A DUAL MECHANISM OF VITAMIN B_{12} PLASMA ABSORPTION [1]

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Recent reports have described two types of plasma absorption curves following the oral administration of vitamin B_{12}. With microbiologic assay methods, after massive doses of vitamin B_{12}, early significant plasma levels have been found indiscriminately in both pernicious anemia patients and control subjects (1, 2). On the other hand, two independent laboratories, using radio-labeled cyanocobalamin, have successfully determined plasma absorption curves after the oral administration of only 0.46 to 1.0 microgram doses (3, 4). With these small amounts of vitamin B_{12} a distinctly different type of absorption curve was found in normal subjects and in pernicious anemia patients when intrinsic factor was added. This curve was characterized by little or no plasma radioactivity during the first four hours of the test and a peak concentration in the 8- to 12-hour interval. Furthermore, there was clear differentiation between control subjects and patients with pernicious anemia, because at these dosage levels the latter without added intrinsic factor showed insignificant plasma radioactivity.

In this investigation plasma absorption curves were obtained in pernicious anemia patients with and without intrinsic factor, as well as in control subjects after the oral administration of test doses of radio-labeled cyanocobalamin which ranged from 0.56 to 500 micrograms. This included a dosage range not previously examined.

MATERIAL AND METHODS

Cobalt^{58}-labeled vitamin B_{12} [2] used in test doses had an initial specific activity of 1,137 microcuries per mg., while that of the Co^{60}-labeled vitamin B_{12} [2] used was 893 microcuries per mg. Crystalline non-radioactive vitamin B_{12} was added as necessary to make up the desired test dosages. In each case the test dose was administered in approximately 100 ml. of water after an overnight fast; breakfast was withheld for two hours. When used, intrinsic factor concentrate (IFC) [3] was added in 100- or 200-mg. amounts to the solution of cyanocobalamin. This material had been found active in pernicious anemia patients when tested by the methods of Heinle, Welch, Scharf, Meacham, and Prusoff (5) and that of Schilling (6). Blood was withdrawn at different time intervals after the test. Radioactivity measurements [4] were made as detailed elsewhere (3). Briefly, a 20-ml. well-type scintillation counter was used with an automatic vial sample-changer and a recording timer. The overall efficiency of this counter was 18.4 per cent for Co^{58} and 24.0 per cent for Co^{60}. A pre-set count of 32,000 was used for each determination in this study. At this count, three times the standard deviation of the background was five counts per minute (cpm). Generally, in each study a pre-test plasma sample was used for the background count. Altogether, eight tests were carried out in eight control subjects and 12 tests in eight patients with pernicious anemia in remission. The diagnosis in each of the latter had been substantiated by absorption tests with and without the addition of IFC.

External monitoring over the liver area was carried out one to two weeks after the test dosages according to the method of Glass, Boyd, Gellin, and Stephanson (7) with the use of a NaI (thallium-activated) probe scintillation counter. The crystal, 1½ inches in diameter and 1 inch in thickness, was mounted on an RCA 5819 photomultiplier tube and shielded by ¾ inch of lead. Ten-minute counts were registered on a Tracerlab Superscaler, model SC 18A.

The amounts of vitamin B_{12} absorbed into the plasma were calculated from the radioactivity measurements with the following formula: micromicrograms of vitamin B_{12} per ml. of plasma equal pr/20 mk; where p is the oral dose of vitamin B_{12} in micromicrograms; r is the experimental net cpm in 20 ml. of plasma; m is the amount of radioactivity in the test dose in microcuries; k is the overall efficiency of the counter times disintegrations per minute per microcurie (2.22×10^6). This calculation was based upon the assumption that the radioactivity represented

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[2] Kindly supplied by Dr. N. S. Ritter, Merck and Co., Rahway, N. J.

[3] Kindly supplied by Dr. R. W. Heinle, The Upjohn Company, Kalamazoo, Michigan.

[4] Facilities of the Radioisotope Unit of the Minneapolis Veterans Administration Hospital were used for radioactivity measurements.

