Electron Microscope Study on Endocrine Cells and Tumor Cells in the Glandular Stomach of Praomys (Mastomys) natalensis

Muneaki Sano

Summary. Endocrine cells in the normal glandular stomach and gastric carcinoids of mastomys were observed by electron microscopy and at least five types of endocrine cells, EC, G, D-like, R (round-granule) and ECL cells were identified. Of these, four types excepting G cells were recognized in the fundic mucosa. Characteristic in mastomys was a scarcity of endocrine cells in the fundic mucosa, where ECL and R cells were predominant types.

Silver impregnation methods including SEVIER-MUNGER's argyrophil reaction of our own modifications were applied to tissue sections and the endocrine cells were examined by electron microscopy. Only EC cells revealed argentaffin granules under the light and electron microscope. R, ECL and some of the G cells were non-argentaffin and argyrophil in reaction and D-like cells and the rest of the G cells failed to show even an argyrophil reaction. Granules of mastomys carcinoid cells, as noted in the previous reports, were non-argentaffin but faintly argyrophil.

Mastomys gastric carcinoids were studied by the same method, with special reference to the parent cells of this particular neoplasia. Noteworthily, mastomys gastric carcinoids arise mostly from the fundus, the area where R and ECL cells mainly occur in normal animals. The neoplasms are composed of cells containing granules resembling partly those of R cells and partly those of ECL cells. ECL cells and neoplastic cells in the present investigation have a similar reactivity to SEVIER-MUNGER's method.

Considering the generally accepted fact that neoplastic cells may not fully duplicate their parent cells in cytological features, it seems reasonable to presume that R and/or ECL cells might be the parent cells of the mastomys gastric carcinoids. In connection with this assumption histamine has been demonstrated to be produced both in mastomys carcinoid cells and normal ECL cells.

Endocrine cells in the gastro-intestinal mucosa and pancreas of various mammals, including man, have been classified by electron microscopy into about thirteen types (PEARSE, 1974). In spite of recent advance in biochemical, histochemical and immunohistochemical studies (CARVALHEIRA, WELSH and PEARSE, 1968; McGUIGAN, 1968; SOLCIA, CAPELLA and VASSALLO, 1969; AURES, 1970; HAKANSON et al., 1970; BUSSOLATI and PEARSE, 1970), the correlation between endocrine cells thus classified morphologically and gastro-intestinal hormones extracted biochemically has not been established as yet, except for that between 5-HT and EC cells (ERSPAMER and ASERO, 1952) and that between gastrin and G-cells (SOLCIA, VASSALLO and SAMPETRO, 1967; BUSSOLATI and CANESI, 1972). Biogenic monoamines such as 5-HT, histamine and dopamine are known to be produced in the gastro-intestinal endocrine cells and stored in the secretory granules of the cells and their possible role in the formation, storage and release of the polypeptide hormones has been suggested (HAKANSON et al., 1970; OWMAN, HAKANSON and SUNDLER, 1973). The correlative roles of polypeptide and amine hormones in endocrine secretory granules remain to be elucidated.
Praomys (Mastomys) natalensis (OETTLE, 1957) is known to produce spontaneous argyrophil carcinoids in the fundus of the glandular stomach (SNELL and STEWART, 1969; SOGA et al., 1969). Following the discovery of round, electron dense, membrane-bounded granules of an endocrine type in the cells of these tumors (SOGA, TAZAWA and Ito, 1969), appreciable amounts of histamine and histidine decarboxylase activity were found in these tumors (Hosoda et al., 1970, 1971). On the basis of these findings, the following hypothesis is formulated. Assuming that a carcinoid tumor arises from an immature or primitive form of a type of endocrine cell normally distributed in the primitive gut system, the parent endocrine cell of a mastomys gastric carcinoid should, hopefully, be a histamine-producing cell with the same histochemical and morphologic characteristics as the tumor cells. Recently, some investigations suggested that the parent cells of carcinoid tumors in the mastomys stomach might be two types of endocrine cells, ECL and round-granule cells in the fundic mucosa or a common progenitor of the two (CAPELLA, SOLCIA and SNELL, 1973; HAKANSON et al., 1973).

