MINIREVIEW

Thiamine Intestinal Transport and Related Issues: Recent Aspects (44538)

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Abstract. In the intestinal lumen thiamine is in free form and very low concentrations. Absorption takes place primarily in the proximal part of the small intestine by means of a dual mechanism, which is saturable at low (physiological) concentrations and diffusive at higher. Thiamine undergoes intracellular phosphorylation mainly to thiamine pyrophosphate, while at the serosal side only free thiamine is present. Thiamine uptake is enhanced by thiamine deficiency, and reduced by thyroid hormone and diabetes. The entry of thiamine into the enterocyte, as evaluated in brush border membrane vesicles of rat small intestine in the absence of H^+ gradient, is Na^+- and biotransformation-independent, completely inhibited by thiamine analogs and reduced by ethanol administration and aging. The transport involves a saturable mechanism at low concentrations of vitamin and simple diffusion at higher. Outwardly oriented H^+ gradients enhance thiamine transport, whose saturable component is a Na^+-independent electroneutral uphill process utilizing energy supplied by the H^+ gradient, and involving a thiamine/ H^+ 1:1 stoichiometric exchange. The exit of thiamine from the enterocyte, as evaluated in basolateral membrane vesicles, is Na^+-dependent, directly coupled to ATP hydrolysis by Na^+-K^+-ATPase, and inhibited by thiamine analogs. Transport of thiamine by renal brush border membrane vesicles is similar to the intestinal as far as both H^+ gradient influence and specificity are concerned. In the erythrocyte thiamine transport is a Na^+-independent, electroneutral process yet with two components: saturable, prevailing at low thiamine concentrations, and diffusive at higher. The saturable (specific) component is missing in patients of the rare disease known as thiamine-responsive megaloblastic anaemia (TRMA), producing a general disturbance of thiamine transport up to thiamine deficiency. The TRMA gene is located in chromosome 1q23.3. Recently, the thiamine transporter has been cloned: it is a protein of 497 aminoacid residues with high homology with the reduced-folate transporter.
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Thiamine (T^+) is required by animal cells to synthesize thiamine pyrophosphate, the coenzyme of the indispensable carbohydrate enzyme transketolase and the dehydrogenase complexes for pyruvate, α-ketoglutarate, and branched-chain keto acids (1). T^+ plasma concentration is regulated both by intestinal and renal mechanisms.

Chemically T^+ is a hydrosoluble organic cation (quaternary ammonium compound) with a high molecular weight (337 Da as hydrochloride). In animal tissues and foods, T^+ is present in four different forms: (i) free (T^+); (ii) monophosphate (TMP); (iii) pyrophosphate (TPP); and (iv) triphosphate (TTP). Total T^+ content (sum of all the forms) is usually in the order of few μg/g, TPP being the most abundant (about 87% of total thiamine) and functionally the best characterized compound. After a normal meal in the intestinal lumen, T^+ is mainly in the free form, since its phosphoesters have probably been completely hydrolyzed

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by different phosphatases of the gastrointestinal tract. The intraluminal concentration has been estimated to be lower than 2 μM (2) in man, and has been found to be less than 2 μM (3) in the proximal small intestine of rats. Hence the physiological intraluminal concentrations of T^+ appear to be very low, and at such concentrations intestinal absorption occurs.

Since the intestinal absorption of T^+ has been the focus of considerable investigation in our laboratory, our own results will be the primary basis for this Minireview. Previously, T^+ intestinal transport has been reviewed both separately (4, 5) and in the context of other water-soluble vitamins (6–8).

Thiamine Intestinal Absorption

Transepithelial Transport. As *in vivo* intestinal absorption of low T^+ concentrations shows, in all animal species there is a rate-limiting step, which suggests that saturable transepithelial transport is taking place (4). In humans, single oral doses of T^+ that are higher than 2.5–5 mg are largely unabsorbed (9, 10), with intestinal uptake following saturation kinetics (11). The process of T^+ intestinal absorption, as studied *in vitro* in human tissue, involves two mechanisms (5, 12, 13). At concentrations lower than 1 μmol/l, T^+ is transported mainly by an active, carrier-mediated system that involves the intracellular phosphorylation of the vitamin (14). At higher concentrations, simple passive diffusion prevails. Absorption takes place primarily in the proximal part of the small intestine (14, 15), with the rate decreasing both in the stomach and the colon.

In vitro, transport of physiological concentrations of T^+ by intact intestinal tissue has been studied mainly in rats using different preparations. The general features of the transport show that T^+ is transferred *via* an active process from mucosa to serosa against a concentration gradient in the rat (3, 16, 17), mouse (18), man (14, 15), chicken (19), and frog (20, 21).

