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Nuclear microanalysis of the monovalent ions distribution in the human amnion
II. Effect of taurine

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Summary. The effect of taurine on the Na^+, K^+, Cl^- concentration and distribution in epithelial and compact layers of the human amniotic membrane had been investigated using the Bordeaux nuclear microprobe. Particle induced X-ray emission and Rutherford backscattering spectrometry techniques had been used to provide quantitative measurements. In physiological medium, the monovalent ions concentrations were identical in epithelial and compact layers. The addition of taurine in Hanks' physiological fluid had no effect on Na^+ concentration, but decreased K^+ and Cl^- concentration in both layers. The quantitative results were related to electrophysiological observations on the effect of taurine on ionic exchanges through channels and paracellular pathways.

Keywords: Amino acids – Chlorine – Human amniotic membrane – Ionic exchange – Nuclear microanalysis – Potassium – Sodium – Taurine

Introduction

Ionic transfer through the human amniotic membrane, a "leaky" epithelium, is regulated by paracellular, through intercellular spaces between two adjacent epithelial cells, and cellular pathways. The total ionic transamniotic conductance is the sum of a cellular, a coupling and a paracellular conductances. Taurine appears to be a powerful membrane stabilizer (Durlach, 1988), the effects of which at physiological doses are comparable to those of magnesium. In previous studies (Bara et al., 1985), it has been shown that the effects of taurine appear to be achieved more by a paracellular action on the physicochemical structure of the amniotic membrane than by an ATPase route. Indeed, the cellular targets of the taurine effects are the Na^+ and K^+ channels and the Na^+ and K^+ paracellular pathways (Bara et al., 1990).

Ultrastructural studies (Guiet-Bara et al., 1991) have indicated that taurine increases the ratio between the intercellular space and the cell volume, but has no effect on the volume of microvilli or podocytes versus the cell volume. These studies do not give informations on the amniotic localization of the ions present in the physiological fluid. Micro-PIXE (Particle induced X-ray emission) is one of the few methods of microanalysis which permit a simultaneous detection of most minerals acting on cellular pathways. It provides unique possibilities to reveal directly the distribution of these elements at the cell scale. Previous studies (Moretto et al., 1993; Razafindrabe et al., 1995) have indicated the first step in the development of these techniques which has been to check the consequences of a simple incubation in a widespread used physiological fluid. These studies have shown the distribution of Na^+, K^+, Cl^- in the epithelial and compact layers of the human amnion.

The aim of this study is to observe the effects of taurine supplementation on the monovalent ions distribution in the amnion layers and to elucidate the previous electrophysiological data.

Material and methods

Tissue sampling

Specimens of human amnion, isolated from the placental zone of the amniotic sac, were obtained after 10 normal deliveries at term. For each specimen, three strips were collected, the first one being immediately quench frozen in isopentane cooled with liquid nitrogen, and this, without any rinsing procedure. This sample was considered as a control sample. The second one was transferred in Hanks' solution, a physiological fluid (composition in mmoles/l: NaCl 150, KCl 6, $MgSO_4$ 0.5, $MgCl_2$ 0.5, $CaCl_2$ 1, glucose 5.5, NaH_2PO_4-KH_2PO_4-$NaHCO_3$ 1) at 37°C and pH 7.4. For the third one, taurine (TA), at 2 mM was added to normal Hanks' solution. After one hour incubation, the two incubated strips were cryofixed as previously. The three strips were kept in liquid nitrogen until sectioned. Sectioning was performed at −30°C using a cryomicrotome (Reichert-jung frigocut 2800). Thin frozen sections, in the thickness range 20–30 μm, were collected on the knife, placed directly on thin formvar^R foils of about 20 $\mu g/cm^2$ and kept in the cryostat for several hours until complete freeze-drying. The slides were then stored in a dessicator over Silica-gel prior to analysis. The morphology of the amnion sections was elucidated using light microscopy of adjoining sections mounted on glass slides and stained with haematoxylin and eosin.

