
ATTI ACCADEMIA NAZIONALE DEI LINCEI
CLASSE SCIENZE FISICHE MATEMATICHE NATURALI
RENDICONTI

PIETRO PALATRONI, ANNA MARIA BONDI, GIOVANNA
MENGHI, MARIA GABRIELLA GABRIELLI

**Morphological observations and histochemical
localization of carbonic anhydrase in the mucosa of
the stomach of *Salmo irideus***

*Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche,
Matematiche e Naturali. Rendiconti, Serie 8, Vol. 69 (1980), n.6, p. 458–462.*
Accademia Nazionale dei Lincei

[<http://www.bdim.eu/item?id=RLINA_1980_8_69_6_458_0>](http://www.bdim.eu/item?id=RLINA_1980_8_69_6_458_0)

L'utilizzo e la stampa di questo documento digitale è consentito liberamente per motivi di
ricerca e studio. Non è consentito l'utilizzo dello stesso per motivi commerciali. Tutte le
copie di questo documento devono riportare questo avvertimento.

*Articolo digitalizzato nel quadro del programma
bdim (Biblioteca Digitale Italiana di Matematica)
SIMAI & UMI*

<http://www.bdim.eu/>

Biologia. — *Morphological observations and histochemical localization of carbonic anhydrase in the mucosa of the stomach of Salmo irideus.* Nota di PIETRO PALATRONI (*), ANNA MARIA BONDI (**), GIOVANNA MENGHI (**) e MARIA GABRIELLA GABRIELLI (*), presentata (***) dal Socio A. STEFANELLI.

RIASSUNTO. — Nello stomaco di *Salmo irideus* è stato eseguito uno studio morfologico ultrastrutturale ed è stata localizzata istochimicamente l'anidrasi carbonica, al microscopio ottico, con il metodo di Hansson.

L'osservazione morfologica ha dimostrato che le cellule di rivestimento della mucosa gastrica sono tipiche cellule mucose. Le ghiandole gastriche sono ghiandole tubulari; nei punti di contatto tra cellule adiacenti sono presenti spazi intercellulari, occupati da numerose evaginazioni citoplasmatiche.

La reazione istochimica per l'anidrasi carbonica è risultata positiva sia nelle cellule di rivestimento della mucosa che nelle ghiandole gastriche.

I risultati sono stati discussi comparandoli con quelli ottenuti nelle corrispondenti strutture del proventricolo del pollo.

In our Institutes studies on the histochemical localization of carbonic anhydrase (CA) in the stomach of various vertebrate species (Palatroni, 1974, 1975; Palatroni *et al.*, 1977, 1980) and histochemical research on the alimentary canal of the trout *Salmo irideus* (Materazzi e Menghi, 1975) are carried out.

In the literature, to the best of our knowledge, there have been no ultrastructural morphological observations of the gastric epithelium of *Salmo irideus* so both this research and the histochemical demonstration of CA in the same tissue seem to be interesting subjects.

It is generally recognized that in the stomach of mammals acid secretion is the result of the catalytic effect of CA on CO₂ hydration (Carter, 1972; Bundy, 1977). This hypothesis agrees with the histochemical findings (Cross, 1970; Palatroni, 1975; Sugai and Ito, 1980) which demonstrate that the enzyme activity in rat and mouse gastric mucous membrane is localized on the surface of the microvilli of the parietal cells.

(*) Istituto di Istologia ed Embriologia dell'Università di Camerino.

(**) Istituto di Anatomia ed Istologia dell'Università di Camerino.

(***) Nella seduta del 6 dicembre 1980.

Other results (Palatroni *et al.*, 1977) have shown that during the embryonic development of the trout CA activity is clearly present on the gastric mucosa at the 4th Prakash (1961) stage, when the gastric glands differentiation begins. In the course of subsequent stages of development the reaction increases in intensity and extension both in the mucous membrane and in the glands.

These observations were carried out using Hansson's method (1967, 1968) which is specific but for sections obtained with a cryostat does not give sufficient resolution of the structures and is difficult to apply when using electron microscopy.

The application of Hansson's method to semi-thin sections obtained after embedding in hydrophilic plastic, which does not interfere with the histochemical reaction, allows a better resolution (Ridderstrale, 1976; Palatroni *et al.*, 1980).

Therefore, to obtain a fine localization of CA in the stomach of *Salmo irideus*, this technique has been used, as previously in fowl proventriculus (Palatroni *et al.*, 1980).

Adults of *Salmo irideus* weighing about 300 g were decapitated and the gastric portion, where the gastric glands are situated, was removed.

