Arch. Biol. Med. Exper. 12:39-48, 1979

# Comparative Cytology of Hydrochloric Acid Secreting Cells

## Citología comparada de células secretoras de ácido clorhídrico

J.D. VIAL, J. GARRIDO

Departamento de Biología Celular, Universidad Católica de Chile, Casilla 114-D, Santiago, Chile

(Recibido para publicación el 19 de diciembre de 1977)

VIAL, J.D., GARRIDO, J. Comparative cytology of hydrochloric acid secreting cells. (Citología comparada de células secretoras de ácido clorhídrico). Arch. Biol. Med. Exper. *12:*, 39-48, 1979.

A comparative study of the oxyntic cells of the stomach of Vertebrates is presented. A system of membranous tubules which is apparently inter-convertible with the luminal plasma membrane is described as an important structural feature in all classes. In all Vertebrates with the sole exception of Mammals, this system shares a common cell with the structures involved in the secretion of zymogen. In Mammals it becomes segregated into a specialized cell type, the parietal cell, characterized in addition by the presence of a long tortuous intracellular canaliculus, which serves as its secretory pole.

The hypothesis is presented and discussed that the parietal cell is capable of achieving a higher efficiency in hydrochloric acid secretion by the existence of the intracellular canaliculus which makes the immediate surroundings of the secretory pole independent of the contents of the gastric lumen.

In Birds (whose gastric glands do not possess parietal cells) a corresponding effect can be achieved only by complex structures such as the compound gastric glands. An explanation is hereby offered for the unique features of the Avian stomach.

VERTEBRATES OXYNTIC CELLS COMPARATIVE CYTOLOGY

The stomach of Vertebrates is capable of secreting a considerable volume of hydrochloric acid at concentrations higher than $10^{-2}$ N. In Fishes, Reptiles, Amphibians and Birds this remarkable property must be attributed to the "principal glandular cells" which are the main components of both the fundic and the proventricular glands (1, 2). In Mammals, however, the fundic glands possess two main cell varieties: the so-called "parietal cells" and the "principal or chief cells" (1). Heidenhain (1) first proposed in 1870 that parietal cells were concerned with acid secretion; this idea had become generally accepted before the evidence in favor of it was in fact conclusive. Before the advent of the electron microscope however, there was no satisfactory explanation for the difference in number of cell categories between Mammals and non-Mammals. The first comparative electron microscopic studies of gastric glands showed that the "principal glandular cells" of Amphibians possess in addition to the organelles that are commonly associated with protein secretion, a peculiar system of vesicles which apparently become incorporated to the apical surface during secretory activity (3). This latter structure was also present in the parietal cell of the Mammalian stomach, while the "principal" or zymogenic cell appeared devoid of it (4). It was inferred the parietal cells "...represent a higher degree of specialization wherein only those functions requiring the presence of the mitochondria and vesicles are retained..." (3).

Evidence was later presented which showed that the Avian oxyntic cell belonged also to the mixed variety which might be assimilated to the Amphibian type (5).

The problem posed to light microscopists as to the role of the parietal cells and the dual function of the "principal glandular cells" of non-Mammals — which appeared responsible for both acid and zymogen secretion — seemed amenable to a new explanation in view of the data obtained by electron microscopy. It was possible to propose that a special cytoplasmic differentiation involved in the secretion of acid was present in the "principal glandular cells" of non-Mammals together with the protein-secreting apparatus. In the Mammalian stomach the special system had become exclusively localized in parietal cells while the zymogen-producing structures were confined to the principal cell.

This type of explanation requires that observations on gastric cell ultrastructure and disposition be extended to all Vertebrate classes. In this work we summarize and compare the results of observations carried out in this laboratory on more than fifteen species; these data are taken together with those published in the literature up to the present time. Evidence is presented which supports the contention that those cells which are responsible for acid production in the Vertebrate stomach possess a peculiar intracytoplasmic membrane system at their apical poles. Important changes occur both in the structure of this intracellular system and in gastric gland topography with the appearance of thermal homeostasis.

*A note on terminology*

The gastric glands of non-Mammalian Vertebrates present a cell type which was designated by Plenk as *principal glandular cells* (1). In this work, these cells will be referred to as *oxynticopeptic cells*. Both oxynticopeptic cells and Mammalian parietal cells belong to the class designated *oxyntic cells*. This term refers to the stainability of these cells by acid dyes. The cells that produce pepsinogen in the Mammalian gastric glands (chief cells or principal cells in Plenk's terminology) will be known here as *zymogenic cells*.