The transport mechanism in rats is saturable and follows Michaelis-Menten kinetics with a maximal flux (J_{max}) of 5.2 $\mu mol \cdot g^{-1} \cdot h^{-1}$ (2), and Michaelis-Menten constant (K_m) values ranging from 0.16 to 0.63 μM (2, 22). The transport is inhibited by metabolic inhibitors, anoxia, low temperature (all suggesting energy dependence), and by different T^+ structural analogs. Interestingly, different animal tissues have been found to share similar transport properties for T^+ with the small intestine (23).

During intestinal transport, T^+ is phosphorylated both in everted jejunal sacs (24) and in isolated enterocytes (25), where T^+ transmembrane transport and its intracellular metabolic transformation form a typical tandem process producing an effective means of favoring membrane crossing. By using labeled T^+, the following forms of thiamine have been found in the intestinal mucosa of the rat (26): TPP (70%), TMP (12%); T^+ (17%) with only traces of TTP. However, 90% of the T^+ transferred to the serosal side is in free form, the remainder being TMP. These values are not very different from those usually found in other animal tissues (1) and show that T^+ during intestinal transport undergoes a phosphorylation-dephosphorylation process. However, during transport, the total (labeled plus unlabeled) mucosal contents of TPP remains virtually unchanged, whereas the labeled forms replace the unlabeled. It should be noted that unchanged TMP can also cross the intestinal mucosa by an active mechanism similarly to T^+, but less efficiently (27), and that TMP transport has been shown to occur as well in the blood-brain barrier (28, 29).

Currently, it is believed that intracellular TMP derives from TPP through enzymatic dephosphorylation catalyzed by a particulate, relatively specific thiamine pyrophosphatase (TPPase) (30, 31). However, in small intestinal brush border membrane vesicles (BBMV) of rats, TMP can also be produced by direct transphosphorylation of T^+ from appropriate phosphate donors normally present in the cell (32). The reaction is catalyzed by intestinal alkaline phosphatase, which is highly concentrated in the enterocyte membrane (33, 34), and involves less than 0.01% of the total inorganic phosphate released by enzyme activity.

In intact intestinal tissue, T^+ transport *in vitro* appears to be a Na^+-dependent process: incubation with a Na^+-free medium blocks transepithelial transfer (2, 21), but does not prevent T^+ entry into the cell and its phosphorylation (21). However, more recent evidence suggests that T^+ transport across brush border membranes is H^+-dependent (*vide infra*).

Membrane Transport. Apart from early investigations on T^+ unidirectional uptake using everted jejunal sacs (2), the particular aspects of transport were suitably studied using BBMV and basolateral membrane vesicles (BLMV), which are well-known preparations excluding metabolic and circulatory influences.

Brush Border Membrane Transport. In BBMV, enterocyte entry has been investigated both in the absence and presence of H^+ gradients. The time course of T^+ transport without H^+ gradient is not influenced by the presence of Na^+ or K^+ or by their absence in the incubation medium (35). At low, physiological concentrations (less than 1.25 μM), T^+ is taken up mainly by a saturable mechanism with apparent K_m = 0.8 μM and J_{max} = 0.35 pmol · mg protein^{-1} · 4 s^{-1}, whereas at higher concentrations a passive diffusion prevails. T^+ is not biotransformed during its transfer into intravesicular space, and the transfer is inhibited competitively by T^+ structural analogs and derivatives, including unlabeled T^+, TMP, pyrithiamine, 2-ethylthiamine, and 5-chloroethylthiamine, amprolium, 4′ -oxythiamine. Unlabeled T^+ and pyrithiamine are the most powerful inhibitors, whereas 4′-oxythiamine is much less effective. Levamisole and inorganic phosphate, which are inhibitors of T^+ phosphate dephosphorylation and T^+ transport (27, 36), are virtually ineffective. These findings suggest that membrane crossing in the enterocyte must be considered as being independent of the phosphorylation-dephosphorylation cou-

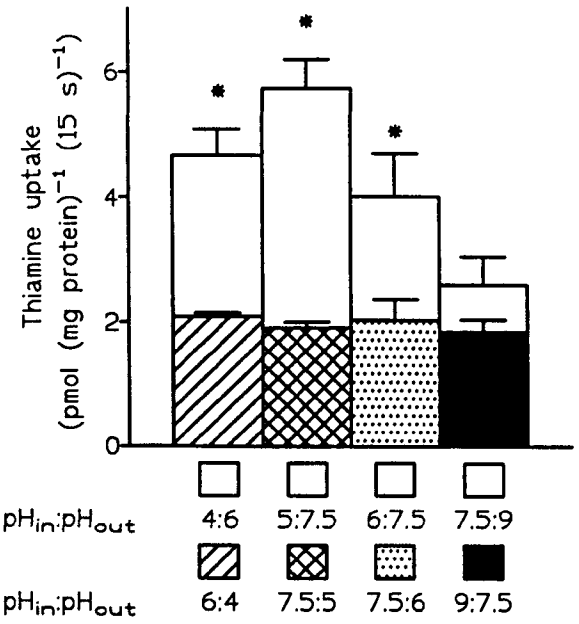

Figure 1. Thiamine uptake by rat proximal small intestinal microvillous membrane vesicles in the presence of an outwardly direct H^+ gradient (open bars) or inwardly directed H^+ gradient (filled bar) (50). Results are the mean ± SE of triplicate determinations for each of at least six different preparations.

pling of T^+, and that the brush border entry of T^+ occurs by Na^+-independent, facilitated diffusion.