Microanalysis and data processing

The analysis were performed using the CENBG microprobe facility in Bordeaux (Llabador et al., 1990). Well defined parts of amnion slides were chosen including epithelial cells and compact lamina. These regions were irratiated with a 1 Mev proton beam focalized down to a 2 μm spot diameter. The beam current, measured on the target was 150 pA and the total collected charge was 0.5 μC. The extension of the scan ranging, according to the sample structure, was chosen between 50 × 50 μm^2 and 100 × 100 μm^2. PIXE and RBS (Rutherford Backscattering Spectrometry) analysis were carried out simultaneously in order to insure the mass standardization. Sodium, potassium and chlorine were determined as well as carbon. X-rays were detected using a 80 mm^2 Si(Li) solid state detector (Link system) fitted with a thin beryllium window (8 μm), which allowed us

to measure the NaKα line with low attenuation. The backscattered particles were detected at 135° of the beam with a Si 20 mm² detector allowing thus the measurement of the organic mass and beam current monitoring.

Elemental mapping of Na, K and Cl revealed a high compartmentalization of ionic species allowing thus a precize delimitation of the epithelial layer (EL) and of the compact layer (CL) on the whole scanned area. An off-line specific treatment of data permitted us to extract X-rays and backscattered particles partial spectra issuing from the previously defined subregions (EL and CL) (Moretto et al., 1993).

Data reduction

Quantitative results expressed in term of dry mass were obtained using the following scheme: all PIXE spectra were fitted with GUPIX software (Maxwell et al., 1989). RBS data were treated using an extension of the RUMP code (Doolittle, 1985), a program developed in our group (Moretto and Razafindrabe, 1995) taking into account the heterogeneity of the sample thickness, non-Rutherford backscattering cross-sections and the autoabsorption of low X-rays. Unfortunately, this program was not available at the beginning of this study. In order to include all experimental data in the reported results, we therefore expressed quantitative values using elemental ratios (Na or K or Cl/reference). The best reference element was estimated to be sulfur because of its unvarying concentration in EL and CL, whether the amnion was incubated or not. This point was checked using the mass normalization procedure above described. Results are expressed as means ± SD. The data statistical processing was made with the one-way analysis of variance test (ANOVA) and the Tukey-Kramer multiple comparisions test. The values of the significance level p of 0.05 and less were considered significant.

Results

Elemental mapping

Previously (Razafindrabe et al., 1995), it has been shown that the EL was clearly define by higher phosphorus level (Fig. 1). Indeed, the phospholipid

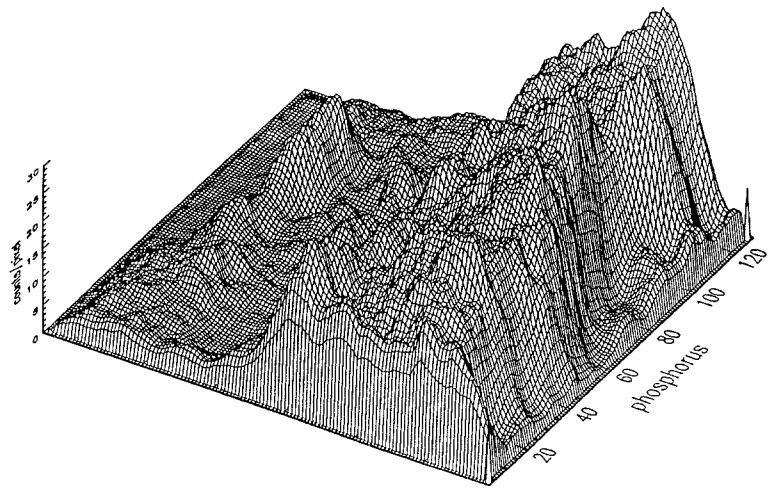

Fig. 1. Three dimensional plotting of the phosphorus distribution defining the epithelial layer. The map was constructed with initial 128 × 128 pixels matrice. Scanning area was 80 × 80 μm^2