## MATERIALS AND METHODS

*Material*

Preparations for electron microscopy were made of the gastric mucosae of the following species: *Halaelurus chilensis* (Chondrichthyes) (6), *Onchorhynchus perca* (Osteichthyes) (7), *Bufo spinulosus* (3, 8), *Caudiverbera caudiverbera, Pleurodema thaul* (Amphibia), *Callopistes maculatus* (Reptilia) (9), *Gallus gallus, Columba livia, Sicales sp.* (10) (Aves), *Tadarida* (Mammalia, Chiroptera) (11), *Marmosa elegans, Dromiciops australis* (Mammalia, Marsupialia) (12), *Rattus, Mus* (Mammalia, Rodentia), *Felis catus, Canis familiaris* (3) (Mammalia, Carnivora), *Homo*.

*Experimental procedures*

Induction of the resting secretory state:

In *Felis* and *Canis* this state was induced by fasting.

The administration of 2,4-dinitrophenol (30 mg/kg) was employed with this object in *Bufo, Columba, Gallus* and *Rattus*.

Metiamide — a generous gift of Dr. R.W. Brimblecombe of Smith, Kline and French Laboratories, Welwyn Garden City, England — at a dose of 5 mg/kg was likewise used in *Felis* and *Columba*.

The *in vitro* incubation of the mucosae in Ussing chambers was employed in the production of anoxia by administration of $O_2$ at low partial pressures.

Stimulation of secretion:

Histamine was used for the stimulation of gastric secretion in *Halaelurus, Bufo, Callopistes, Gallus, Felis* and *Canis*.

Electron microscopy:

Over the period covered by the observations reported here, several fixatives have been employed. First, the osmium tetroxide-veronal acetate fixative of Palade was used. It was then modified by the addition of 0.3 M sucrose (11). Later specimens were processed in phosphate or cacodylate-buffered glutaraldehyde, pH 7.2-7.4, additioned of 0.1 to 0.3 M sucrose.

All of the material presented was embedded in Epon.

Scanning electron microscopy of the luminal surface was performed in *Rattus* under normal feeding conditions; in *Columba* before and after treatment with 2,4-dinitrophenol; in *Bufo* before and after the injection of a maximal dose of histamine.

Special techniques:

Silver methanamine impregnation (13) was employed in *Bufo* and *Columba* for the study of the tubular system. A colloidal iron-copper-uranyl technique (14) was similarly used in *Bufo*. Treatment of gastric cells with heavy meromyosin for actin filament decoration was carried out as described previously (8) in *Bufo, Rattus, Gallus* and *Columba*.

## RESULTS

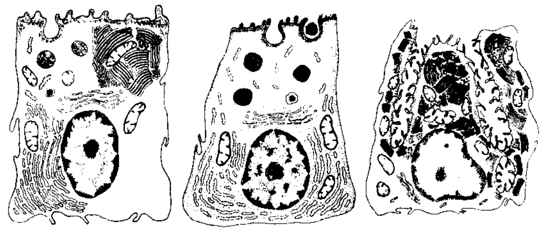
It would appear convenient to divide the description in three principal parts:

*a)* The oxynticopeptic cell in Fishes, Amphibians and Reptiles.
*b)* The Avian oxynticopeptic cell, and
*c)* The Mammalian parietal cell.

### a) *The oxynticopeptic cell in Fishes, Amphibians and Reptiles*

In all of these Vertebrate classes, the luminal pole of the cell presents a variable number of plicae less than 0.1 um in thickness and which project for 1 um or more above the apical plasmalemma. Directly beneath the region of the plicae a zone some 0.25 um wide is found where filamentous structures form a dense network. Beneath this layer a system composed of smooth-walled vesicles and tubules is found. Between the membranous elements and also below them, numerous mitochondria are present. The development of the membranous tubular and vesicular system bears an inverse relation to the number and complication of the apical processes. As it has been show in Amphibians the integrity of the membranous system can be easily altered by improper osmolality of the fixatives employed. In the material we consider here, optimal fixation conditions have been obtained for Amphibian cells. In cells so fixed, the system is seen to be a tubular system (Fig. 1); in material obtained from other Vertebrate classes the vesicular component is abundant. The relation that exists between the apical plasma membrane and the tubulo-vesicular system can be understood from the results of experimental work carried out in *Bufo* (Amphibia).

In the Bufo gastric mucosa the administration of histamine *in vitro* or *in vivo* results in a cell which presents very little or no tubulo-vesicular system while the apical plasma membrane surface is enormously increased by long and complicated processes that project toward the glandular lumen. The injection of 2,4-dinitrophenol *in vivo* or mucosal anoxia *in vitro* determine the inverse changes: the tubular system is highly developed and tightly packed while only a few apical processes are seen. These results, together with those obtained in other laboratories (15) confirma the idea that "...the abundant membranous material lining the vesicles is used by the ecell to increase its external surface during activity" (3).



1

*Fig. 1.* Diagramatic representation of the evolution of the gastric gland cells. From left to right:

Non-Mammalian gastric gland cell. The drawing depicts on the left zymogenic structures and on the right the tubular system.

