

acidic groups or the combinations -OH and -COOH on the amino acid are essential in stabilizing the bacteriophage.

The separate effects of calcium and aspartate are suppressed by combination of the two at certain levels. The binding of metals by amino acids is a well established fact and appears a reasonable explanation for the mutual suppression.

Summary. 1. T1r⁺ phage is rapidly and irreversibly inactivated at 37°C in distilled water, .15 N sodium or potassium chloride or in Davis medium. 2. Addition of calcium,

magnesium or the amino acids serine, threonine, homoserine, aspartate or glutamate results in stabilization of phage in the diluents mentioned above.

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Limits of Absorption of Orally Administered Vitamin B₁₂: Effect of Intrinsic Factor Sources.* (21246)

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Studies of patients having pernicious anemia(2,3) and of persons with total gastrectomy(4,5) have shown that when vit. B₁₂ is given orally in doses of 0.5 µg its absorption from the gastrointestinal tract is dependent upon the presence of a gastric substance, presumably the Castle-defined intrinsic factor(6). However, the role of intrinsic factor in facilitating absorption of vit. B₁₂ has not as yet been clarified and, moreover, the pattern of absorption of this vitamin in subjects with normal gastric secretion has not been defined.

In the present investigation, a study has been made of the absorption of vit. B₁₂ in individuals with clinically normal gastric function. Graded doses of Co⁶⁰-labeled vit. B₁₂[†] were administered orally and the total amount of radioactivity excreted in stool samples measured by methods previously reported(2,5).

Results. 0.5, 2, 5, and 10 µg amounts of labeled B₁₂ were given in a series of doses at

TABLE I. Fecal Excretion of Co⁶⁰ following Graded Doses of Co⁶⁰ Vit. B₁₂ Given to Normal Subjects.

Dose in µg	No. of tests	% Co ⁶⁰ excreted		µg vit. B ₁₂ absorbed (calculated)	
		Range	Avg	Range	Avg
.5	15	57-19	35	.22-.41	.34
2.0	12	69-30	48	.61-1.44	1.03
5.0	15	87-50	67	.73-2.50	1.65
10.0	5	93-70	84	.68-2.97	1.63

various time intervals to subjects who were either fasting or between meals. Each dose contained 0.110 µc of Co⁶⁰. No test was repeated with a given subject using the same dose, although most subjects received more than one dose level. It was found (Table I) that as the amount of administered vit. B₁₂ was increased, the per cent of Co⁶⁰ excreted in the stools increased. The amount of vit. B₁₂ presumed to be absorbed, a figure obtained by subtracting the apparent B₁₂ excreted from the total amount administered (Table I), was no greater with 10 µg than with a 5 µg dose, an average value of approximately 1.6 µg being the calculated absorption at both dose levels.

The effect of a test meal on vit. B₁₂ absorp-

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[†] Generously supplied by Dr. Charles Rosenblum of Merck & Co., N. J.

TABLE II. Effect of Test Meal on Fecal Excretion of Co⁶⁰ at Varying Doses of Co⁶⁰ Vitamin B₁₂.

Dose of Co ⁶⁰ B ₁₂ (μg)	% Co ⁶⁰ excreted	
	Fasting	With test meal
.5	42	19
	50	26
	52	20
	65	28
	31	27
	25	28
	27	25
5	74	86
	57	51
	78	67
	50	58

tion was then investigated. The meal, given as breakfast, contained no sources of vit. B₁₂. It was found (Table II) that after 0.5 μg of the vitamin was administered in 4 of 7 subjects, food decreased the excretion of Co⁶⁰ and it may be significant that the 3 in whom no effect of food was found excreted considerably less radioactive material when fasting than the other 4. These results suggest that secretion of intrinsic factor may be stimulated by food. However, in subjects given 5 μg vit. B₁₂ (Table II), no difference was obtained in Co⁶⁰ excreted in the fasted as compared with the fed state. It should be pointed out that in all these tests, stools were collected until radioactivity no longer appeared.

In further studies of the absorption of vit. B₁₂ at the 5 μg dose level, relatively large amounts of intrinsic factor were administered simultaneously. In 4 individuals who were given either gastric juice or intrinsic factor concentrate from hog stomach mucosa together with 5 μg vit. B₁₂ it was found that the addition of these substances did not enhance vitamin absorption (Table III). In 3 instances an inhibitory effect seems to have been obtained.