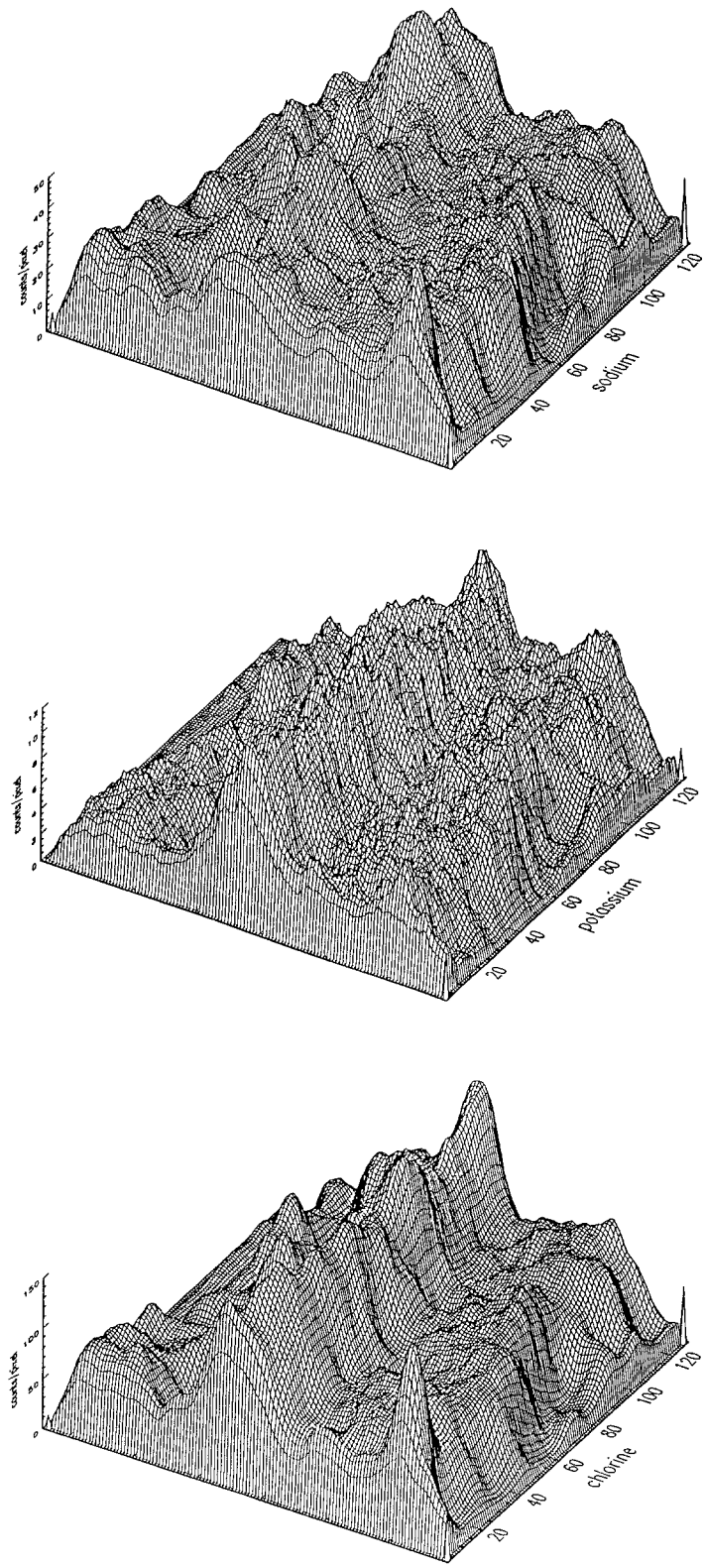

Fig. 2. Three dimensional plotting of the sodium, potassium and chlorine distributions after analysis of amniotic strips incubated in Hanks' solution + 2 mM taurine. The maps were constructed with initial 128 × 128 pixels matrice. Scanning area was 80 × 80 μm^2