As a quaternary ammonium compound, T^+ is positively charged (organic cation). It is well known that endogenous and exogenous organic cations can be transported into BBMV by organic cation/H^+ antiport systems both in the small intestine (37, 38) and in the renal tubule (39–41). However, T^+ can compete for transport with some organic cations, especially choline (another quaternary ammonium compound) in different preparations (42–47). Moreover, lipophilic organic cations almost completely inhibit T^+ uptake in isolated hepatocytes (48), whereas choline and other quaternary ammonium compounds are inhibitory of T^+ transport (49).

Recently, the problem of the behavior of T^+ as an organic cation has been investigated in a study of its transport by BBMV taken from the small intestine of rats (50). The presence of H^+ gradient across the vesicle membrane enhances T^+ uptake only when the gradient direction is from inside to outside ($pH_{in} < pH_{out}$), the transport rate increasing with the gradient value (Fig. 1). This suggests that the entry of T^+ into the enterocyte depends on countertransport of H^+ (T^+/H^+ antiport). In fact, the stoichiometry of T^+/H^+ exchange was found to be 1:1, indicating that T^+ intestinal transport is an electroneutral process. In line with this evidence, the antiportal H^+ exchange mechanism for T^+ transport has been demonstrated in rat liver sinusoidal membranes (51) and in human placental epithelium (52). In small intestinal BBMV, T^+ uptake is not influenced by changes in membrane electrical potential, thus confirming that T^+ transport is an electroneutral process as previously reported for intact intestinal tissue (2), basolateral membrane vesicles (53), and erythrocytes and ghosts (54). Electroneutral H^+/organic cation antiport systems have been reported in intestinal and renal vesicles of different animal species (37, 39, 40).

In BBMV the pH gradient (5_{in}:7.5_{out}) influences the relationship between membrane binding and intravesicular translocation of T^+ during transport. The intactness of the gradient mainly favors translocation, whereas its dissipation partially enhances membrane binding most likely at the external membrane face. In any case, T^+ antiportal transport maintains a defined saturable component, whose apparent kinetic constants are much higher than those recorded in the absence of H^+ exchange. At $pH_{in} = 5$ and $pH_{out} = 7.5$, J_{max} increases about five times, and the K_m about three times. Since passive permeability does not change, as shown by the unaltered value of its coefficient (K_D), one must conclude that H^+ gradient energizes T^+ intestinal transport.

To conclude, the saturable component of T^+ transport, as evaluated in BBMV, appears to be a Na^+- independent uphill process (35), which can use the energy supplied by the H^+ gradient (Fig. 2), and involves a 1:1 stoichiometry exchange of T^+ and H^+ (50).

As to the specificity of T^+/H^+ intestinal antiport (Table I), different T^+ analogs (especially pyrithiamine and amprolium) are strong inhibitors of transport, whereas organic cations like choline, tetraethylammonium (TEA), and creatinine, which are typical substrates of renal organic cations/H^+ antiport, are ineffective. This substrate specificity of T^+ intestinal T^+/H^+ antiport is different from that of hepatic T^+/H^+ antiport (51), which is unaffected by pyrithiamine and amprolium and is inhibited by choline and TEA. However, T^+/H^+ intestinal antiport also appears to be distinct from renal organic cation/H^+ antiport (Table I) and partially similar to renal Na^+/H^+ antiport (55, 56). T^+/H^+ intestinal antiport shares some substrate specificity with the intestinal guanidine/H^+ antiport, since T^+ and some analogs are able

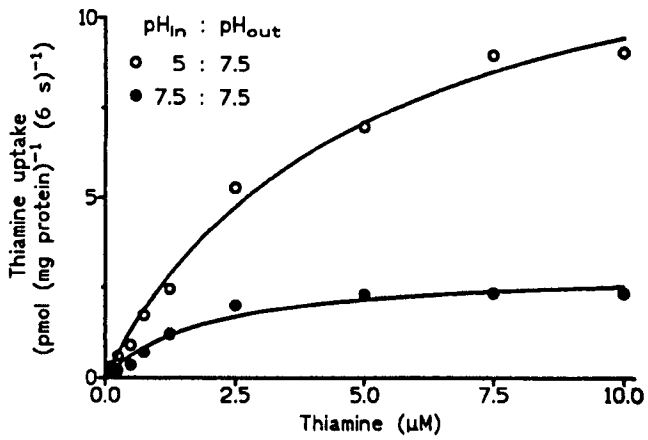

Figure 2. Saturable component of thiamine uptake by rat proximal small intestinal microvillous membrane vesicles in the presence of a pH gradient (pH_{in} 5: pH_{out} 7.5) (open symbols) and in the absence of a pH gradient ($pH_{in} = pH_{out} = 7.5$) (filled symbols) (50). Results are the mean of triplicate determinations for each of five different preparations. SE is within 10% of the mean values.