For morphological observation with the electron microscope, small pieces (1 mm wide) of tissue were prefixed in a glutaraldehyde solution, post-fixed in osmium tetroxide, stained with uranyl acetate and embedded in Araldite. Ultra-thin sections were cut with the LKB ultramicrotome mod. Ultratome III and, after staining with lead citrate, examined with a Hitachi HS-8 electron microscope.

For histochemical research the tissue was fixed for 2 hours at 4 °C in 0.1 M pH 7.4 Millonig buffer containing 0.5 percent glutaraldehyde and 4 percent formaldehyde. After fixing small pieces (1–2 mm wide) of tissue were embedded in type JB-4 (Polysciences) plastic; semi-thin sections of 1–2 μ m were transferred, using Millipore filters, into Hansson's incubation medium, and were left floating for 8–10 minutes at room temperature (22–24 °C). After rinsing with distilled water the samples were treated with $(\text{NH}_4)_2\text{S}$ (1 %) for 3 minutes, rinsed again and then, still floating, stained with hematoxylin/eosin. Then the sections were dried and placed on slides with Technicon mounting medium.

For the controls samples were incubated in the presence of 10^{-5} M acetazolamide, which acts as a specific inhibitor of CA.

The validity of this technique, and more generally of histochemical methods for CA demonstration, has been questioned by some authors who do not accept its specificity (Muther, 1972, 1977; Churg, 1973). Other authors, such as Musser and Rosen (1972) and Lightfoot and Cassidy (1973), have a different opinion. The subject has already been treated by one of us (Palatroni, 1974, 1975) and an extensive critical study on the histochemistry of CA was made by Lonnerholm (1974) in support of the specificity of Hans-

son's method. Recent works by Lonnerholm (1980) and Sugai and Ito (1980) give further evidence supporting the histochemical method.

Plate I, Fig. 1 shows the cells of the surface epithelium of the gastric mucosa of *Salmo irideus* as they appear at the electron microscope. They are typical mucous cells which possess basal nuclei and contain masses of soprannuclear mucin granules; a well developed rough endoplasmic reticulum, concentric in shape, is present.

The gastric glands are tubular and mostly localized in the cardiac region of the stomach. In Plate I, Fig. 2, the reconstruction, at ultrastructural level, of a part of a gastric gland is depicted and parts of two cells with zymogen granules are observable. At the points of contact between adjacent cells there are intercellular spaces occupied by extensive cytoplasmic evaginations, as shown in Plate I, Fig. 2. Near these spaces there are zymogen granules and mitochondria; the rough endoplasmic reticulum is wide; the nucleus situated in the basal region of the cell has a finely diffuse chromatin and a voluminous nucleolus.

The histochemical reaction for CA shows the presence of the enzyme at cellular level both in the surface epithelium of the mucosa and in the gastric glands (Plate II, Fig. 4-5).

In the surface epithelium of the mucosa CA activity is present more or less diffusely, on the luminal surface; the enzyme is mostly localized on the contact surface between adjacent cells (Plate II, Fig. 4).

In the cells of the gastric glands (Plate II, Fig. 5) the CA activity appears mostly on the lateral surface, between cells, where the ultrastructural morphological observation has shown the presence of an intercellular space (Plate I, Fig. 2); the positive reaction occurs also in the luminal surface of the secreting cells. Therefore these results are similar to those obtained in the proventricular mucosa of the fowl, both in the morphological picture described by Toner (1963) and in the histochemical localization of CA as revealed by Palatroni *et al.* (1980). These observations agree more generally with the characteristics ascribed, in mammalia and in other vertebrates, to cellular surfaces competent for an acid secretion. Hence as Toner did for the fowl, we should define the cells of the gastric glands of *Salmo irideus* as "oxinticpeptic cells".

REFERENCES

- BUNDY H. F. (1977) - *Carbonic anhydrase*, «Comp. Bioch. Physiol.», 57 B, 1.
CARTER M. J. (1972) - *Carbonic anhydrase: isoenzymes, properties, distribution and functional significance*, «Biol. Rev.», 47, 465.
CHURG A. (1973) - *Carbonic anhydrase histochemistry: evidence for non enzymatic reaction and artifact production*, «Histochemie», 36, 293.
CROSS S. A. M. (1970) - *Ultrastructural localisation of carbonic anhydrase in rat stomach parietal cells*, «Histochemie», 22, 219.
HANSSON H. P. J. (1967) - *Histochemical demonstration of carbonic anhydrase activity*, «Histochemie», 11, 112.

