REVISTA ESPAÑOLA DE FISIOLOGIA, 50 (3), 167-174, 1994

Inhibition of D-Galactose and L-Phenylalanine Transport by $HgCl_2$ in Rat Intestine *in vitro*

A. Lugea*, A. Barber and F. Ponz

Departamento de Fisiología y Nutrición
Universidad de Navarra
31008 Pamplona (Spain)

(Received on May 13, 1994)

A. LUGEA, A. BARBER and F. PONZ. *Inhibition of D-Galactose and L-Phenylalanine Transport by $HgCl_2$ in Rat Intestine* in vitro. Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (3), 167-174, 1994.

The effect of Hg^{2+} on galactose and phenylalanine uptake has been studied in rat everted intestinal rings incubated for 2 minutes. The presence of 0.5 mM Hg^{2+} in the incubation medium inhibited the total galactose uptake from 30 % to 40 % and that of the phenylalanine about 70 %. The inhibition was due to a reduction of galactose transport and Na^+-dependent phenylalanine transport. Hg^{2+} inhibited the galactose transport in a non-competitive way, with a Vmax diminution without Km modification. The Na^+-dependent phenylalanine transport was totally blocked in the presence of 1 mM Hg^{2+}. The washing of the intestinal rings with 5 mM EDTA slightly decreased the inhibition produced by 0.5 mM Hg^{2+} on phenylalanine uptake whereas it did not modify the inhibition of galactose uptake. However, the inhibition of galactose uptake was completely reversed after washing with 10 mM cysteine. Therefore, phenylalanine transport seems to be more sensitive to $HgCl_2$ than galactose transport. The inhibition of these intestinal transport systems by Hg^{2+} might be due to its interaction with ligands of the transport proteins located in the luminal membrane of enterocytes.

Key words: Mercury, Galactose, Phenylalanine, Intestinal transport.

Sugars and amino acids are absorbed through the small intestinal epithelium both by diffusional passive processes and by active transport systems most of them Na^+-dependent. The active transport is selective, saturable and mediated by specific carriers located at the brush border membrane of the enterocyte. The required energy is obtained from the coupling of the Na^+-gradient across the brush-border membrane created by the activity of the Na^+/K^+ ATPase located at the basolateral membrane of the enterocytes (13). Moreover, the amino acids can be

Correspondence to A. Barber.
* Actual address: Unidad de Investigación del Sistema Digestivo, Hospital General Vall d'Hebron, Universidad Autónoma de Barcelona, Barcelona 08035 (Spain). (Fax: 343- 428 18 83).

absorbed by Na^+-independent transport systems (18).

Inhibition by $HgCl_2$ and other mercury compounds of the intestinal absorption of nutrients has been reported in various animal species and in different intestinal preparations (1, 7, 8, 11, 20, 21). The inhibition produced by mercury and other heavy metals is complex because they can interact with different proteins and enzymatic systems, altering both metabolic processes and cellular permeability. The toxic effect of Hg^{2+} is related to its ability to interact with sulfhydryl groups but also imidazole and carboxyl groups (16). At the intestinal epithelium level, the inhibition induced by Hg^{2+} on the transport of sugars and amino acids might be due to the interaction of the metal with functional groups localized in the transporters of the apical membrane of the enterocyte, or to an impairement of the Na^+/K^+ ATPase activity decreasing the Na^+ gradient (15).

Previous works carried out in our laboratory showed that Hg^{2+} inhibits galactose transport in rat intestine *in vivo* (17). This study was undertaken to examine the effects of Hg^{2+} on the intestinal transport of galactose and phenylalanine *in vitro*.

Materials and Methods

Chemicals.– $HgCl_2$, D-galactose (Gal), L-phenylalanine (Phe), L-methionine (Met), L-cysteine (Cys) and disodium ethylenediamine-tetraacetic acid (EDTA) were obtained from Merck. Phlorizin (Phlor) and urethane were from Sigma. All other reagents were of analytical grade and used without further purification. D-(1-^{14}C) galactose (61 mCi/mmol) and L-(U-^{14}C) phenylalanine (504 mCi/mmol) were obtained from Du Pont. A physiological solution Krebs-Ringer-Tris (KRT) containing 140 mM NaCl, 5.6 mM KCl, 3 mM $CaCl_2$, 1.4 mM $MgSO_4.7\ H_2O$, 6.1 mM Tris and 4.9 mM HCl, pH 7.4 was used. In Na^+-free solution Na^+ was osmotically substituted by Tris. $HgCl_2$ and the other reagents were added to the physiological solution just before each period of incubation.