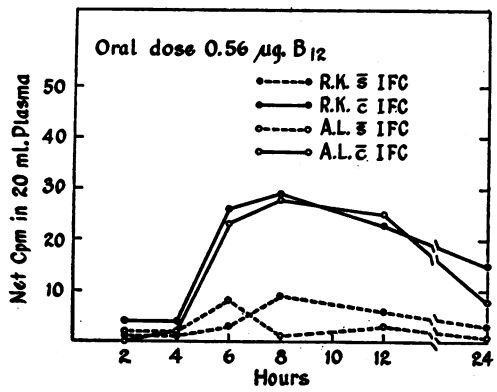

FIG. 1. RADIOACTIVITY OF PLASMA IN TWO PATIENTS WITH PERNICIOUS ANEMIA IN REMISSION AFTER THE ORAL ADMINISTRATION OF 0.56 MICROGRAM VITAMIN B_{12} CONTAINING 0.5 MICROCURIE Co^{60} WITH AND WITHOUT INTRINSIC FACTOR CONCENTRATE (IFC)

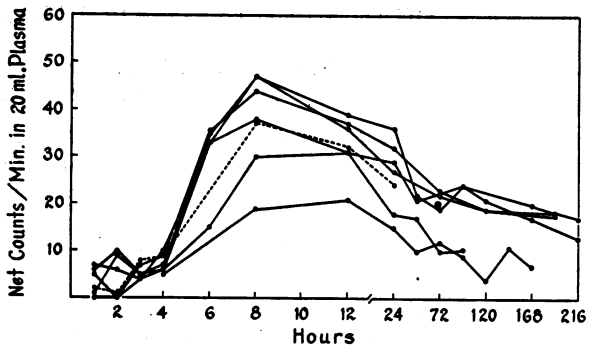

FIG. 2. RADIOACTIVITY OF PLASMA IN SIX CONTROL SUBJECTS (SOLID LINES, TEST DOSE 0.46 MICROGRAM VITAMIN B_{12}, 0.5 MICROCURIE Co^{60}) AND IN ONE PATIENT WITH LAENNEC'S CIRRHOSIS AFTER A PORTACAVAL SHUNT OPERATION (BROKEN LINE, TEST DOSE 0.56 MICROGRAM VITAMIN B_{12}, 0.5 MICROCURIE Co^{60})

cyanocobalamin and not a split-off cobalt salt. This was a reasonable presumption because the radioactivity is stored in the same way after oral and parenteral administration of Co^{60}-labeled vitamin B_{12}, but in a completely different manner from that observed after parenterally injected $Co^{60}Cl_2$ (8).

In a few instances the large oral doses of vitamin B_{12} contained radioactivity in amounts above those calculated to be safe (Co^{58}, 8 microcuries; Co^{60}, 3 microcuries) if entirely absorbed. This was justifiable because only a small fraction of these test doses was expected to be absorbed (9). Moreover, the highest liver radioactivity found in any of the patients in this study was within the general range reported by others after Co^{60} (7, 10).

RESULTS

Two male patients with pernicious anemia, aged 61 and 64, were given oral test doses of 0.56 microgram (0.5 microcurie Co^{60}), first without and later with the addition of 100 mg. IFC. Four weeks elapsed between the repeated tests. Figure 1 shows that there was but little radioactivity in the plasma unless intrinsic factor was given with the test dose. When IFC was added, absorption curves were obtained similar to those found in control subjects given test doses of like amount (3) (Figure 2); negligible radioactivity appeared in the plasma during the first four hours of the test, and peak concentrations in the plasma were not attained until eight hours following dosage.

Differing absorption curves were observed in five of six pernicious anemia patients given from 50- to 300-microgram doses of radio-labeled vitamin B_{12} without IFC. The results are summarized in Table I and are shown graphically in Figure 3. One patient with the 50-microgram test dose had little plasma radioactivity, but each of the other five had relatively high values by three hours with peak concentrations usually in four to six hours.