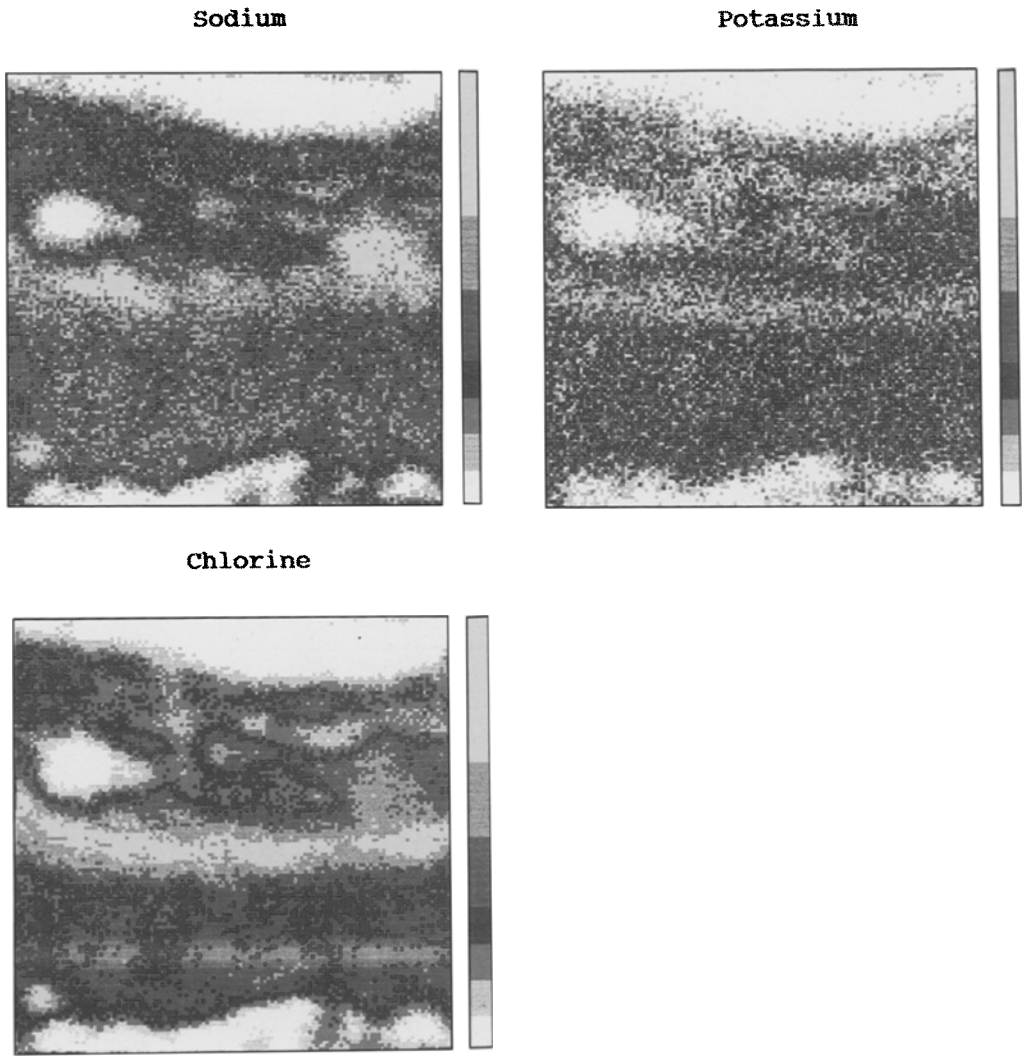

Fig. 3. Elemental maps of Na^+, K^+ and Cl^- of amniotic sample. The scan extend was 80 μm. The concentration increased, according the grey scale, from white to black

bilayer of the plasma membrane contributed to this high phosphorus level because the numerous convolutions which increased the surface of cellular exchanges. An example of the minerals distribution in a sample incubated in Hanks' solution + taurine was displayed in Fig. 2. The delineated subregions used for the determination of the quantitative values and the RBS energy spectra were visible in Fig. 3. The related PIXE partial spectra were presented in Fig. 4.

