary B_{12} deficiency did not produce megaloblastic changes in the bone marrow, whereas folic acid deficiency did.

Dr. D. L. Horrigan (Cincinnati, Ohio): Certainly Dr. Will's data on the different ratios in pernicious anemia marrows are very interesting. I wonder how much of that might be due to simple immaturity of the marrow. And I wonder if he has any data on an iron deficiency marrow or one that is hyperplastic due to acute hemolytic anemia, to show that the immaturity itself is not responsible for these changes.

Dr. J. J. Will (Cincinnati, Ohio): Yes, we feel this is not evidence of mere immaturity, but is a specific change that took place in the megaloblastic type cells.

Dr. B. Connor Johnson (Urbana, Ill.): As many of you know, we have been working with vitamin B_{12} deficiency, using the baby pig as experimental animal, for the past five years. B_{12}-deficient baby pigs do not show a megaloblastic anemia, even though they do die of the deficiency, usually within two to four weeks.

A severe choline deficiency can also readily be produced in the baby pig. As in the rat, this choline requirement can be replaced by dietary methionine. In order to study the interrelationship between this choline requirement and vitamin B_{12}, a series of *in vivo* and *in vitro* experiments have been carried out. The first group of experiments were designed to find out whether vitamin B_{12} is involved in direct transmethylation reactions. *In vivo*

it was found that baby pigs on a B_{12}-deficient, choline-free diet, containing sufficient methionine to provide both sulfur amino acid and methyl requirements of the animal, were able to transmethylate from this methionine to make choline for prevention of fatty livers just as well as were B_{12}-adequate animals. There was no evidence of choline deficiency even in animals dying of B_{12} deficiency in this experiment. This fact, that B_{12} is not involved in direct transmethylation from methionine to form choline, was confirmed in *in vitro* studies with liver homogenates of B_{12}-deficient as compared to normal pigs and chickens. Differences in direct transmethylating ability were found in rats, on the other hand, and may be related to apoenzyme formation, or to some other effect, I am not sure. I would be interested in hearing Dr. Williams' comments on this.

Since we didn't find vitamin B_{12} involved in transmethylation, we of course examined methyl synthesis, following the line of approach used by Dr. Stekol and Dr. Arnstein. In *in vivo* experiments we fed baby pigs glycine as a possible methyl precursor. On choline-free diets containing only enough methionine to satisfy the animals' needs for sulfur amino acids, the B_{12}-deficient pigs on these diets all showed a severe choline deficiency; however, with vitamin B_{12} added, there were no fatty livers and no evidence of choline deficiency, indicating, therefore, by a straight nutrition experiment, that B_{12} is required for methyl synthesis from, in this case, glycine; that is, in the presence of vitamin B_{12} glycine completely replaced choline in the diet. This was substantiated by tracer experiments in these pigs in which the C^{14} of α-labeled glycine was found to be incorporated into the methyl groups of choline in the presence of B_{12}, while only to about a tenth as much in the absence of vitamin B_{12}.

Dr. J. N. Williams (Madison, Wis.): These experiments actually seem to point up exactly what I was trying to bring out this morning: that there are many reactions in some way associated with folic acid and vitamin B_{12}, but the *exact* steps at which the vitamins are involved cannot be stated with any certainty. An easy explanation is that in the case of the pig one is dealing with a different type of enzyme than in the rat. However, I wouldn't like to make that statement without equivocation. I am able to think of an example of an enzyme that is different in its mechanics of action in one animal as compared to another. That is xanthine oxidase in the rat as compared to xanthine dehydrogenase of the chick. That is one possibility, but beyond that I have no other explanation.