Two of these patients were retested with the same doses of vitamin, but with the addition of 200 mg. IFC. The early rise in plasma radioactivity was abolished in each case (Figure 4). Instead there was a gradual rise in radioactivity to peak values at 12 hours. While there was inhibition of that phase of absorption not mediated by intrinsic factor, radioactivity measurements over the livers

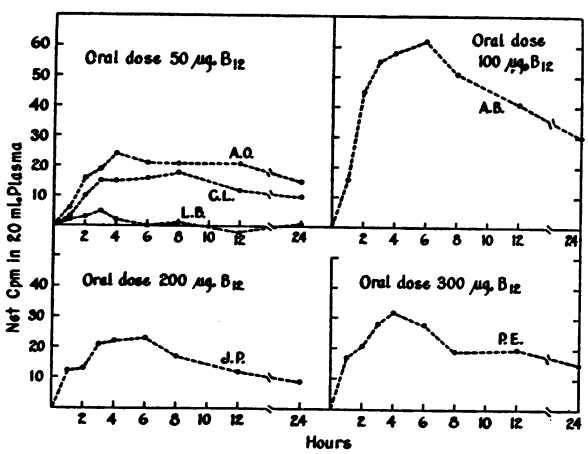

FIG. 3. RADIOACTIVITY OF PLASMA IN SIX PATIENTS WITH PERNICIOUS ANEMIA AFTER THE ORAL ADMINISTRATION OF 50 TO 300 MICROGRAMS OF RADIOACTIVE VITAMIN B_{12}

TABLE I

Radioactivity (cpm) in 20 milliliters of plasma at different time intervals after the oral administration of various test doses of Co^{58} vitamin B_{12}

Name*	Age	Diagnosis	Vit. B_{12} μg.	Radio-activity μc.	Cpm at different hours after oral test dose 1	2	3	4	5	6	8	10	12	24	36	Max. calculated amount labeled vitamin B_{12} μμg./ml. plasma	Radio-activity over the liver net cpm
H. M.	23	No disease	10	5.0	8	8	8	13		38	66		56		31	16	
G. B.	21	No disease	50	8.0	7	9	11	11	15	25	34	32	29	23		26	1,125
R. R.	40	Psychoneurosis	50	8.0	3	7	6	9	15	23	24			16		18	
E. J.	25	Appendicitis	100	8.0	1	2	4	9	12	14	16		14	11		24	
E. E.	59	Ess. hypertension	100	8.0	13	9	17	20		20	34		23	21		51	
A. H.	58	Emphysema	200	16.0	11	15	19	26		27	36		33	26		54	1,123
M. F.	44	Duodenal ulcer	300	16.0	14	21	20	17	20	22	25		27	21		61	
G. G.	40	Br. asthma, diabetes	500	16.0	7	15	19	18	19	22	21		21	19		83	
A. O.†	60	Pernicious anemia	50	8.0	6	16	19	24		21	21		21	15		18	670
L. B.†	63	Pernicious anemia	50	8.0	2	3	5	2		0	1		−2	1		4	118
C. L.†	64	Pernicious anemia	50	8.0	3	10	15	15		16	18		12	10		14	254
C. L.†‡	64	Pernicious anemia	50	8.0	2	3	4	6		8	14		21	14		16	721
A. B.	68	Pernicious anemia	100	9.8	16	45	55	58		62	51		41	30		76	760
A. B.‡	68	Pernicious anemia	100	8.9	5	4	6	10		15	20		22	11		30	946
J. P.	45	Pernicious anemia	200	16.0	12	13	21	22		23	17		12	9		35	170
P. E.	66	Pernicious anemia	300	16.0	17	21	28	32		28	19		20	15		72	370

* All were male patients.
† Approximately 4.0 microcuries CO^{60} B_{12} included.
‡ Two hundred mg. intrinsic factor concentrate added.

after IFC showed higher counts in each instance (Table I).