This paper deals with an ultrastructural investigation of normal endocrine cells and carcinoid cells in mastomys stomach and attempts: 1) classification of endocrine cells in the mastomys glandular stomach in reference to the previous reports, and 2) identification of the neoplastic cells of carcinoid with any type of the normal endocrine cells. This study is characterized by the intensive use of silver stainings, which at the electron as well as light microscopic level, may often serve in facilitating and confirming the diagnosis of endocrine cell types.

**Materials and Methods**

Eight mastomys aged from 10 to 22 months were used for this study. Of these, four had grossly detectable tumors neither in the stomach nor in the other organs, three had palpable gastric tumors in the glandular portion and the last was an animal that had a transplanted gastric tumor in the thigh muscle. Under methoxyflurane anesthesia, gastric (oxyntic and pyloric) and duodenal mucosa and the pancreas were excised. The submucosal neoplastic nodules and transplanted tumors were also dissected out if the animal had them. All these materials were fixed for 2 hrs in a 2.5% glutaraldehyde solution buffered at pH 7.4 by 0.2 M sodium cacodylate, and postfixed for the next 2 hrs in an s-collidine-buffered 1% osmium tetroxide. After dehydration in a graded series of ethanol baths, the specimens were embedded in epoxy resin (Epon 812). The blocks were sectioned on a Porter-Blum ultramicrotome. Semi-thin sections were stained with toluidine blue and observed for orientation by light microscopy.

Ultra-thin sections trimmed according to light microscopic orientation were placed on both copper and titanium grids. For conventional electron microscopy, ultra-thin sections on copper grids were stained with uranyl acetate and lead citrate. To the ultra-thin sections on titanium grids, two methods of silver staining were applied: MASSON-HAMPERL’s procedure modified by SINGH (1964) and SEVIER-MUNGER’s method (1965). Uran and lead staining was omitted.

As the original SEVIER-MUNGER’s method caused too heavy silver grain deposition for electron microscope observation, the concentration of the silver solution and the soaking duration were partly modified. In the modified SEVIER-MUNGER’s method, solutions named by us S–M I, II and III were prepared by the following procedure:
Cold 28% ammonium hydroxide was added drop by drop to 50 ml of a 3% silver nitrate solution, which was shaken vigorously, until a slight precipitate remained. To this a 10% sodium carbonate solution was added, and then seven drops of 28% ammonium hydroxide were added until this solution became crystal clear. To this ammoniacal silver solution, 2% formalin was added while shaking gently. The amount of the silver deposits in the sections could be changed by the volume of this formalin. Thus, three solutions were prepared: S-M I solution was that with four drops of formalin added, S-M II had five drops, and S-M III six drops.

Sections were immersed for 15 min in 10% silver nitrate at 40°C, left for 5-7 min until they became gray to light yellow, and rinsed in distilled water. The sections were then transferred to one of the solutions, S-M I, II and III, and developed for 5-8 min until a golden brown color appeared. After being rinsed in distilled water, the sections were placed in a 2% sodium thiosulfate for 2 min and then rinsed again in distilled water.

All sections were photographed on a JEM-T7 (Japan Electron Optic Co., Ltd.) electron microscope.

Observations

I. Endocrine Cells in Normal Gastric Mucosa of Mastomys

General ultrastructure of endocrine cells in the mucosa of the mastomys glandular stomach displayed characteristics of the corresponding cells in the other rodents reported up to date (FORSSMANN, et al., 1969; HAKANSON et al., 1971b).

The endocrine cells were found in the deeper half of the gastric glands. The basal part of the cells was in diffuse contact with the basement membrane of the gastric glands. The apical part of some cells apparently reached the gastric lumen with a narrow cytoplasmic process covered with microvilli. The cytoplasm of the cells was generally clearer than that in the adjacent exocrine cells and contained secretory granules found mainly in the infranuclear regions, a predominant Golgi apparatus usually in the supranuclear regions, narrow cisterns of endoplasmic reticulum, small and elongate mitochondria, and free ribosomes and polysomes dispersed in the cytoplasm. Lysosomes, lipid deposits and bundles of microfilaments were occasionally observed in certain cell types. At least five types of endocrine cells were identified.

