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THE SODIUM ACTIVATION OF BIOTIN ABSORPTION IN HAMSTER SMALL INTESTINE *IN VITRO*

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SUMMARY

1. The uptake (equated, under our experimental conditions, to the medium→cell unidirectional flux) of biotin in hamster small intestine has been investigated under various conditions. The transport shows saturation kinetics ($v_{max} = 2.24\ (\pm 0.2) \cdot 10^{-2}$ μmoles·min^{-1}·ml^{-1} tissue water $\pm$ S.E. ($n = 5$); apparent $K_t = 1.0$ mM) and Na^+ dependence.

2. Preincubation in Na^+-free medium which presumably lowers the intracellular Na^+ content has no effect on the uptake of biotin during incubation in normal Krebs–Henseleit solution, suggesting a dependence on the extracellular rather than on intracellular Na^+ concentration.

3. The activation of biotin uptake by Na^+ is compatible with the formation of a ternary carrier–biotin–Na^+ complex.

4. The effect of various biotin analogues on biotin transport has been investigated, most of them showing a pattern of competitive inhibition. The molecular implications of this inhibition type are briefly discussed.

INTRODUCTION

To the best of our knowledge, biotin absorption in the small intestine has only been investigated by two research groups, namely TURNER AND HUGHES[1] and SPENCER AND BRODY[2]. They both measured the transmural flux of biotin during incubation periods of 1 h. TURNER AND HUGHES measured the relative decrease of the substrate concentration in the incubation medium by a microbiological technique. Over the concentration range investigated (10^{-6}–$7 \cdot 10^{-5}$ M), they found a reasonably linear relationship between biotin concentration and net biotin absorption. They concluded that this vitamin is absorbed by diffusion. SPENCER AND BRODY, on the other hand, used radioactive biotin and broadened the concentration range from 10^{-6} to 10^{-3} M, thereby finding saturation kinetics. The "apparent Michaelis constant", as determined from steady-state concentrations in the serosal fluid after 1 h incubation time, was $6 \cdot 10^{-5}$ M.

Our purpose was to investigate whether: (i) the transport of biotin in the small intestine follows saturation kinetics and is inhibited competitively by biotin analogues (and thus may be presumed to be carrier-mediated); (ii) it is stimulated by extracellular Na^+ as is known for a number of other transport systems[3].

Our data answer both questions positively; in addition, they permit some inference on the molecular requirements of the biotin transport system in hamster small intestine.

MATERIALS AND METHODS

Hamsters of average weight 100 g were fed ad libitum. They were killed by a blow on the neck. The intestine was removed and cleared of mesentery and adipose tissue, then everted on a metal rod and rinsed with either 0.9 % NaCl or isotonic choline chloride. Pieces of small intestine some 1–2 cm long were randomized and mounted on the apparatus described by SEMENZA[4] in order to expose only the mucosal face to the medium. The incubation was performed in a Dubnoff shaking water bath at 37° in a Krebs–Henseleit[5] buffer previously gassed for 1 h with a mixture of O_2–CO_2 (95:5, by vol.) and containing, in addition to the substrate, inulin as extracellular marker (5 mg/ml). The incubation was stopped by immersing the gut segments in ice-cold buffer for 15 sec; the intestine was then punched out, rinsed with ice-cold mannitol, gently blotted on filter paper, immediately weighed and homogenized in 1 ml water. The homogenate was then used for determining the biotin influx and the magnitude of the extracellular volume.

[^{14}C]Biotin was measured with a Nuclear-Chicago liquid scintillation counter after digesting an aliquot of the tissue homogenate in 2 ml NCS-Solubilizer overnight. The counting efficiency was 90 $\pm$ 2 %. Inulin was determined according to ROE *et al.*[6]. The inulin space varied between 3 and 7 %, when the endogenous inuloid material was subtracted. Mannitol as extracellular marker was abandoned after a few initial experiments since there is growing evidence of intracellular penetration of this compound[7–10].

The net amount of biotin accumulated was computed according to the following formula:

$$\frac{\mu\text{moles}}{\text{min}\cdot\text{ml tissue}} = \frac{(\text{dpm}\cdot\text{ml}^{-1}\text{ tissue accumulated}) - (f\cdot\text{dpm}\cdot\text{ml}^{-1}\text{ medium})}{\text{dpm}\cdot\mu\text{mole}^{-1}\text{ biotin}}$$

$$f = \frac{\text{inulin}\cdot\text{ml}^{-1}\text{ tissue}}{\text{inulin}\cdot\text{ml}^{-1}\text{ medium}}$$

dpm = decomp/min.

The tissue water has been assumed to be 80 % of tissue weight[11].

Substances

Biotin and thioctic acid were supplied by Fluka AG Switzerland. [*Carbonyl*-^{14}C]-Biotin, homobiotin, (—)-biotin, and biotin hydroxamic acid were a generous gift of Prof. Wiss and Dr. Weber, Hofmann-LaRoche, Basel, Switzerland.

The stability of biotin has been checked by paper chromatography (butanol–methanol–benzol–water (2:1:1:1, by vol.) and autoradiography according to RUIS *et al.*[12].

RESULTS

Time course of biotin uptake

The rate of biotin uptake in hamster intestine *in vitro* is almost linear during the first 15 min (see Fig. 1). The 15-min uptake was thus taken as a reasonably good measure of the lumen to cell unidirectional flux. In view of the low capacity of the biotin transport system, 15-min incubations were used routinely. Histological examination[13] did not show damage of the tissue up to 15 min incubation.

