

Magnesium absorption from distal part of swine digestive tract under isotonic and isoionic conditions from both sides of the epithelium

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ABSTRACT

The effect of low magnesium concentrations in composed isotonic and isoionic Tyrode's solution on its absorption from the distal part of the swine digestive tract was investigated *in vitro* on bags made of survived epithelia of the swine caecum and proximal colon. The epithelial side of either epithelium was always turned into the bags that were filled with Tyrode's solution containing 0.5, 1.0 or 2.5 mmol/L magnesium. Tyrode's solutions were always situated outside the bags, i.e., from submucosal side of the epithelium. At the beginning of the experiment and after 30, 60, 120 and 180 minutes of the experiment samples were taken from the solution outside the bags and analyzed spectrophotometrically for magnesium concentration. The excess of magnesium contents in the solution obtained in each experimental interval represents "the net flux" of magnesium in the direction of absorption. According to the results of the experiment, magnesium may be absorbed from both caecum and proximal colon epithelia against the concentration gradient, as well as under isotonic and isoionic conditions from both sides of the epithelium. The intensity of magnesium absorption depended on its concentration in the contents, on the epithelium used, and on the time of sampling. Results indicate the distal part of the swine digestive tract as the important part for magnesium absorption, especially under low concentrations in the contents. Under these experimental conditions the passive transport of magnesium played the major part.

Key words: magnesium absorption, caecum, proximal colon, swine

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Introduction

Magnesium absorption has an important role in organism to maintain necessary magnesium concentration (GÜNTHER, 1990). According to hitherto prevailing experimental results, the site of the most efficient magnesium absorption in the digestive tract depends on animal species, as well as on the anatomical and physiological peculiarities of their digestive tract. Thus, in equines magnesium is absorbed mainly in the small intestine, in omnivore and carnivore in the large intestine, and in ruminants the main site of magnesium absorption is the fore stomach (MENEELY et al., 1982; MEYER and ZENTEK, 1990; RAHNEMA et al., 1994). An important role in overall mineral absorption, especially bivalent cations, is attributed to the colon (DEMIGNÉ et al., 1989; HARDWICK et al., 1990; YOUNES et al., 1996). Sparse data in the literature as a main site of magnesium absorption in the swine digestive tract pointed to its distal parts: ileum and large intestine (PARTRIDGE, 1978).

The complexity of the epithelium of digestive tract, as an absorptive membrane, represents an important model for investigation of magnesium transport means through the biological membranes. Although many authors consider that magnesium is primarily transported through the biological membrane by solvent drug, the possibility of other means of transport in the intestines, such as simple and facilitated diffusion and active transport, could not be excluded (ALDOR and MOORE, 1970; BEHAR, 1974; EBEL and GÜNTHER, 1980; MENEELY et al., 1982).

BEHAR (1974) has established a positive correlation between net water transport and net magnesium transport in the rat ileum and colon, concluding that magnesium is transported in these organs by solvent drug. The main moving power for passive diffusion was the existence of concentration gradient, whilst the increase of magnesium concentration from the luminal side of the epithelium linearly increased magnesium passage through the epithelium in the direction of absorption. However, because of diminution of magnesium transport in the case of its high concentration in the contents, and the presence of metabolic inhibitors, HAYASHI and HOSHI (1992) concluded that normal intracellular accumulation of magnesium depends, at least partly, on present energy originating from oxidation of glucose.

All cells in the organism maintain intracellular concentration of ionized magnesium (Mg^{2+}) at slightly lower concentrations than in the extracellular space. The intracellular concentration of magnesium ions is kept a little below 1 mmol/L, while in the extracellular space it amounted to about 1 mmol/L (BEYENBACH, 1990).

Given the above-mentioned facts, the aim of this research was to investigate the intensity of magnesium absorption from the epithelium of the distal part of the swine digestive tract under conditions of its low concentrations in the contents, as well as under isotonic and isoionic conditions. At the same time, by monitoring “net passage” of magnesium in the direction of absorption, we sought to elucidate the means of magnesium transport through the epithelia of caecum and proximal colon.