EC (enterochromaffin) cells

The cells of this type were found predominantly in deeper portions of the pyloric gland but rarely in the fundic gland as well. In the pyloric region, their apical ends frequently reached the lumen. Granules of this cell type were osmiophilic and pleomorphic: round, oval, dumbbell-shaped, kidney-shaped and rod-like. They measured approximately 250 mμ (180-350 mμ) in the greatest dimension. An electron-dense content bounded by a limiting membrane was generally homogeneous but occasionally granular (Fig. 1). This cell type reacted constantly to MASSON-HAMPERL's method (Fig. 3) and showed the strongest reaction to SEVIER-MUNGER's method (Fig. 4) of all the endocrine cell types. They were stained faintly even by S-M I and reacted so heavily to S-M II that silver particles in a cluster were crowded within and occasionally even outside the granules (Fig. 6). The granules of the duodenal EC cells were
Fig. 1 and 2. Two types of granules in EC cells ×15,000. In Fig. 1, the granules are typical with an electron-dense, pleomorphic, homogeneous core that is closely bounded by a limiting membrane. They are smaller than those of the duodenal EC cells. In Fig. 2, the granules are vacuolated with a widened space beneath the limiting membrane and the central cores are irregular in shape.

Fig. 3. Masson-Hamperl's argentaffin reaction showing precipitation of the silver particles on the limiting membrane and the core of EC cell granules. Glutaraldehyde-osmium-fixed and uranyl stained. ×15,000
larger than those of the gastric EC cells and reacted positively to both silver methods.

In the pyloric glands, the cells with vacuolated granules (Sasagawa, Kobayashi and Fujita, 1970) whose core was electron-dense, pleomorphic and reactive to Masson-Hamperl's method were occasionally found together with the typical EC cells (Fig. 2).

G cells

This cell type well known as gastrin-producing cells (Solcia, Vassallo and Capella, 1969; Pearse and Bussolati, 1972) was found only in the pyloric gland. The apical end of this cell type was observed also to reach the glandular lumen. Round basal granules of the cell displayed variable appearances: a relatively electron-dense core bounded by a limiting membrane (180 mµ), a roughly granular or vague core in a membrane sac (230 mµ) or only a membrane sac (250 mµ) without discernible contents. The granules of the latter two types were observed only in G cells as a rather specific characteristic of this cell type (Fig. 5).

G cells were usually non-reactive to both Masson-Hamperl’s and Sevier-Munger’s methods. Some of them, however, reacted strongly to Sevier-Munger’s whereas even the adjacent G cells in the same section were non-reactive (Fig. 7).

D-like cells

Although D-like cells were found both in the pyloric and fundic glands, they were very few as compared with other endocrine cell types. They were difficult to distinguish from the D cells in the pancreas of mastomys (Fig. 9). The granules of this cell type were characteristically polygonal and measured approximately 250 mµ (150-350 mµ) in the greatest dimension. Their content was closely bounded by a limiting
Fig. 5. Granules of a G cell in a pyloric gland. Note the various appearances of the granules; some are solid enough but others are more or less vesicular in appearances. ×15,000

Fig. 6. A G cell and an EC cell in the pyloric gland of a mastomys. Glutaraldehyde-osmium fixation followed by Sevier-Munger's (S-M II) method. Silver deposits are evident in the EC cell granules; a weak reaction may be seen on the G cell granules. ×9,000
Fig. 7. Comparison of two types of G cells stained with the S-M II method. The one on the left is not reactive to this reaction while the other on the right is heavily reactive. Glutaraldehyde-osmium-fixed, without electron staining. ×15,000

Fig. 8. A D-like cell in the fundic mucosa with its characteristic granules. Compare this cell with the pancreatic D cell shown in Figure 9. ×15,000
Fig. 9. An A cell and a D cell in the pancreatic islet of a mastomys. The A cell on the left shows broad-haloed, round granules containing homogenous, electron dense cores. Note the difference in this cell from an R cell in Figure 10. The D cell on the right contains granules similar in appearance to those of the D-like cell in the mastomys' stomach (Fig. 8). ×15,000
Fig. 11. A portion of an R cell stained with Sevier-Munger's (S-M II) method. Silver grain deposits are seen in all the granules. Note also the limiting membrane and halo of the granules. Gutaraldehyde-osmium-fixed, without electron staining. ×15,000

Fig. 12. An ECL cell of flattened shape extending along the basement membrane and surrounded by the chief cells. Note the broad-haloed granules and central cores of varying density. ×5,000

Fig. 10. An R cell among parietal cells in the fundic mucosa. R cells contain round and electron-dense granules. The organelles are well-developed, particularly with dilated endoplasmic reticulum and prominent Golgi apparatus. ×7,500
membrane, poorly osmiophilic and finely granular in texture (Fig. 8).