Mammalian zymogenic cell. The structures represented on the left hand side of the first drawing have become segregated in this cell variety.

Mammalian parietal cell. The tubular system and mitochondria are the main structures in the cytoplasm, from which elements involved in zymogen secretion are absent. The intracellular canaliculus appears as a profoundly in-, vaginated secretory pole.

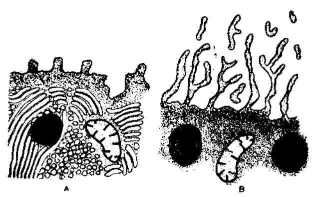
The modifications observed in the *Callopistes* (Reptilia) gastric mucosa after histamine injection are esssentially similar to the ones described above (9). No material is available of Osteichthyes treated with histamine. In *Halaelurus* (Chondrichthyes) similar observations have been recorded with the exception that the apical surface is smoother than what would be expected after histamine (6). This observation, however, is difficult to evaluate considering that no data are available on the secretory response of these muchosae to histamine. No data are available either on possible vascular reactions to histamine, which might conceivably modify secretion.

It would thus appear that the oxynticopeptic cell of these Vertebrates possesses a unique subplasmalemmal membranous system which can interconvert reversibly with the apical plasma membrane. In view of the importance of this system in the subsequent discussion it seems appropriate to describe it carefully, as it is seen in *Bufo*, our best studied species (Figs. 4-8).

In the resting state (Fig. 2A) the tubular system is composed of bundles of 10 to 50 tightly packed smooth-walled tubules which are 400 A° in diameter and at least 1 um in length. These bundles are connected to the extracellular space in contact with the apical membrane by a small number of tubules of scanty diameter. The interior of these tubules can be shown to possess a polysaccharide which probably presents acidic groups as revealed by staining with the technique introduced by Belmar (14).

This description basically coincides with one given by other authors (16) in *Rana* with the exception that in their material the polysaccharide seems to be neutral in reaction.

In the secreting stomach after histamine treatment the plasmalemma of the apical processes is associated with a network of actin filaments which are connected to the apical membrane by bridges spaced some 300 A° apart. It can be surmised that the tubular system membrane becomes associated with actin as at traverses the filamentous network which is interposed between the tubular system and the plasma membrane. This network is composed mainly of filaments 50 to 80 A° in diameter which can be decorated with heavy meromyosin (8) (Fig. 2B).




2

*Fig. 2.* Schematic representation of the changes occuring at the apical pole of the oxyntic cell of *Bufo* at different stages of secretory activity.

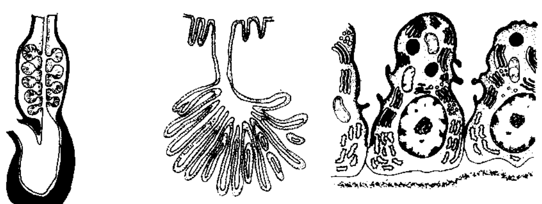
A) Resting state. The tubular system is tightly packed, and separated from the apical membrane by a well-defined filamentous layer.

B) Cell secreting under maximal histamine stimulation. The tubular system has disappeared. The apical membrane is folded into complex processes and underlined by a closely adherent filamentous network.

Scanning electron micrographs of histamine-stimulated material show that the apical processes are essentially plate-like in shape.

b) *The Avian oxynticopeptic cell*

In the Vertebrate classes we have up to now described, the gastric glands are tubular and unbranched; they occupy the mucosal layer of the stomach wall. In Birds, as it is well known, the topography of these glands is fundamentally different (17). The gastric glands are compound glands which are placed deeply within the proventricular wall. Each gland is formed by a very large number of tubules which converge from all directions to a common duct which opens in turn into the gastric lumen. Each of the tubules of these glands possesses a lining of mucous cells near its opeing into the common duct while its distal four fifths are lined by oxynticopepctic cells. The most striking feature of these cells is that their junctional complexes are placed very near their basal poles; in this manner, each cell is surrounded by a circular cleft which separates the main portion of the cell body from the adjoining cells (Fig. 3). The cytoplasm of these cells



3

*Fig. 3.* Schematic representation of some structural features of the Avian stomach. From left to right:

The bulky compound glands are grouped together, allowing for a more distensible muscular portion of the stomach, which is the main digestion chamber of the Avian gastric complex.

