Normal and Promoted GI Absorption of Water-Soluble Substances II: Absorption of Vitamin B_{12} from Ligated Stomach and Intact Intestine of the Rat

W. W. DAVIS and C. J. KREUTLER

Abstract □ Both normal and promoted absorption of vitamin B_{12} in the doubly ligated stomach and the intact GI tract of the rat were investigated using ^{57}Co-labeled cyanocobalamin. Very little absorption of vitamin B_{12} occurs normally from the ligated stomach of the rat. Much more absorption is seen from the intact intestine. Both gastric and intestinal absorption are markedly increased by addition of a nonionic surface-active agent, polyoxyethylene-20-oleyl ether, to the aqueous solution of the vitamin. The effect of *ad libitum* feeding upon either normal or promoted absorption is minor. At high vitamin B_{12} levels, somewhat more normal absorption occurs in fasted than in fed animals. The results reported here are interpreted to be similar to the effects of surface-active absorption promoters on the absorption of other water-soluble substances.

Keyphrases □ Vitamin B_{12}—stomach and GI absorption, surfactant effect, rats □ Cyanocobalamin-^{57}Co—stomach and GI absorption, surfactant effect, rats □ Absorption, stomach and GI—vitamin B_{12}, rats

The normal occurrence of vitamin B_{12} in the food of all animals is small, and no active transport system is recognized for its absorption. The absorption from normal food sources would be inadequate but for the occurrence and special role of intrinsic factor. This relationship was extensively reviewed by Castle (1).

In the human, the condition known as pernicious anemia is one in which inadequate intrinsic factor is secreted in the stomach to permit the absorption of physiologically adequate amounts of vitamin B_{12}. Adequate oral therapy may be provided either by supplementing the intrinsic factor generally with supplementary vitamin B_{12} (2) or by administering large amounts of vitamin B_{12} without supplementing intrinsic factor (3).

In the rat, as in man, intrinsic factor is secreted in the stomach, binds vitamin B_{12} in the stomach and the intestine, and, as a complex in the intestine, is somehow responsible for improved efficiency of the passive absorption of the vitamin from dilute solutions (1). The quantity of intrinsic factor produced in the stomach is normally sufficient to allow the absorption from the food of physiologically adequate amounts of vitamin B_{12}.

Glass (4) reviewed a variety of extragastric factors that influence the absorption of vitamin B_{12} and discussed their possible modes of action. Okuda *et al.* (5) administered a number of surfactants in undiluted form to rats and observed an increase in absorption of vitamin B_{12}. Their experimental evidence supported their suggestion that increased absorption was due to delayed intestinal transit of the highly viscous mixture, a mechanism devoid of therapeutic interest. Preliminary experience in this laboratory employing dilute surface-active absorption promoters (6) indicated that the efficiency of absorption of vitamin B_{12} in test animals might be increased by the use of dilute solutions of selected surface-active agents, acting either in the stomach or in the intact intestine by inducing a reversible hyperabsorptive state of the GI mucosa.

EXPERIMENTAL

Male rats of the CFE strain[1], weighing 150–275 g., were used. The rats were caged individually and fasted for 15–20 hr. before beginning the experiment.

Rats with Ligated Stomach—The animal was anesthetized with ether, and the abdominal area was exposed through a midline incision. A pyloric ligation was performed, and the 2-ml. aqueous test solution was introduced into the stomach by a stomach tube prior to esophageal ligation. Care was taken not to occlude blood vessels unnecessarily. The abdominal incision was closed with wound clamps and coated with collodion. A gauze dressing was applied and the animal was placed in a holding cage where it recovered from the anesthesia within 5 min. Two hours after dosing, the animals were sacrificed and their liver and kidneys were excised for assay.

Intact Rats—Intact rats were lightly anesthetized with ether and were given 2 ml. of the aqueous test solution by stomach tube. These animals were held for 2, 6, 16, or 24 hr. before they were sacrificed, and their liver and kidneys were excised for assay. After 2 hr. postdosing, food[2] and water were provided *ad libitum* to some animals, while other animals were fasted throughout the 24-hr. experiment.