Materials and methods

Investigations were carried out *in vitro* on bags made of the surviving epithelium of the swine caecum and proximal part of colon. Swine originated from a commercial pig farm, were reared by common pig-breeding technology, and fed by commercial mash for pig fattening. At the time of experiment their average body mass amounted to between 90 and 110 kg.

Material for the experiment was collected from the abattoir at the slaughter line. Immediately after exenteration of the animal, caecum and colon were removed, placed in a thermos bottle and transported to the laboratory for further procedure. The caeca and colon were opened with scissors and the remnants of digests were removed from the mucous membrane, first mechanically and then by repeated rinsing the membrane with warm Tyrode's solution. Thereafter, the epithelium was carefully peeled from the muscular layer and stretched on special funnels with a diameter of 4 cm. The epithelial side of the mucous membrane was always turned inside the bag.

Two identical series of experiments were performed: one with the bags made of the mucous membrane of swine caecum (12 animals per each concentration) and the other with bags made of mucous membrane of the proximal colon (12 animals per each concentration).

The bags were filled with 25 ml of Tyrode's solution containing 1.0 mmol/L of magnesium (standard Tyrode's solution), or with the same Tyrode's solution in which the magnesium concentration was adjusted to 0.5 or 2.5 mmol/L by the sodium chloride concentration in the solution. Tyrode's solution was always situated outside the bags, i.e., from the submucosal side of the epithelium. Thus prepared, the bags were immersed in low beakers a 400 ml containing 50 ml of Tyrode's solution and mounted into the thermostat at 38 °C; permanent aeration of the solution outside the bags was provided by small aquarium pumps. Particular care was taken in adjusting the levels of the solutions outside and inside the bags in order to equalize hydrostatic pressures from both sides of the epithelium. The experiments lasted 3 hours. Immediately before the experiment began, as well as after 30, 60, 120 and 180 minutes of the experiment, the samples of Tyrode's solution from the beaker, i.e., from the submucosal side of the epithelium, were collected and the concentration of magnesium determined spectrophotometrically by the method after BASINSKI (1965). Results are expressed as the "net flux" of magnesium that passed from the epithelial side of the epithelium to its submucosal side. This represents the difference between the magnesium concentration immediately before the experiment began and the magnesium concentration obtained in the sample at the actual sampling interval during the experiment.

Data were statistically analyzed and shown as a mean \pm standard error of mean (M \pm S.E.M.). The significance of all differences between the experimental groups was checked by Student's t-test.

Results

In the series of experiments in which caecum epithelium was used as absorptive membrane, in all experimental intervals and at all magnesium concentrations in the contents used, a significant increase ($P < 0.001$) of magnesium concentration from the submucosal side of the epithelium was achieved in relation to initial magnesium concentration in Tyrode's solution before the beginning of the experiment (Fig. 1).

When Tyrode's solution containing 0.5 mmol/L magnesium was introduced from the epithelial side of the epithelium, net flux of magnesium

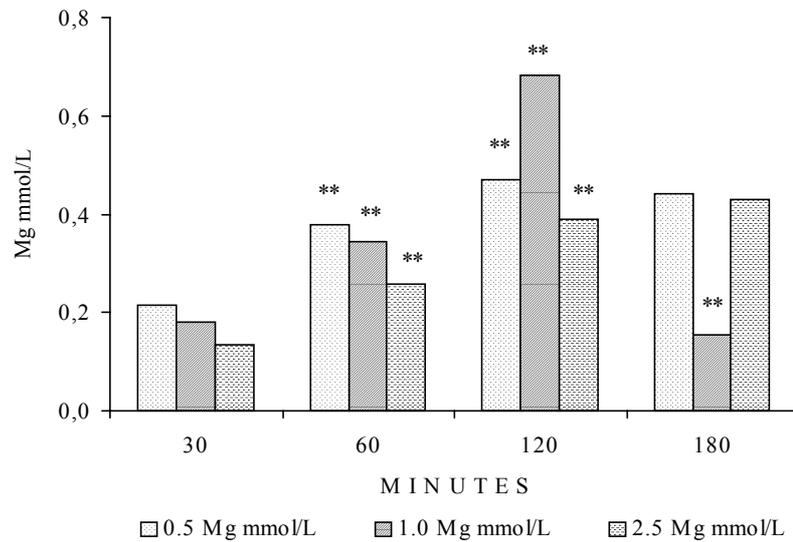


Fig.1. Net flux of magnesium to submucosal side of the epithelia of swine's caecum. Asterisks indicate significant differences as compared with the previous experimental interval. ** P < 0.001

