Ethanol selectively affects Na^+-gradient dependent intestinal transport systems

Brigitte O'Neill, Fritz Weber*, Dietrich Hornig* and Giorgio Semenza+

Laboratorium für Biochemie der Eidgenössischen Technischen Hochschule, ETH-Zentrum, CH-8092 Zürich and **Abtlg für Vitamin- und Ernährungsforschung, F. Hoffmann-La Roche & Co. AG., CH-4002 Basel, Switzerland*

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Moderate concentrations of ethanol reduce the velocity of uptake of three representative Na^+-symport systems (D-glucose, L-alanine, L-ascorbate), whether electrogenic (the first two) or electroneutral (L-ascorbate). This 'inhibition' is observed only if these transport systems are tested in the presence of an initial Na^+ gradient (out > in); no inhibition is found in tracer-equilibrium exchange measurements. A representative Na^+-*in*dependent system (D-fructose) is not inhibited by ethanol. 'Passive diffusion' (measured as uptake of L-glucose) is increased somewhat by alcohol. All these observations can be rationalized [as suggested by Tillotson et al. (1981) Arch. Biochem. Biophys. 207, 360–370] by an effect of ethanol on passive diffusion, which leads to a faster collapse of the Na^+ gradient, with the resulting reduction of the uptake velocities of Na^+-dependent transport systems when tested with the added driving force of an Na^+ out → in gradient.

Ethanol effect *Na^+ symport* *Membrane permeability* *D-Glucose transport* *Amino acid transport* *Ascorbate transport*

1. INTRODUCTION

Ethanol, at the concentrations reached in the jejunal lumen after moderate liquor intake, inhibits the absorption of a number of solutes (for review, see [1]). This inhibition can be reproduced in vitro with 2–3% ethanol, using surviving intestine [2], or brush border membrane vesicles [3,4]. It has been suggested [3,4] that the inhibition of Na^+-dependent D-glucose uptake is due to a faster collapse of the Na^+ gradient (out → in) across the membrane [3,4]. Additional, but unconvincing mechanisms include a decrease of the vesicular volume [4] (which, however, is unlikely since the amount of D-glucose associated with the vesicles at equilibrium is not decreased by ethanol [3,4]) [The small decrease by ethanol in the amount of taurocholate associated with the vesicles at equilibrium [4] cannot be taken as an indication of a decrease in the intravesicular volume, since the amount of taurocholate associated with the vesicles far surpasses (by a factor of nearly 200) the amount occurring in free solution in the intravesicular volume. Thus, most taurocholate must be associated with the membrane, presumably in the lipid bilayer. For this reason, also the observed decrease, by ethanol, of the velocity of taurocholate 'uptake' in the presence of an initial Na^+ gradient, may refer more to the association of taurocholate with the membrane than to its transport across it.] and changes in the fluidity of the lipid bilayer [4] (which, however, are not or hardly detectable at ethanol concentrations already affecting transport [3,4]). Higher alcohol concentrations do affect membrane fluidity (e.g. [6]).

The suggestion that the 'inhibition' of Na^+-dependent D-glucose transport by ethanol is

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+ To whom correspondence and reprint requests should be addressed

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due to a faster collapse of the initial Na^+ gradient rests on the observation that alcohol (~3%) inhibits the Na^+-dependent D-glucose uptake only when tested in the presence of an initial Na^+ gradient (out → in) [3,4], but has no effect on the same transport system when tested in the absence of an Na^+ gradient, i.e. in counterflow [4] or under conditions in which $Na^+_{in} = Na^+_{out}$ [3]. Unfortunately, direct measurements of the effect of ethanol on the uptake of Na^+ by brush border membrane vesicles (such as attempted in [4]) are inherently inadequate for solving this problem, because too much of the Na^+ taken up by these vesicles is bound to, rather than transported across, the membrane [5].