Preparation of Oral Dose—The 2-ml. aqueous test solution administered to the rats contained 0.4 $\mu c.$ ^{57}Co-vitamin B_{12}[3] in pH 7.0 isosmolar sodium phosphate buffer. Additional unlabeled vitamin B_{12} was added so that the standard solutions contained 400, 2000, or 6000 ng. of the vitamin. The nonionic surfactant, polyoxyethylene-20-oleyl ether[4], when administered, was present at a 1% concentration in the 2-ml. dose.

Assay of Tissue Radioactivity—The excised livers and kidneys were placed in wheaton vials for γ-counting. The radioactivity was determined using an autogamma spectrometer[5]. Computations of the vitamin B_{12} content of the tissues were made, and the results were expressed in nanograms. The total uptake by liver and kidneys was also expressed as percent of the administered dose and referred to as the "efficiency of absorption."

RESULTS

Absorption from Ligated Stomach—Results of the experiments with gastric ligated animals are presented in Table I. In the absence of an absorption promoter, the absorption of the drug was extremely limited. When the absorption promoter was present, much more absorption occurred. Both with and without promoter, the increasing dose of the vitamin (400, 2000, and 6000 ng./animal) resulted in an approximately proportionate increase in absorption at the higher concentrations. Less than proportionate absorption occurred at the lowest dose level.

[1] Carworth Farms, New York City, Rockland County, N. Y.
[2] Purina Laboratory Chow.
[3] Supplied by Merck and Co., Inc., Rahway, N. J.
[4] Brij-98, Atlas Chemical Industries, Wilmington, Del.
[5] Model 410A, Packard Instrument Co., Inc., LaGrange, Ill.

Table I—Normal and Polyoxyethylene-20-oleyl Ether-Promoted Absorption of Vitamin B_{12} in Ligated Rat Stomach[a]

Treatment Group	Sacrifice Time after Dosing, hr.	Weight, g. Mean	Weight, g. Range	Liver and Kidney Uptake of Vitamin B_{12}: Nanograms of Vitamin B_{12} Liver, Mean	Kidney, Mean	Total Mean	Total Range	Percent of Dose, Total Mean
400-ng. dose								
Control[b] (vitamin B_{12} only)	2	207	2	0.30	0.17	0.5	0.7	0.1
Promoted[c] (with 1% promoter)	2	196	13	1.60	1.08	2.7	0.4	0.7
2000-ng. dose								
Control[b] (vitamin B_{12} only)	2	259	14	2.18	0.85	3.0	1.5	0.2
Promoted[c] (with 1% promoter)	2	248	5	25.03	19.41	44.4	2.3	2.2
6000-ng. dose								
Control[b] (vitamin B_{12} only)	2	184	3	8.23	3.53	11.8	21.5	0.2
Promoted[c] (with 1% promoter)	2	190	4	73.49	62.29	135.8	34.9	2.3

[a] Values are recovered vitamin B_{12} calculated from the means of total radioactivity recovered. Each treatment group consisted of two animals. [b] The 2-ml. dose contained 0.4 μc. ^{57}Co–vitamin B_{12} with a total of 400, 2000, or 6000 ng. vitamin B_{12} in pH 7.0 isosmolar sodium phosphate buffer. [c] The 2-ml. dose contained 0.4 μc. ^{57}Co–vitamin B_{12} with a total of 400, 2000, or 6000 ng. vitamin B_{12} and 1% polyoxyethylene-20-oleyl ether in pH 7.0 isosmolar sodium phosphate buffer.

Absorption from Intact GI Tract—Experiments were performed in intact rats with the same three dose levels of vitamin B_{12}. The accumulation of absorbed radioactivity in the organs of the intact animals was followed over periods of up to 24 hr. The influence of *ad libitum* food and water was examined.

Three tables present the data: Table II for 400 ng., Table III for 2000 ng., and Table IV for 6000 ng. The data presented allow several comparisons of single parameter effects. First, the presence of promoter produced an increase over normal absorption. There are 18 such direct comparisons of the averages of pairs of animals. Except for one of these comparisons (Table II, Footnote *d*), all showed greater absorption and accumulation when polyoxyethylene-20-oleyl ether was added.

At 2 hr., the accumulation in the liver was greater than in the kidneys. At later times, the accumulation in the kidneys was greater than in the liver. A minor exception appears (Table III) for the 24-hr. fasted normal data.

