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In Vitro Transport of Radiolabeled Vitamins By the Small Intestine[1,2]

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In vitro studies have enhanced our understanding of the intestinal absorption of amino acids, carbohydrates and lipids (1). Such investigations have not previously been extended, to any degree, to the vitamins. One of the principal reasons is that for such experiments to have biological validity, they must be performed at vitamin concentrations which approach *in vivo* levels. For example, previous studies on riboflavin absorption by the small intestine had been carried out at a concentration of 4×10^{-4} M, a figure set by the demands of the spectrophotometric procedure employed (2). In the present study, riboflavin was followed at a concentration of 7×10^{-6} M by use of the radiolabeled vitamin; this represents a 57 fold lowering of the concentration, and approaches the actual level of the vitamin found in the gut wall. Radiolabeled vitamins can be detected and quantitated in low concentrations, and their metabolic products can be followed through blood and tissues. The present report covers a survey of the *in vitro* absorption of 16 radiolabeled vitamins by the hamster small intestine.

METHODS

Vitamin concentrations within the small intestine were calculated from literature data (3, 4) in order to determine the range of concentrations to be utilized (Table I). Everted sacs of small intestine, three from each adult golden hamster, were filled with 1 ml of fluid, and incubated (in 25 ml Erlenmeyer flasks) in 5 ml of the same fluid (5). That is, the vitamins were dissolved in pH 7.4 Krebs-Henseleit buffer (without calcium or magnesium) which was then used for both mucosal (outer) and serosal (inner) fluids. Upon gassing with 95% O_2 + 5% CO_2, the flasks were incubated for one hour at 37°C in an oscillating

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water bath. After incubation, gut sacs were removed and drained of the serosal fluid. The sacs were weighed to the nearest milligram. In all subsequent calculations the sacs were assumed to be 80 per cent water (repeated studies have shown that the hamster small intestine is 80± 3% water) (6). Following centrifugation to remove sloughed tissue, the mucosal and serosal solutions, as well as standards, were counted for radioactivity. Beta emitting radioisotopes were assayed by use of a liquid scintillation counter, while Co^{60} was quantitiated through use of a well type gamma counter. The vitamins employed, position of the radiolabel, and sources of the compounds are listed in the Appendix. We are grateful to the research laboratories that made these radiolabeled vitamins available. Radiopurity of the vitamins, as determined chromatographically, was over 90 per cent in each case. The radiolabeled biotin and thiamine were eluted from paper chromatograms and were over 98 per cent radiopure. The fat soluble vitamins (A, D, tocopherol) were used in concentrations on the order of 10^{-6}M.

TABLE I

APPROXIMATE CONCENTRATION OF VARIOUS VITAMINS IN THE SMALL INTESTINE. VALUES WERE CALCULATED FROM DATA IN REFERENCES (3) AND (4).

Vitamin	*Species*	*Concentration in 10^{-6} Moles/Liter Tissue Water*
Ascorbic acid	Rat	3,400
	Cow	1,560
	Guinea Pig	920
Biotin	Rat	0.6
	Human	0.3
Folic acid	Guinea Pig	0.001
	Rat	0.008
myo-Inositol	Rat	2,400
	Dog	2,500
	Human	5,200
Nicotinic acid	Rat	380
	Human	200
Pantothenic acid	Human	30
Pyridoxine	Rat	9
	Human	3
Riboflavin	Rat	17
	Cow	31
	Human	14
Thiamine	Rat	7
	Human	2
Vitamin A	Rat	under 1
Vitamin B_{12}	Dog	0.004
	Human	0.02
Vitamin D	Dog	0.3

At this concentration, in the presence of traces of propylene glycol, benzene, and ethanol, respectively, they are water soluble (and all were doubly filtered prior to use).

Experiments were then performed with the vitamin in the mucosal fluid, and buffer alone as the serosal fluid. Similarly, the experiments were done with the vitamin in the serosal fluid and buffer as the mucosal fluid. These experiments approximated the unidirectional vitamin fluxes. In the previous experiments plus these, at least 2 hamsters (6 intestinal sacs) were utilized for each determination. Subsequent values listed are the mean of each experiment.

RESULTS

When initially present on both sides of the *in vitro* hamster small intestine at the same concentration, biotin was the only vitamin to move against a concentration gradient (7). Down to, and including, the lowest concentrations employed, none of the other vitamins could be shown to accumulate in the serosal fluid (that is, they were not transported against a concentration gradient, Table II). Results are further expressed in Table III, where the final ratio of serosal concentration/mucosal concentration is given. From the radioactivity disappearing from the mucosal and serosal solutions, and the weight of the gut wall (assuming the gut to be 80% water), the concentration of radioactivity per unit of tissue water can be calculated. This was done in each case, and the result expressed in the second column of Table III as the ratio of tissue concen-

TABLE II

SPECIFIC ACTIVITY AND LOWEST CONCENTRATION OF THE VITAMINS STUDIED. ONLY BIOTIN WAS TRANSPORTED AGAINST A CONCENTRATION GRADIENT.