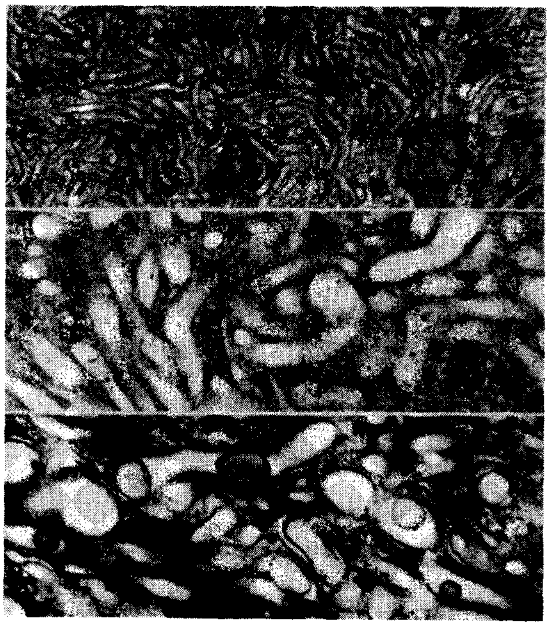
The glands are grouped into compoud structures, removed from the gastric lumen, and connected to it by a slender excretory duct.

The oxynticopeptic cells are surround by a pericellular cleft which amounts to an invagination of the apical pole far less complete than the one observed in Mammals (See Fig. 1).

presents the structures which are associated with zymogen secretion. The organization of the secretory pole is somewhat variable according to the species considered.

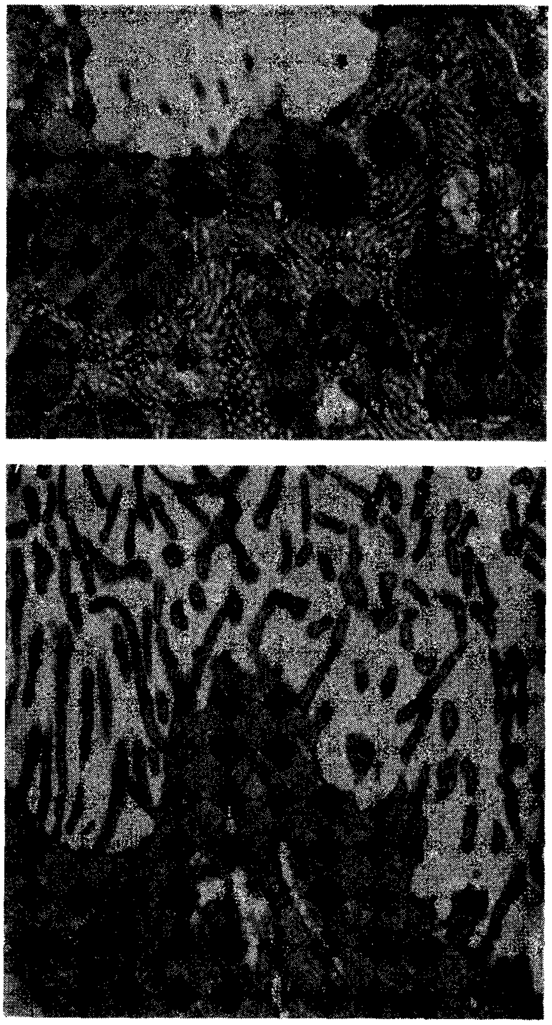
In *Sicales* and in the newborn chick (below 100 g body weight) the intercellular cleft is completely filled by a complex interweaving of membranous processes while the cytoplasm shows the presence of a very small number of vesicular or tubular elements. Fasting adult fowl, on the other hand, present a large intercellular cleft with only a few processes while the immediate cytoplasm shows many tubular and vesicular profiles. In *Columba* (200 g body weight) the image resembles the one described for small birds although the number of vesicular elements present is larger. In *Columba* specimens of 400 g body weight the number of vesicular profiles is still larger while the apical processes are distinctly fewer.

The histamine-stimulated fowl stomach possesses cells which show only a few tubular and vesicular profiles together with a high surface elaboration. The administration of 2,4-dinitrophenol to *Columba* and to the newborn chick produces a smooth apical surface and an increase of the tubular system. Metiamide ($H_2$ histamine receptor antagonist) given to *Columba* results in similar images.



*Figs. 4, 5, 6:* Electron micrographs of the tubular system in *Bufo* (4) (60000 ×), *Columba* (5) (120000 ×) and *Felis catus* (6) (75000 ×).

Fixation artifacts may partly account for differences in diameter of tubules between different classes (See text).



*Figs. 7, 8.* Electron micrographs of oxynticopeptic cells of *Bufo*, Fig. 7 after 2,4-dinitrophenol. Fig. 8 after histamine stimulation.

The elaboration of the apical surface and the disappearance of the tubular system that accompany secretory activity are readily apparent. Note highly ordered array of tubules in the resting state.

It would then seem that in Birds a membranous system is present at the apical pole of the oxynticopeptic cell which behaves in a way that is basically similar to the system observed in Fishes, Amphibians and Reptiles. This system would be almost always in activity in small sized birds, probably in relation to their feeding frequency; in spite of this, the tubular component and its interconvertibility with the apical membrane have been demonstrated experimentally both in large and in small birds.