to the submucosal side 30 minutes after beginning of the experiment amounted on average to 0.22 ± 0.01 mmol/L with a coefficient of variability (C.V.) of 14.57%. After 60 minutes, net flux amounted on average to 0.38 ± 0.02 mmol/L (C.V. = 13.80%). This increase in net flux was statistically significant ($P < 0.001$) in relation to values obtained in the previous time interval. A significant increase of net flux of magnesium in the direction of absorption was continued, and after 120 minutes of the experiment amounted on average to 0.47 ± 0.02 mmol/L (C.V. = 11.70%; $P < 0.001$). At the end of the experiment, i.e., after 180 minutes, net flux declined, amounting on average to 0.44 ± 0.01 mmol/L (C.V. = 10.03%).

In the case where standard Tyrode's solution containing 1.0 mmol/L of magnesium was introduced from both sides of the caecum epithelium, net flux of magnesium in the direction of absorption 30 minutes after the

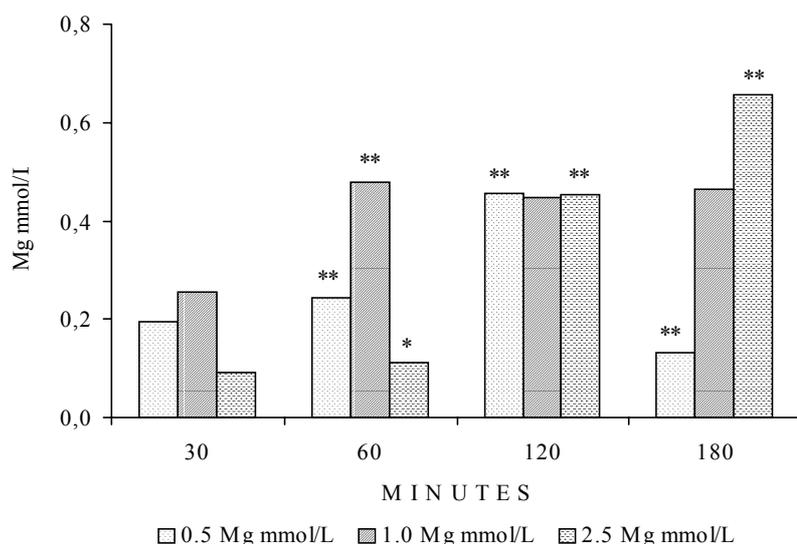


Fig. 2. Net flux of magnesium to submucosal side of the epithelia of swine's proximal colon. Asterisks indicate significant differences as compared with the previous experimental interval. * $P < 0.05$, ** $P < 0.001$.

beginning of experiment amounted on average to 0.18 ± 0.02 mmol/L (C.V. = 45.53%). After 60 minutes of the experiment net flux of magnesium almost doubled, amounting on average to 0.34 ± 0.02 mmol/L (C.V. = 20.84%). In relation to the previous time interval this increase was highly significant ($P < 0.001$). This significant increase of net magnesium flux was continued in the next time interval, and after 120 minutes amounted to an average of 0.68 ± 0.02 mmol/L (C.V. = 12.43%). At the end of the experiment, after 180 minutes, net flux of magnesium declined, amounting on average to 0.15 ± 0.02 mmol/L (C.V. = 58.29%). This diminution of magnesium net flux was statistically highly significant in comparison with values obtained after 60 and 120 minutes of the experiment ($P < 0.001$).