Form of biotin absorbed

The biotin recovered from the tissue homogenate has the same R_F value in *n*-butanol–acetic acid–water (4:1:1, by vol.) as that of the biotin in incubation medium and in the original solution. It is the only radioactive spot detected. Thus there is no demonstrable biological conversion of this compound during the incubation.

Saturation kinetics

Fig. 2 shows the Lineweaver–Burk plot of the biotin uptake in segments of hamster small intestine, with the indication of the S.E. of 5 experiments. The apparent K_t is 1.0 mM. The maximal velocity of biotin tissue uptake ($2.24\ (\pm 0.2) \cdot 10^{-2}$ μmoles·ml^{-1} tissue water·min^{-1}) is one order of magnitude higher than the value found by SPENCER AND BRODY[2] for transmural steady-state accumulation.

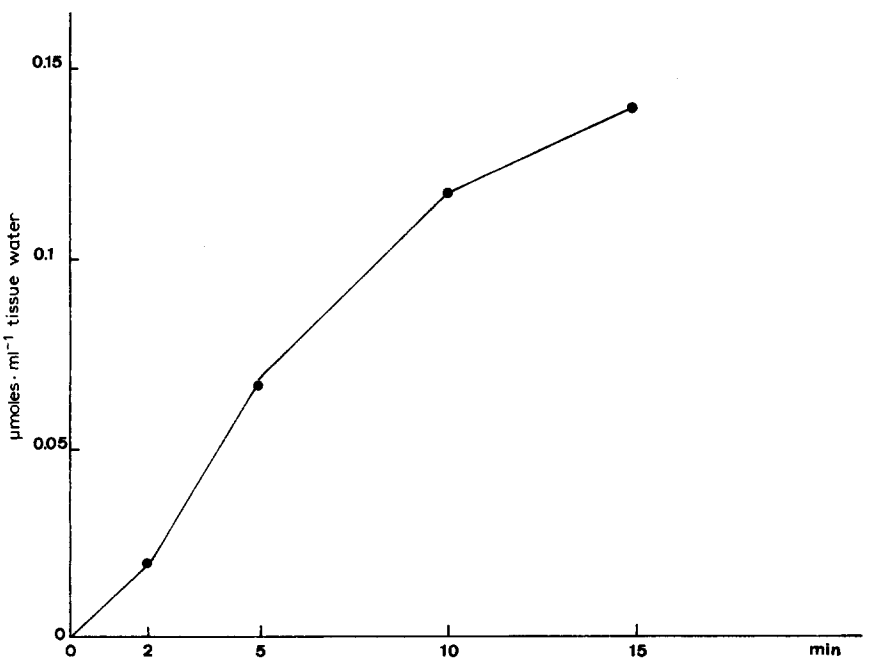

Fig. 1. Time course of biotin uptake in hamster small intestine segments. Biotin concentration, 1 mM.

Localization of maximal transport along the small intestine

Fig. 3 shows the absorption pattern of hamster intestine cut at the pyloric region and the ileocoecal valve. Our findings which refer to the uptake of biotin from the mucosal side agree with those obtained by SPENCER AND BRODY[2] for the transmural transport in everted sacs.

Dependence of Na^+ concentration

Total or partial substitution of Na^+ in the incubation medium by choline$^+$ reduces the amount of biotin transported by increasing the apparent K_t without influ-

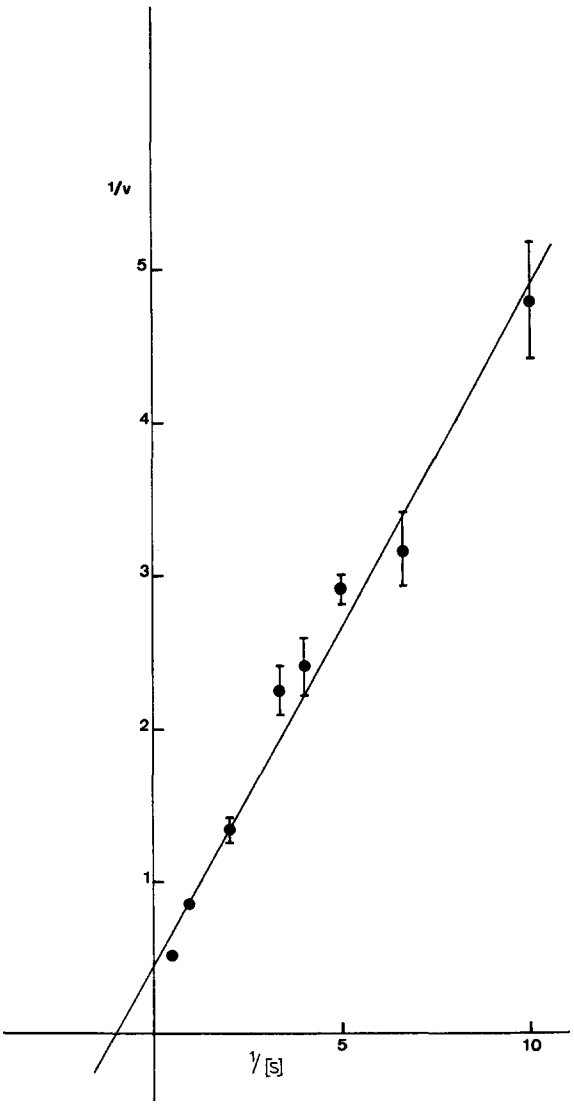

Fig. 2. Lineweaver–Burk plot of biotin uptake in hamster small intestine. Average of 5 experiments; incubations at 37° for 15 min; the bars indicate the S.E. K_t = 1.0 mM; v_{max} = $2.24 \cdot 10^{-2}$ μmoles·min^{-1}·ml^{-1} tissue water. v = μmoles ($\times 10^{-2}$) biotin taken up·min^{-1}·ml^{-1} tissue water; [S] = biotin concentration in the medium (mM).

encing the maximal velocity within the limits experimental error. The value found for $1/v_{max}$ computed by the least-squares fit method amounts to 0.444 $\pm$ 0.048 S.E. ($n = 5$). Fig. 4 shows the Lineweaver–Burk plot of biotin uptake plotted against the biotin concentration at various Na^+ concentrations in the incubating medium. Fig. 5 shows the secondary plots of $1/K_t$ *versus* [Na^+] and K_t *versus* $1/[Na^+]$.