A detailed description of the system shows some essential facts:

*1.* The intracytoplasmic membranes are organized in tubules which are shorter and more sinuous than those present in Amphibians;
*2.* The disposition in bundles of these tubules is not always evident;
*3.* Although some vesicular profiles can be seen, well-fixed material produces images where tubular elements predominate;
*4.* The folds of the intercellular cleft are plate-like; filaments are seen inside these structures although the association between these filaments and the plasma membrane is less evident than in Amphibians.

c) *The Mammalian parietal cell* (Fig. 1)

In Mammals, the general plan of the gastric glands resembles the one seen in Fishes, Aphibians and Reptiles. The difference resides in that instead of oxynticopeptic cells, two types of cells are found: zymogenic or chief cells and parietal cells. The first type presents a highly developed protein synthesizing apparatus while the second type possesses membranous elements which are akin to those we have described before.

Parietal cells are often placed in the outer aspect of the glandular tube; they void their secretion through a fine tubule which traverses the space between neighboring cells. They possess a highly branched, deep intracellular canaliculus whose lumen is partly filled with fingerlike processes, while tubular and vesicular membranous elements are seen in the cytoplasm adjacent to the plasma membrane which forms the canalicular wall.

The relation between the secretory state of the mucosa and the body size of the animals is quite similar in Mammals and Birds. Small animals present a highly developed system of processes that practically fill the canalicular lumen; the intracytoplasmic membranous system is, in correspondence, poorly developed. In those larger animals which present intermittent HCl secretion *(Canis* and *Felis)* the resting stomach shows cells presenting canaliculi with few processes accompanied by a well-developed tubular system. When these cells are stimulated by histamine a development of intracanalicular pr( :esses is accompanied by a loss of intracellular membrane. Treatment with 2,4-dinitrophenol in the rat provokes a reduction in the number of canalicular processes and a corresponding increase of the tubulovesicular intracytoplasmic profiles. An analogous effect has been demonstrated in the mouse stomach in the resting state (18).

The rat secretes HCl continuously (19, 20). The mouse secretes intermittently but it is relatively difficult to obtain a stomach in a true resting state (21). No data are available on other small mammals studied in this work but it seems reasonable to suppose that given their metabolic requirements, their gastric HCl secretion must resemble the continuous one present in the rat rather than the intermittent one recognizedly present in larger mammals.

Thus in the Mammalian parietal cell, a system of memhranes which resembles the one shown by other HCl secreting cells is present. The intracellular membranous system presents distinct similarities with the one seen in Birds. It is formed by short, closely packed tubular elements which tend to be sinuous rather than straight. The intracanalicular processes are for the most part, finger-like. Their membrane is constantly associated with actin in a manner which closely resembles this type of association in Amphibians (8).

## DISCUSSION

a) *The acid-producing apparatus*

In all of the cell types we have studied, a membranous intracytoplasmic tubular system is a central feature. This system adopts two different forms according to the secretory state of the cell. The structure and individuality of the system have been often misinterpreted and even overlooked due to the fact that this system is not easy to preserve structurally. Frequently the tubular elements become fragmented into vesicles; cells often swell during the preparative procedures. The result of these artifacts is a cell whose apical pole is filled with vesicles which present no specific order or pattern. The tubular system is particularly sensitive to low osmolality fixatives, especially if they are based on osmiun tetroxide (11). This sensitivity appears to be characteristic of the system because it is apparent even in conditions which result in excellent preservation of other membranous organelles.

In the resting state, well-preserved cells show a tightly packed tubular system. The highest degree of order in this packing occurs in Amphibians; this order is almost as striking in Birds and Mammals and only slightly less evident in Fishes and Reptiles. It is interesting to note that 2,4-dinitrophenol and anoxia in Amphibians, Birds and Mammals result in the adoption of a highly ordered disposition. Thus the assembly of extremely ordered arrays of elements does not seem to be directly dependent on the metabolic energy of the cell, a fact which suggests the possibility that components of the tubular membranes themselves may be responsible for it. This possibility seems interesting because it may represent an example of spontaneous assembly of a supramolecular arrangement.

Besides the evidence we have presented here there is ample evidence in the literature (5, 15, 18, 22) to show that this system presents a second structural state which corresponds to the secreting cell. A distinction might be made here between the plate-like expansions seen in non-Mammals and the finger-like processes present in the Mammalian cell. However, these different structures appear to correspond to functional equivalents as their association with actin filaments suggests. This association with actin has been best studied in Amphibians and Mammals (8) where filaments of actin run parallel to the plane of the membrane and are connected to it by periodically spaced bridges. The presence of this association seems to be important in the genesis of the structure that the system adopts during active secretion. In the resting state no association exists between actin filaments and the tubular elements of the system. This suggests the general possibility that the assembly of membranous structures of the type presented by these cells may be conditioned by the state of the cytoplasmic matrix. The tubular system we have described reacts with some histochemical techniques for the demonstration of polysaccharides. This has been shown in Amphibians (16), Mammals (23) and Birds. The identity of the reactive polysaccharide is doubtful; results published for *Rana* (16) indicate that a neutral polysaccharide is the main component while our results in *Bufo* seem to evidence an acidic nature for it. From the point of view of this discussion however, it must be noted that the tubular system and the apical plasma membrane react similarly with these techniques, which constitutes further indication that they represent two states of the same system.

