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Vitamin B_{12} Absorption from Fish (41201)

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Abstract. The main purpose of this investigation was to study the assimilation of cyanocobalamin from fish. For this reason rainbow trout were injected with ^{57}Co-vitamin B_{12} and sacrificed 2 to 3 weeks later. The radioactivity was found to be evenly distributed in the muscle mass. In contrast to findings in birds and mammals the liver contained on the average less radioactivity than did the kidneys, head, skin, and muscle mass. The radiolabeled fish was ingested by three healthy subjects in 50- to 300-g portions and the absorption measured by the stool excretion method. The study shows that from an average of 2.07 μg of vitamin B_{12} (50 g of fish), 4.05 μg (100 g), 9.2 μg (200 g), and 13.3 μg (300 g) the assimilation of cobalamin averaged 0.87, 1.55, 3.90, and 3.98 μg, respectively. In two of the subjects, an upper limit of absorption was reached, while in one it was still climbing. A standard urinary excretion test using 100 g of fish, eaten by healthy subjects and by patients who presented with low serum vitamin B_{12} levels, showed very low absorption values after the fish and after the crystalline radiocyanocobalamin in patients with pernicious anemia. Subnormal assimilation of cobalamin from the fish with normal absorption of crystalline radio-B_{12} was noted in subjects with simple gastric achlorhydria and in patients who had undergone partial gastrectomy.

Vitamin B_{12} absorption has been extensively studied after oral administration of crystalline radiocyanocobalamin (1, 2). In contrast, very little is known about the assimilation of this vitamin from natural food sources. Furthermore, the few studies which have been performed have shown contradictory results. Thus, the assimilation of the vitamin from mutton (3) and chicken meat (4) was reported to be equal to that observed after administration of comparable amounts of labeled crystalline vitamin B_{12} whereas that from eggs was far inferior (5). It is possible that poor assimilation of vitamin B_{12} is unique for eggs only. However, further absorption studies using various other food-B_{12} sources are required in order to prove this contention.

In this report, we present the results of vitamin B_{12} absorption studies performed in healthy subjects and in patients with vitamin B_{12} deficiency using rainbow trout labeled *in vivo* with radiocyanocobalamin.

Materials and Methods. Twelve rainbow trout (*Salmo gairdneri*) weighing about 2–4 kg each were used for these experiments. The meat of the fish was labeled with radioactive vitamin B_{12} as follows: 15–25 μCi ^{57}Co-vitamin B_{12} (sp act 220 μCi/μg, Amersham/Searle Corp., Arlington Heights, Ill.) was given im in two to four divided doses over a period of 2 days into both sides of the fish, about 25 mm posterior to the operculum and equidistant ventral to the dorsal crest. The fish which were kept in suitable tanks in the Fish Hatchery, wet laboratory, Minnesota Department of Natural Resources, St. Paul, Minnesota, were sacrificed 2–3 weeks later, immediately dissected and then frozen. A control fish was kept in a similar tank but never given vitamin B_{12} injections. The injected fish as well as the noninjected control were fed a special grower diet (P.R. 11) prepared by Glencoe Mills, Glencoe, Minnesota. The radioactivity of the various parts of the fish was determined in a modified Packard small animal gamma scintillation counter with an average total recovery of 35.3% (range 17.3–54.9%) of the injected radioactivity. Table I shows three representative examples of the relative distribution of radioactivity in the various parts of the fish. Depending upon the size, the fish yielded four

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TABLE I. RELATIVE DISTRIBUTION OF RADIOACTIVITY IN VARIOUS PARTS AND ORGANS OF THREE REPRESENTATIVE RAINBOW TROUT INJECTED WITH ^{57}Co-VITAMIN B_{12}

Parts of fish	Percentage distribution			
	Fish A	Fish B	Fish C	Average
All fillets	24.6	13.1	20.5	19.3
Liver	6.0	16.6	9.3	10.6
Kidneys	23.8	20.4	19.7	21.3
Intestines	5.6	5.4	7.5	6.2
Testes	4.5	2.8	3.2	3.5
Spleen	0.5	0.5	0.3	0.4
Backbone	6.3	2.3	2.6	3.7
Head	17.8	26.0	16.0	19.9
Skin	8.8	12.9	18.8	13.5
Heart	2.1	ND[a]	2.1	2.1