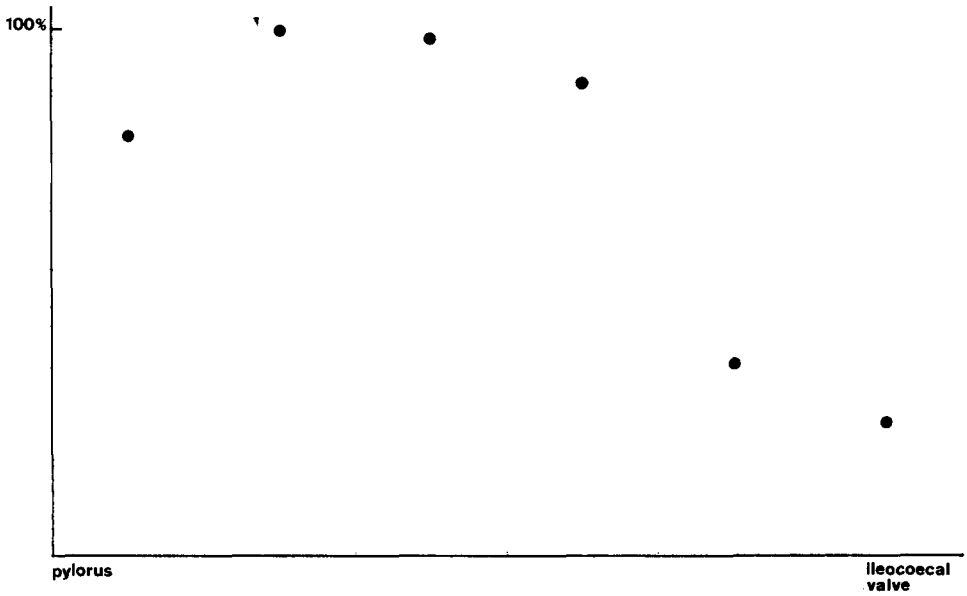

Fig. 3. Biotin absorption along the small intestine (as percent of the highest value; average of 2 experiments). The intestine was cut into 6 segments of equal length; incubation under same conditions as indicated on Fig. 2. Biotin concentration, 1 mM.

In some experiments, Na^+ was replaced by K^+ instead of by choline$^+$; the uptake of biotin under this condition after correction for the extracellular space was essentially zero. Li^+ inhibits biotin uptake less strongly than K^+.

Extracellular versus intracellular Na^+

The Na^+ dependence of biotin transport being clearly established, it seems important to examine whether the transport depends on the extracellular or on the intracellular Na^+ concentration. According to BOSAČKOVÁ AND CRANE[14] and to SCHULTZ *et al.*[15], preincubation in Na^+-free medium for 20 min lowers the intracellular Na^+ concentration; subsequent incubation in buffers of standard composition restores the original intracellular Na^+ concentration after not less than 30 min (ref. 14).

Experiments of this kind have been performed under the conditions specified in Table I. A possible inhibition of metabolic processes by high intracellular K^+ is not ruled out. It is significant, however, that both K^+ and choline$^+$ substitution produced similar results.

The following conclusions can be drawn:

(i) Without Na^+ in both preincubation and incubation, the uptake of biotin is the lowest ($P < 0.001$).

(ii) When Na^+ is present in the incubation, the uptake exceeds the value obtained in Na^+-free medium (K^+: $P < 0.02$; choline$^+$: $P < 0.001$).

(iii) Preincubation in Na^+-free medium, when Na^+ is replaced by choline$^+$, does not affect the uptake in normal medium ($P > 0.4$).

(iv) Biotin uptake in the presence of K^+ is lower than in the presence of choline$^+$. The difference, however, is not statistically significant, which is probably due to the large scatter at this low level of uptake.

Therefore, biotin uptake is clearly more sensitive to extracellular than to intracellular Na^+.