- HANSSON H. P. J. (1968) - *Histochemical demonstration of carbonic anhydrase activity in some epithelia noted for active transport*, «Acta Physiol. Scand.», 73, 427.
- LIGHTFOOD F. G. and CASSIDY M. M. (1973) - *Characteristics of the staining reaction for carbonic anhydrase in light and electron microscopy*, «J. Histochem. Cytochem.», 21, 402.
- LONNERHOLM G. (1974) - *Carbonic anhydrase histochemistry, a critical study of Hansson's cobalt-phosphate method*, «Acta Physiol. Scand. Suppl.», 418, 1.
- LONNERHOLM G. (1980) - *Carbonic anhydrase in rat liver and rabbit skeletal muscle; further evidence for the specificity of the histochemical cobalt-phosphate method of Hansson*, «J. Histochem. Cytochem.», 5, 427.
- MATERAZZI G. and MENGHI G. (1975) - *Il canale alimentare in embrioni ed avannotti di Salmo Iriseus*, «Ann. Ist. e Mus. Zool.», Napoli 21, 21.
- MUSSER G. L. and ROSEN S. (1972) - *Observations on the specificity of newer histochemical methods for the demonstration of carbonic anhydrase activity*, «J. Histochem. Cytochem.», 20, 951.
- MUTHER T. F. (1972) - *A critical evaluation of the histochemical methods for carbonic anhydrase*, «J. Histochem. Cytochem.», 20, 319.
- MUTHER T. F. (1977) - *On the lack of specificity of the cobalt-bicarbonate method for carbonic anhydrase*, «J. Histochem. Cytochem.», 25, 1043.
- PALATRONI P. (1974) - *Osservazioni istochimiche sulla anidrasi carbonica nello stomaco ghiandolare dell'embrione del pollo*, «Rend. Acc. Naz. Lincei», 56, 249.
- PALATRONI P. (1975) - *Osservazioni ultrastrutturali sulla localizzazione dell'anidrasi carbonica e sulla morfologia delle cellule parietali dello stomaco del topo*, «Rend. Acc. Naz. Lincei», 58, 797.
- PALATRONI P. and MENGHI G. (1977) - *Localizzazione istochimica della anidrasi carbonica durante lo sviluppo embrionale e post embrionale dello stomaco di Salmo irideus*, «Riv. Ist. Norm. Pat.», 21, 7.
- PALATRONI P., GABRIELLI G. and SCATTOLINI B. (1980) - *Histochemical localization of carbonic anhydrase in fowl proventriculus*, «Experientia», 36, 678.
- PRAKASH A. (1961) - *Distribution and differentiation of alkaline phosphatase in the gastro intestinal tract of steel head trout*, «J. Exp. Zool.», 146, 237.
- RIDDERSTRALE Y. (1976) - *Intracellular localization of carbonic anhydrase in the frog nephron*, «Acta Physiol. Scand.», 98, 465.
- SUGAI N. and ITO S. (1980) - *Carbonic anhydrase, ultrastructural localization in the mouse gastric mucosa and improvements in the technique*, «J. Histochem. Cytochem.», 28, 511.
- TONER P. G. (1963) - *The fine structure of resting and active cells in the submucosal glands of the fowl proventriculus*, «J. Anat. Lond.», 97, 575.

EXPLANATION OF PLATES I-II

PLATE I

- Fig. 1. - Electron micrograph of the cells of the gastric mucosa epithelium in the stomach of *Salmo irideus*. Reconstruction. $\times 3500$.
- Fig. 2. - Electron micrograph showing a part of a gastric gland in the stomach of *Salmo irideus*. The intercellular spaces, the glandular lumen, the various cytoplasmic structures and the zymogen granules are clearly observable. Reconstruction $\times 5500$.

PLATE II

- Fig. 3. - Electron micrograph of the lumen, filled with microvilli, in a gastric gland of *Salmo irideus*. $\times 12000$.
- Fig. 4. - Gastric mucosa epithelium of the stomach of *Salmo irideus*. Histochemical reaction for carbonic anhydrase activity by Hansson's method. The positive reaction, indicated by dark lines, is present in the cellular membranes, mostly on the contact surfaces between adjacent cells. Light micrograph. $\times 1200$
- Fig. 5. - Gastric gland of the stomach of *Salmo irideus*. Histochemical reaction for carbonic anhydrase activity by Hansson's method. The positivity, indicated by dark lines, results on the lateral surfaces of the cells where the ultrastructural observation has shown the presence of intercellular spaces; the reaction is also present on the luminal surface of the secreting cells. Light micrograph. $\times 1200$