In the case where Tyrode's solution containing 2.5 mmol/L of magnesium was introduced from the epithelial side of the caecum epithelium, after 30 minutes of the experiment net flux of magnesium

amounted on average to 0.14 ± 0.02 mmol/L (C.V. = 43.85%). In second experimental period, i.e., after 60 minutes, net flux of magnesium amounted on average to 0.26 ± 0.02 mmol/L (C.V. = 25.82%), which was statistically highly significant increase compared with that from the previous experimental interval ($P < 0.001$). A significant increase of net flux of magnesium in the direction of absorption was continued during the next experimental period, amounting after 120 minutes to an average of 0.39 ± 0.02 mmol/L (C.V. = 18.09%; $P < 0.001$). Net flux of magnesium was maintained at this level up to the end of the experiment, and after 180 minutes of the experiment amounted on average to 0.43 ± 0.02 mmol/L (C.V. = 14.79%).

In a series of experiments, when the epithelium of the swine proximal colon was used as absorptive membrane, a significant rise ($P < 0.001$) of net flux of magnesium was achieved in all experimental intervals and at all magnesium concentrations used, compared with magnesium concentration in Tyrode's solution outside the bags obtained at the beginning of the experiment (Fig. 2).

When Tyrode's solution containing 0.5 mmol/L of magnesium was introduced from the epithelia side of the epithelium, net flux of magnesium to the submucosal side of the epithelium 30 minutes after the beginning of the experiment amounted on average to 0.20 ± 0.01 mmol/L (C.V. = 19.28%). After 60 minutes, net flux of magnesium amounted on average to 0.24 ± 0.03 mmol/L (C.V. = 35.14%), and after 120 minutes 0.47 ± 0.02 mmol/L (C.V. = 15.94%). In both cases this rise was statistically highly significant ($P < 0.001$) in relation to the previous experimental interval. At the end of the experiment, i.e., after 180 minutes, net flux of magnesium significantly declined ($P < 0.001$) in the relation to previous experimental interval, amounting on average to 0.13 ± 0.01 mmol/L (C.V. = 34.39%).

In the case where Tyrode's solution containing 1.0 mmol/L of magnesium was situated on both sides of the colon epithelium, 30 minutes after the beginning of experiment, net flux of magnesium from the epithelial to submucosal side amounted on average to 0.26 ± 0.03 mmol/L (C.V. = 44.95%). After 60 minutes of the duration of the experiment, net flux of magnesium in the direction of absorption significantly increased, amounting

on average to 0.48 ± 0.03 mmol/L (C.V. = 18.39%; $P < 0.001$). From then, up to the end of the experiment, net flux of magnesium remained at roughly the same values: 0.45 ± 0.03 mmol/L (C.V. = 24.47%) after 120 minutes, and 0.46 ± 0.03 mmol/L (C.V. = 22.99%), respectively.

When Tyrode's solution containing 2.5 mmol/L of magnesium was used for filling the bags, net flux of magnesium to submucosal side of the colon epithelium amounted on average to 0.09 ± 0.01 mmol/L (C.V. = 23.83%). This rise continued up to the end of the experiment, amounting on average to 0.11 ± 0.01 mmol/L (C.V. = 20.35%; $P < 0.05$) after 60 minutes, 0.45 ± 0.03 mmol/L (C.V. = 23.38%; $P < 0.001$) after 120 minutes, and 0.66 ± 0.02 mmol/L (C.V. = 12.60%; $P < 0.001$) after 180 minutes of the experiment, respectively.

Discussion

According to results of the presented investigation one may assume that both investigated epithelia, swine caecum and proximal colon, are permeable for the transport of magnesium in the direction of absorption under all experimental conditions applied. However, variations in the dynamics and intensity of magnesium absorption occurred between the epithelia used, depending on its concentration in the contents, i.e., from epithelial side of the epithelium, and depending on the duration of the experiment (Fig. 1).