Animals.– Albino Wistar rats of either sex weighing 150 to 250 g were obtained at the CIFA (Centro de Investigación Farmacológica Aplicada) breeding center at the University of Navarra (Pamplona, Spain). The animals were housed under controlled conditions of temperature, humidity and illumination (from 7 am to 7 pm) and maintained on standard rodent chow and with tap water *ad libitum*.

Measurements of D-galactose and L-phenylalanine uptake.– The influx of D-galactose and L-phenylalanine into rings of everted rat jejunum was determined according to the tissue accumulation method (2). After 24-hours fast, rats were anesthetized with urethane (1.25 g/kg s.c.) and the abdomen was incised. A 20 cm length of proximal jejunum was rapidly removed and rinsed gently with cold physiological solution (see above) and everted. Rings of about 0.5 cm in length and 25-30 mg in weight were cut. Three rings of everted intestine were incubated in each flask for 2 or 5 min at 37 °C with shaking in 10 ml oxygen-saturated incubation medium (KRT) containing D-galactose or L-phenylalanine and radioactive tracers. Hg^{2+} was added to the medium as $HgCl_2$. Samples were taken from the incubation medium initially and after the period of incubation for radioactive counting. At the end of the incubation period rings were rapidly removed, rinsed

in cold KRT, weighed and kept overnight in 0.5 ml 0.1 N HNO_3 at 4 °C. Aliquot samples were taken for liquid scintillation counting (LS 1800, Beckman). The uptake of D-galactose and L-phenylalanine were estimated from the relation between the cpm of the incubation medium samples and the cpm of the HNO_3 aliquots. The results were expressed as nmoles of substrate/100 mg wet weight. In some experiments, 0.5 mM phlorizin or 40 mM L-methionine were added to the incubation medium for total inhibition of intestinal transport of D-galactose and L-phenylalanine respectively.

Reversibility of the inhibitory effect of Hg^{2+} on D-galactose and L-phenylalanine uptake.– After 2 min preincubation of the intestinal rings in the physiological solution containing Hg^{2+} only, the rings were washed twice for 5 min in KRT solution in the absence or in the presence of either 5 mM EDTA or 10 mM L-cysteine. Thereafter, the rings were incubated for 2 min in the physiological solution with D-galactose or L-phenylalanine without Hg^{2+}.

Statistical methods.– Results of substrate uptake are expressed as means ± SEM. Statistical differences between groups were determined using the Analysis of Variance for multiple comparisons and the Student's *t* test for single comparisons. The linear regression analyses were used for the estimation of kinetic parameters.

Results

Effect of Hg^{2+} on galactose uptake.– The 0.5 mM galactose uptake was inhibited in a dose dependent manner by Hg^{2+} (fig. 1). The degree of inhibition increased with the incubation time. Considering these results, 0.5 mM Hg^{2+} was chosen to study the effect of Hg^{2+} on D-galactose uptake as function of galactose concentration (0.5 mM to 20 mM) in experiments of 2 min duration. Galactose uptake includes a passive diffusional component and a transcellular saturable process mediated by specific phlorizin-sensitive transporters. Galactose uptake was significantly inhibited by 0.5 mM Hg^{2+} at every galactose concentration studied (fig. 2). Nevertheless, the passive sugar uptake, obtained in the presence of 0.5 mM phlorizin, was not altered by the metal, indicating that the Hg^{2+} effect was exerted only on the galactose transport system. The transport values were estimated from the difference between the uptake values obtained in the presence of phlorizin (diffusion) or in its absence (total uptake). Hg^{2+} inhibited in a non-competitive way galactose transport, decreasing V_{max} about 40 % without modifying apparent K_m (fig. 3). From these data Hg^{2+} appears to have an inhibition constant K_i of about 0.8 mM.