Figure 5 depicts the absorption curves in eight control subjects given 10- to 500-microgram test doses of radio-labeled cyanocobalamin. Three kinds of curves were obtained: the familiar delayed type; a new, somewhat biphasic, plateau-shaped configuration; and curves intermediate between these two types. None of the curves were the same as that found with comparable dosages of vitamin without addition of IFC in patients with pernicious anemia. The early, proportionately large amount of radioactivity in the plasma found in pernicious anemia was not obvious until the 200- to 300-microgram dosage. Then, in contrast to the con-

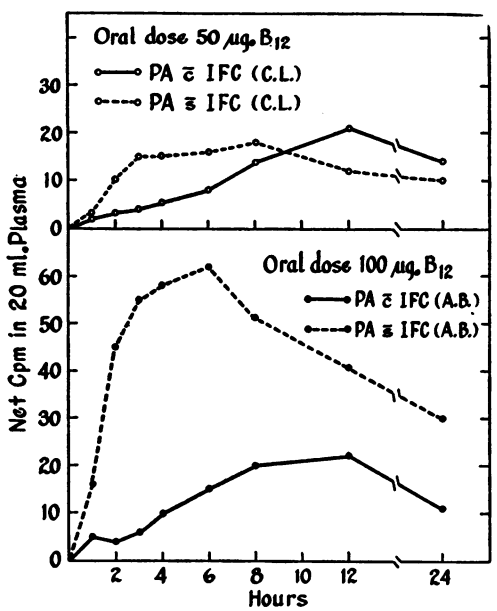

FIG. 4. COMPARISON OF THE PLASMA RADIOACTIVITY IN TWO PATIENTS WITH PERNICIOUS ANEMIA AFTER THE ORAL ADMINISTRATION OF 50 AND 100 MICROGRAMS OF RADIOACTIVE VITAMIN B_{12} WITH AND WITHOUT INTRINSIC FACTOR CONCENTRATE (IFC)

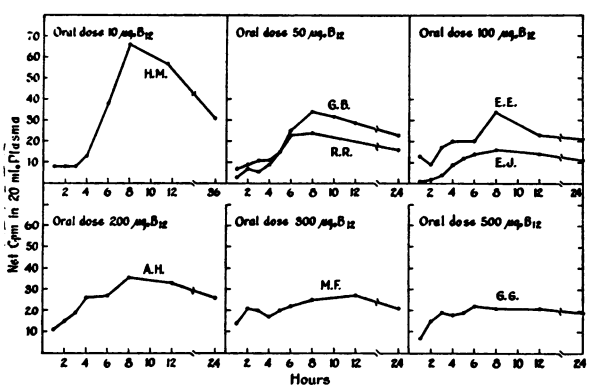

FIG. 5. RADIOACTIVITY OF PLASMA IN EIGHT CONTROL SUBJECTS AFTER THE ORAL ADMINISTRATION OF 10 TO 500 MICROGRAMS OF VITAMIN B_{12} CONTAINING 5.0 TO 16.0 MICROCURIES OF Co^{58}

tinued fall-away after the early peak in the pernicious anemia patients, a second phase was observed in the control subjects, in which the plasma radioactivity increased and persisted for many hours. At dosage levels of 50 to 100 micrograms intermediate types of curves were found.

Table I includes the maximum amounts of vitamin B_{12} absorbed into the plasma. Sixteen micromicrograms per ml. of plasma were found when the 10-microgram test dose was given to a control subject. In general, with increasing size of test doses larger amounts of cyanocobalamin were found, 83 micromicrograms per ml. being observed in the subject who received the 500-microgram test dose. This same tendency was seen in the six pernicious anemia patients to whom IFC was not given. However, the individual results showed wide variation.

The results of measurements of radioactivity over the liver one to two weeks after dosage are recorded in Table I. Consistently higher values were obtained in normal subjects and in patients with pernicious anemia when IFC accompanied the test dose, than in patients with pernicious anemia without IFC. This was true even when higher peak plasma radioactivity had been found in the latter. The effect of intrinsic factor can be illustrated by comparison of ratios of liver radioactivity to the peak radioactivity of 20 ml. of plasma. The average ratio was 16 in six patients with pernicious anemia without the addition of IFC to the test dose (range, 7 to 28). On the other hand, the ratio was raised to 34 and 43 in two pernicious anemia patients retested with added IFC, and was 31 and 33, respectively, in two normal subjects.

DISCUSSION

These studies show that patients with pernicious anemia absorb significant amounts of radio-labeled cyanocobalamin in the dosage range of 50 to 300 micrograms of the vitamin. The resulting plasma absorption curves were unique with rapid rises in plasma radioactivity to peaks usually in four to six hours. The peaks were followed by relatively more rapid declines of radioactivity than in control subjects given comparable dosages. For example, in the pernicious anemia patients the average plasma concentration after 24 hours was 45.4 per cent of the peak plasma level, compared to 71.6 per cent in control subjects. The difference is statistically significant, $t = 3.9$, $p < 0.01$.