The problem was thus tackled again indirectly, but by investigating the response to ethanol of a broader range of transport systems. Specifically, the questions asked and the answers obtained were: (i) does alcohol inhibit, in addition to Na^+-dependent D-glucose symport, other Na^+-symport systems of the same membrane, such as that of L-alanine and that of L-ascorbate? We find that it does, but again only if these transport systems are tested in the presence of an initial Na^+ gradient (out > in); (ii) is the ethanol inhibition due to a faster collapse of $\Delta\psi$? We find that the Na^+-dependent *non*-electrogenic L-ascorbate system [7] is likewise inhibited by ethanol, which shows that a system need not be electrogenic to be inhibited by ethanol; (iii) does ethanol inhibit non-Na^+-dependent, electroneutral transport systems? If the inhibitions observed are due to a faster collapse of the initial Na^+ gradient, it should not. We chose to investigate D-fructose uptake, which is, in terms of capacity, the major non-Na^+-dependent, non-electrogenic transport system in this membrane, and find no effect of alcohol; (iv) does ethanol increase 'diffusion' through this membrane (as measured by the uptake of a neutral marker, such as L-glucose)? We find that it does, which indicates a possible moderate increase in non-specific permeability.

2. MATERIALS AND METHODS

Chemicals: All reagents were of the highest purity available. L-[^{3}H]Glucose, D-[^{3}H]glucose, L-[^{3}H]alanine and L-[1-^{14}C]ascorbic acid were purchased from New England Nuclear, Boston, MA; D-[^{14}C]fructose from the Radiochemical Centre, Amersham (England). All chemicals used were reagent grade and were obtained from Merck, Darmstadt (FRG), Sigma, St. Louis, MO (USA) or Fluka, Buchs (Switzerland).

Vesicle preparation: The vesicles of small intestinal brush border membrane of guinea pig were prepared from fresh intestine by the Ca^{2+} precipitation method ([9], as modified in [5,7]).

Transport measurements were carried out at 20°C with vesicles at 7–10 mg protein · ml^{-1}. (i) In the presence of an initial inwardly directed Na^+ gradient: unless stated otherwise, the outer medium had the following composition at zero time: 100 mM NaCl, 0.150 mM dithiothreitol, 300 mM D-mannitol, 10 mM Hepes-Tris (pH 7.5) and 0.3 mM labeled substrate; the medium inside the vesicles (i.e. the solution in which the last washing of the vesicles was carried out) was 300 mM D-mannitol, 10 mM Hepes-Tris (pH 7.5). (ii) Under conditions of tracer equilibrium exchange: the vesicle suspension was preincubated on ice for 90 min in 100 mM NaCl, 0.150 mM dithiothreitol, 300 mM D-mannitol, 10 mM Hepes-Tris (pH 7.5) and 0.3 mM cold substrate; an additional 15–25 min preincubation period at 20°C followed in the absence or presence of ethanol (1–3%). Incubation proper (at 20°C) was started by mixing 10 μl of the preincubated vesicle suspension with 10 μl of a solution of the same composition plus the tracer.

The incubations were stopped at selected times by addition of ice-cold 250 mM NaCl in 1 mM Tris-HCl (pH 7.5), the suspension filtered through a Sartorius filter (0.65 μm pore size) under suction, and washed twice with the same ice-cold stop solution. Radioactivity was measured in a scintillation counter; protein was determined according to Lowry et al. [10].

All experiments were repeated at least 3 times, with consistent results. Only individual experiments are shown; each point is the mean of 3–4 determinations; the bars indicate the SD, whenever it was larger than the symbol used.

3. RESULTS

Since, of the commonly used experimental animals, only guinea pigs are known to possess in

the small-intestinal brush border membrane the non-electrogenic Na^+-dependent transport system for L-ascorbate (e.g. [7] and earlier references quoted therein), guinea pigs were used throughout.

Ethyl alcohol, even at the highest concentration tested (3%), does not fragment the brush border membrane, as shown by the essentially unchanged amount of substrate associated with the vesicles at equilibrium (see most figures).