Table I. Effect of Some Cations and Thiamine Analogs on the Saturable Component of the Thiamine/H^+ Antiport in Rat Proximal Small Intestine Brush Border Vesicles (50)

Cation	Thiamine transport (% activity) Mean[a] ± SEM	Cation	Thiamine transport (% activity) Mean[a] ± SEM
None (control)	100.00	Organic II	
Inorganic (chloride salts)		Acetylcholine	93.3 ± 3.8
Na^+	103.7 ± 5.9	Histamine	89.4 ± 10.4
K^+	91.6 ± 11.4	Serotonin	63.6 ± 8.5
Li^+	90.4 ± 5.4	Spermidine	53.6[b] ± 16.3
NH_4^+	103.1 ± 11.1	Organic III	
Thiamine analogs		Guanidine	57.9[b] ± 10.5
4'-oxythiamine	49.0[b] ± 9.6	Amiloride	75.6 ± 11.8
Thiamine (unlabeled)	31.4[b] ± 4.8	Phenformin	37.3[b] ± 8.8
Amprolium	26.5[b] ± 2.4	Metformin	96.2 ± 7.0
Pyrithiamine	20.8 ± 6.9	Organic IV	
Thiochrome	70.3 ± 7.2	Harmaline	23.6[b] ± 7.2
Organic I		Clonidine	31.3[b] ± 6.7
Choline	92.4 ± 12.2	Imipramine	32.3[b] ± 2.9
Tetraethylammonium	98.2 ± 8.8	Organic V	
Creatinine	82.8 ± 8.3	Omeprazole	436.2[b] ± 84.2

Note. [^{3}H]Thiamine concentration, 1 μ*M*. Incubation time, 6 sec. H^+ gradient, pH_{in} 5 : pH_{out} 7.5. Cations and thiamine analogs were added to the incubation medium at an initial 0.1 m*M* concentration. Organic I, typical substrates of renal organic cation/H^+ antiport. Organic II, endogenous organic cations, inhibitors of the intestinal guanidine/H^+ antiport. Organic III, guanidine and derivatives. Organic IV, inhibitors of intestinal guanidine/H^+ antiport. Organic V, inhibitor of gastric (H^+–K^+)-ATPase.

[a] Mean of at least triplicate determinations for each of five different preparations, [b] $P \leq 0.05$ vs controls before transformation of data as percentage activity.

to inhibit guanidine/H^+ exchange, and guanidine and some derivatives can inhibit T^+/H^+ antiport (50). In placental brush border membrane, three distinct antiporters are present: for T^+, for guanidine, and for amiloride (52).

Interestingly the T^+/H^+ antiport mechanism can explain both the intestinal absorption and the secretion of T^+, as proposed by Aronson (57) for the Na^+-H^+ exchanger. From a thermodynamic point of view, the direction of T^+ flux, mediated by the T^+/H^+ exchange mechanism, depends on whether the electrochemical proton gradient ($\Delta\eta_{H+}$) across the membrane is higher or lower than the T^+ gradient ($\Delta\eta_{T+}$). Both gradients can be calculated with the following equation:

$$\Delta\eta_S = RT \cdot \ln[S]_{out}/[S]_{in} + F \cdot \Delta\varphi \cdot Z_S$$

where S is the cation transported, $\Delta\varphi$ is the electrical potential difference across the luminal cell membrane (50 mV) (58), and R, T, F,and Z have their usual meaning.

In the proximal jejunum, where the acidic microclimate at the luminal surface has the lowest pH, $[H^+]_{out} = 0.63$ μ*M* (59), and $[H^+]_{in} = 0.1$ μ*M* (60), $\Delta\eta_{H+}$ corresponds to 9.5 KJ/mol. This is the same tract, where, $[T^+]_{out} = 2$ μ*M* (3) and $[T^+]_{in}$ 0.17 μ*M* (36), and hence $\Delta\eta_{T+}$ corresponds to 11.2 KJ/mol. Moreover, both $\Delta\eta_{H+}$ and $\Delta\eta_{T+}$ are directed from lumen to cell. Under these assumptions, $\Delta\eta_{T+}$ is higher than $\Delta\eta_{H+}$, and T^+ can be absorbed in exchange with a H^+ that is secreted in the lumen.

In the distal small intestine, where the pH of the microclimate increases, reaching a value similar to the intracellular pH, $\Delta\eta_{H+}$ vanishes, whereas $\Delta\eta_{T+}$ remains unmodified and is the only driving force behind the T^+/H^+ antiport, determining T^+ entry.