[a] Not done.

to six fillets weighing approximately 80–120 g with a uniform distribution of radioactivity throughout the muscle mass. For example, in three fish with four, five, and six fillets, the average content of radioactivity ± SEM was found to be 222 ± 1.49, 836 ± 3.66, and 1264 ± 11.02 cpm/g, respectively. Occasionally, the injections led to an intramuscular hemorrhage with resultant increased radioactivity in the corresponding fillet, and this was therefore discarded and not used in any absorption study. The fillets were cooked in a Teflon pan, transferred in 50-g portions to plastic containers and, after radioactivity determination in the scintillation counter, stored in a freezer at −20° until served, usually within 2–8 weeks. The radioactivity of the 50-g portions of fish ranged from 0.05 to 0.17 μCi, which was quite satisfactory for radioactivity counting purposes.

The vitamin B_{12} content of the cooked fish was determined by an isotope method (6) with sensitivity equal to that of the *Euglena gracilis* assay (7), the fish having been prepared according to the method of Biggs *et al.* (8) as previously described (5, 9, 10).

The vitamin B_{12} content of the 12 injected fish averaged 4.7 μg (range 3.5–5.5 μg)/100 g with a corresponding value of 5.3 μg for the control fish. These figures are in close agreement with previously published values for fish of the family *Salmonidae* (11). The total amount of vitamin B_{12} ingested was derived from the amount of fish served and its known vitamin B_{12} content.

In the morning, the fish was reheated in a microwave oven for 45 sec and served in 50- to 300-g portions to fasting volunteers with coffee and toast. No other food was allowed for the next 4 hr. The residual radioactivity in the empty containers was measured in the isotope counter. Fourteen volunteers, fully informed of the nature of the test, consented to participate in these studies. Eight were healthy normal subjects (serum vitamin B_{12} concentrations 200 to 700 ng/liter) and two had pernicious anemia in remission (serum vitamin B_{12} concentrations of 40 and 56 ng/liter). Two had simple gastric achlorhydria (serum vitamin B_{12} of 60 and 120 ng/liter); two had Billroth II-type gastric resection (serum vitamin B_{12} of 120 and 140 ng/liter). All sera were assayed prior to vitamin B_{12} absorption studies.

The absorption of the radioactive vitamin B_{12} was measured by the fecal excretion method (12) or the urinary excretion test (13) as previously described (5, 9, 10). The net ingested radioactivity for the stool excretion method was derived from the activity of the fish with correction for decay and the remaining activity in the empty containers. Stool was collected for at least 7 days and counted in the small body counter. The absorption, expressed in percentage, was calculated from the net intake and the 7-day excretion of radioactivity. The assimilated amount of the vitamin B_{12} was obtained from the known amount of cobalamin ingested and the percentage of radioactivity absorbed. The net administered radioactivity for the urinary excretion test was deduced from the radioactivity present in a 1-g portion of the fish and the known amount of fish ingested with correction for the percentage left in the containers. Before counting, the fish was digested with acid and pepsin in 10-ml volumes in the counting vial. The total radioactivity in the 24-hr urine was derived from the radioactivity of a 10-ml sample of urine, determined in a gamma scintillation counter, and the total urine volume. Correction for decay was avoided by counting the radioactivity of the 1-g sample of fish and urine

TABLE II. Vitamin B_{12} Assimilation from Varying Amounts of Fish Ingested by Three Normal Subjects as Studied by the Stool Excretion Method

Amount of fish ingested (g)	Subject No. 1 Amount fed (μg)	Subject No. 1 Amount absorbed (%)	Subject No. 1 Amount absorbed (μg)	Subject No. 2 Amount fed (μg)	Subject No. 2 Amount absorbed (%)	Subject No. 2 Amount absorbed (μg)	Subject No. 3 Amount fed (μg)	Subject No. 3 Amount absorbed (%)	Subject No. 3 Amount absorbed (μg)
50	2.18	38.1	0.83				1.95	46.4	0.90
100	3.90	32.9	1.28	3.90	47.2	1.84	3.90	33.3	1.29
100							4.50	39.7	1.79
200	10.90	33.5	3.65	7.80	48.5	3.78	10.40	41.4	4.31
200				7.80	49.6	3.87			
300	12.0	28.6	3.43	11.70	25.3	2.97	15.60	35.5	5.54

together on the same day. The urinary excretion test after administration of crystalline radiocyanocobalamin was performed as previously described (4, 5, 9, 10).