3.1. *Inhibition of Na^+-dependent transport systems tested in the presence of an initial $\Delta\tilde{\mu}Na^+$*

Ethyl alcohol (2–3%) inhibits the velocity of uptake of all Na^+-dependent transport systems tested (i.e. in addition to that of D-glucose, those of L-alanine, L-ascorbate (figs 1 and 4). At lower concentrations (i.e. 1%) ethanol has little or no effect (e.g. fig.1A). We thus observe inhibition at the

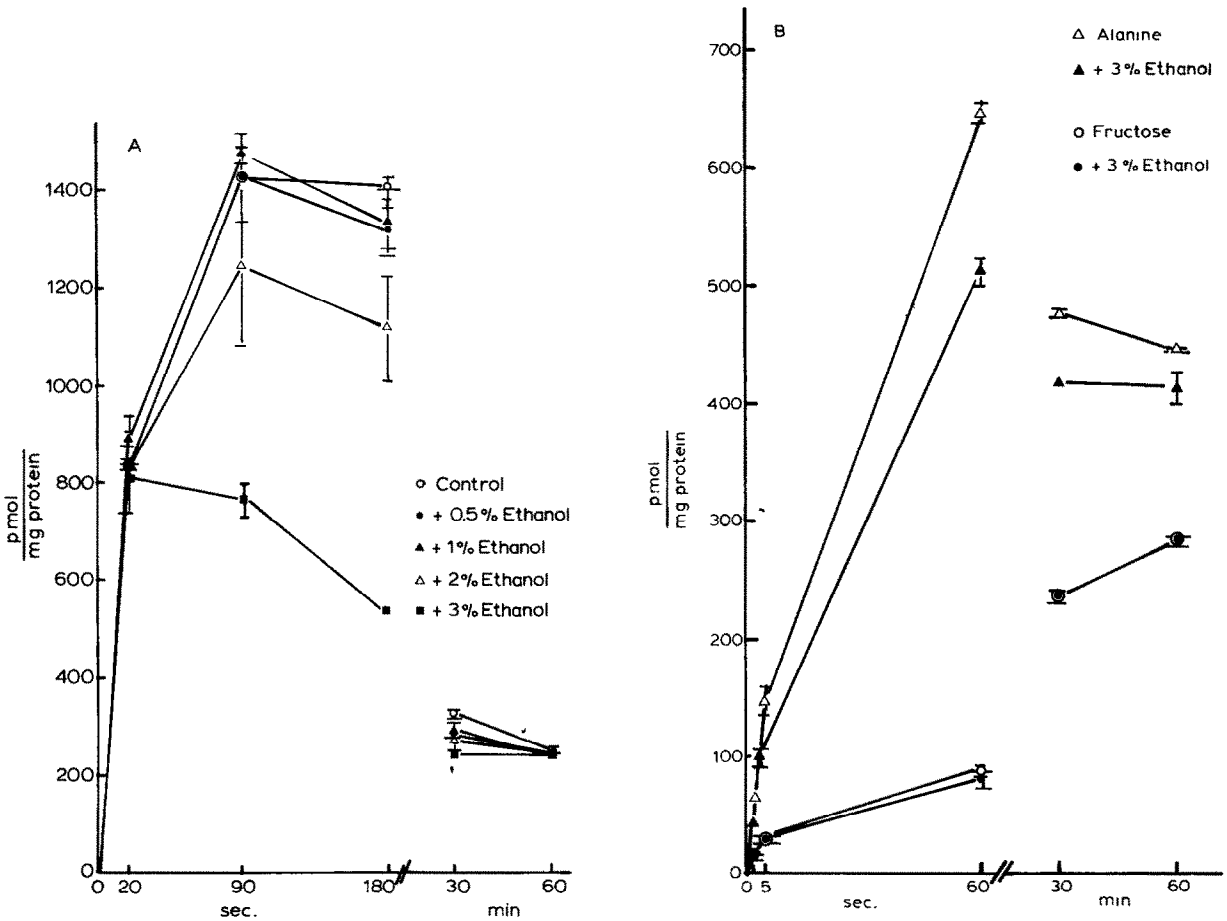

Fig.1. Time course of uptake into brush border membrane vesicles from guinea pig small intestine, of various solutes, in the presence of an initial Na^+ gradient (out → in) and in the presence or absence of ethanol. The concentration of substrate was in all cases 0.3 mM (in the outer medium only). (A) Uptake of D-glucose in the presence of an initial NaCl gradient, at 20°C. (B) Uptake of L-alanine and of D-fructose, in the presence of an initial NaCl gradient, at 20°C. At the beginning of the incubation proper (time zero) the outer medium had the following composition: 0.3 mM substrate, 100 mM NaCl, 10 mM Hepes-Tris (pH 7.5), 0.150 mM dithiothreitol, 300 mM D-mannitol. The medium inside the vesicles was composed of buffer and D-mannitol, at the same concentrations as in the outer medium. The vesicles were preincubated for 20 min and incubated either in the absence of ethanol (○), or in the presence of ethanol, at the concentrations given. For further details, see section 2. The bars indicate the SD; if not given, the SD was smaller than the symbol used. (The uptake of D-fructose had not reached equilibrium after 60 min.)