Whenever $[T^+]_{in}$ increases, as may happen when large amounts of vitamin, especially in its lipophilic form, are ingested or intramuscularly injected, T^+ may also be secreted into the lumen. Actually the chick small intestine is not only able to absorb, but also to secrete T^+ into the lumen (61), particularly when high levels of vitamin are administered.

Monocationic T^+ entry into the enterocyte along its electrochemical gradient causes an outflow of H^+ according to a 1:1 stoichiometric ratio, thereby preserving cellular neutrality and maintaining the electroneutrality of T^+ transport by BBMV. As a whole, the T^+/H^+ antiport can mediate the uphill extrusion of H^+ driven by the downhill flow of T^+ into the enterocyte, where low intracellular concentration of T^+ is maintained by active basolateral extrusion of the vitamin *via* the Na^+-K^+-ATPase (53) (*vide infra*). This mechanism of transepithelial transport of the T^+ should show some analogies with that of Na^+.

In an attempt to obtain information on the molecular characteristics of the T^+ transporter, the effects of some reagents that modify the residues of specific aminoacids of protein were investigated on T^+ binding in the small intestinal (and renal) BBMV (62, 63). In both types of vesicles, the aminoacid residues apparently involved in T^+ binding are histidine, lysine, serine, tyrosine, and possibly arginine. In addition, carboxylic and sulfhydryl groups are also involved. These results fit in well with the conceptual model

of the intestinal T^+ transport system depicted by Komai and Shindo (64) and with the T^+ binding site of the soluble T^+-binding protein from *S. cerevisiae* proposed by Iwashima *et al.* (65).

Basolateral Membrane Transport. Small intestinal BLMV of rats with 63.8% inside-out sidedness have been used to investigate the *in vitro* enterocyte exit (53). The time course of T^+ uptake is hyperbolic, reaching equilibrium after 60–90 min of incubation. However, it is affected neither by Na^+ or K^+ in the incubation medium nor by modifications of the transmembrane electrical potential. At physiological concentrations (less than 1.25 μM), T^+ is transported mainly by a saturable mechanism with apparent Michaelis constant K_m = 1.32 μM and J_{max} = 1.93 pmol · mg protein^{-1} · 4 s^{-1}: 61% of the T^+ taken up is translocated into the vesicular space without any biotransformation. At concentrations higher than those indicated, an unsaturable uptake mechanism gradually prevails. Considering that the physiological intraluminar concentration of T^+ is considered usually lower than 2 μM (2, 3), it is apparent that the intestinal transport of T^+ is accounted for predominantly by saturable mechanisms at both the luminal (35) and contraluminal sides (53) of the enterocyte.

Na^+-K^+-ATPase appears to be directly involved in T^+ transport across BLMV (53) as the following results show. Different inhibitors of Na^+-K^+-ATPase activity are able to inhibit T^+ uptake effectively by BLMV. In the absence of ATP, ouabain, frusemide, and vanadate reduce uptake by 35%, 30, and 15%, respectively. The inhibition caused by ouabain is specific for both BLMV and T^+ uptake, and involves displacement of T^+ from its binding sites on the membrane, as suggested by results obtained with basolateral membrane sheets. Possibly, the much greater T^+ binding capacity of BLMV compared with BBMV may be ascribed to the almost exclusive presence of Na^+-K^+-ATPase in BLMV, to whose external configuration ouabain is able to bind.

The direct intervention of Na^+-K^+-ATPase in BLMV T^+ transport can be demonstrated by reproducing *in vitro* those conditions known to operate in the intact enterocyte. In the presence of Na_2ATP, and Na^+, K^+, and Mg^{2+}, appropriately distributed across the cellular membrane to allow efficient Na^+-K^+-ATPase activity, the rate of T^+ uptake shows an overshoot with an ≈ 85% increment (Fig. 3) compared with the control. Thus, under the experimental conditions associated with complete activation of Na^+-K^+-ATPase in the intact tissue, BLMV are able to transport T^+ actively as shown by the overshoot. Importantly, the overshoot disappears after altering the distribution of ions or changing the type of high-energy phosphate used, or by using BBMV. Since only T^+ structural analogs like pyrithiamine, amprolium, and 4′-oxythiamine, but not the organic cation choline, are able to decrease T^+ uptake significantly by BLMV, the strictly ATP-dependent uptake appears to be structurally specific for the vitamin.

The mechanism that is coupled directly to the hydrolysis of ATP (primary active transport) may account both for the well-known T^+ transport against a concentration gradient (16, 17) and for its overall Na^+-dependence (2, 21).