D-like cells failed to react to either MASSON-HAMPERL’S or SEVIER-MUNGER’S method. Only when stained with the S–M III reaction which had an intense reducing ability, they reacted faintly.

**Round-granule (R) cells**

The cells of this type, provisionally called “R cells” in the present paper, were occasionally found both in the fundic and pyloric glands (Fig. 10). R cells were almost oval and no apical process was found to reach the glandular lumen. In their clear cytoplasm, organelles were well-developed; prominent Golgi apparatus, dilated endoplasmic reticulum and lipid droplets were constantly observed. The granules were peculiarly round and measured about \(250 \mu m (200-300 \mu m)\) in diameter. Their content, bounded by a limiting membrane with a narrow halo, was homogeneous and highly electron-dense.

The granules of this cell type never reacted to MASSON-HAMPERL’S method, whereas they did strongly to SEVIER-MUNGER’S method (Fig. 11). They reacted variably even to S–M I with a low reducing ability.

**EC-like (ECL) cells**

The cells of this type were relatively rare and found only in the deeper two thirds of the fundic glands. They extended along the basement membrane and were located generally among chief cells (Fig. 12). The cells were characterized by broad-haloed granules with a coarsely granular core of variable size. In the same single cell, various granules were intermingled: round and slightly irregular shaped, haloed granules containing more or less electron-dense, coarsely granular core; vesicles with a variably electron-dense, coarsely granular core, and vesicles with a slightly electron-dense core or even without a core (Fig. 13).

The granules of these cells failed to react to either MASSON-HAMPERL’S or SEVIER-MUNGER’S (S–M II) method, except for occasional, small granules that were found to react slightly to the S–M III method. In the latter method, the cytoplasm and its organelles were extensively damaged (Fig. 14).

In addition to the five types of endocrine cells described above, cells with small, round and broad-haloed granules were observed in the pyloric region. They were, however, so rare that a detailed analysis has not been attained to decide whether or not they might be the sixth cell type or simply a variant of any one of the five described types.

To sum up, EC, G, R and D-like cells were found in the pyloric glands of the mastomys, the first two being predominant. On the other hand, in the fundic glands in which endocrine cells were primarily very rare, R and ECL cells were found almost with an equal frequency. As shown in Table 3, only EC cells showed a positive argentaffin as well as an argyrophil reaction, whereas other endocrine cells were non-argentaffin and either argyrophil or non-argyrophil. Among the argyrophil cells, R cells showed the strongest reaction. A part of the G cells was reactive to argyrophil reaction, while their remainder and D-like cells were non-reactive. ECL cells were reactive only weakly to argyrophil reaction.
Fig. 13. and 14. Two of serial ultra-thin sections from the same block, one after conventional electron staining (Fig. 13) and the other after silver impregnation (S-M III) (Fig. 14). Silver precipitation occurs diffusely in the cytoplasm and nucleus, it is weak on ECL granules while very intense on R cell granules. Glutaraldehyde-osmium-fixed, without electron staining. \( \times 15,000 \)
Fig. 15. A portion of a tumor cell from a mastomys stomach with broad-haloed granules, the majority of which simulate those of ECL cells. Compare them with the granules in Figure 12. Occasional granules are elongate or rather stretched, as indicated by the arrow. ×15,000
Endocrine and Tumor Cells in Mastomys Stomach

Fig. 17. Granules of a tumor cell from a mastomys stomach show positive argyrophil reaction (S–M III). Silver grain deposit in the substance of the granules in varying density. ×15,000

Fig. 16. A tumor cell from a mastomys stomach containing pleomorphic non-argentaffin granules. ×9,000  Inset: The granules with a discrete limiting membrane are shown at a higher magnification. ×15,000
II. Carcinoid Tumor Cells of the Mastomys Stomach

In the submucosal nodules of primary carcinoids, in the metastatic lesions of lymph nodes or in the first transplanted neoplastic nodules in the thigh muscle, the neoplastic tissue showed, as a rule, a medullary pattern and the cells were characterized by well-developed organelles: numerous mitochondria varying in shape and size, Golgi apparatus with abundant lamellae, excessively dilated endoplasmic reticulum, lysosomes, lipid deposits and specific secretory granules.