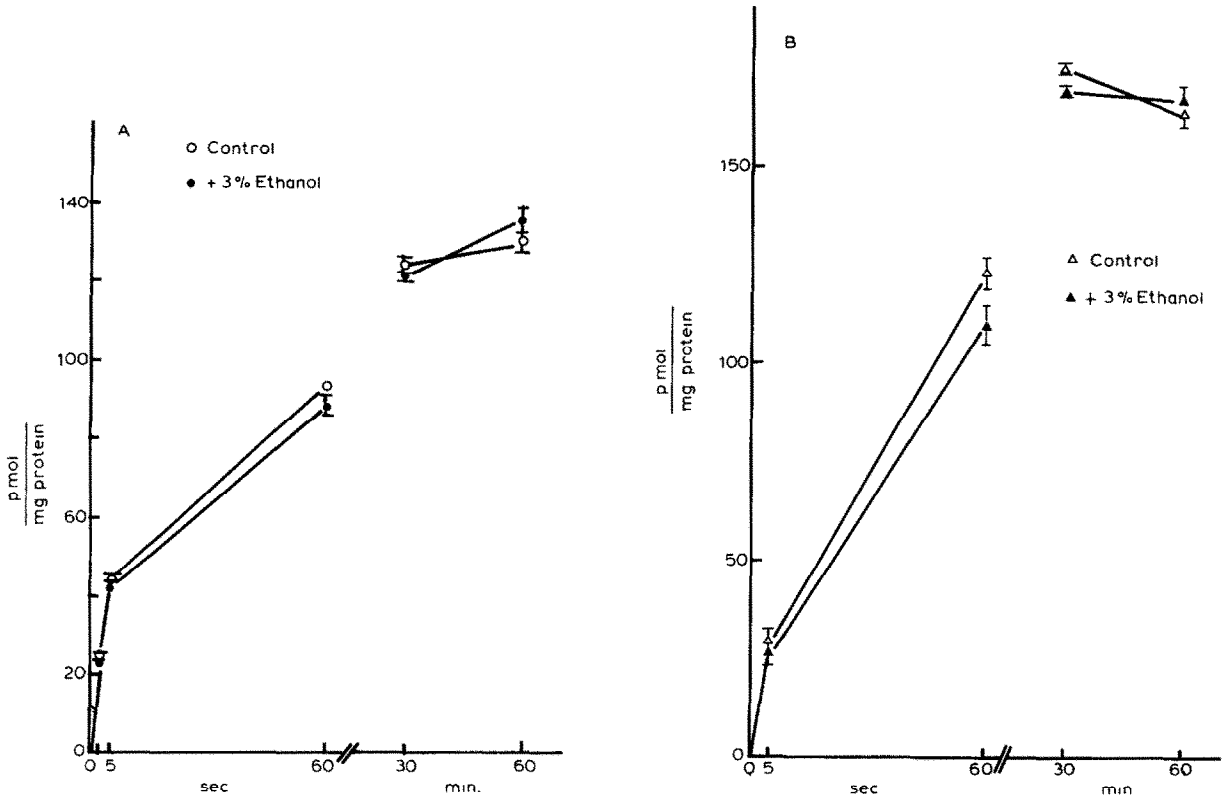

Fig.2. Time course of uptake of D-glucose (A) and of L-alanine (B) under equilibrium tracer-exchange conditions. The vesicles were first preincubated on ice for 90 min in 0.3 mM cold substrate, 100 mM NaCl, 10 mM Hepes-Tris (pH 7.5), 0.150 mM dithiothreitol, 300 mM D-mannitol; they were then equilibrated for an additional 20 min at 20°C without (○) or with 3% ethanol. Incubation proper, again at 20°C and in the absence or presence of ethanol, was started with labelled substrate, as described in section 2.

same concentrations which produced inhibition of D-glucose uptake in rat brush border vesicles in another study [3] and of amino acids in rat surviving intestine (everted sacs) [2].

3.2. *Lack of inhibition of Na^+-dependent transport systems when tested in the absence of an initial $\Delta\tilde{\mu}Na^+$, under tracer-equilibrium exchange conditions*

If the same Na^+-dependent systems are tested in the absence of an initial Na^+ out → in gradient (i.e. in vesicles which had been pre-equilibrated in Na^+-containing buffers with no substrates, as in [3], or in buffers containing both Na^+ and substrates, such as in the tracer-equilibrium exchange experiments of fig.2A,B), alcohol no longer inhibits the uptake. This shows that these cotransporters per se are not sensitive to ethanol at the concentrations tested.

3.3. *Lack of inhibition of the electroneutral Na^+-independent uptakes of D-fructose*

D-Fructose is transported across the brush border membrane by a non-accumulating Na^+-independent carrier ([8] and previous papers quoted therein). If the effect of ethanol on the Na^+-dependent systems investigated above is solely due to an accelerated collapse of the initial $\Delta\tilde{\mu}$ or $\Delta\mu$ of Na^+ across the membrane, D-fructose uptake should not be affected by alcohol. Indeed, it is not, whether tested in the presence of an initial NaCl out → in gradient (fig.1B), or in its absence (not shown).

3.4. *Effect of ethanol on diffusion*

Is passive diffusion (defined operationally as the uptake of a neutral compound not interacting with carriers) increased by ethanol, or is the effect of ethanol confined to increasing the permeability of