Results. Three normal subjects ingested from 50 to 300 g of trout fillet with at least a week between servings. Table II shows the amount of fish and vitamin B_{12} ingested as well as the percentage of radioactivity and the amount of vitamin B_{12} absorbed. The vitamin assimilated from 50, 100, 200, and 300 g of fish averaged 0.87 μg (range 0.83–0.90), 1.55 μg (range 1.28 to 1.84), 3.90 μg (3.65 to 4.31), and 3.98 μg (range 3.0 to 5.54), respectively. On repeat studies of 100 g (subject No. 3) and 200 g (subject No. 2) there was a smaller difference in the stool excretion of radioactivity (6.4 and 1.1%) than those reported when duplicate or triplicate tests were performed with a uniform dose of crystalline radiocyanocobalamin (14–16).

Table III shows the results of the urinary excretion tests after administration of crystalline radio-B_{12} and the ingestion of 100 g of fish followed by an im injection of 1000 μg of nonlabeled vitamin B_{12} 6 hr later. The table reveals the average values with upper and lower limits in the eight normal subjects and the individual values for the six patients. The urinary radioactivity in patients with pernicious anemia was negligible after the fish and very low after crystalline radio-B_{12}. The patients with simple gastric achlorhydria and those with Billroth II-type gastric resection had subnormal values after ingestion of the fish, but normal excretion after the crystalline radiocyanocobalamin.

Discussion. Rainbow trout were selected for this study as they can well tolerate the stress associated with being handled out of water, as in the im injections of the radiocyanocobalamin. It was interesting to note that the muscle mass, the kidneys, head, and skin showed on the average more

TABLE III. Urinary Excretion Values after Ingestion of 100 g of Fish and Crystalline Radiocyanocobalamin[a]

Subjects	Percentage 24-hr urinary radioactivity: Fish[b]	Percentage 24-hr urinary radioactivity: Crystalline ^{57}Co-B_{12}[c]
Normals (8)		
Average	6.5	25.7
Range	4.3–9.4	15.8–33.0
Pernicious anemia		
R.O.	0.2	1.5
L.M.	0.1	0.1
Gastric achlorhydria		
M.F.	3.0	36.3
A.P.	3.3	19.3
After Billroth II		
V.A.	1.4	13.9
A.K.	1.2	22.4

[a] A "flushing" dose of 1000 μg of nonlabeled vitamin B_{12} was administered 6 hours after ingestion of fish and of oral crystalline radiocyanocobalamin.
[b] Fish dose contained 3.9 to 5.2 μg of vitamin B_{12}.
[c] Crystalline dose of radio-B_{12} contained 0.56 μg of vitamin B_{12}.

radioactivity than did the liver. This is in contrast to what is seen in mammals and birds where most of the injected radiocyanocobalamin accumulates in the liver (4, 17). These findings suggest that the liver in fish plays a less important role for the storage of vitamin B_{12} than it does for mammals and birds. This is also consistent with earlier findings showing less vitamin B_{12} per gram of liver in fish than in other organs (18).

The study shows that from a mean of 2.07 μg of vitamin B_{12} (50 g of fish), 4.05 μg (100 g), 9.2 μg (200 g), and 13.3 μg (300 g), the assimilation of cobalamin averaged 0.87, 1.55, 3.90, and 3.98 μg, respectively. These findings indicate that the assimilation of cobalamin from fish rises as the amount of fish cobalamin ingested increases. The average absorption values may indicate an upper limit to the intestinal uptake, similar to what is known for the absorption of crystalline vitamin B_{12} (19). However, the individual absorption values substantiate an upper limit theory in only two of the three volunteers. Unfortunately, we were unable to add more fish to the dose in subject No. 3 as the amount of fish would be more than the volunteer could eat at one meal. In order to assess these absorption results, they must be compared with those obtained after ingestion of comparable amounts of crystalline radiocyanocobalamin and radio-B_{12} incorporated into other foods.