Considering that in both examined compartments of the swine large intestine, magnesium absorption was determined at a concentration of 0.5 mmol/l in the contents, results undoubtedly show that in the distal part of the swine digestive tract, magnesium may be transported against a concentration gradient. The obtained results are in agreement with the opinion expressed by BEHAR (1974) and KARBACH (1989) that under such conditions the "solvent drag" is indicated as the main mode of magnesium transport against a concentration gradient. In the present study, the magnesium transport against a concentration gradient may also be correlated to water transport, meaning that magnesium was absorbed, at least partially, by the solvent drag. Under isotonic and isoionic condition, in series with Tyrode's solution containing magnesium at 1.0 mmol/L,

which corresponds to the level of intracellular ionized magnesium, “net transport” of magnesium to the submucosal side of the mucous membrane probably occurred by simple diffusion, mainly due to the electrochemical potential and to a lesser degree to the concentration gradient. However, under conditions of low magnesium concentrations in the contents (0.5 and 1.0 mmol/L) we were not able to exclude the possibility of the existence of its active transport across the mucous membrane. When magnesium concentration in Tyrode’s solution was increased to 2.5 mmol/L, its absorption through both examined epithelia also occurred by simple diffusion according to concentration gradient and electrochemical potential, as was previously shown in some other parts of the digestive tract (BEHAR, 1974; BEHAR, 1975; HEINONEN and AKERMAN, 1987; STOJEVIĆ et al., 1993; MILINKOVIĆ-TUR et al., 2000).

It is clear that magnesium was transported mainly passively from both epithelia and from all magnesium concentrations used in these investigations. The differences in dynamics of changes in magnesium fluxes at different experimental conditions and in different epithelia may be explained by differences in physic-chemical properties of a particular biological membrane. If one regards the epithelium as an ionic exchanger, as propounded by EISENMAN et al. (1967), this may be a way to explain the peculiarities of epithelial resistance to magnesium passage at different concentrations in the contents. Namely, the caecum and colon epithelium possess electrochemical properties specific to the particular epithelium, which may be changed by altering the magnesium concentration in the solution.

It can be concluded that the swine caecum and proximal colon represent the important sites of magnesium absorption under its low concentrations in the contents as under isotonic and isoionic condition, where magnesium was mostly absorbed by passive transport. The participation of the mentioned mechanisms of magnesium transport pointed out by this research remains to be solved through future investigations.

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SAŽETAK

Istražen je utjecaj niskih koncentracija magnezija u složenoj izotoničnoj i izoioničnoj Tyrodeovoj otopini na njegovu resorpciju u distalnom dijelu svinjskog probavila. Istraživanja su obavljena *in vitro* metodom vrećica, načinjenih od preživjele sluznice svinjskog slijepog crijeva i proksimalnog kolona. Epitelnom stranom sluznica je bila okrenuta u unutrašnjost vrećice koje su potom bile napunjene Tyrodeovom otopinom u kojoj je koncentracija magnezija iznosila 0,5, 1,0 ili 2,5 mmol/L. S vanjske, submukozne, strane sluznice crijeva redovito se nalazila Tyrodeova otopina iz koje su, neposredno prije početka pokusa i nakon pokusnog razdoblja od 30, 60, 120 i 180 minuta uzeti uzorci za spektrofotometrijsko određivanje magnezija. Višak magnezija u svakom pokusnom razdoblju predstavljao je »neto prolaz« magnezija smjerom resorpcije. Prema dobivenim rezultatima magnezij se u svinjskom slijepom crijevu i proksimalnom kolonu može resorbirati nasuprot koncentracijskom gradijentu, kao i u obostrano izotoničnim i izoioničnim uvjetima. Intenzitet resorpcije magnezija ovisio je o njegovoj koncentraciji u sadržaju, upotrijebljenoj sluznici te o vremenskom razmaku. Dobiveni rezultati upućuju da distalni dio svinjskog probavila predstavlja važno mjesto resorpcije magnezija pri njegovim niskim koncentracijama u sadržaju, pri čemu se magnezij u tim uvjetima najvećim dijelom resorbirao pasivnim prijenosom.

Ključne riječi: resorpcija, magnezij, slijepo crijevo, proksimalni kolon, svinja