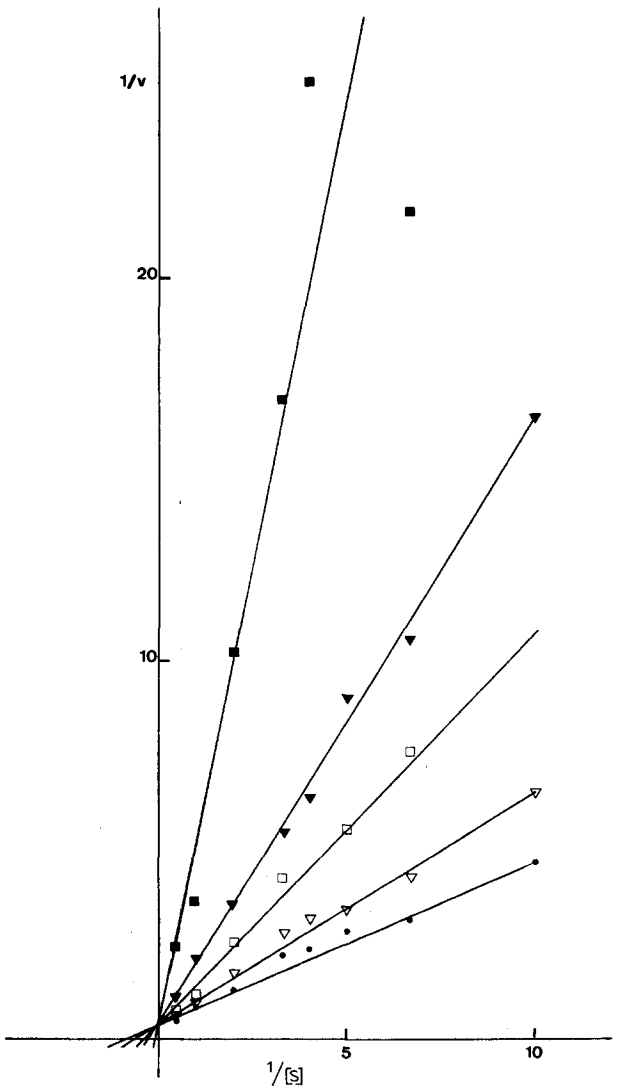

Fig. 4. Na^+ dependence of biotin transport. For incubating conditions, see legend of Fig. 2. Na^+ concentration (mequiv/l): ●, 140 ($n = 5$); ▽, 45 ($n = 2$); □, 30 ($n = 1$); ▼, 15 ($n = 4$); ■, 0 ($n = 1$).

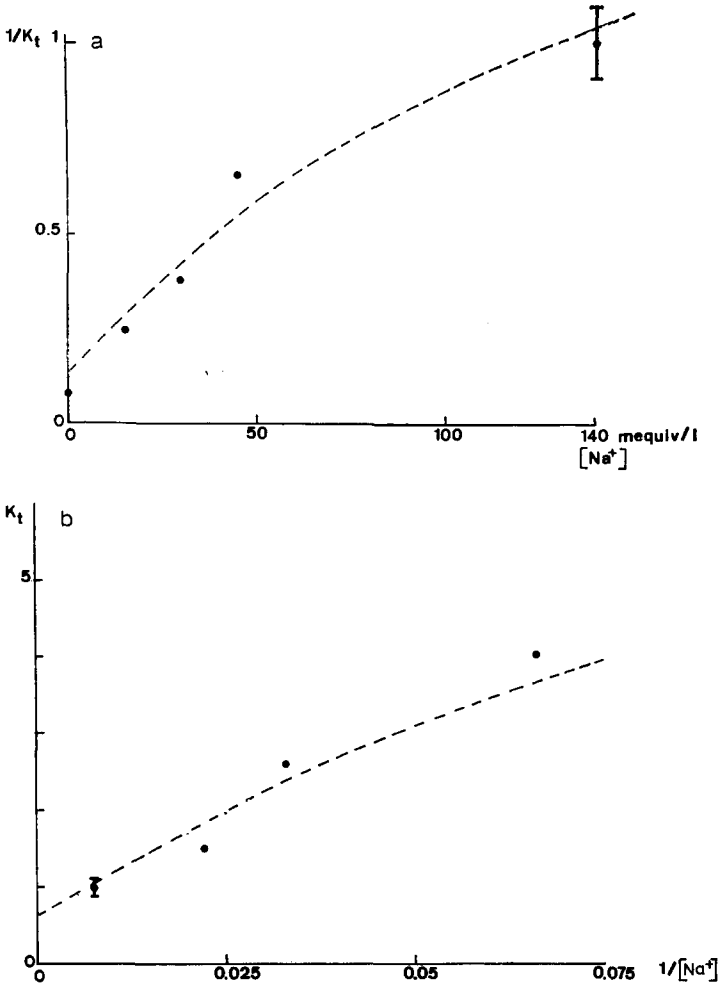

Fig. 5. Secondary plots of the data shown in Fig. 4. (a) $1/K_t$ *vs.* [Na^+]. (b) K_t *vs.* $1/[Na^+]$. The dotted line was computed for the model of non-compulsory reaction sequence, using the K values reported in APPENDIX.

Effect of biotin analogues on biotin uptake

The following substances were tested: thioctic acid, (—)-biotin, homobiotin and biotin hydroxamic acid. Fig. 6 reports an example of the inhibition of biotin uptake by thioctic acid. Experiments at various concentrations of the inhibitor indicated a competitive type and a K_i of approx. 0.35 mM. (—)-Biotin also appears to be a competitive inhibitor (Fig. 7), the K_i varying between 1 and 4 mM. Homobiotin does inhibit; but both the nature of inhibition and the K_i could not be determined with security. If a competitive inhibition is also assumed for homobiotin, its K_i must be larger than 15 mM. Biotin hydroxamic acid was not found to inhibit up to a concentration of 4.5 mM. It should be noted that an accurate kinetic investigation of these inhibitors was hampered by the small capacity of the biotin transport system and by the limited solubility of biotin and of its analogues.

TABLE I

EFFECT OF PREINCUBATION IN Na^+-FREE MEDIA

(*a*) *Na^+ replaced by K^+*: The amount of biotin accumulated given in % of control ± S.E. ($n = 4$). All data were corrected for the extracellular space. Preincubation for 20 min at 37°; incubation for 15 min at 37°. Biotin concentration, 1 mM. (*b*) *Na^+ replaced by choline$^+$*: The amount biotin accumulated given in % of control ± S.E. ($n = 6$). Preincubation for 15 min at 37°; incubation for 15 min at 37°. Biotin concentration, 0.5 mM.