The absorption by the intrinsic factor mechanism of crystalline vitamin B_{12} also increases with increments in the oral doses until an average maximum uptake of 1.6 μg is reached after ingestion of 5–10 μg of the vitamin (19). For example, after 2.0 μg the absorption was 0.92 μg which is quite comparable to the value of 0.87 μg after ingestion of 2.07 μg in 50 g of fish. After 5.0 μg of crystalline vitamin B_{12} the average uptake was 1.4 μg as tabulated from the literature by Chanarin (19) and 1.6 μg as determined by Chanarin himself (19), values which are quite comparable to our finding of 1.55 μg after ingestion of 4.05 μg cobalamin in 100 g of fish. After 10 μg of crystalline vitamin B_{12} the average uptake was still only 1.6 μg (19), which is lower than the 3.90- and 3.98-μg average values after ingestion of 9.2 to 13.3 μg cobalamin in 200 and 300 g of fish. Assimilation of cobalamin from fish in normal subjects, therefore, seems comparable to that from crystalline vitamin B_{12} after ingestion of smaller doses and better after larger doses.

Heyssel *et al.* administered mutton labeled *in vivo* with radio-B_{12} to normal volunteers and found average absorption values of 0.62, 2.5, and 2.65 μg after ingestion of 0.95, 3.03, and 5.11 μg of vitamin B_{12}, respectively (3). These findings also show that absorption of vitamin B_{12} from food in normals equals that from crystalline vitamin B_{12} when small doses are ingested, but with larger doses food-B_{12} absorption appears superior.

Assimilation of radio-B_{12} incorporated into chicken meat was also found to be comparable to that from crystalline radiocyanocobalamin (4), but owing to the low vitamin B_{12} content of chicken meat the maximum uptake from a single ingestion could not be tested. The good assimilation of cobalamin from mutton, chicken meat, and fish is in contrast to the poor assimilation of radio-B_{12} from eggs labeled *in vivo* with radiocyanocobalamin (5, 9, 20). Eggs seem to be a unique type of food possessing an inhibitory effect on the absorption of vitamin B_{12} as well as on other substances such as iron and biotin (10).

The reason for the superior intestinal absorption after higher doses of food-B_{12} compared with crystalline radiocyanocobalamin is not known. Although the conclusion is based on a small number of observations it is unlikely the result of errors such as deficient stool collection or inaccurate radioactivity counting. Furthermore, a similar conclusion can be drawn from the study by Heyssel *et al.* in which the absorption was determined by whole body counting (3). Most likely the upper limit of the gastrointestinal absorption of vitamin B_{12} is underestimated when the vitamin is administered in a crystalline form. This may be due to a relatively higher binding of the vitamin to the inactive R-binder in the gastric juice than to the intrinsic factor. The fasting gastric juice has a greater percentage of R-binder than does the stimulated juice

(21). Therefore, when crystalline radiocyanocobalamin is administered, far more of the vitamin may be bound to this inactive binder than when food-B_{12} is ingested. A further contributing factor may be a lack of pancreatic juice in the intestine after ingestion of crystalline radio-B_{12} but not after food-B_{12} since the pancreatic juice is secreted after a stimulating meal and is capable of degrading the R-binder with release of vitamin B_{12} to the intrinsic factor (22).

After having established a normal range for the urinary excretion method using 100 g of fish, the test was applied to patients who presented with low serum vitamin B_{12} concentration. Patients with pernicious anemia had very low absorption values both with fish and with crystalline radiocyanocobalamin. Subjects with simple gastric achlorhydria and patients with partial gastrectomy had impaired assimilation of cobalamin from fish despite a normal absorption of crystalline radiocyanocobalamin. These findings confirm our previous observations (4, 5) about impaired assimilation of food-B_{12} in these two latter groups.

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