As the dose of vitamin B_{12} was increased, the percent of the dose absorbed by the normal intrinsic factor-assisted process diminished. This decrease is seen in 11 of 12 comparisons. This decrease in efficiency of normal absorption was most prominent in progressing from the lowest dose to the intermediate dose. With promoter present, absorption approached a constant efficiency as the concentration of vitamin B_{12} increased (Tables II–V). This efficiency was lower than in the intrinsic factor-assisted process at the lowest concentration of the vitamin. With high doses of the vitamin, the limiting efficiency of absorption in the presence of promoter was much higher than in the absence of promoter.

Table VI presents the influence of promoter as ratios of efficiency in the presence *versus* the absence of promoter. At low concentrations of the vitamin, where the normal intrinsic factor-assisted absorption was most efficient, the addition of promoter added little further efficiency. The average ratios for promoted *versus* normal absorption was only 1.5 and 1.6 for fasted and fed animals, respectively. At high concentrations of the vitamin, this ratio was higher, averaging 2.0 and 4.1 for the fasted and fed animals, respectively.

The effect of feeding *versus* fasting is shown clearly in Table V. Efficiency of normal absorption at the low concentration of vitamin B_{12} was approximately equal in fasted and fed animals. Efficiency of normal absorption at the high concentration was lower in the presence of food. When promoter was present, no prominent difference in absorption was observed between fed and fasted animals, either at low or high concentrations of the vitamin.

DISCUSSION

Reynell *et al.* (7) found little or no absorption of vitamin B_{12} in the stomach of the rat. Okuda (8) found no absorption from a dose of 50 ng. of vitamin B_{12} in the pyloric-ligated stomach of the rat. The data presented here show that 0.12% of a dose of 400 ng. of vitamin B_{12} is absorbed normally from the doubly ligated stomach of the rat after 2 hr. When the dose of vitamin is increased to 6000 ng., the percent of the dose absorbed normally is somewhat higher—

Table II—Normal and Polyoxyethylene-20-oleyl Ether-Promoted Absorption of Vitamin B_{12} (400 ng.) in Intact Rats[a]

Treatment Group	Sacrifice Time after Dosing, hr.	Weight, g. Mean	Weight, g. Range	Liver and Kidney Uptake of Vitamin B_{12}: Nanograms of Vitamin B_{12} Liver, Mean	Kidney, Mean	Total Mean	Total Range	Percent of Dose, Total Mean
Fasted animals								
Control[b] (vitamin B_{12} only)	2	192	26	1.07	0.37	1.4	1.5	0.4
	6	233	60	3.87	7.04	10.9	2.8	2.7
	24	170	6	5.99	8.68	14.7	0.5	3.7
Promoted[b] (with 1% promoter)	2	222	13	2.38	1.29	3.7	4.3	0.9
	6	169	7	7.15	7.26	14.4	2.2	3.6
	24	164	1	9.97	15.31	25.3	1.0	6.3
Fed animals								
Control[b] (vitamin B_{12} only)	6	171	1	2.58	5.92	8.5	3.4	2.1
	16	176	11	3.68	8.21	11.9	1.2	3.0
	24	217	18	4.90	11.57	16.5	0.1	4.1
Promoted[b] (with 1% promoter)	6	157	11	3.71	10.59	14.3	4.8	3.6
	16	180	3	5.65	13.54	19.2	4.4	4.8
	24	209	49	3.48	10.44	(13.9)[d]	3.4	(3.5)[d]

[a] Values are recovered vitamin B_{12} calculated from the means of total radioactivity recovered. Each treatment group consisted of two animals. [b] The 2-ml. dose contained 0.4 μc. ^{57}Co–vitamin B_{12} with a total of 400 ng. vitamin B_{12} in pH 7.0 isosmolar sodium phosphate buffer. [c] The 2-ml. dose contained 0.4 μc. ^{57}Co–vitamin B_{12} with a total of 400 ng. vitamin B_{12} and 1% polyoxyethylene-20-oleyl ether in pH 7.0 isosmolar sodium phosphate buffer. [d] These figures resulted from combination of data from two animals, giving total recoveries in 12.20 and 15.64 ng. vitamin B_{12}. Several comparisons indicate these figures to be inconsistent with related data. They were accordingly ignored in all comparisons and summary generalizations of data in the text.