Although neoplastic cells did not always contain demonstrable granules, most of the cells contained membrane-bounded granules varying in shape and size. Most granules were round and moderately haloed, measuring approximately 200 m\(\mu\) (150–250 m\(\mu\)) in the greatest dimension, with a poorly osmiophilic, coarsely granular central.

Table 1. Schematic diagram of granules in endocrine cells and carcinoid cells of the mastomys stomach and pancreas.

<table>
<thead>
<tr>
<th>cell</th>
<th>granule</th>
<th>size (m(\mu))</th>
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<tbody>
<tr>
<td>pylorus</td>
<td>G</td>
<td>150–250</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>200–300</td>
</tr>
<tr>
<td></td>
<td>D-like</td>
<td>150–350</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>150–300</td>
</tr>
<tr>
<td>fundus</td>
<td>ECL</td>
<td>150–500</td>
</tr>
<tr>
<td></td>
<td>Tumor</td>
<td>150–250</td>
</tr>
<tr>
<td>pancreas</td>
<td>A</td>
<td>200–250</td>
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<tr>
<td></td>
<td>D</td>
<td>200–250</td>
</tr>
</tbody>
</table>
Fig. 18. A tumor cell (right) transplanted from the mastomys stomach to the thigh muscle (a muscle fiber on the left). The tumor cell contains granules reacting to the S–M III method. Glutaraldehyde-osmium-fixed, without electron staining. ×7,500

Fig. 19. A tumor cell from a mastomys stomach, showing strongly argyrophil granules. Formaldehyde fixation followed by SIEVER-MUNGER’s stain (S–M III) of ultra-thin section. Without electron stain. ×15,000
core (Fig. 15), while some granules were narrow-haloed with an osmiophilic, homogeneous substance, measuring about 200 mµ. There also occurred a few pleomorphic granules measuring up to 300 mµ in the maximum dimension (Fig. 16).

The granules reacted weakly to S-M II and heavily to S-M III (Fig. 17). Since, under the light microscope, neoplastic cells were stained strongly with SEVIER-MUNGER’s method, ultra-thin sections fixed with 10% formaldehyde alone or 10 formaldehyde–2.5% glutaraldehyde were used for the S-M II method. In this method, the granules were strongly argyrophil with heavily deposited silver grains within their cores (Fig. 19). No granules of the neoplastic cells, even the pleomorphic ones, reacted to MASSON-HAMPERL’s argentaffin method.

The light microscopic examination of a gastric carcinoid in a mastomys that died from perforation of a duodenal ulcer showed numerous granules reacting heavily to SEVIER-MUNGER’s method (Fig. 18), while the same carcinoid transplanted to the thigh muscle of a second mastomys consisted of neoplastic cells whose granules were relatively few in number but clearly argyrophil after the SEVIER-MUNGER’s procedure, both at the light and electron microscope level.

The observation on the granules of endocrine cells and carcinoid cells of the mastomys stomach and pancreas are summarized in Table 1.

**Discussion**

**Endocrine cells**

Electron microscopy of the conventional and silver-stained preparations revealed the presence of at least five types of endocrine cells, EC, G, D-like, R and ECL, in the normal glandular stomach of the mastomys. The granule morphology of individual cell types is shown in Table 1. The criteria for classification of the cells were mainly based on those previously reported in other mammals including man (VASSALLO, SOLCIA and CAPELLA, 1969; PEARSE et al., 1970; SASAGAWA, KOBAYASHI and FUJITA, 1970; KOBAYASHI, FUJITA and SASAGAWA, 1971; VASSALLO, CAPELLA and SOLCIA, 1971b; SOLCIA et al., 1973). The distribution and types of endocrine cells in the mastomys were essentially identical with those in rats (FORSSMANN et al., 1969; CAPELLA, VASSALLO and SOLCIA, 1971; HAKANSON et al., 1971b), in the fundic mucosa of the mastomys endocrine cells were unexpectedly low in population as compared with other species (HAKANSON et al., 1973).