Quantitative results

The resulting concentrations of Na^+, K^+ and Cl^- were displayed as a Na/S, K/S and Cl/S ratios because sulfur was the sole element with sufficient stability, even after incubation, to be used as simultaneous reference for EL and

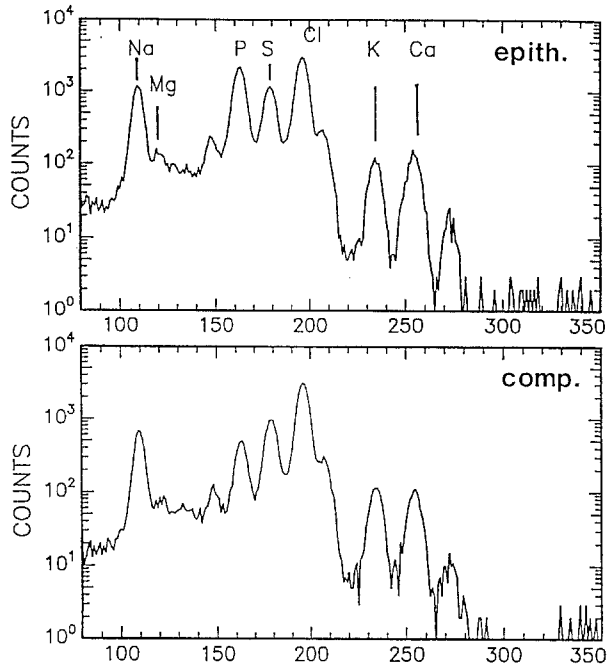

Fig. 4. PIXE partial spectra of epithelial and compact layers

1) Sodium (Fig. 5)

CL. In unwashed control samples, the Na^+ concentration was not statistically different in EL and CL ($p = 0.07$). Concentrations of sodium were strongly increased ($p < 0.001$) in EL (×3) and CL (×4) after incubation in Hanks' solution without modification between EL and CL. The addition of taurine had no significant effect on the Na^+ concentration in EL and CL.

2) Potassium (Fig. 6)

In unwashed control samples, the K^+ concentration was not statistically different in EL and CL ($p = 0.57$). Incubation in Hanks' solution had no effect on the K^+ concentration in EL and CL. However, the addition of taurine induced a very significant decrease of the K^+ concentration in EL and CL ($p < 0.001$).

3) Chlorine (Fig. 7)

In unwashed control samples, the Cl^- concentration was not statistically different in EL and CL ($p = 0.13$). After incubation in Hanks' solution, the Cl^- concentration was increased significantly in EL and CL ($p < 0.001$) without modifications between these two layers. The addition of taurine in Hanks' solution implicated a significant decrease of the Cl^- concentration in EL ($p < 0.01$) and in CL ($p < 0.05$). In this case, the Cl^- concentration was higher in CL than in EL ($p < 0.05$).