A hypothetical scheme for the transport of low (physiological) amounts of T^+ by the enterocyte is depicted in Figure 4. At low concentrations, T^+ is transported mainly by transcellular means. Entry at the luminal side is largely by exchange with H^+ (A) and very little by enzymatic transphosphorylation to TMP. Cellular crossing is associated with intracellular enzymatic phosphorylation to TPP and

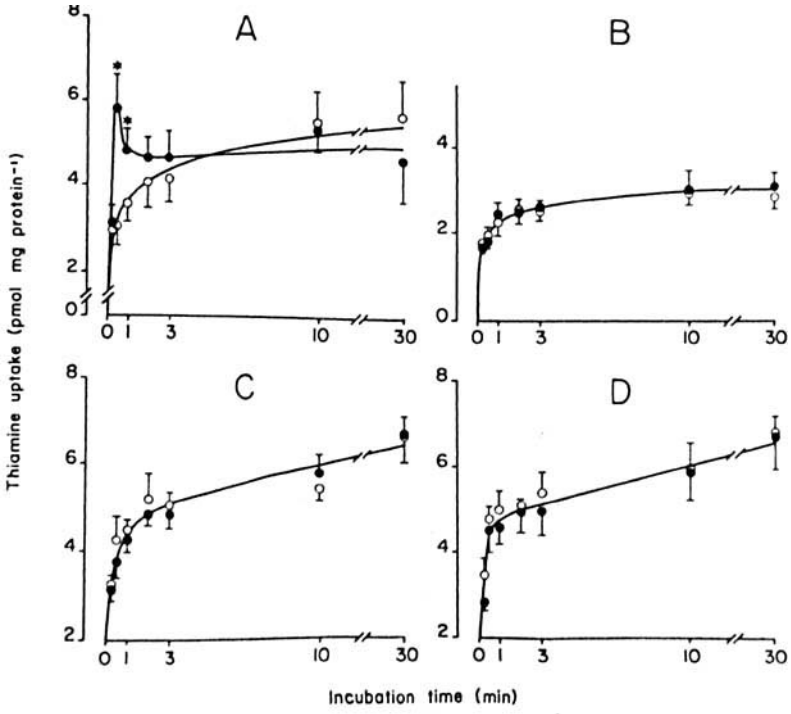

Figure 3. Specificity of the Na^+-K^+-ATPase–dependent transport of thiamine by rat small intestinal basolateral membrane vesicles (53). (A) basolateral membrane vesicles and (B) microvillous membrane vesicles; presence of Na_2-ATP (filled symbols) or Na_2SO_4 (controls; open symbols). C and D, same experimental conditions as in A, but Na_2-ATP was substituted with phosphocreatine (disodium salt) (C) or NaCl was substituted with isosmotic KCl (D). Control (open symbols) is Na_2SO_4. Results are the mean ± SE of five different preparations.

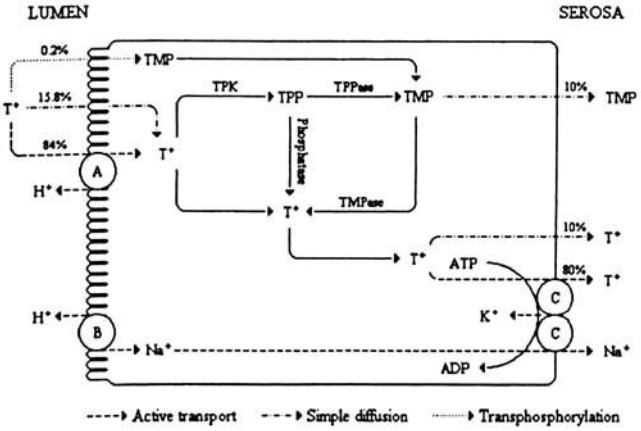

Figure 4. Transcellular thiamine transport by rat enterocyte: an updated model. The entry T is largely by exchange with H^+ (A) and very little by enzymatic transphosphorylation to TMP. Cellular crossing is associated with intracellular enzymatic phosphorylation to TPP. The exit is directly dependent on the activity of Na^+-K^+-ATPase (C). The numerical values are approximate and obtained from references: (32, 50) for the luminal entry; (21, 26, 53) for the serosal exit. T: thiamine; TMP, TPP: thiamine mono-, pyrophosphate; TPK: thiamine pyrophosphokinase; TMPase, TPPase: thiamine mono-, pyrophophatase. (A) T^+/H^+ antiport; (B) Na^+/H^+ antiport; (C) Na^+-K^+-ATPase.

dephosphorylation of TPP to TMP and T^+. The exit at the serosal side, apart from the small amounts diffusing as TMP and T^+, is mainly directly dependent on the activity of Na-K-ATPase (C).

Factors Influencing Thiamine Intestinal Transport. Factors influencing T^+ intestinal transport can be distinguished into hormonal and nonhormonal and may operate in the membrane and/or intracellular phase of transport (Fig. 4). In the membrane phase, as evaluated in small intestinal BBMV, a preparation involving only membrane processes, ethanol administration, and aging is effective.