Table III—Normal and Polyoxyethylene-20-oleyl Ether-Promoted Absorption of Vitamin B_{12} (2000 ng.) in Intact Rats[a]

Treatment Group	Sacrifice Time after Dosing, hr.	Weight, g. Mean	Weight, g. Range	Liver and Kidney Uptake of Vitamin B_{12}: Nanograms of Vitamin B_{12} Liver, Mean	Kidney, Mean	Total Mean	Total Range	Percent of Dose, Total Mean
Fasted animals								
Control[b] (vitamin B_{12} only)	2	170	10	3.72	0.87	4.6	1.0	0.2
	6	208	4	9.63	16.07	25.7	5.3	1.3
	24	165	13	18.51	17.65	36.2	9.0	1.8
Promoted[c] (with 1% promoter)	2	250	0	8.41	4.31	12.7	5.5	0.6
	6	239	58	12.93	23.65	36.6	11.3	1.8
	24	165	18	34.80	47.64	82.4	0.1	4.1
Fed animals								
Control[b] (vitamin B_{12} only)	6	167	0	6.00	10.13	16.1	4.2	0.8
	16	179	3	11.31	27.01	38.3	7.1	1.9
	24	242	72	9.56	32.57	42.1	23.5	2.1
Promoted[c] (with 1% promoter)	6	164	9	13.27	22.93	36.2	1.6	1.8
	16	169	5	19.49	51.95	71.4	17.5	3.6
	24	231	58	26.55	96.26	122.8	35.2	6.1

[a] Values are recovered vitamin B_{12} calculated from the means of total radioactivity recovered. Each treatment group consisted of two animals. [b] The 2-ml. dose contained 0.4 μc. ^{57}Co–vitamin B_{12} with a total of 2000 ng. vitamin B_{12} in pH 7.0 isosmolar sodium phosphate buffer. [c] The 2-ml. dose contained 0.4 μc. ^{57}Co–vitamin B_{12} with a total of 2000 ng. vitamin B_{12} and 1% polyoxyethylene-20-oleyl ether in pH 7.0 isosmolar sodium phosphate buffer.

Table IV—Normal and Polyoxyethylene-20-oleyl Ether-Promoted Absorption of Vitamin B_{12} (6000 ng.) in Intact Rats[a]

Treatment Group	Sacrifice Time after Dosing, hr.	Weight, g. Mean	Weight, g. Range	Liver and Kidney Uptake of Vitamin B_{12}: Nanograms of Vitamin B_{12} Liver, Mean	Kidney, Mean	Total Mean	Total Range	Percent of Dose, Total Mean
Fasted animals								
Control[b] (vitamin B_{12} only)	2	233	23	6.06	3.47	9.5	1.5	0.2
	6	204	16	43.34	55.90	99.2	68.9	1.7
	24	169	0	48.64	54.76	103.4	31.4	1.7
Promoted[c] (with 1% promoter)	2	237	22	40.88	27.71	68.6	13.4	1.1
	6	226	4	44.00	117.31	161.3	2.3	2.7
	24	167	15	100.28	145.85	246.1	1.6	4.1
Fed animals								
Control[b] (vitamin B_{12} only)	6	167	9	12.69	19.21	31.9	12.6	0.5
	16	180	9	16.14	35.92	52.0	27.4	0.9
	24	214	30	13.53	35.27	48.8	19.3	0.8
Promoted[c] (with 1% promoter)	6	169	12	36.55	61.46	98.0	32.0	1.6
	16	178	4	54.10	142.79	196.9	21.3	3.3
	24	237	54	57.71	201.59	259.3	138.5	4.3

[a] Values are recovered vitamin B_{12} calculated from the means of total radioactivity recovered. Each treatment group consisted of two animals. [b] The 2-ml. dose contained 0.4 μc. ^{57}Co–vitamin B_{12} with a total of 6000 ng. vitamin B_{12} in pH 7.0 isosmolar sodium phosphate buffer. [c] The 2-ml. dose contained 0.4 μc. ^{57}Co–vitamin B_{12} with a total of 6000 ng. vitamin B_{12} and 1% polyoxyethylene-20-oleyl ether in pH 7.0 isosmolar sodium phosphate buffer.

viz., 0.20%. This observation appears consistent with the proposal that normal passive absorption from the stomach is proportional to the concentration of free uncomplexed vitamin B_{12}. The fraction of unbound vitamin was very small in the experiments of Okuda (8), in which only 50 ng. was used; this fraction was larger in the present experiment in which 400 ng. was used, and it approached substantially all free vitamin in the 6000-ng. experiments. Efficiency of promoted gastric absorption also increases with the fraction of uncomplexed vitamin. With increasing dose and fraction in the free form, the efficiency increases to about 2.3% of the dose.