Preincubation	*Incubation*	*Biotin accumulated* (%)
(*a*) *Na^+ replaced by K^+*		
Na^+	Na^+	100*
Na^+	K^+	52.5 ± 2.0
K^+	Na^+	62.7 ± 2.4
K^+	K^+	10.2 ± 4.0
(*b*) *Na^+ replaced by choline*		
Na^+	Na^+	100**
Na^+	Choline$^+$	32.5 ± 6.5
Choline$^+$	Na^+	114.1 ± 15.3
Choline$^+$	Choline$^+$	22.4 ± 6.0

* 100 % = 9.0 nmoles·min^{-1}·ml^{-1} tissue water.
** 100 % = 3.9 nmoles·min^{-1}·ml^{-1} tissue water.

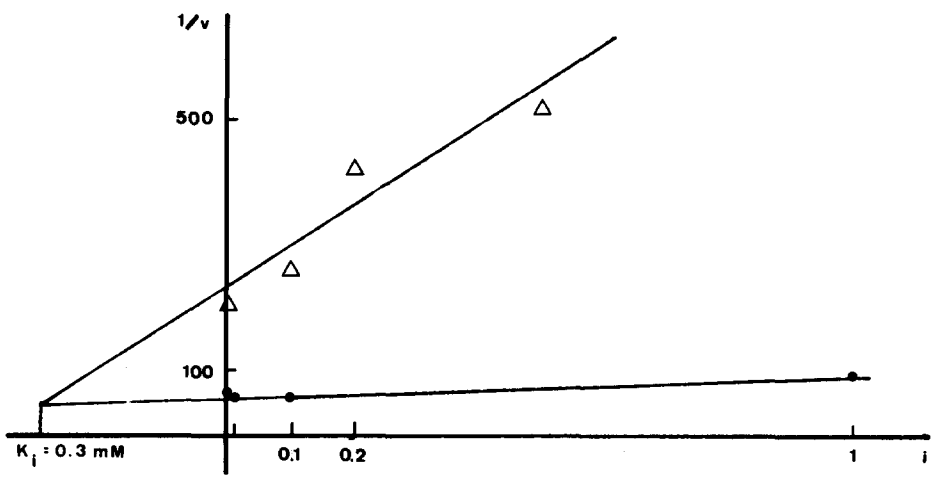

Fig. 6. Inhibition of biotin uptake by thioctic acid. Dixon plot at 0.1 (△) and 1 (●) mM biotin. [*I*] = concentration of thioctic acid (mM).

Effect of sulphate

When the isotonic chloride in the Krebs–Henseleit buffer was substituted with 0.111 M Na_2SO_4, a 10 to 40 % increase of biotin uptake was found.

DISCUSSION

The present investigation was undertaken in the hope of finding an intestinal transport system having a very low K_t. A water-soluble vitamin occurring in trace

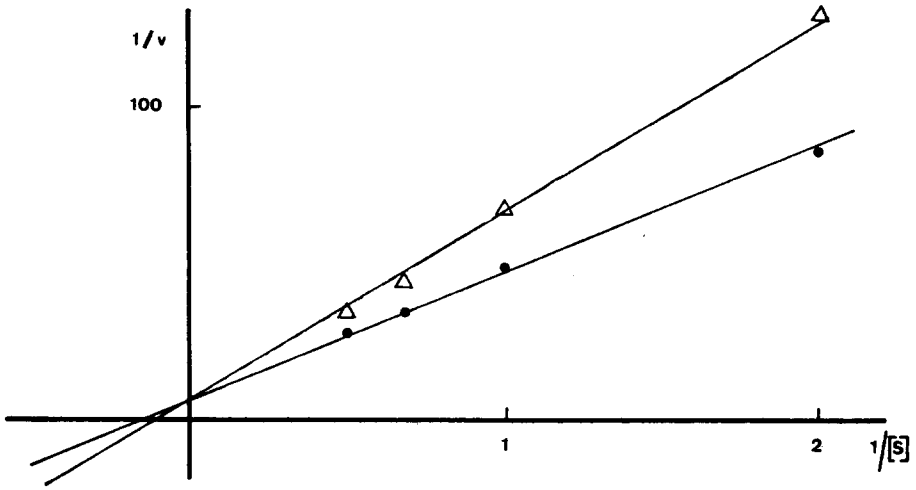

Fig. 7. Inhibition of biotin uptake by (—)-biotin. Lineweaver–Burk plots without (●) or with (△) 2.5 mM (—)-biotin.

amounts in the diet, such as biotin, was the obvious candidate. Much to our disappointment, the biotin transport system turned out to have an apparent K_t not much smaller than that of the glucalogue system, and to have instead a much lower v_{max}, namely, 2 orders of magnitude smaller than that of glucalogues. This is probably the reason why previous authors renounced the investigation of the uptake of biotin and preferred to measure the transmural transport. Our task was rendered possible by the availability of a procedure for the study of intestinal uptake which requires a very small correction of the extracellular space, and exposes the mucosal side alone to the medium[4]; the uptake values have only to be corrected for the little fluid trapped at the mucosal surface.

Substitution of sulphate for chloride in the incubation media did increase biotin uptake. However, we chose not to investigate this effect further and routinely used Krebs–Henseleit buffer of standard composition.

Na^+ activation

Our data clearly show that biotin intestinal uptake depends on the extracellular Na^+ concentration (Fig. 4, Table I). The present paper, therefore, adds one more solute to the list of substrates the transport of which is Na^+-dependent in the small intestine. (For a review, see ref. 3.)