Vitamin	*Specific Activity* μ*curies/*μ*mole*	*Lowest Concentration Used (in* 10^{-6} *M)*
p-Aminobenzoic acid-C^{14}	3.39	8.9
Ascorbic acid-C^{14}	1.25	0.5
Biotin-C^{14}; H^3	3.56; 90	5.0
Foli acid-H^3	340.	0.064
myo-Inositol-H^3	14.	2.9
N-methylnicotinamide. Cl-C^{14}	1.9	6.6
Nicotinamide-C^{14}	5.32	5.7
Nicotinic acid-C^{14}	10.	2.8
Pyridoxine-H^3. HCl	166.	0.59
Riboflavin-C^{14}	1.0	7.0
Thiamine-S^{35}; H^3	8.45; 33.2	7.1; 5.4
Thioctic acid-H^3; S^{35}	18.6; 11.0	1.0; 1.6
d, 1-α-Tocopherol-H^3	49.	6.6
Vitamin A-H^3 acetate	34.5	0.9
Vitamin B_{12}-Co^{60}	1054.	0.017
Vitamin D_3-H^3	57.7	0.34

tration of radioactivity/original standard concentration of radioactivity. For several vitamins, there was concentration of radioactivity within the intestinal wall (although we are as yet uncertain as to the chemical form of the species accumulating intramurally). For two vitamins, nicotinic acid and vitamin A acetate, the tissue accumulation was considerable. The tissue concentration of the vitamin is being maintained against the final mucosal concentration. Hence, the ratio of the tissue concentration of radioactivity/final mucosal concentration of radioactivity is shown in the third column of Table III.

Experiments in which the vitamin was placed on one side of the gut wall, and buffer on the other, are documented in Tables IV and V. These conditions allowed an approximation of the unidirectional fluxes of the vitamins across the gut wall (an approximation only, since the volumes on the 2 sides of the sacs were not identical; the mucosal volume was 5.0 ml while the serosal volume was 1.0 ml in all cases). The mucosal to serosal flux is tabulated in Table IV.

TABLE III

DATA INDICATING THE DISTRIBUTION OF THE VITAMINS AFTER 1 HOUR OF INCUBATION. FROM THE RADIOACTIVITY LOST FROM THE BATHING SOLUTIONS, AND THE WEIGHT OF THE GUT SACS, THE CONCENTRATION OF RADIOACTIVITY PER UNIT OF GUT WATER WAS CALCULATED. IN THE SECOND COLUMN THIS VALUE IS COMPARED WITH THE ACTIVITY OF THE ORIGINAL STANDARD. IN THE LAST COLUMN IT IS COMPARED WITH THE RADIOACTIVITY IN THE MUCOSAL SOLUTION AT THE END OF 1 HOUR.

Vitamin	*Final Ratio: Serosa Conc./ Mucosa Conc.*	*Tissue Conc./ Std. Conc.*	*Tissue Conc./ Final Mucosa Conc.*
p-Aminobenzoic acid-C^{14}	0.87	1.8	1.9
Ascorbic acid-C^{14}	0.95	1.2	1.3
Biotin-C^{14}	1.20	0.49	0.52
Foli acid-H^3	0.94	0.65	0.67
myo-Inositol-H^3	1.00	2.5	3.1
N-methylnicotinamide-C^{14}.Cl	0.85	0.96	0.99
Nicotinamide-C^{14}	1.00	1.7	1.9
Nicotinic acid-C^{14}	0.99	7.8	13.
Pyridoxine-H^3.HCl	0.68	1.8	1.8
Riboflavin-C^{14}	0.78	1.7	1.8
Thiamine-H^3	0.97	2.8	3.2
Thioctic acid-H^3	0.99	0.29	0.29
d, 1-α-Tocopherol-H^3	0.83	1.1	1.2
Vitamin A-H^3 acetate	1.00	12.	18.
Vitamin B_{12}-Co^{60}	0.92	0.41	0.41
Vitamin D_3-H^3	1.09*	4.3	5.3

*The mucosal solution in this case lost more radioactivity to the gut wall than the serosal solution. Hence, the ratio was greater than 1.0, but did not represent transport against a concentration gradient.