In the intact rat, the experimental data on normal absorption of the vitamin are consistent with the following interpretation. The intrinsic factor–vitamin complex is formed in the stomach to the limit of occurrence of intrinsic factor. This is less than enough to complex all of the 400-ng. dose, so that part of the 400-ng. dose is free. The complexed portion is strongly adsorbed on the intestinal wall, and the vitamin is more efficiently absorbed from this adsorbed complex than from the noncomplexed, nonadsorbed portion. When the total dose of vitamin is increased without increasing the quantity of intrinsic factor, the contribution of the intrinsic factor-assisted process becomes negligible, and the overall efficiency of absorption falls toward a characteristic value. In the absence of promoter, this value is roughly 1.7% of the dose in the fasted animal or 0.8% of the dose in the fed animal over 24 hr.

Administration of promoter with the vitamin in the intact rat does not affect formation of the vitamin–intrinsic factor complex. Again, high efficiency of absorption is seen at a low concentration of vitamin where the intrinsic factor-assisted process is maximally effective. The increased efficiency of absorption of the relatively small amount of uncomplexed vitamin in the presence of promoter results in an overall high efficiency of absorption.

Table V—Efficiency of Normal and Promoted Absorption Expressed as Percent of Dose Absorbed

Organ	Hours	Vitamin B_{12} Administered, ng. 400	2000	6000
Stomach (normal)	2	0.1	0.2	0.2
Stomach (promoted)	2	0.7	2.2	2.3
Intestine (normal)	2	0.4	0.2	0.2
Intestine (promoted)	2	0.9	0.6	1.1
Intestine, fasted (normal)	6	2.7	1.3	1.7
Intestine, fasted (promoted)	6	3.6	1.8	2.7
Intestine, fed (normal)	6	2.1	0.8	0.5
Intestine, fed (promoted)	6	3.6	1.8	1.6
Intestine, fasted (normal)	24	3.7	1.8	1.7
Intestine, fasted (promoted)	24	6.3	4.1	4.1
Intestine, fed (normal)	24	4.1	2.1	0.8
Intestine, fed (promoted)	24	(−)	6.1	4.3

Table VI—Ratios of Polyoxyethylene-20-oleyl Ether-Promoted Absorption to Normal Absorption Efficiencies in the Rat

Time of Sacrifice	Vitamin B_{12} Dose, ng. 400	2000	6000
Ligated stomach animals			
2 hr.	5.6	14.8	11.3
Fasted intact animals			
2 hr.[a]	3.3	2.8	7.1
6 hr.	1.3	1.4	1.6
24 hr.	1.7	2.3	2.4
Average	1.5	1.8	2.0
Fed intact animals			
6 hr.	1.7	2.2	3.3
16 hr.	1.6	1.9	3.8
24 hr.	—	2.9	5.3
Average	1.6	2.3	4.1

[a] Excluded from averages.

At a higher concentration of the vitamin, the intrinsic factor-assisted absorption contributes little and the efficiency of absorption falls and approaches a value characteristic of the uncomplexed vitamin in the "hyperabsorptive" intestine. This value is above 4.0% of the dose absorbed whether the animal is fed or fasted.

Previous work in these laboratories demonstrated a hyperabsorptive state of the dog stomach which resulted specifically from a surfactant-organ interaction (6). This hyperabsorptive state subsequently returned to a normal absorptive state after removal of the promoter. Unpublished studies in these laboratories also demonstrated similarly hyperabsorptive states of segments of the GI tract of dogs, rats, and chickens. The present study indicates that the ligated stomach and the intact GI tract of the rat becomes hyperabsorptive for uncomplexed vitamin B_{12} under the influence of polyoxyethylene-20-oleyl ether.