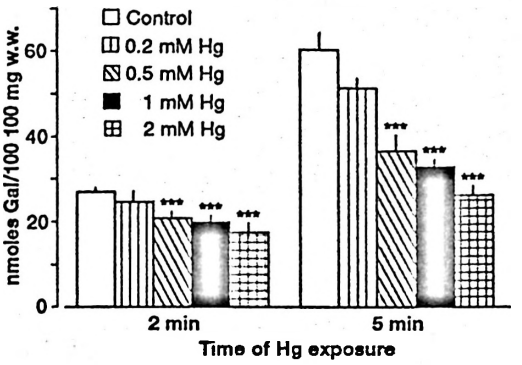

Fig. 1. *Influence of several concentrations of Hg^{2+} on 0.5 mM galactose uptake in rat intestinal rings.* The tissue was incubated for 2 or 5 min in the presence of Hg^{2+}. Each value represents the mean ± SEM of six intestinal rings. ***$p < 0.001$ vs control group.

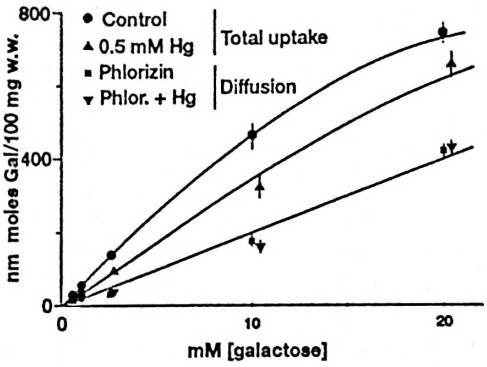

Fig. 2. *Effect of 0.5 mM Hg^{2+} on galactose uptake in intestinal rings.*
The tissue was incubated for 2 min without (total uptake) or with 0.5 mM Phlorizin (diffusion). Each value represents the mean ± SEM of twelve intestinal rings.

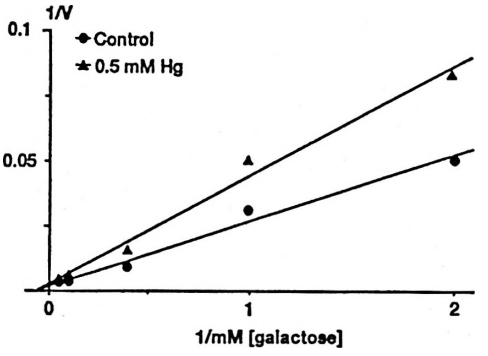

Fig. 3. *Lineweaver-Burk plot of the inhibition of galactose transport by 0.5 mM Hg^{2+} in rat intestine.*
Each value represents the mean of twelve intestinal rings. V, nmoles galactose/100 mg w. w. Kinetic parameters estimated: Control, K_m = 16.3 mM, V_{max} = 660 nmoles/100 mg w. w. Hg^{2+}, K_m = 16.7 mM, V_{max} = 397 nmoles/100 mg w.w.

Effect of Hg^{2+} present in the serosal or mucosal solution on galactose uptake.– The galactose inhibitory effect could be attributed to the binding of Hg^{2+} to ligands in the mucosal membrane, inside the enterocyte or in the basolateral membrane, especially Na^+/K^+ ATPase. To study the interaction site of Hg^{2+}, everted

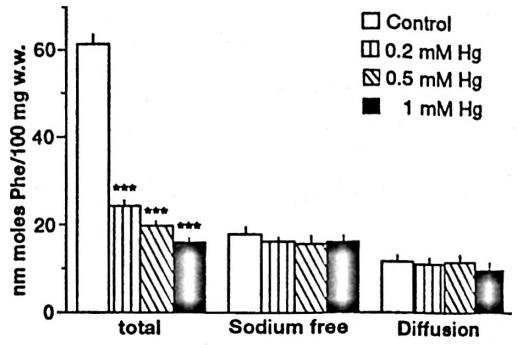

Fig. 4. *Inhibition of 0.5 mM phenylalanine uptake by Hg^{2+} in presence of Na^+ in the incubation medium (Total uptake) and in Na^+-free medium.*
Diffusion of Phe was estimated in Na^+ containing medium with 40 mM Met added. Each bar represents the mean ± SEM of twelve experimental values. ***p < 0.001 vs control group.

intestinal sacs of rat jejunum were prepared according to the technique of WILSON and WISEMAN (23). These sacs were preincubated for 2 min in physiological solution which contained 0.5 mM Hg^{2+} in either the mucosal or serosal solution. Then the sacs were sliced into rings which were incubated as usual for 2 min with the galactose and in the absence of the metal. Results show that sugar uptake decreased significantly ($p < 0.01$) with respect to the control (33.3 ± 1.7, n = 6) when Hg^{2+} was found in the mucosal solution (19.9 ± 1.5, n=6) but not in the serosal solution (28.9 ± 1.6, n = 6). Therefore, the Hg^{2+} inhibitory effect was related to its presence at the mucosal side.