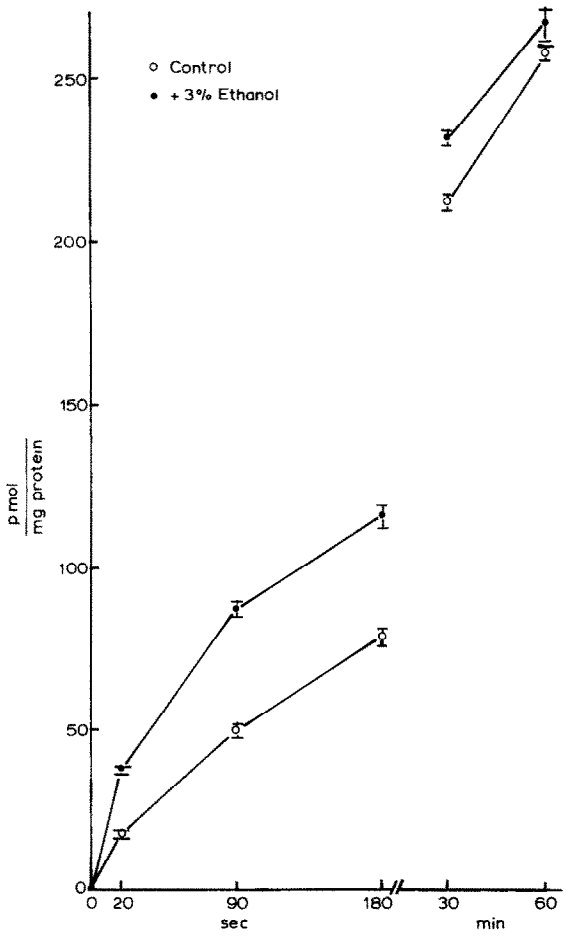

Fig.3. Time course of uptake of 0.3 mM L-[^{3}H]glucose in the presence of an initial NaCl out → in gradient. Same conditions as in fig.1A. Note that the uptake of L-glucose is accelerated by 3% ethanol (●), as compared to vesicles in the absence of alcohol (○).

Na^+? L-Glucose, which interacts little [11], if at all, with the Na^+, D-glucose cotransporter, is often used as a marker for passive diffusion (e.g. [12]). Its uptake is accelerated by 3% ethanol (1 or 2% concentrations have no effect, not shown) (fig.3), which shows that the effect of the alcohol is not confined to Na^+ permeability.

The lack of *increase* of D-fructose uptake by ethanol probably indicates that the contribution of diffusion to the uptake of this sugar is only marginal, which is consistent with the notion that D-fructose uptake is carrier-mediated [8].

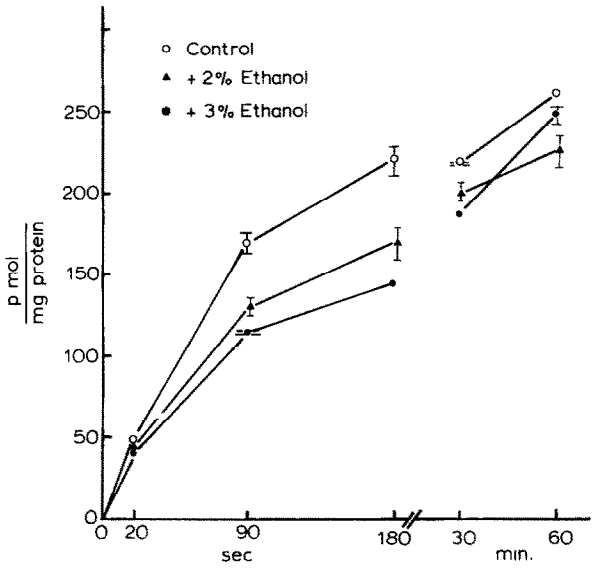

Fig.4. Time course of uptake of 0.3 mM L-[^{14}C]ascorbate in the presence of an initial NaCl out → in gradient, in the absence of ethanol (○) or in 2% (▲) or 3% (●) ethanol. (The uptake values in the presence of 0.5 or 1% ethanol were not different from the control, and are thus not given.) Same conditions as in fig.1A.

4. DISCUSSION

Summing up the results in section 3, ethanol at concentrations $\geqslant$ 2% inhibits all the Na^+-dependent transport systems in the small-intestinal brush border which have been tested – D-glucose (fig.1A and [3,4]), L-alanine (fig.1B), L-ascorbate (fig.4), taurocholate (see [4]) – be they electrogenic (D-glucose, L-alanine) or not (L-ascorbate). This inhibition by ethanol is observed only when these transport systems are driven by an initial Na^+ gradient (out > in), no inhibition being detected when they are tested in the presence of Na^+, but in the absence of a *gradient* of Na^+ (fig.2A,B). No effect of ethanol is observed on the transport of D-fructose (fig.1B) which is carrier-mediated but not Na^+-dependent (e.g. [8]). These effects of alcohol on some systems but not on others, and on some systems only under certain conditions, are fully rationalized by ethanol producing a faster collapse of $\Delta\mu Na^+$ (or of $\Delta\bar{\mu}Na^+$) across the brush border membrane. This mechanism had already been suggested by others [3] for the ethanol inhibition of

Na^+-dependent D-glucose transport and is reinforced by our investigation which includes more systems having different properties.