Strong although unconclusive evidence is available which indicates that the tubular system is derived from the Golgi apparatus. In the first place, it has been shown that the first appearance of the tubular system in gastric cells during ontogeny is preceded by a great development of the Golgi apparatus (24). Secondly, histochemical reactions which are positive for the tubular system also strongly stain the Golgi membranes (23, 16). Given the fact that the membranous elements of the system interconvert with the plasma membrane, a possible derivation of the system from the Golgi apparatus does not appear surprising.

From physiological evidence it seems probable that both the hydrogen ion and the chloride ion secretions occur across the luminal membranes of the gastric cells. Those agents that stimulate secretion as well as the more specific blocking agents (histamine $H_2$ receptor antagonists) produce clear and characteristic effects on the membranous systems of the apical pole. In Amphibians, the presence of a $HCO_3^-$-activated adenosinetriphosphatase activity has been demonstrated in these membranous systems (25). Up to the present time, changes in acid secretion have been possible to correlate with structural changes at the apical pole. These data appear sufficient to propose that the function of the system under discussion is the secretion of HCl. It is important to remark that the elements of the system are always associated with the abundant mitochondria present in these cells (already described by classical histologists as "oxyphyle granules"). The secretion of hydrochloric acid involves an increase in concentration ranging from $10^5$ to better than $10^6$ times; this implies a free energy change of considerable magnitude (22). Thus the constant association of mitochondria with the membranous system is an additional argument which supports its acid-secreting function.

The bulk of the evidence available is then in favor of the contention that in gastric glands a *sui generis* apparatus exists. The structure, behavior and function of this apparatus set it apart from other membranous systems of the cell and warrant its designation as the "acid-producing apparatus".

b) *The Mammalian parietal cell*

The parietal cell represents an evolutionary acquisition which is characteristic of Mammals. For the first time, the acid-producing apparatus appears completely separated from the zymogen-producing structures. In this sense, as it has been pointed out before (3), the parietal cell represents a higher degree of specialization. It must be remembered, however, that in some Amphibians —of which *Rana* is a salient example— a very poor development of the zymogen producing structures exists, the secretion of zymogen being carried out mainly by esophagic glands. On the other hand, in some Mammalian parietal cells, especially in small animals, rough endoplasmic reticulum cisternae are found (12). Nevertheless, the parietal cell never possesses secretion granules or a complex ergastoplasm. This cell is easily recognizable with the light microscope and its general organization appears to be structured around the acid-producing apparatus. This component and the abundant mitochondria are the principal cytoplasmic organelles.

A peculiar aspect of the parietal cell is the intracellular canaliculus. It is thin (less than 1 um), long, branched and sinuous. The membrane which forms its wall is the apical mem-, brane of the cell, where the characteristic changes of the acid-producing apparatus occur. It is well known that the Mammalian gastric mucosa is able to secrete HCl at volumes and concentrations which are considerably higher than those possible in Fishes, Reptiles and Amphibians. The evolutionary signification of this capability should probably be sought in the increased metabolic requirements which accompany thermal homeostasis. It is possible that the elimination of zymogenic structures from the parietal cell may be related to the adoption of a thin canaliculus which would possibly hinder the elimination of a proteinaceous secretion. An interesting speculation is to try to correlate the appearance of the canaliculus with the secretory efficiency of the parietal cell. In *Bufo*, the surface changes seen in oxynticopeptic cells are often accompanied by the formation of an excavated or concave apical pole; this change was first observed by Tornier (26) in 1886. Studies on the embryogenesis of the parietal cell show that the canaliculus is first seen as a depression of the apical pole which becomes gradually more conspicuous in the neighborhood of the elements of the acid-producing apparatus (27).

In Birds, the profound pericellular cleft where the changes typical of the function of the acid-producing apparatus occur is analogous to the parietal cell canaliculus, even though the separation of the secretory pole from the glandular lumen in evidently much less complete.

Thus it would seem that the parietal cell canaliculus is an extreme case of a tendency of oxyntic cells to withdraw their secretory poles from direct contact with the glandular lumen.

Some of the available data point out that the secretion of the parietal cell is a HCl solution isotonic with blood plasma (22). The different models which have been proposed for transport in isotonic conditions (28, 29) require the presence of some type of structural organization of the secretory surface which may segregate this area from the bulk solution that would otherwise freely bathe it.