The first column give the ratio of the final serosal concentration/final mucosal concentration. In each case there was vitamin passage not only into the gut wall, but all the way across it into the serosal fluid. The second column lists the fraction of original material on the mucosal side that ended in the serosal fluid. The third column in Table IV lists the fraction of the original amount on the mucosal side that was still in the mucosal fluid. The sum of the figures in the second and third column, for each vitamin, equal 1.0 if there was full recovery, and less than 1.0 if some was present in the gut wall under these conditions (these conditions of vitamin on only one side of the sacs originally, of course, differ from the experiments which had the vitamin at the same concentration on both sides initially). Table V presents similar data for the serosa to mucosa flux. Again, each of the vitamins passed all the way across the gut wall, although d, 1-α-tocopherol and vitamin D_3 passed minimally. There was also a marked difference in the passage of nicotinamide and nicotinic acid.

DISCUSSION

One could reason, teleologically, that because vitamins occur in low concentrations in foods, and are necessary for body functions, efficient mechanisms might have evolved for their absorption from the small intestine. Of all the vitamins tested, only biotin moved against a concentration gradient under the experimental conditions. The mode of absorption of the other vitamins is sug-

TABLE IV

DATA ON THE FLUX OF VITAMINS FROM THE MUCOSAL FUID INTO THE SEROSAL FLUID, AFTER 1 HOUR OF INCUBATION. STANDARD DEVIATIONS WERE ABOUT 15 PER CENT OF THE REPORTED VALUES.

	Mucosa to Serosa Flux		
Vitamin	*Final Serosa Conc./ Final Mucosa Conc.*	*Fraction in Serosal Fluid*	*Fraction in Mucosal Fuid*
p-Aminobenzoic acid-C^{14}	0.46	0.08	0.89
Ascorbic acid-C^{14}	0.49	0.09	0.88
Biotin-C^{14}	0.72	0.12	0.84
Folic acid-H^3	0.33	0.06	0.88
myo-Inositol-H^3	0.50	0.08	0.75
N-methylnicotinamide-C^{14}.Cl	0.34	0.06	0.90
Nicotinamide-C^{14}	0.65	0.11	0.84
Nicotinic acid-C^{14}	0.41	0.05	0.62
Pyridoxine-H^3.HCl	0.35	0.07	0.92
Riboflavin-C^{14}	0.20	0.03	0.87
Thiamine-H^3	0.39	0.06	0.83
Thioctic acid-H^3	0.61	0.10	0.85
d, 1-α-Tocopherol-H^3	0.10	0.02	0.97
Vitamin A-H^3 acetate	0.53	0.08	0.74
Vitamin B_{12}-Co^{60}	0.17	0.03	0.84
Vitamin D_2-H^3	0.12	0.02	0.72

gested by the observation that they entered the gut wall by diffusion. In vivo this might be sufficient to allow translocation to the blood stream or lymphatics, and transfer throughout the body; this could be particularly true of vitamins which were bound to blood components, and hence had their free (uncombined) concentration reduced.

In vitro, 2 of the vitamins (nicotinic acid and vitamin A acetate) accumulated in especially high concentrations within the intestinal wall. This does not mean that they were in their original chemical form. Indeed, we have chromatographic evidence that nicotinic acid is converted to a different chemical species within the gut wall. Studies of the individual vitamins and their metabolic fates in the small intestine will be reported in subsequent communications.

Vitamins are known to occur within the lumen of the small intestine. They likely enter *via* the food, and are contained in bile, gastric juice and pancreatic fluid. The observations recorded here as to the serosal to mucosal passage of vitamins across the small gut suggest that intestinal secretion or "leakage" of vitamins (from the milieu interieur of the lumen) might be another means of entry. Studies on the outward fluxes would be of special interest in cases of intestinal malabsorption, since there is likely a component of malsecretion or increased gut permeability in these disorders.

TABLE V

DATA ON THE FLUX OF VITAMINS FROM THE SEROSAL FLUID INTO THE MUCOSAL FLUID, AFTER 1 HOUR OF INCUBATION. STANDARD DEVIATIONS WERE ABOUT 15 PER CENT OF THE REPORTED VALUES.