Long-term ethanol administration to rats reduces T^+ uptake by BBMV (66). In this way ethanol could impair the intestinal absorption of T^+, as was shown both in rats (67) and humans (68, 69). However, Hoyumpa *et al.* (70) reported no modification of T^+ transport by everted jejunal sacs, a preparation mainly involving the intracellular phase of transport, from chronically ethanol treated rats. This apparent discrepancy may be ascribed exclusively to the type of preparation and experimental conditions.

Aging is associated with intrinsic alterations of the enterocyte plasma membrane resulting in a decreased affinity for T^+ (K_m increased) and an increased number of T^+ carriers (J_{max} increased) of the saturable component of T^+ transport, and a greatly reduced passive permeability to T^+ (K_D decreased) (71). The decreased diffusion could explain the reduced T^+ absorption *in vivo* by old rats (72, 73) receiving rather high doses of T^+, whose intestinal transport is predominantly a diffusive process (5, 35).

In the intracellular phase of T^+ transport, T^+ body status seems to be important. T^+ deficiency increases T^+ uptake as evaluated in everted jejunal sacs (74, 75). According to Patrini *et al.* (75), the net transport of labeled T^+ is enhanced in T^+-deficient rats. Everted jejunal sacs take up and phosphorylate labeled T^+ at a rate that is inversely related to the endogenous cellular content of phosphorylated T^+. All this suggests that the intestinal transporters of T^+ are downregulated by T^+ body concentrations (76). T^+ deficiency enhances the capacity of T^+ intestinal uptake in man as well (15).

The problem of the hormonal control of T^+ intestinal absorption is more complex and may involve both membrane and intracellular phases of transport. Judging from the results obtained with rat BBMV, it does not seem that thyroid hormone and insulin can control membrane T^+ uptake directly (77). However, thyroid hormone and insulin can modify T^+ intestinal absorption indirectly by influencing intestinal tissue, as has been shown for rat everted jejunal sacs. Whereas hypothyroidism does not modify T^+ transport, hyperthyroidism (T_3 administration) reduces both T^+ compound content in the intestinal tissue and net transport, probably as a consequence of a reduced phosphorylation rate of T^+ to TPP due to a reduced pyrophosphokinase activity (78). However, insulin deficiency (diabetes) increases TPP levels in the intestinal tissue, while reducing the net transport of T^+ and TMP (79).

Renal Transport of Thiamine

As could be expected, T^+ transport in the kidney tubule shows many analogies with intestinal transport. Reabsorptive T^+ uptake, as evaluated in the BBMV of the rat kidney cortex (80), is a Na^+-independent, electroneutral process, showing a biphasic course that is nonlinear (saturable) at physiological concentrations and linear at higher ones. T^+ uptake is enhanced several-fold by an outwardly directed H^+ gradient, and yet maintains a biphasic course. The saturable component has kinetic constants that are 12-fold compared with those in the absence of gradient, and is inhibited by T^+ structural analogs. Renal T^+/H^+ antiport has a stoichiometric ratio 1:1 and specificity similar to the intestinal one (80).

Thiamine Transport in Erythrocytes and Ghosts

It is generally accepted that the transport of T^+ at low concentrations by rat erythrocytes is a carrier-mediated process (81, 82). Recently T^+ transport was thoroughly investigated in human erythrocytes and ghosts, demonstrating its similarities with enterocyte transport (54). As in enterocytes, the transport of T^+ in erythrocytes is a Na^+-independent, electroneutral process, exhibiting two components (saturable and nonsaturable) with the former prevailing at T^+ concentrations less than 0.5 μM. The saturable component is a carrier-mediated process and follows Michaelis-Menten kinetics with high affinity and low capacity for T^+ (facilitated diffusion). The comparison between the vesicular membrane structures of BBMV and erythrocyte ghosts (54) shows that in both types of membranes, the saturable component of transport, which is prevalent at low (physiological) concentrations, displays affinities for T^+ of

the same order of magnitude and similar molecular specificities, because it is inhibited to approximately the same extent by unlabeled T^+ and some structural analogs. However, transport capacities seem to be quite different, being much lower for the ghosts (54).

It is noteworthy that in thiamine-responsive megaloblastic anaemia (TRMA), a rare disease also known as Rogers syndrome (OMIM 249270), associated with diabetes mellitus and sensorineural deafness, the saturable, specific component in T^+ uptake, normally prevailing at physiological concentrations of vitamin, is absent in erythrocytes, ghosts, and possibly other cells (83–85) (Fig. 5). This produces a generalized disturbance of T^+ uptake and cellular transport leading to a T^+ deficiency and thus to the above mentioned symptoms. The nonsaturable (diffusive) component of uptake is normally present, allowing the partial correction of clinical and biochemical abnormalities by administration of pharmacological doses of T^+. However, this does not affect T^+ transport, suggesting that a transport defect is the true cause of vitamin deficiency. Recently, the *TRMA* gene was localized on the long arm of chromosome 1, 1q23.3 (86). Moreover, fibroblasts from patients with TRMA lack high-affinity T^+ transport, and without a T^+ supplementation (10–30 n*M* T^+) they die in 5–14 days (87).