The model of solvent-solute coupling proposed by Hill (29) which postulates an electro-osmotic theory of fluid transfer is particularly interesting in this respect. The condition for isotonic fluid transfer according to this model would seem to be that the secretory pole membrane be organized structurally in such a way that it remains bathed by the fluid being secreted. A direct way of obtaining this is to make the apical membrane so highly convoluted that the flow out of the spaces so generated prevents the diffusive entry of solution from the gastric lumen. Similar conditions would apply if the model chosen were that of Diamond and Bossert (28) in which the secretory pole must remain in contact with its own secretion.

These considerations afford an interesting explanation for the parietal cell. In Fishes, Amphibians and Reptiles the broad lumen of the gastric glands greatly hinders any control that the oxynticopeptic cell may exert on the fluid which bathes its apical pole. The development of the parietal cell would permit that a gland with a histologically similar structural plan might achieve a considerably higher secretory efficiency. The crucial element is then the segregation of the apical surface by its invagination into the cell. This brings of necessity a reordering of the acid-producing apparatus in relation to the invaginated apical pole.

c) *The Avian oxynticopeptic cell*

The preceding discussion brings us to the necessity of considering the Avian oxynticopeptic cell. It seems that the Avian gastric mucosa can secrete HCl at volumes and concentrations wich are quite similar to those attained in mammalian stomachs (30). From the data we have presented and those in the literature it can be concluded that the pericellular cleft in the Avian mucosa is less elaborate a structure than the Mammalian canaliculus; for this reason it might be expected to comply less fully with the condition of spatial confinement of apical pole secretion. The Avian cell is however located in the compound proventricular glands, which are unique in structure and cell disposition among Vertebrate gastric glands. They are deeply placed in the proventricular wall and are connected to the gastric lumen by a thin excretory duct. In these conditions the contents of the gastric lumen can have but little influence on the concentration of the fluid bathing each apical pole. Thus it would appear that the requirements for isotonic secretion might be met by entirely different structural arrangements. It must be noted that the bulky conformation of these glands seems to impose some restrictions on the anatomical shape of the stomach, which is different in Birds from the one seen in the rest of the Vertebrate classes; these differences might be related to the division of the Avian stomach into a glandular and a muscular portion.

The profound dissimilarities in structural plan of the Avian stomach may represent then a means of obtaining the highly efficient HCl secretion required for thermal homeostasis in the absence of parietal cells: it is obtained by the cooperative action of many less efficient cells which are organized into distinctive compound glandular structures.

d) *Parietal cell differentiation*

Studies carried out and reviewed by Plenk (1) indicate that the parietal cell is the first cell type to differentiate in Mammalian gastric glands. During development, this cell type coexists with undifferentiated cells for considerable periods; in Man it is only about 5 months of gestation time later that differentiated zymogenic cells make their appearance. The work done by Nomura (27) with the electron microscope also shows the presence of unequivocal parietal cells at an age in which zymogenic cells are still absent.

Studies done in Amphibians have not been directed to the elucidation of the point that interests us here, namely whether the acid-producing apparatus or the zymogenic apparatus appears first. From the description available however (24) one may infer that here the acid-producing apparatus differentiates first. This interpretation, if confirmed, might furnish a clue toward explaining the phenomenon by which the same anlage results in non-Mammals in one cell type, while in Mammals it results in two cell types which retain exclusively either the acid-producing apparatus or the zymogenic apparatus. It might then be proposed that: 1) The acid-producing apparatus is expressed earlier than the zymogenic apparatus; 2) In Mammals, the expression of other characters of the parietal cell (as the canaliculus, the peculiar intercellular adhesiveness, etc.) impedes the expression of the zymogenic apparatus.

We might in concluding say that in Vertebrate gastric glands a morphogenetic process occurs which affects the relationships between Golgi-derived membranes and cytoplasmic matrix elements (such as actin filaments). Furthermore, this process has a distinctiveness or individuality which makes its result, the acid-producing apparatus, easily identifiable in all members of the Phylum. One of the oustanding characteristics of this process is the constitution of supramolecular assemblies of two distinct kinds in relation to the functional state of the cell; the interconvertibility of these supramolecular assemblies appears to be one of their essential features and seems to be present in all classes of the Vertebrate Phylum.

## ACKNOWLEDGEMENTS

This work has been helped by the Dirección de Investigación de la Universidad Católica de Chile, Grants 52/77 and 51/77, and by Grant N° 20 of the PNUD/UNESCO RLA 76/006 project.

The scanning electrom microscopy was carried out at the Molecular, Cellular and Developmental Biology Department, University of Colorado thanks to the hospitality extended by Prof. K.R. Porter.