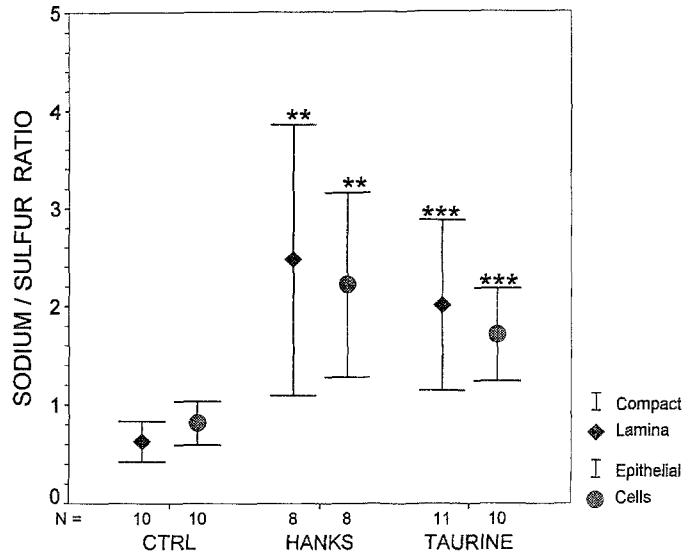

Fig. 5. Sodium/sulfur ratio (means ± SD) in unwashed control samples (*CTRL*), in samples incubated in Hanks' solution (*Hanks*) and in Hanks' solution + 2mM taurine (*Taurine*) (**p < 0.01, ***p < 0.001)

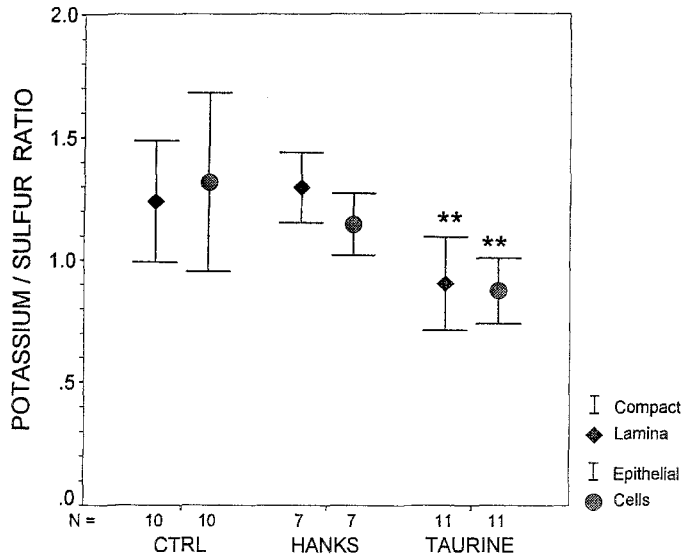

Fig. 6. Potassium/sulfur ratio (means ± SD) in unwashed control samples (*CTRL*), in samples incubated in Hanks' solution (*Hanks*) and in Hanks' solution + 2mM taurine (*Taurine*) (**p < 0.01)

Discussion

According to the quantitative results, the monovalent ions (Na^+, K^+, Cl^-) are distributed in the "exchanging" layer (EL) and in the "supporting" layer (CL). The distribution of these ions is equivalent in the two layers. This fact

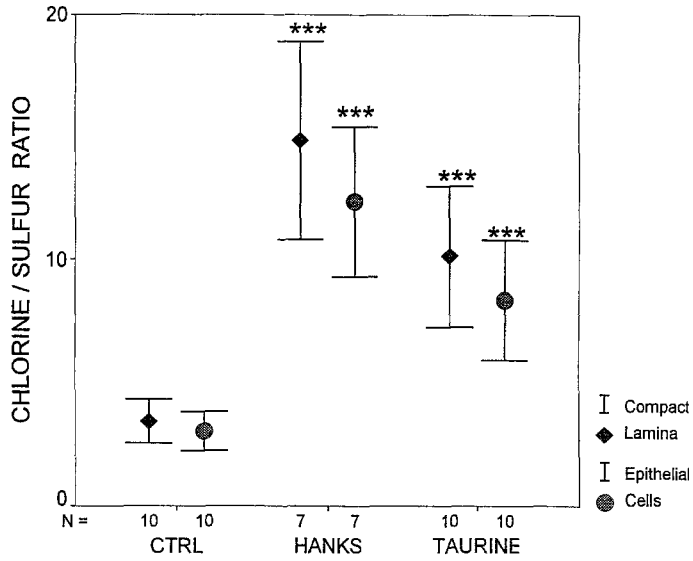

Fig. 7. Chlorine/sulfur ratio (means ± SD) in unwashed control samples (*CRTL*), in samples incubated in Hanks' solution (*Hanks*) and in Hanks' solution + 2mM taurine (*Taurine*) (***$p < 0.001$)

is observed in unwashed control samples and in incubated samples in Hanks' solution. These data demonstrate the importance of the compact layer.