In our kinetic analysis it was assumed that our measurements are directly proportional to the unidirectional flux from the lumen into the mucosal cells, and that no transinhibition is brought about by either intracellular biotin or Na^+. (The intracellular concentration of biotin has been measured by TURNER AND HUGHES[1] using a microbiological technique: it is $27 \cdot 10^{-13}$ M/cm intestine.) That this assumption is probably valid, is shown by the linear time course of biotin uptake under our conditions (Fig. 1).

Extracellular Na^+ activates biotin transport by lowering the apparent K_t without effecting the apparent maximum velocity. Similar kinetic patterns were described earlier for sugar uptake in hamster small intestine[17] and for amino acid

uptake in rabbit small intestine, recently reviewed by SCHULTZ AND CURRAN[3]. Fig. 5 shows how the apparent K_t changes with Na^+. Whatever the model, our data on the lumen to cell unidirectional flux (saturation, Na^+ activation, inhibition by biotin analogues, see next paragraph) are clearly compatible with a carrier-mediated transport mechanism. However, a minor "diffusional" component in biotin intestinal uptake cannot be excluded (and might actually be responsible for much of the uptake in the absence of Na^+). At very high concentrations of biotin, its uptake seems to keep on increasing slowly (E. Long, unpublished experiments). The limited solubility of biotin prevented a careful study of this phenomenon.

The kinetic models for Na^+ activation of biotin transport will be discussed on the basis of the equations reported in APPENDIX. Two experimental observations indicate that the "permeability coefficients" of the various forms of the carrier (or their physical equivalents) are essentially equal: (i) the v_{max} values are the same, irrespective of whether Na^+ is present or not (see Fig. 4): thus, $P_3 = P_4$; (ii) the time-course of the uptake is essentially linear for quite some time (see Fig. 1): thus, during this time, no trans-inhibition of either substrate or Na^+ is detectable. This in turn indicates that the P values are equal. (For the pertinent equations, see ref. 21).

In equilibrium carrier kinetics $1/K_t$ is a linear function of Na^+ in the compulsory reaction sequence (X: carrier; S: substrate):

$$S + X \rightarrow XS$$
$$XS + Na^+ \rightarrow XSNa^+$$

The data shown in Fig. 5a tend to exclude this reaction sequence. The opposite reaction sequence, namely:

$$Na^+ + X \rightarrow XNa^+$$
$$XNa^+ + S \rightarrow XSNa^+$$

predicts instead that K_t is a linear function of $1/[Na^+]$.

Our data shown in Fig. 5b, would be compatible with this kinetic model. However, they are also compatible with a model of non-compulsory sequence of activation, as shown by the dotted lines in Figs. 5a and 5b. Since some biotin uptake, albeit small, is observed even in the absence of Na^+ (but some Na^+ may have been present in the "Na^+-free" media, originating from the tissue) we favour the model of non-compulsory reaction sequence. The "dissociation constants" given in APPENDIX, and used in computing the dotted lines in Figs. 5a and 5b, were calculated from Eqn. A-4. They are valid as long as the model chosen is actually correct. Furthermore, these constants are based on experiments carried out in media containing 5.9 mequiv/l K^+. The effect of this cation (competition with Na^+ (refs. 15 and 16) but also of other kinds[3]) is included in their numerical values.

Summing up, the data are compatible mainly with a non-compulsory reaction sequence, but also with one of the compulsory reaction sequences. A final choice between these alternatives was hampered by the low capacity of this transport system.

The effect of biotin analogues

The following compounds were tested: thioctic acid (Fig. 6), (—)-biotin (Fig. 7), homobiotin and biotin hydroxamic acid. Their effect is compatible with the hypothesis

that the side chain and more precisely, the free carboxyl group is involved in the carrier–substrate interaction. All inhibiting compounds have similar side chains with a free carboxyl group; when this function is substituted to form the hydroxamate of biotin, no inhibition is demonstrable. Our results extend those of SPENCER AND BRODY[2]. They tested actithiazic acid, desthiobiotin, diaminobiotin and thioctic acid, all of them having the free carboxyl group and all of them showing an inhibitory effect. However, they also observed that two compounds having no free carboxyl group did inhibit biotin uptake: biotin methyl ester and biocytin. KOIVUSALO and co-workers[18,19] described an enzyme (biotinidase), which is capable of cleaving these substrates, releasing free biotin. They demonstrated this enzyme activity in rat intestinal homogenates. As SPENCER AND BRODY[2] pointed out, it is possible that the inhibiting effect of these two compounds is due rather to the dilution of the substrate by admixture of the released biotin moieties. This argument, of course, is valid only if biotinidase is located externally to the biotin transport system, which is not known; nor it is known whether biotinidase cleaves biotin hydroxamate or not.

Finally, it remains to be examined whether the biotin analoga tested are competitive inhibitors or substrates for biotin's transport system.

APPENDIX

The rate equations for unidirectional flux in Na^+-activated membrane transport systems

Symbols: they are defined as given in Fig. 8 and as follows:

$$\frac{X\cdot Na}{XNa}=K_1 \qquad \frac{X\cdot S}{XS}=K_2 \qquad \frac{XNa\cdot S}{XNaS}=K_3 \qquad \frac{XS\cdot Na}{XNaS}=K_4$$

$$\frac{X'\cdot Na'}{XNa'}=K_5 \qquad \frac{X'\cdot S'}{XS'}=K_6 \qquad \frac{XNa'\cdot S'}{XNaS'}=K_7 \qquad \frac{XS'\cdot Na'}{XNaS'}=K_8$$

X_t is the total carrier concentration, the P's are the permeability coefficients (or their physical equivalents) of the various forms of carrier. J_s^i is the unidirectional flux of the substrate across the membrane. $J^i_{s\,(S',\,Na^+=o)}$ is the unidirectional flux across the membrane at zero trans-concentrations of both substrate and Na^+. The velocity of uptake measured in the present paper (v, in μmoles$\cdot$min$^{-1}\cdot$ml^{-1} tissue water) is proportional to it.

