Histochemical localization of carbonic anhydrase in fowl proventriculus

P. Palatroni, Gabriella Gabrielli and B. Scattolini

Institute of Histology and Embryology, Faculty of Science, University of Camerino, I-62032 Camerino (Italy), 4 October 1979

Summary. The carbonic anhydrase activity in fowl proventriculus was studied by the histochemical method of Hansson. The activity was observed in the mucose membrane cells and in proventricular gland cells. These results, about which there is disagreement in the literature, are discussed in the text.

The problem of secretion of hydrochloric acid by the cells of zymogenic glands in fowl proventriculus is still being debated in the literature. Some authors, such as Hill[1] and Hodges[2] claim that these cells are oxynticopeptic and ascribe to them the double function of production of hydrochloric acid and the pepsinogen precursor enzyme. Some others, such as Gay et al.[3,4] think that only the cells covering the gastric mucous membrane are responsible for hydrochloric acid production.

It is generally recognized that in the stomach of mammals acid secretion is the result of the catalytic effect of carbonic anhydrase (CA) on CO_2 hydratation[5,6]. This hypothesis agrees with the histochemical findings[7,8] 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.

Other results[9] have shown that during the embryonic development of a chicken (from 8-day-old up to hatching) the CA activity is clearly present in the proventriculus both in the cells covering the gastric membrane and in the typical proventricular glands.

These observations were carried out using Hansson's method[10,11] which is specific but, for sections obtained with a cryostat, does not give sufficient resolution of the structures and is difficult to apply for observation with the electron microscope. The application of Hansson's method to thin sections obtained after embedding in hydrophilic plastic, which does not interfere with the histochemical reaction, allows a better resolution of the different structures[12]. This method was applied to localize the CA in fowl proventriculus in order to try to clarify the contradictory findings in the literature on the functional activity of the proventricular glands.

Materials and methods. 15-day-old Golden Comet chickens were used in this research: small pieces (1-2 mm wide) of proventriculus were fixed for 3 h at 4 °C in a 3% glutaraldehyde solution in 0.2 M cacodylate buffer (pH 7.4) in presence of 7% sucrose. After this treatment the tissue was embedded in type J. B-4 (Polysciences) plastic. The sections, 1-2-μm-thick, were transferred, using Millipore filters, into the incubation medium where they stayed, floating, for about 10 min at room temperature (22-24 °C). After rinsing with distilled water the samples were treated with $(NH_4)_2S$ (1%) for 3 min, rinsed again, and then dryed and placed on slides by means of a Technicon mounting medium. The controls were performed by incubating the samples in the medium with the addition of 10^{-5} M acetazolamide, a specific inhibitor of CA.

The histological structure of the proventriculus was studied with the light microscope using samples stained with hematoxylin/eosin and with the PAS reaction. The ultrastructure was studied with the electron microscope using ultra-thin sections of fragments fixed in glutaraldehyde, post-fixed in osmium tetroxide, and embedded in araldite.

Results. Figure 1 shows a partial section of the proventriculus and some of the characteristic proventricular glands located on the wall of the organ. These glands have only a small number of cells that react with silver ions, and seem to contain mainly cubic cells. Aitken[13], Toner[14], Menzies and Fisk[15], on the basis of the fine structure and cytochemical characteristics of these cells, concluded that they secrete hydrochloric acid and the enzyme precursor of pepsinogen, and for this reason are called 'oxynticopeptic'.

CA activity is present on the surface of the mucous membrane and oriented toward the lumen (figure 2), this is located in the apex of the cells; the enzyme is also present