Of particular interest was the finding that IFC abolished this early type of absorption curve and gave rise instead to gradual increases of radioactivity in the plasma with delayed peaks (Figure 4) such as found with smaller test doses with intrinsic factor present. Although excessive amounts of intrinsic factor can inhibit the absorption of small test doses (*e.g.*, 0.5 microgram) of vitamin B_{12} (11, 12), inhibition of the absorption of larger test doses has rarely been found (13). The retarded absorption presently observed might be due to interference with absorption by some nonspecific material in the IFC (11). However, the intrinsic factor may have been responsible, since the resulting absorption curves were similar to those observed in control subjects given comparable amounts of cyanocobalamin. Moreover, the addition of IFC resulted in each instance in higher hepatic radioactivity. This suggests that more, rather than less, cyanocobalamin was absorbed in spite of the change in the contour of the absorption curves.

Another possibility must be considered to explain the delayed rise of radioactivity in peripheral blood samples in the presence of intrinsic factor. With intrinsic factor there could be early removal of the absorbed vitamin B_{12} by peripheral tissues or organs such as the liver. Simultaneous radioactivity measurements of blood samples from the portal vein would be most helpful to place the cause of the slower rise in plasma radioactivity. Such studies are not available. However, Booth and Mollin (4) found maximum radioactivity in the plasma 8 to 12 hours after dosage while the highest counts over the liver were not obtained until two to six days later. On the other hand, we have obtained a plasma absorption curve in one patient with an effective portacaval shunt. The subject, a 46-year-old man with Laennec's cirrhosis, was studied 17 months after operation. As seen in Figure 2, there was a delay in the peak of radioactivity in the plasma identical to that found in normal subjects. These observations make it unlikely that the liver immediately removes cyanocobalamin from the blood under the influence of intrinsic factor and thereby causes the delay in the rise of radioactivity in the plasma. Moreover, we are not aware of any evidence which indicates

that peripheral tissues selectively remove and temporarily store newly absorbed vitamin B_{12} after oral administration. Therefore, it seems more likely that the intestine is in some way responsible for the slower rise in radioactivity in the plasma in the presence of intrinsic factor. This view is in agreement with that suggested by Booth and Mollin (4).

Tests in control subjects showed that the inhibition of the early rise of radioactivity in the plasma could be overcome by larger doses of vitamin B_{12}. Examination of Figure 5 shows that in one of two control subjects given 100 micrograms and in the subjects tested with 200 micrograms there was considerable plasma radioactivity by four hours, as compared with the results at the 10- and 50-microgram levels. With 300- and 500-microgram doses relatively high plasma radioactivity was found at two and three hours. Then, in contrast to the results obtained in patients with pernicious anemia, the peak was followed by a plateau-like curve. This prolongation was probably due to the superimposition of absorption mediated by intrinsic factor.

The results of these studies lend support to the hypothesis that there are two modes of intestinal absorption of cyanocobalamin: one, mediated by intrinsic factor, is characterized by more gradual and prolonged plasma absorption curves; the other, found with much larger doses of cyanocobalamin, is characterized by early rises in plasma levels of vitamin B_{12}, as indicated by measurements of radioactivity. It has been suggested that this latter mechanism is due to a passive diffusion across the intestinal barrier (9). However, in pernicious anemia the four- to six-hour peak amounts of plasma radioactivity (Figure 3) appeared later than would be expected from a process of simple diffusion. The particular curves can be compared to the plasma absorption curves of iron which are believed to be related to an *active* process of absorption. Oral administration of iron results in peak plasma concentrations two-and-one-half to five hours after test doses (14). Until more details are known it is perhaps best to refer to the two mechanisms of absorption of vitamin B_{12} as the one mediated by intrinsic factor, and the other as absorption independent of this factor.