Assumptions

In deriving the equations of Table II the following assumptions were made:

(a) The carrier binds one substrate and one Na^+.

(b) The rate-limiting step in the unidirectional flux is the translocation of the carrier through the membrane (or its physical equivalent), so that carrier, substrate and Na^+ can be considered as being always in equilibrium at each face of the membrane.

(c) In the steady state there is no buildup of carrier anywhere in the membrane (*i.e.* that the total velocity of translocation of carrier molecules in its various forms is the same in both directions).

(d) The permeability coefficients (P, or their physical equivalents) for a given carrier form are equal in both directions.

It was not assumed that necessarily $K_1 = K_5$, $K_2 = K_6$, *etc.*; from the definitions of the K's, however, it follows that $K_1K_3 = K_2K_4$ and that $K_5K_7 = K_6K_8$.

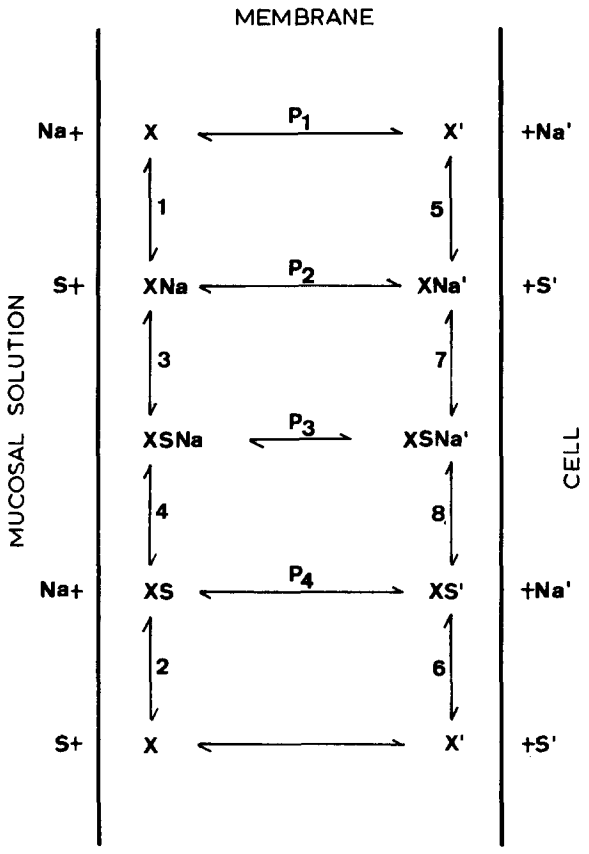

Fig. 8. A general model for the formation of a tertiary complex comprising a membrane site ("carrier" X), a substrate (S) and Na^+.

Equations

The rate equations for both J_s^i and $J_{s\,(S',Na'=0)}^i$ are given in Table II for the three main models: that of non compulsory reaction sequence, that in which the carrier must bind first the substrate and then Na^+, and that in which the carrier must bind first Na^+ and then the substrate. The K_t values for $J_{s\,(S',Na'=0)}^i$ plots are also given. (K_t is defined as "apparent Michaelis constant" or the negative reciprocal of the intercept on the $1/[S]$ axis in $1/J_{s\,(S',Na'=0)}^i$ plots). It will be noted that the $K_t = f[Na^+]$ functions are characteristically different in the three models. In fact, no simple linearity is found in the non-compulsory reaction sequence. If the carrier binds the substrate first, $1/K_t = a + b\ Na^+$ ($a,b > 0$); if the carrier binds Na^+ first, $K_t = a' + b'/Na^+$ ($a',b' > 0$). See Eqns. A-7 and A-11.

Each equation can easily be changed slightly if one assumes a given relationship among the P values or among the K values. For example, the rate equation for the model best describing the absorption of sugars in rabbit ileum is obtained from

TABLE II

RATE EQUATIONS OF Na^+-ACTIVATED UNIDIRECTIONAL FLUXES

(*A*) *Noncompulsory reaction sequence* ($o < K's < \infty$)

$$J_s^i = \frac{X_t(P_3 + P_4K_4/Na)}{1 + \frac{K_3}{S} + \frac{K_4}{Na} + \frac{K_3K_1}{S \cdot Na} + \left(1 + \frac{K_7}{S'} + \frac{K_8}{Na'} + \frac{K_7K_5}{S' \cdot Na'}\right) \frac{P_3 + P_2\frac{K_3}{S} + P_4\frac{K_4}{Na} + P_1\frac{K_3K_1}{S \cdot Na}}{P_3 + P_2\frac{K_7}{S'} + P_4\frac{K_8}{Na'} + P_1\frac{K_7K_5}{S' \cdot Na'}}} \quad \text{(A-1)}$$

Idem, with negligibly small trans concentrations of both Na^+ and substrate

$$J^i_{s(S',Na'=0)} = \frac{X_t(P_3 + P_4K_4/Na)}{1 + \frac{P_3}{P_1} + \frac{K_4}{Na}\left(1 + \frac{P_4}{P_1}\right) + \frac{K_3}{S}\left(1 + \frac{P_2}{P_1} + 2\frac{K_1}{Na}\right)} \quad \text{(A-2)}$$