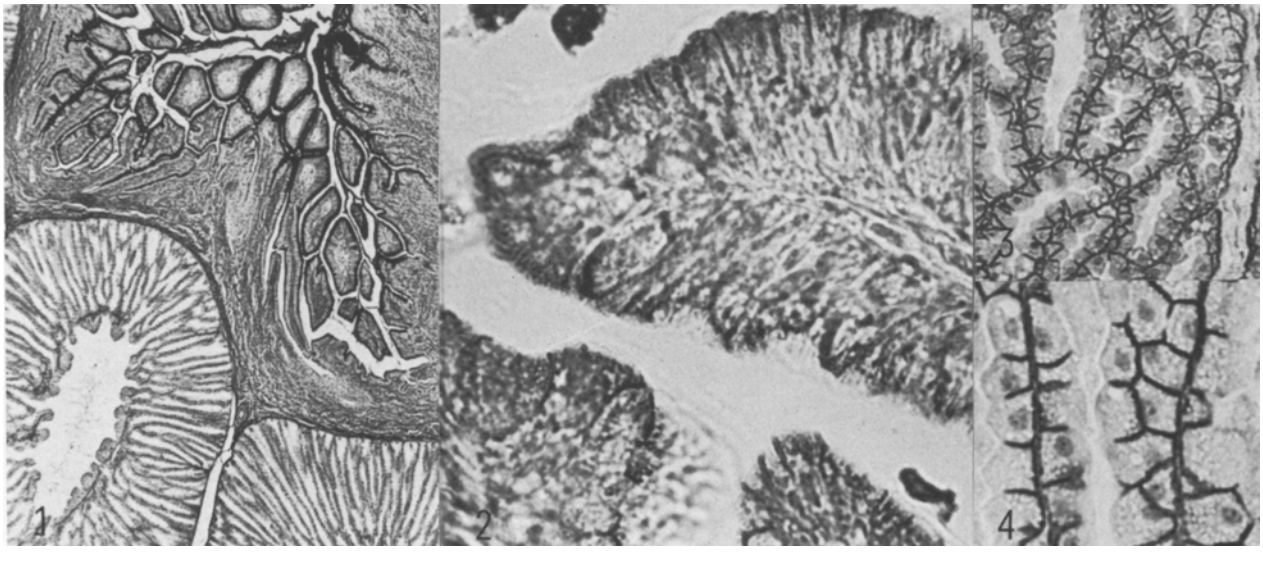

Fig. 1. Fowl's proventriculus, showing both the luminal surface and the glands on the wall of the organ. PAS reaction. × 30. Fig. 2. Fowl's proventriculus, folds of the gastric mucosae. Hansson reaction for CA. × 1600. Fig. 3 and 4. Fowl's proventriculus, oxynticopeptic cells of the glands, observed both in transversal (figure 3) and in longitudinal (figure 4) sections in respect to the lumen. Hansson reaction for CA. × 650 and × 1600, respectively.

on the side surfaces of adjacent cells. In the oxynticopeptic cells of the glands, observed both in transverse (figure 3) and in longitudinal (figure 4) sections with respect to the lumen of the tubular glands, the CA is localized on the basal and lateral surfaces of the cells. The dark lines, in conclusion, clearly indicate where the reaction is present and where it occured.

These results disagree with the findings of Gay et al.[3,4] who, by means of immunohistochemistry, concluded that the CA activity was localized only in the mucous membrane cells and that hydrochloric acid is not produced by the gland cells. These results are therefore consistent both with the Toner[14] hypothesis and with ours[9].

The electron microscope observations show, in fact, that the apical cell membrane of the glandular cells is smooth, without microvilli. In addition the junctional complex among neighbouring cells is localized close to the base of the cells and the basement cell membrane shows invaginations whose extention increases after stimulation with histamine[14]. The lateral surface which is also the boundary surface, in addition, contains several microvilli which increase the surface. Furthermore this surface may be used for secretion owing to the basal position of the junctional complex. By mean of this mechanism a surface increase (almost six times according to Toner) is obtained. This is almost twice as great as the one which is caused by the presence of intracellular canaliculi in the mammalian parietal cells (3 times according to Hally[16]). For this reason Toner[14] considers the space between the oxynticopeptic cells as equivalent to the intracellular canaliculi of the oxyntic cells.

It is also worth while pointing out that an increased surface has been observed also in the oxynticopeptic cells of other vertebrates[17–19], and that this increased surface is considered to be fundamental for an acid secretion to take place.

In the mammalian oxyntic cells, which certainly produce HCl, the CA activity is typically localized in the intracellular canaliculi at the level of microvilli. For this reason it may be concluded that the distribution of the enzyme on the surface of the oxynticopeptic cells of fowls, described in the present paper, is a histochemical proof of the fact that the 'intracellular space' between the oxynticopeptic cells and the 'intracellular canaliculi', characteristic of the oxyntic cells, play the same role. This, in conclusion, shows that the oxynticopeptic cells determine acid secretion.

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Effect of tranexamic acid on progress of experimental tumours and on DNA-synthesis[1]

B. Åstedt and C. Tropé

Research Laboratory of the Department of Obstetrics and Gynecology, and the Department of Oncology, University Hospital, S-221 85 Lund (Sweden), 16 October 1978

Summary. The fibrinolytic inhibitor tranexamic acid affects certain experimental tumours; it prolongs survival (Lewis lung adenocarcinoma), decreases tumour weight (C3H breast carcinoma), and inhibits ascitic production (AH 130 rat hepatoma). However, it does not significantly influence DNA-synthesis in cell suspensions prepared from the same tumours.

Cytostatic drugs directly or indirectly impair the DNA-synthesis of malignant cells. They also inevitably act on the normal cells, which limits their therapeutic value and makes the search for points of attack in other enzyme systems necessary. Certain cells of mammalian tissues in culture produce hardly-detectable amounts of stable plasminogen activator or none at all, but if they are transformed by oncogenic viruses or carcinogenic substances they release abundant amounts of this activator[2,3]. Furthermore, it has been shown that in organ cultures of normal human ovarian tissue only trace amounts of plasminogen activator are released, while malignant tumours originating from the same organ release large amounts in such cultures[4]. This activator has been shown to be immunologically identical with the plasminogen activator in urine, i.e. urokinase[5], but not with the activator released from the vessel walls into the blood stream[6]. Such activators have been found in the advancing front of invasive carcinoma[7–9] and are thought to be of importance for the fibrinolytic process necessary for the proliferation of tumour vessels[9].

We report here that the fibrinolytic inhibitor tranexamic acid given to animals with certain experimental tumours prolongs survival, decreases the tumour weight and inhibits ascitic production, but does not significantly influence DNA-synthesis in cell suspensions prepared from the same tumours.

Material and methods. 20 C57 black mice were given 5 million cells of Lewis lung adenocarcinoma s.c. resulting in a 100% tumour take. To 10 of them tranexamic acid was given in the drinking water, 0.5 g/kg b. wt/day, and their survival was compared with that of the controls.

20 C3H mice were given 3 million cells of C3H breast carcinoma s.c. From the day of inoculation tranexamic acid was given to 10 of them in their drinking water (0.1 g/kg/day for 20 days). Compared to the C57 black mice the dosage was reduced because of diarrhoea as a side effect. After this period the animals were sacrificed and the circumscript tumours dissected out.

20 Sprague Dawley rats were inoculated with AH 130 rat hepatoma cells. From the day after the inoculation tranex-