	Serosa to Mucosa Flux		
Vitamin	*Final Mucosa Conc./ Final Serosa Conc.*	*Fraction in Serosal Fluid*	*Fraction in Mucosal Fluid*
p-Aminobenzoic acid-C^{14}	0.18	0.50	0.46
Ascorbic acid-C^{14}	0.15	0.52	0.40
Biotin-C^{14}	0.10	0.68	0.32
Folic acid-H^3	0.26	0.64	0.36
myo-Inositol-H^3	0.13	0.57	0.38
N-methylnicotinamide-C^{14}.Cl	0.13	0.55	0.35
Nicotinamide-C^{14}	0.26	0.38	0.50
Nicotinic acid-C^{14}	0.07	0.50	0.17
Pyridoxine-H^3.HCl	0.09	0.49	0.21
Riboflavin-C^{14}	0.10	0.55	0.25
Thiamine-H^3	0.19	0.56	0.44
Thioctic acid-H^3	0.20	0.49	0.50
d, 1-α-Tocopherol-H^3	0.05	0.77	0.21
Vitamin A-H^3 acetate	0.07	0.45	0.16
Vitamin B_{12}-Co^{60}	0.05	0.73	0.02
Vitamin D_2-H^3	0.03	0.63	0.10

We are of the opinion that studies on the absorption of C^{14} or H^3 labeled vitamins in selected patients are feasible. There are, at least, two possible approaches. The first is to administer the vitamin orally and follow its appearance in the blood stream, as well as excretion in urine and feces. The second is to utilize segments of intestinal tissue obtained by means of per oral biopsy devices. We have noted that such tissue fragments accumulate amino acids and glucose when incubated *in vitro* (8). Such a procedure is being tried with selected vitamins. For the *in vivo* studies, we must pause a moment to consider the radiation dose being delivered to the patient. In terms of whole body radiation, let us look at the most unfavorable situation, that is one in which the vitamin distributes itself into all of the body water. A 70 kg man has 56 kg body water. Administration of 500 microcuries of a tritiated vitamin would produce 0.009 microcuries/ml body water. Sampling 0.1 ml of blood, with a counting efficienty of 10 per cent would produce 222 counts/minute. The beta radiation dose delivered, assuming the 0.009 microcurie/ml value, with a beta energy of 0.01 MEV and an effective half-life of one week (perhaps a reasonable value for the majority of vitamins), would be under 0.5 rads. For many of the vitamins the situation may be more favorable, and the radiation dose delivered would be less. For example, Johns and co-workers (9) found that intravenously injected folic acid-H^3 disappeared from the blood stream of man rapidly (only 0.1% per liter remained at 30 minutes) and that 1 to 70 per cent of the radioactivity appeared in the urine within 12 hours.

CONCLUSIONS

1. Of the radioactively labeled vitamins tested only biotin was transported by the *in vitro* hamster small intestine against a concentration gradient.
2. All of the vitamins entered the gut wall. Some (particularly nicotinic acid and vitamin A) achieved high concentrations within the wall, but may have been in altered chemical form.
3. All of the vitamins passed from the mucosal to serosal side when placed on the mucosal side alone (and from serosal fluid to mucosal fluid under the reverse conditions). The gut wall was relatively impermeable to vitamin D_3 and to d, 1-α-tocopherol.
4. The results suggest that vitamin entry into the body may be the result of diffusion (perhaps followed by tissue binding or chemical modification).
5. Because of the known rapid turnover of most vitamins, H^3 or C^{14} labeled vitamins can likely be used to follow vitamin absorption and metabolism in man without excessive radiation of the host.

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APPENDIX.

RADIOACTIVELY LABELED VITAMINS EMPLOYED IN THE PRESENT INVESTIGATION.

Vitamin	*Position of Radiolabel*	*Source*
p-Aminobenzoic acid	7-C^{14}	New England Nuclear Co.
Ascorbic acid	1-C^{14}	New England Nuclear Co.
Biotin	Carbonyl-C^{14}	New England Nuclear Co.; Amersham
	Carboxyl-C^{14}	Hoffman-LaRoche (Prof. O. Wiss)
	Tritiated	Isotope Specialty Co.
Folic acid	Tritiated	Amersham
myo-Inositol	2-H^3	New England Nuclear Co.
N-methylnicotinamide.Cl	methyl-C^{14}	New England Nuclear Co.
Nicotinamide	7-C^{14}	New England Nuclear Co.
Nicotinic acid	7-C^{14}	New England Nuclear Co.
Pyridoxine.HCl	Triatiated	Amersham
Riboflavin	Isoalloxazine-2-C^{14}	Canadian Dept. National Health (Dr. E. J. Middleton)
Thiamine	S^{35}	Hoffman-LaRoche (Dr. W. E. Scott)
	Tritiated	Vanderbilt Univ. (Dr. R. A. Neal)
Thioctic acid	Tritiated	Wilzbach labeling and purification
	S^{35}	Oklahoma State Univ. (Dr. F. R. Leach)
d, 1-α-Tocopherol	Tritiated	Amersham
Vitamin A acetate	15, 16-H^3	Hoffman-LaRoche (Prof. O. Wiss)
Vitamin B_{12}	Co^{60}	Abbott Laboratories
Vitamin D_3	Tritiated	Univ. Calif. (Dr. K. G. Scott)