Effect of Hg^{2+} on L-phenylalanine uptake.– Phenylalanine uptake by the intestinal tissue represents at least the sum of three components: Na^+-dependent transport, Na^+-independent transport and passive diffusional uptake. The effect of $HgCl_2$ (0.2 to 1 mM) on the different components of 0.5 mM phenylalanine uptake has been studied. After a 2 min incubation in the presence of Hg^{2+} the

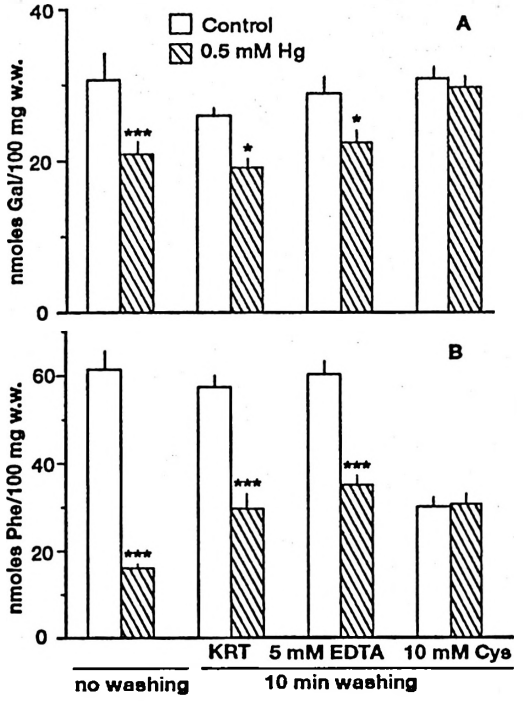

Fig. 5. *Reversibility of 0.5 mM galactose uptake inhibition by 0.5 mM Hg^{2+}.*
After a 2 min pre-incubation period without (Control) or with the inhibitor (Hg^{2+}), the rings were incubated with galactose (A) or phenylalanine (B): a) without intermediate washing; b) with 10 min washing in physiological solution KRT; c) with washing in the presence of 5 mM EDTA or d) 10 mM Cys. Each bar represents the mean ± SEM of nine experimental values. *p < 0.05; ***p < 0.001 vs control group.

total uptake of the amino acid was inhibited about 60 % by 0.2 mM Hg^{2+} and about 70 % by 1 mM Hg^{2+} (fig. 4). On the other hand, phenylalanine uptake in the absence of Na^+ or in the presence of 40 mM L-methionine (diffusion) was not affected by the metal. A similar inhibition was observed at 1 mM Hg^{2+} using different phenylalanine concentrations. As shown in table I, Na^+-dependent phenylalanine transport was almost completely blocked by 1 mM Hg^{2+} which did not modify Na^+-independent transport.

Reversibility of the Hg^{2+} inhibitory effect on galactose uptake.– The presence of 0.5 mM Hg^{2+} in the physiological solution during a 2 min preincubation period inhibited 0.5 mM galactose uptake in the intestinal tissue about 30 % (fig. 5, A). It is important to remark the absence of the metal during the incubation period. Neither 10 min washing of the intestinal rings with KRT solution nor addition of 5 mM EDTA reduced the inhibitory effect produced by the metal. Nevertheless, the washing of the intestinal tissue with 10 mM L-cysteine for 10 min reversed the inhibition completely.

Reversibility of the Hg^{2+} inhibitory effect on L-phenylalanine uptake.– After preincubation of the intestinal rings for 2 min in the presence of 0.5 mM Hg^{2+} and subsequent incubation in its absence, a 72 % inhibition of the 0.5 mM phenylalanine uptake by the intestinal tissue was observed (fig. 5, B). The 10 min intermediate washing with physiological solution KRT or containing 5 mM EDTA significantly increased the amino acid uptake, without reaching the control values. Washing of the intestinal rings with 10 mM Cys significantly reduced phenylalanine uptake under control conditions, probably due to the competition of both amino acids for the transport system. For this reason Cys was not effective to study the reversibility of the phenylalanine uptake inhibition due to Hg^{2+}.