It can be concluded from Tables II–IV that the absorption in intact animals continued beyond 16 hr., because the fraction of the dose absorbed continued to increase between 16 and 24 hr. Roughly one-half of the total absorption occurred during the first 6 hr. A check of fecal and urinary radioactivity indicated that at 6 hr. very little excretion had occurred, while by 16 hr. most of the unabsorbed radioactivity had passed out of the GI tract.

The ratios of efficiency of promoted *versus* normal absorption in the intact intestine (Table VI) vary with dose and time of sacrifice. The highest ratios are for the fed animals at high dose and for the animals sacrificed at 2 hr. The 2-hr. results may well include some of the influence of the high ratio of promoted *versus* normal absorption characteristic of the stomach. It was concluded that the effect of the absorption promoter, polyoxyethylene-20-oleyl ether, is to render the stomach and intestine hyperabsorptive, improving the efficiency of absorption of only the uncomplexed portion of the vitamin. The ability of the endogenous intrinsic factor to enhance the absorption of small doses of vitamin B_{12} is not impaired by the presence of polyoxyethylene-20-oleyl ether.

It is not intended in this paper to consider or to imply the therapeutic usefulness of promoted absorption in vitamin B_{12} therapy. Data from clinical reports were cited because much of the knowledge of vitamin B_{12} absorption was obtained from clinical experience. The surprising effectiveness of polyoxyethylene-20-oleyl ether in promoting absorption of vitamin B_{12} of molecular weight 1355 suggests that passive absorption mechanisms may extend to substances of even higher molecular weights.

REFERENCES

(1) W. B. Castle, *New Engl. J. Med.*, **249**, 603(1953).

(2) G. B. Walden and G. H. A. Clowes, *Proc. Soc. Exp. Biol. Med.*, **29**, 873(1932).

(3) S. O. Waife, C. J. Jansen, R. E. Crabtree, E. L. Grinnan, and P. J. Fouts, *Ann. Int. Med.*, **58**, 810(1963).

(4) G. B. Glass, *Physiol. Rev.*, **93**, 529(1963).

(5) K. Okuda, E. V. Duran, and B. F. Chow, *Proc. Soc. Exp. Biol. Med.*, **103**, 588(1960).

(6) W. W. Davis, R. R. Pfeiffer, and J. F. Quay, *J. Pharm. Sci.*, **59**, 960(1970).

(7) P. C. Reynell, G. H. Spray, and K. B. Taylor, *Clin. Sci.*, **16**, 663(1957).

(8) K. Okuda, *Amer. J. Physiol.*, **199**, 84(1960).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 9, 1971, from *The Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46206*

Accepted for publication July 16, 1971.

The authors thank Mrs. Jean Pieper of the radiochemical counting group of these laboratories for all counting sample preparation and all radiochemical counting.

In Vitro Metabolism of Certain Nornuciferine Derivatives

ROBERT V. SMITH* and S. PAUL SOOD

Abstract □ The metabolism of nornuciferine and seven of its derivatives by rat, rabbit, and guinea pig liver microsomes was studied. Nornuciferine was found as a metabolite of the *N*-alkylated analogs. A sensitive method for analyzing nornuciferine was developed based on reverse TLC fluorimetry. By using this method, relative *N*-dealkylation rates were studied with guinea pig microsomes. The conversion of nornuciferine to the oxoaporphine, lysicamine, was observed photolytically and metabolically with rat and rabbit microsomes. Chromatographic and spectral analyses of lysicamine are discussed.

Keyphrases □ Nornuciferines—*in vitro* metabolic and photolytic conversions to lysicamine □ Lysicamine—metabolic and photolytic conversion from nornuciferines, identification □ Aporphine biotransformations—nornuciferine metabolism □ TLC fluorimetry, reverse—analysis, nornuciferines □ Photolysis—conversion of nornuciferines to lysicamine □ TLC—identification, lysicamine □ Mass spectroscopy—identification, lysicamine

The metabolism of apomorphine was studied by Kaul *et al.* (1–4). Besides these investigations, however, no studies have appeared relative to the biological fate of aporphine alkaloids. This is somewhat surprising since aporphines occur widely in the plant kingdom (5, 6). In addition, naturally derived aporphines, as well as many synthetic analogs, possess interesting biological activities (6–10). Of particular significance are reports