Recently, it has been estimated that 100 grams of undried beef liver may contain from 60 to 118 micrograms of vitamin B_{12} (15, 16). Figure 3 includes plasma absorption curves with dosages in this general range. Such absorption suggests the possibility that the success of the dietary liver therapy of Minot and Murphy (17) was due to the cyanocobalamin content itself, and not necessarily due to other factors present in the liver which may have promoted hematopoiesis or vitamin B_{12} absorption.

Clinical experience has indicated a wide variation in results in the treatment of pernicious anemia patients with oral doses of cyanocobalamin. Whereas some may show an almost optimal response to daily doses of 15 micrograms (18) and some can be successfully treated with daily oral doses of 50 micrograms (19), failures have been reported with daily doses of as much as 250 micrograms (20–22). This variation is well illustrated in Figure 3 at the 50-microgram dose. Two patients had significant plasma absorption curves and hepatic radioactivity (Table I), while a third patient showed little absorption and only one-half to one-sixth the liver radioactivity of the other two. These findings indicate great individual variation in the absorption gradient in patients with pernicious anemia for doses of vitamin B_{12} above the physiologic range. Such a variation in the absorption gradient is well known also for test doses of a more physiologic magnitude, when a source of intrinsic factor is added (23).

The plasma absorption curves determined by bioassay of vitamin B_{12} after the administration of massive doses of the vitamin have shown peak plasma concentrations 1 to 24 hours after the administration of the tests, and no definite separation between patients with pernicious anemia and control subjects has been claimed (1, 2). This is in contrast to the definitive results obtained when test doses of 50 to 300 micrograms were given. However, with the larger test doses there was less difference between the absorption curves of pernicious anemia patients and normal subjects. This was probably due to the fact that as the test doses were increased, the absorption mechanism independent of intrinsic factor gradually became the more dominant also in the control subjects.

The calculated amount of vitamin B_{12} absorbed was found to range from about 18 micromicrograms per ml. of plasma in a control subject given the 50-microgram test dose to 83 micromicrograms

per ml. of plasma when 500 micrograms were administered. The figures were not too different in the patients with pernicious anemia, except in the patient who absorbed only a minor amount when the 50-microgram test dose was given. Our data are too few to make a more detailed comparison between the amounts calculated to have been absorbed in each group.

Because such small amounts of cyanocobalamin were absorbed it is understandable that bioassay has failed to reveal definite changes in the concentration of vitamin B_{12} in the serum after single oral test doses of less than 500 micrograms (9, 18). Tracer techniques, being more sensitive, have the further advantage that they can determine absorption without measurable changes in the total amount of vitamin B_{12} in the serum.

SUMMARY AND CONCLUSIONS

1. Studies of the rate of entrance of vitamin B_{12} into the plasma have been carried out in eight pernicious anemia patients and eight control subjects after the oral administration of test doses varying from 0.56 to 500 micrograms of Co^{58}- or Co^{60}-labeled vitamin B_{12}.

2. Negligible or no plasma absorption was observed in two patients with pernicious anemia when oral test doses of 0.56 microgram of vitamin B_{12} were administered without intrinsic factor concentrate. When the latter was administered, absorption curves were obtained which were similar to those previously found in control subjects given test doses in the same range. They were characterized by delayed plasma radioactivity appearing after four hours, with a peak concentration at eight hours following dosage.

3. Oral test doses of 50 to 300 micrograms of vitamin B_{12}, given without IFC to patients with pernicious anemia, were followed by differing absorption curves characterized by an early rise in plasma radioactivity and peak concentrations four to six hours after the test. The addition of IFC abolished the early rise in plasma concentration and resulted in a delayed peak concentration at 12 hours. Although IFC delayed the appearance of vitamin B_{12} in the plasma, it enhanced hepatic uptake of radioactivity.

4. Control subjects given test doses of 200 to 500 micrograms of vitamin B_{12} displayed a slightly diphasic absorption into the plasma, with an early rise in the radioactivity. A test dose of 10 micrograms caused a delayed absorption similar to the results observed with the much smaller test doses, while doses of 50 to 100 micrograms resulted in absorptions of an intermediate nature.

5. The present findings lend support to the concept of a dual mechanism of absorption of vitamin B_{12} from the gastrointestinal tract.

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