In incubated samples, the Na^+ and Cl^- concentrations are strongly elevated in both layers, while the K^+ concentration remains identical. This observation is correlated with the concentration of ions in the Hanks physiological fluid: Na^+ and Cl^- are the more important ions. In these samples, the monovalent concentration rests equal in the two layers. Also, the ions of the physiological fluid are distributed in the same manner in the two layers. The compact layer acts as a buffer, which can fix minerals. No significant variation in the K^+ concentration of EL and CL is observed after incubation. We could expect that an efficient physiological fluid would preserve the level of the main intracellular cation.

The addition of taurine in the Hanks' solution implicates several constatations: taurine has no effect on the Na^+ concentration in EL and CL, but decreases the K^+ and Cl^- concentrations in both layers comparatively to the values observed in Hanks' solution only.

Electrophysiological studies (Bara et al., 1990) have indicated that taurine addition increases the movement of Na^+ ions across the amniotic membrane. The nuclear microanalysis data show that the Na^+ concentration remains constant after addition in both layers. The presence of a Na/taurine symport, in the epithelial cells, observed in other cells (Moyer et al., 1992; Suleiman et al., 1993) could explain this result. The symport induces an efflux of Na^+ when the concentration is higher than the physiological level. Previous results (Bara et al., 1990) suggest that the addition of taurine implies an efflux of Na from the epithelial cells; indeed the electrophysiological studies show that taurine increases the Na^+ transport through the Na^+ channels and the Na^+

paracellulare pathway. Also, the concentration in EL decreases and the concentration in CL increases. Consequently, numerous movements of Na^+ take place from CL towards EL, and finally, the concentration in EL and CL remains constant with regard to the values in Hanks' solution after an Na^+ ions turnover.

The addition of taurine implicates also a decrease in the K^+ concentration in EL and CL. In this case, taurine acts as a magnesium-like: there is a leak of K^+ from exchanging (EL) and supporting (CL) layers. This effect, "Mg-like", is confirmed by previous studies (Bara, 1976) which indicate a competition between Mg^{2+} ions and K^+ ions in various membranes.

A decrease of Cl^- concentration is observed in EL and CL after addition of taurine in Hanks' solution. Electrophysiological studies (Bara et al., 1990) have indicated that taurine has no effect on Cl^- channels and Cl^- paracellular pathway. The present microanalysis appears in opposition. Indeed, taurine induces a decrease of Cl^- concentration which should be correlated with an increase of the Cl^- conductance. The increase of Cl^- conductance has been observed in other tissues (Huxtable, 1992; Pierno et al., 1994). In the case of epithelial amniotic cells, the effect is indirect on Cl^- conductance and taurine may interfere with transport systems, Cl/HCO_3 antiport, particularly (Bara and Guiet-Bara, 1994). In the compact layer, an hypothesis may be suggested: CL acts as a buffer matter and interferes also in the exchange regulation. The following mechanism may be considered: Cl^- ions get out from EL, CL regulates the internal medium and Cl^- ions re-enter in EL (observation of Cl^- ions decrease in CL). In EL, the exchanges begin again (leak of Cl^- ions). This mechanism corresponds to an infinite feed-back effect.

Taurine may be considered as acting like a true "magnesium sparing" hormone (Durlach, 1988) in several biological processes. The nuclear microanalysis (Bara et al., 1995) indicate that Mg^{2+} ions decrease the Na^+, K^+ and Cl^- concentrations in EL and CL, except Na^+ concentration in EL. These data are similar to that observed with taurine. It would be very interesting to study other amino acids, taurine analogues or zwitterionic molecules to compare theirs effects with those obtained with taurine.

The microanalysis observations demonstrate the relationship between addition of taurine and monovalent ions concentration and distribution in human amniotic membrane layers. These results may be correlated and may explain previous electrophysiological data.

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