Apparent Michaelis constant (K_t) of Eqn. A-2:

$$K_t = K_3 \frac{P_1 + P_2 + 2P_1K_1/Na}{P_1 + P_3 + (P_1 + P_4)K_4/Na} \quad \text{(A-3)}$$

For $P_1 = P_2 = P_3 = P_4$,

$$K_t = K_3 \frac{Na + K_1}{Na + K_4} \quad \text{(A-4)}$$

(*B*) *Compulsory reaction sequence in which the carrier binds first the substrate and than* Na^+ (K_1 and $K_5 = \infty$; thus K_3 and $K_7 = o$)

$$J_s^i = \frac{X_t(P_4 + P_3Na/K_4)}{1 + \frac{Na}{K_4} + \frac{K_2}{S} + \left(P_4 + P_3\frac{Na}{K_4} + P_1\frac{K_2}{S}\right) \times \frac{(1 + K_8/Na' + K_8K_6/Na' \cdot S')}{(P_3 + P_4K_8/Na' + P_1K_8K_6/Na' \cdot S')}} \quad \text{(A-5)}$$

Idem, with negligibly small trans concentrations of both Na^+ and substrate:

$$J^i_{s(S',Na'=0)} = \frac{X_t(P_4 + P_3Na/K_4)}{1 + P_4/P_1 + Na/K_4(1 + P_3/P_1) + 2K_2/S} \quad \text{(A-6)}$$

Apparent Michaelis constant (K_t) of Eqn. A-6:

$$K_t = \frac{2K_2}{1 + \frac{P_4}{P_1} + \frac{Na}{K_4}\left(1 + \frac{P_3}{P_1}\right)} \quad \text{(A-7)}$$

(*Table II continued*)

For $P_1 = P_3 = P_4$,

$$K_t = \frac{K_2}{1 + \mathrm{Na}/K_4}; \qquad \frac{1}{K_t} = \frac{1 + \mathrm{Na}/K_4}{K_2} \tag{A-8}$$

(C) *Compulsory reaction sequence in which the carrier binds first Na^+ and than the substrate* (K_2 and $K_6 = \infty$; thus K_4 and $K_8 = 0$)

$$J_s^i = \frac{X_t P_3}{1 + \frac{K_3}{S}\left(1 + \frac{K_1}{\mathrm{Na}}\right) + \left[1 + \frac{K_7}{S'}\left(1 + \frac{K_5}{\mathrm{Na}'}\right)\right] \frac{P_3 + \frac{K_3}{S}\left(P_2 + P_1 \frac{K_1}{\mathrm{Na}}\right)}{P_3 + \frac{K_7}{S'}\left(P_2 + P_1 \frac{K_5}{\mathrm{Na}'}\right)}} \tag{A-9}$$

Idem, with negligibly small trans concentrations of both Na^+ and substrate:

$$J^i_{s(S', \mathrm{Na}' = 0)} = \frac{X_t P_3}{1 + \frac{P_3}{P_1} + \frac{K_3}{S}\left(1 + \frac{P_2}{P_1} + 2\frac{K_1}{\mathrm{Na}}\right)} \tag{A-10}$$

Apparent Michaelis constant (K_t) of Eqn. A-10:

$$K_t = K_3 \frac{1 + \frac{P_2}{P_1} + 2\frac{K_1}{\mathrm{Na}}}{1 + \frac{P_3}{P_1}} \tag{A-11}$$

For $P_1 = P_2 = P_3$,

$$K_t = K_3(1 + K_1/\mathrm{Na}) \tag{A-12}$$

Eqn. A-2 assuming that P_2, $P_4 \ll P_3$, *i.e.* that neither the XS or the XNa form of carrier can cross the membrane to any significant extent[20]. Eqn. A-6 was previously given by SCHULTZ AND CURRAN[3]; some others were derived by W. Wilbrandt (personal communication) also.

The particular model best describing the Na^+ activation of biotin intestinal absorption

As pointed out in DISCUSSION, the model of non-compulsory reaction sequence best describes the Na^+ activation of biotin intestinal absorption, as judged from both the $K_t = f[Na^+]$ functions and the experimental observation that $K_2 \neq \infty$. In addition, it was found that the apparent maximum velocities are the same irrespective of the Na^+ concentrations in the medium (Fig. 4). Thus, $P_3 = P_4$.

From the $K_t = f[Na^+]$ function for the non-compulsory reaction sequence and for $P_1 = P_2 = P_3 = P_4$ (*i.e.* from Eqn. A-4), the best values for K_1, K_2, K_3 and K_4 were estimated: $K_1 = 140$; $K_2 = 12$; $K_3 = 0.5$; $K_4 = 5.9$ (all mM).

The dotted lines in Figs. 5a and 5b were calculated from these values. It should be noted that, all experiments having been carried out in the presence of 5.9 mM K^+, the effect of this cation is included in the numerical values of the constants.

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Biotinyl-*p*-nitrophenyl ester inhibits irreversibly, presumably by affinity labeling, the biotin transport system in yeast[22]. We have now tested this compound on hamster small intestine. (Preincubation for 30 min at 30° in Krebs–Henseleit buffer, 10% ethanol with 80 μM biotinyl-*p*-nitrophenyl ester; incubation at 37° in 2 mM biotin in Krebs–Henseleit buffer.) No inhibition was detected, perhaps because of the much larger K_t value of the small intestinal system, or because of its different substrate specificity.

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