Discussion

$HgCl_2$ reduced galactose and phenylalanine uptake in everted rings of rat jejunum. This reduction was likely due to Hg^{2+} interaction with the Na^+-galactose and Na^+-phenylalanine cotransporters.

Phenylalanine uptake was more sensitive to the metal than galactose uptake. Galactose uptake was inhibited from the initial concentration of 0.5 mM Hg^{2+}, whereas phenylalanine uptake was reduced even to a greater extent from 0.2 mM Hg^{2+}. Uptake inhibition also increased with exposure time of the rings to the Hg^{2+}, especially for galactose. Similar results had been found by Stirling (20) for galactose and alanine in rabbit ileum *in vitro* and by MILLER (12) for glucose and cycloleucine in intestine of various teleost species. The present results suggest that Hg^{2+} interacts with residues located in the mucosal membrane or in the cytosolic side, probably with distinct affinity for the metal. The slight reversibility of the inhibition by washing with KRT only or with 5 mM EDTA also supports that suggestion.

Inhibition was exclusively exerted on the transport system as no effect was observed on the passive permeability of the intestinal tissue. Similar results have been widely reported for Hg^{2+} and for other heavy metals. The inhibition produced by Hg^{2+} could be due to metal interactions with the transport mechanisms decreasing the affinity of the solute or Na^+ for the cotransporter or altering the substrate transfer across the mucosal side of the tissue. In the present study, Hg^{2+} decreased the V_{max} of the galactose transport without changing the K_m. Such changes suggest that Hg^{2+} does not modify the affinity of galactose to its cotransporter. A similar non-competitive inhibition for mercury has also been described in other species (20, 21) as well as in rat intestine for other heavy metals (10). Miller (12) reported different values for the kinetic parameters of glucose and cycloleucine transport using membranes or slices from killfish intestine.

Hg^{2+} affected only the galactose and phenylalanine Na^+-dependent transport systems, suggesting that the metal interacts with functional groups related to Na^+-coupled processes. However, the metal could also modify the intestinal permeability to Na^+ reducing the Na^+ gradient across the luminal membrane. Nevertheless, it has been described a non-modification of the Na^+ permeability by Hg^{2+} (12, 14, 22). On the other hand, Hg^{2+} is known to inhibit Na^+/K^+ ATPases (6, 9). In our experiments it could alter the intestinal Na^+ gradient and the Na^+-coupled transport systems. However, the results of the present work do not support this hypothesis. First, because Hg^{2+} inhibited galactose transport when it is present at the mucosal side and not at the serosal one. Secondly, the short incubation period and the low absorption rate of the metal (4) hinder its accessibility to the enterocyte basolateral membrane. Therefore, Hg^{2+} seems to interact with groups in the luminal membrane related to the Na^+-substrate cotransport systems.

Our results do not allow to establish the nature of the functional groups involved in the inhibition. Washing with 10 mM cysteine completely reversed the Hg^{2+} inhibition on galactose transport. Cysteine is often used to protect enzymes against the inactivation caused by the oxidation of -SH groups, suggesting that -SH groups could be involved in this inhibition. In the same way, the inhibition by Hg^{2+} observed in this study is similar to that found with sulfhydryl reagents (3). Moreover, inhibition of Na^+-dependent alanine transport and galactose transport by Hg^{2+} was reversed by the thiol reducing agents dithiothreitol (19) and dithioerythritol (17) respectively. Therefore, although the interaction of Hg^{2+} with other chemical groups cannot be discard-

ed, the metal might bind to different -SH groups in the mucosal membrane related to sugars or amino acids transport systems.

Acknowledgements

The investigation was supported by grant PB 86-0407 from the Spanish DGICYT (Dirección General de Investigación Científica y Tecnológica).

A. LUGEA, A. BARBER y F. PONZ. *Inhibición por Cl_2Hg del transporte de D-galactosa y L-fenilalanina en intestino de rata* in vitro. Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (3), 167-174, 1994.