In additional experiments with 2 gastrectomized subjects, the effect of graded doses of the intrinsic factor sources mentioned above was compared at both the 0.5 μg and 5 μg of vit. B₁₂ dose levels (Table IV). When 0.5 μg B₁₂ was administered, increasing the amount of intrinsic factor increased the amount of B₁₂ absorbed. At 5 μg, absorption was also increased by intrinsic factor addition. However, a larger amount of intrinsic factor

supplement did not further increase B₁₂ absorption and the B₁₂ absorbed was within the range obtained in subjects with normal gastric function. Similar results were obtained when gastric juice was used as a source of intrinsic factor.

Glass and coworkers have reported(7) that in normal subjects the per cent of vit. B₁₂ taken up by the liver varies inversely with the oral dose when the vitamin is given in amounts of 0.5 to 50 μg. Hence the available evidence points to the extreme limitation of vit. B₁₂ absorption even in normal individuals.

In individuals with a functioning gastric mucosa, when the amount of vit. B₁₂ absorbed is not increased by a larger dose, gastric juice or hog mucosal extracts have no effect in increasing vitamin absorption. It would appear that here some factor other than intrinsic factor is operating to limit the passage of B₁₂ across the intestinal membrane. The results indicate that the materials used—gastric juice and hog stomach extracts—might actually inhibit B₁₂ absorption. An intrinsic factor concentrate has been shown to inhibit vit. B₁₂ absorption in rats also(8,9). The inhibitory effect observed in these various experiments is not necessarily due to the intrinsic factor *per se*, since the administered substances were not pure. In this regard, Wijmenga and coworkers(9) have isolated from combinations of hog mucosa extracts and crystalline vit. B₁₂ a cobalamin protein with a high molecular weight which does not by itself have hemopoietic activity. In the pres-

TABLE III. Co⁶⁰ Excretion following Administration of Graded Doses of Intrinsic Factor and Labeled Vit. B₁₂ to Normal Subjects.

Sub- ject	Dose		Co ⁶⁰ ex- creted, %	Vitamin B ₁₂ absorbed (calculated) μg
	Vit. B ₁₂ , μg	Intrinsic factor		
Ma	5		81	.94
	5	400 mg conc.*	91	.45
Mc	5		60	1.98
	5	<i>Idem</i> *	91	.45
Ha	5		57	2.15
	5		51	2.40
	5	150 ml gastric juice	90	.48
Ga	5		74	1.32
	5	200 mg conc.*	71	1.43

* Hog gastric mucosa fraction, kindly supplied by Dr. R. W. Heinle of Upjohn Co.

TABLE IV. Co^{60} Excretion following Administration of Graded Doses of Intrinsic Factor and Labeled Vitamin B_{12} to Patients with a Total Gastrectomy.

Subject	Dose		Co^{60} excreted, %	Vitamin B_{12} absorbed (calculated) μg
	Vit. B_{12} , μg	Intrinsic factor		
Mo	.5		100	0
	.5	2.5 mg conc.*	64	.18
	.5	5.0 *	28	.36
	5.0		91	.45
	5.0	200 †	65	1.74
	5.0	400 †	62	1.93
St	.5		95	.03
	.5	25.0 mg conc.†	74	.13
	.5	50.0 †	28	.36
	5.0		87	.65
	5.0	150 ml gastric juice	40	3.00
	5.0	300	38	3.10

* Supplied by Merek & Co., Inc.

† " " Upjohn Co.

ent experiments, the formation of a similar compound which is not readily absorbed might explain the inhibitory results and serve also as a basis for explaining the limitation of absorption observed when doses of the order of 5 to 10 μg of B_{12} alone were given. At this concentration of vitamin the cobalamin protein might be formed within the gastrointestinal tract.

Summary. The results show the extreme limitation of vit. B_{12} absorption from the gastrointestinal tract of individuals with normal gastric function. Within the dose range used,

an upper level of absorption appears to have been reached which averages 1.6 μg vit. B_{12} . This level is not increased by giving the vitamin with a test meal or in conjunction with intrinsic factor sources. In patients with a total gastrectomy, a graded response to intrinsic factor is obtained using 0.5 μg vit. B_{12} but at a 5 μg level, increasing the amount of intrinsic factor does not increase B_{12} absorption beyond levels obtained in normal subjects.

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Regeneration of Tissue Nonprotein Sulfhydryl Compounds in Rats After Exposure to Cold and Restraint.* (21247)

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It has been previously reported(1-4) that cold and restraint lower the concentration of

liver nonprotein sulfhydryl compounds. Since Waelsch and Rittenberg(5,6) and Block(7,8) reported a rapid turnover of liver glutathione, this study was done to determine if the regeneration of these sulfhydryl compounds would be equally fast after the concentration was lowered by exposure to cold and restraint. To observe the effect of caloric intake on the

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