The (main) effect of ethanol is on Na^+ permeability, rather than on Cl^- permeability, since the uptake of D-glucose is also affected when tested in the presence of an initial NaSCN gradient (out → in, see [3]). The P_{SCN^-} of this membrane is more than 10-times larger than its electrogenic P_{Na^+} [13]. (We mostly used NaCl, rather than NaSCN in the experiments reported here, because thiocyanate inhibits the Na^+,D-glucose cotransporter and thus cannot be used in tests involving preincubations; M. Kessler, 1982, unpublished)

Direct inhibition of the cotransporters by alcohol (including conceivable 'uncoupling' of the site binding the organic solute with that binding Na^+), or changes in the fluidity of the lipid bilayer are thus very unlikely as additional mechanisms in the inhibition of transport systems by the concentrations of ethanol used in the present study.

The effect of alcohol on intestinal Na^+-dependent transport systems may well have 'physiological' significance, since the ethanol concentrations producing the in vitro effects studied by us and by others [2–4] are likely to be attained at least in the upper jejunum during ingestion of hard liquor. It remains to be seen whether this effect of alcohol is one of the reason(s) for the poor vitamin C status of humans drinking more than 70 g ethanol daily [14,15].

Although coincidental, it is also interesting to note that among the substrates the uptake of which is not influenced by ethanol is D-fructose, a sugar known to mitigate the effects of acute alcohol intoxication [16–18].

REFERENCES

[1] Wilson, F. and Hoyumpa, A. (1979) Gastroenterology 76, 388–403.
[2] Chang, T., Lewis, J. and Glazko, A.J. (1967) Biochim. Biophys. Acta 135, 1000–1007.
[3] Tillotson, L.G., Carter, E.A., Inui, K.-I. and Isselbacher, K. (1981) Arch. Biochem. Biophys. 207, 360–370.
[4] Hunter, C.K., Treanor, L.L., Grey, J.P., Halter, S.A., Hoyumpa, A. and Wilson, F.A. (1983) Biochim. Biophys. Acta 732, 256–265.
[5] Kessler, M., Acuto, O., Storelli, C., Murer, H., Müller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 506, 136–154.
[6] Mitjavila, S., Fernandez, Y. and Biogegrain, R.A. (1984) 6th Meet. Eur. Intest. Transp. Group, Pamplona (Spain), Sept. 27–29, 1984, Abstr.18.
[7] Siliprandi, L., Vanni, P., Kessler, M. and Semenza, G. (1979) Biochim. Biophys. Acta 552, 129–142.
[8] Sigrist-Nelson, K. and Hopfer, U. (1974) Biochim. Biophys. Acta 367, 247–254.
[9] Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B.K., Cerda, J.J. and Crane, R.K. (1973) Biochim. Biophys. Acta 323, 98–112.
[10] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[11] Caspary, W.F. and Crane, R.K. (1968) Biochim. Biophys. Acta 163, 395–400.
[12] Hopfer, U., Nelson, K., Perrotto, J. and Isselbacher, K.J. (1973) J. Biol. Chem. 248, 25–32.
[13] Kessler, M. and Semenza, G. (1979) FEBS Lett. 108, 205–208.
[14] Lemoine, A., Monges, A., Codaccioni, J.L., Bermond, P. and Körner, W.F. (1972) Rev. Alcoolisme 18, 199–217.
[15] Bonjour, J.P. (1979) Int. J. Environ. Nutr. Res. 49, 434–441.
[16] Merry, J. and Marks, V. (1967) Lancet ii, 1328–1330.
[17] Pawan, G.L.S. (1968) Nature 220, 374–376.
[18] Soterakis, J. and Iber, F.L. (1975) Am. J. Clin. Nutr. 28, 254–257.