The Gene Cloning and Expression of the Human Thiamine Transporter

The gene for the solute T^+ transporter has recently been cloned (88–90) in the course of the study of the gene mutated in TRMA and of the human placental organic cation transporters. The *SLC19A2* gene (about 22 kb), also called *THTR-1* (88) or *ThT1* (90), maps to chromosome 1q24 and consists of six exons and five introns (89, 90). The gene encodes a protein of 497 amino acids (55.4 kDa and pI of 6.35) containing: 12 transmembrane domains, 3 potential N-glycosylation sites, 3 further sites, intracellularly located, with a consensus sequence for protein kinase C-dependent phosphorylation and an amino acid sequence (17 residues) that is a characteristic sign of G-protein-coupled receptors (90). This protein has high sequence homology with the reduced-folate transporter (40% identity and 55% similarity), but no significant homology with the yeast T^+ transporter Tri10 (91, 92). Thus T^+ transporter can be considered the second member of the folate transporter family. The expression of T^+ transporter in normal tissues, studied by Northern blot analysis, revealed that transcript is very high in skeletal muscle followed by heart and placenta, low in liver and kidney and almost undetectable in the brain and intestine (88–90). HeLa cells transient transfected with cloned cDNA showed that the T^+ transporter is Na^+-independent, stimulated by an outwardly directed H^+ gradient and has a high T^+ specificity (90).

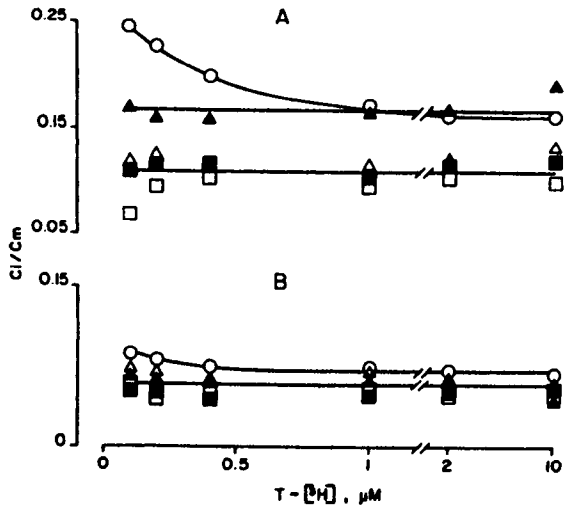

Figure 5. Effect of thiamine concentrations on the equilibrium (30 min) uptake of thiamine by (A) erythrocytes and the (B) ghosts in two cases of thiamine-responsive megaloblastic anaemia (TRMA) (84). Results are expressed as the ratios between [^{3}H]thiamine ([^{3}H-T])radioactivity in the cell (C_i) and that in the medium (C_m), and represent the mean of triplicate determinations for each of the last two experiments. The standard errors (SE) were within 10%. Erythrocytes and ghosts were obtained from: ■,□ Case 1: during S-benzoylmethyl (BOM)-thiamine treatment (■) and 3–5 days after temporary discontinuation of treatment (□). ▲, Δ Case 2: during BOM-thiamine treatment (▲) and 3–5 days after temporary discontinuation of treatment (Δ). O, aged-matched controls of either sex, from the same area.

Perspectives

Some basic questions remain to be addressed regarding T^+ intestinal transport. As suggested by Diaz *et al.* (89), since serum T^+ contents are normal in TRMA patients (93), an additional T^+ intestinal transporter not affected by SLC19A2 defects should exist and could be cloned. Moreover, as indicated above, the expression of cloned T^+ transporter is very high in skeletal muscle and almost absent in the intestine, kidney, and brain. These results are inconsistent with those of *in vivo* T^+ uptake, determined in turnover experiments followed by a compartmental model analysis, showing significantly higher values in the intestine and kidney than in skeletal muscle (94). The values of fractional rate constants and turnover rates of T^+ uptake are 40- and 25-fold higher in the small intestine and in the kidney, respectively. All this seems to suggest the presence of another T^+ transporter.

Moreover, the relationship between the transport of T^+ and the activity of the Na^+-K^+-ATPase, that appears to be directly involved in T^+ basolateral crossing (21, 53, 70), will be considered in further studies. In particular, does T^+ substitute one Na^+ or one K^+ from their sites in the Na^+-K^+-ATPase molecule, or does T^+ have its own specific site? Is the transport mechanism T^+ specific or can it be used by other organic cations?

Furthermore, the relationship between T^+ and folate absorption warrants investigation since the cloned T^+ transporter represents the second member of the folate transporter family (88–90), and previous investigations suggest reduced T^+ absorption occurring in the presence of folate malabsorption (95–97).

Finally, the issue of the identification of the amino

acids involved in the T^+ binding site in the cloned T^+ transporter by site-directed mutagenesis experiments (98) needs to be addressed.

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