Se estudia el efecto del Hg^{2+} sobre la penetración de galactosa y fenilalanina en anillos intestinales evertidos de rata en experimentos de 2 min. La presencia en el medio de incubación de Hg^{2+} 0,5 mM inhibe la penetración total de galactosa entre 30 y 40 % y la de fenilalanina sobre el 70 %. La inhibición se debe a ladisminución del transporte de galactosa y del de fenilalanina dependiente de Na^+. El Hg^{2+} inhibe el transporte de galactosa de forma no competitiva, con disminución de la Vmax y sin modificación de la Km. El transporte de fenilalanina se anula totalmente en presencia de Hg^{2+} 1 mM. El lavado de los anillos intestinales con EDTA 5 mM disminuye la inhibición producida por el Hg^{2+} 0,5 mM sobre el transporte de fenilalanina, pero no la que ejerce sobre el transporte de galactosa. El lavado con cisteína 10 mM revierte totalmente la inhibición del transporte de galactosa. Los resultados sugieren que la inhibición por Hg^{2+} de los sistemas de transporte intestinal puede ser debida a su interacción con ligandos de las proteínas transportadoras de la membrana luminal de los enterocitos.

Palabras clave: Mercurio, Galactosa, Fenilalanina, Transporte intestinal.

References

1. Chauncey, B., Schimid, E. C. and Goldstein, L. (1988): *J. Toxicol. Environ. Health*, 23, 257-265.
2. Crane, R. K. and Mandelstam, P. (1960): *Biochim. Biophys. Acta*, 45, 460-467.
3. Diez, N., Barber, A., Ponza nd F. (1991): *Rev. esp. Fisiol.*, 47, 69-74.
4. Endo, T., Nakaya, S. and Kimura, R. (1988): *Pharmacol Toxicol*, 63, 361-368.
5. Farmanfarmaian, A. and Socci and R. (1985): *Aquat. Toxicol.*, 5, 21-23.
6. Hajjar, J. J., Dobish, M. P. and Tomicic, T. K. (1989): *Proc. Soc. Exp. Biol. Med.*, 190, 35-41.
7. Iturri, S. J., Peña and A. (1986): *Comp. Biochem. Physiol.*, 84, 363-368.
8. Klip, A., Grinstein, S., Biber, J. and Semenza, G. (1980): *Biochim. Biophys. Acta*, 598, 100-114.
9. Kramer, H. J., Gonick, H. C. and Lu, E. (1986): *Nephron*, 44, 329-336.
10. Lugea, A., Barber, A. and Ponz, F. (1988): *Rev. esp. Fisiol.*, 44, 121-126.
11. Miller, D, S., Shehata, A. T. and Lerner, J. (1980): *J. Pharmacol. Exp. Ther.*, 214, 101-105.
12. Miller, D. S. (1981): *J. Pharmacol. Exp. Ther.*, 216, 70-76.
13. Murer, H.. and Kinne, R. (1977): In: "Biochemistry of Membrane Transport" (Semenza, G. and Carafoli, E., eds.), Springer-Verlag. Berlin. 292-304.
14. Murer, H., Eveloff, J., Kinne, W. B. and Field, M. (1977): *Bull. M. T. Desert Isl. Biol. Lab.*, 17, 52-56.
15. Mush, M. W., Chauncey, B., Schimid, E. C., Kinne, R. K, H. and Goldstein, L. (1990): *Toxicol. Appl. Pharmacol.*, 104, 59-66.
16. Pritchard, J. B. (1979): *Fed. Proc.*, 38, 2220-2225.
17. Rodríguez-Yoldi, M. J., Lluch, M. and Ponz, F. (1987): *Rev. esp. Fisiol.*, 43, 239-244.
18. Schultz, S. G. (1979): In "Membrane Transport in Biology" (Giebisch, G., Tosteson, D. C. and Ussing, H. H., eds.). Springer-Verlag. Berlin. 749-780.
19. Sellinger, M., Ballatori, N. and Boyer, J. L. (1988): *Toxicol. Appl. Pharmacol.*, 95, 279-283.
20. Stirling, C. E. (1975): *J. Membr. Biol.*, 23, 33-56.
21. Tsuchiya, W. and Okada, Y. (1982): *Experientia*, 38, 1073-1075.
22. Watkings, D. W., Chenu, C. and Ripoche, P. (1989): *Eur. J. Physiol.*, 415, 165-171.
23. Wilson, Th., Wiseman, G. (1954): *J. Physiol.*, 239, 116-125.