Support for many years from the Fundación Gildemeister is gratefully acknowledged.

## RESUMEN

Se presenta un estudio comparativo de las células oxínticas del estómago de los Vertebrados. Ellas presentan como rasgo común, un sistema de túbulos membranosos intracitoplásmicos cuya membrana parece ser interconvertible con la membrana plasmática del polo luminal. En todos los Vertebrados con la excepción de los Mamíferos, este sistema comparte una misma célula con las estructuras relacionadas con la secreción de cimógeno. En los Mamíferos, el sistema tubular referido se halla limitado en forma exclusiva a una variedad de célula, la célula parietal, que no presenta estructuras relacionadas con la síntesis de cimógeno. La célula parietal presenta además un largo y tortuoso canalículo intracelular en el cual se halla su polo secretor. Se presenta y se discute la hipótesis de que la célula parietal es capaz de alcanzar una alta eficiencia en secreción de ácido clorhídrico por la existencia del canalículo intracelular que hace que la zona del polo secretor se mantenga separada del lumen glandular e independiente de los cambios de concentración del contenido de éste. En las Aves, cuyas glándulas no presentan células parietales, es posible que un efecto análogo en cuanto a la eficiencia de la secreción se alcance por la compleja estructura de las glándulas compuestas. Es posible entonces que la ausencia de células parietales en esta clase sea un factor determinante en el desarrollo del plan estructural del estómago de Aves, único entre todas las clases de Vertebrados.

## REFERENCES

1. Plenk, H., *Der Magen. In Handbuch der Mikroskopischen Anatomie des Menschen,* Von Möllendorff W., Ed. Verlag von Julius Springer, Berlín, 5. Band 2. Teil: 1, 1932.
2. Manfredi-Romanini, M.G., Bollettino di Zoologia, xxxi:239, 1964.
3. Vial, J.D. Orrego, H., J. Biophys. Biochem. Cytol, *7:* 367, 1960.
4. Ito, S., Winchester, R., J., J. Cell Biol., *16:*541, 1963.
5. Toner, P.G., J. Anat., *97:*775, 1963.
6. Rebolledo, I.M., Vial, J.D., Arch. Biol. Med. Exp., *10:* R66, 1976.
7. Santelices, C.L., Vial, J.D., Arch. Biol. Med. Exp., *10:* R71, 1976.
8. Vial, J.D., Garrido, J., Proc. Natl. Acad. Sci. USA, *73:* 4032, 1976.
9. Rebolledo, I.M., Unpublished data.
10. Lazo, A.R., Thesis. Facultad de Filosofía y Ciencias de la Educación. Universidad Católica de Chile, 1965.
11. Vial, J.D., Orrego, H., Protoplasma, *LXI:*164, 1966.
12. Gneis, C.C., Raggio, M.A.B., Thesis. Facultad de Filofía y Ciencias de la Educación, Universidad Católica de Chile, 1965.
13. Rambourg, A., J. Histochem. Cytochem., *15:*409, 1967.
14. Belmar, M., Arch. Biol. Med. Exp. In Press.
15. Sedar, A.W., Fed. Proc., *24:*1360, 1965.
16. Forte, T.M., Forte, J.G., J. Cell Biol., *47:*437, 1970.
17. Farner, D.S., *In Biology and Comparative Physiology of Birds,* A.J. Marshall, Ed. Academic Press, Vol. *1:*411, 1960.
18. Ito, S., Schofield, G.C., J. Cell Biol., *63:*364, 1974.
19. Friedman, M.H.F., Proc. Soc. Exp. Biol., *54:*42, 1943.
20. Komarov, S.A., Shay, H., Rayport, M., Fels, S., Gastroenterol., *3:*406, 1944.
21. Berenblum, L., Fogel-Kaufman, H., Gastroenterol, *32:*279, 1957.
22. Forte, J.G., *Hydrochloric acid secretion by gastric mucosa.* In Membranes and Ion Transport. E.E. Bittar, Ed., Wiley Interscience, Vol., *3:*111, 1970.
23. Sedar, A.W., J. Ultrastruct. Res., *28:*112, 1969.
24. Forte, G.M., Limlowongse, L., Forte, J.G., J. Cell Sci. *4:*709, 1969.
25. Koenig, C.S., Vial, J.D., J. Histochem. Cytochem., *18:*340, 1970.
26. Tornier, O., Arch. Mikrosk. Anat., *27:*181, 1886.
27. Nomura, Y., Ztschr. f. Anat. u. Entwicklunsges., *125:*316, 1966.
28. Diamond, J.M. Bossert, W.H., J. Gen. Physiol., *50:* 2061, 1967.
29. Hill, A.E., Proc. Roy. Soc. Lond. Ser. B., *190:*115, 1974.
30. Burhol, P.G., Hirschowitz, Am. J. Physiol., *222:*308, 1972.