Gastrin-producing G cells found only in the pyloric mucosa. Although it was

**Table 2.** Comparison of endocrine cell population in the mastomys stomach as shown in this study and that in other mammals defined by Bologna’s criteria (SOLCIA et al., 1973)

<table>
<thead>
<tr>
<th>Stomach</th>
<th>EC</th>
<th>G</th>
<th>D</th>
<th>D₁</th>
<th>A-like</th>
<th>ECL</th>
<th>R</th>
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<tbody>
<tr>
<td>Mastomys</td>
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<tr>
<td>pylorus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>fundus</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Bologna</td>
<td></td>
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</tr>
<tr>
<td>pylorus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>fundus</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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reported by Forssmann and Orci (1969) that the electron density of the contents in
the G cell granules of the cat was influenced by the feeding conditions indicating a
functional secretory cycle, granules with variably appearing cores were constantly
observed in the G cells of mastomys even after food administration. Most of the 5-
HT storing EC cells were distributed in the pyloric mucosa but a small number of
them were in the fundic mucosa. Serial sections of the mastomys stomach, Soga et
al. (1974) showed at the light microscopic level that argentaffin cells were sparsely
distributed in the fundic area. These cells corresponds to the EC cells. D-like cells
were found rarely both in the pyloric and fundic mucosa. Although the D-like cells
of mastomys are almost identical in morphology and distribution to the gastro-
intestinal D-like cells in other mammals, they are apparently different from the D-
cells initially reported by Vassallo, Capella and Solcia (1971b) and confirmed in
the Bologna Meeting (Solcia et al., 1973). In addition, D-like cells were negative to
the S-M II method, but faintly positive to the S-M III method. Based on the results
of histochemical findings (Erspamer and Asero, 1952; Solcia, Capella and Vassallo,
1969a; Pearse and Bussolati, 1972) and cell distribution, it is difficult to con-
sider the G, EC or D-like cells as the parent cells of the mastomys gastric carcinoid.

In the fundic mucosa of the mastomys Capella et al. (1973) reported the occur-
cence of cells which they called “round-granule cells”. From all morphological
respects, especially the size, shape, density and fine structure of the granules, it is
most probable that they are identical with our R cells. As a matter of fact, our
designation of “R” refers to “round” of Capella et al. A discrepancy between the
description by Capella et al. and our finding is that they did not find the round-
granule cells in the mastomys pyloric mucosa whereas we found numerous R cells
there. The I cells found in the intestine of a dog by Bussolati et al. (1971) or the M
cells described in the human duodenum by Sasagawa, Kobayashi and Fujita (1973)
may possibly correspond to our R cells. It is worthy to mention in this connection
that neither I nor M cells have been reported to occur in the stomach as far as the
dog and man are concerned. The problem of the identity of I, M and R cells must
be elucidated but, for the time being, it seems safe and practical to use the name of
R or round-granule cells for the elements found in the mastomys stomach.

Besides in the fundic, a few ECL cells have been demonstrated, in the pyloric
mucosa of the rat (Capella and his associates, 1971), whereas in the mastomys
stomach ECL cells seem to occur exclusively in the fundic mucosa. Though in some
cells the granule cores are mostly lower in density as shown in Figures 12 and 13,
others contain typical granules with a small, dense core surrounded by a large ves-
cular space, thus supporting the identification of the cells with the ECL cells reported
in other mammals.

Silver reactions of cells

Several silver stainings for endocrine cells (Sinth, 1964; Grimelius, 1968;
Capella, Solcia and Vassallo, 1969) have recently been applied to electron-micro-
scopic investigations (Battaglia, 1969; Vassallo, Solcia and Capella, 1969; Capella
and Solcia, 1972). In some studies, silver stainings were performed en bloc and the
tissue was later sectioned. Different silver stainings were applied to ultra-thin serial
sections and the results were compared (Black, 1968; Chang and Bencosme, 1968;
It has been recommended that the fixatives formaldehyde or glutaraldehyde be used for argentaffin reaction but not osmium tetroxide (Masson-Hamperl's method) and formaldehyde but not glutaraldehyde for argyrophil reaction (Grimelius's method) to produce favorable results (Hakanson et al., 1971a). One of the advantages in this method is that the fine structure which is damaged by silver nitrate can be examined without damage in an adjacent section stained with the usual heavy metals.

As shown in Table 3, some endocrine cells displayed reactions different from the previous reports (Vassallo, Capella and Solcia, 1971a, b; Capella, Solcia and Snell, 1973; Hakanson et al., 1973). Only EC cells were positive to argentaffin reaction, while the core of vacuolated EC cell granules also reacted heavily to Masson-Hamperl's method. Except for D-like cells, most of the endocrine cells in the mastomys stomachs reacted with variable intensity to Sevier-Munger's argyrophil method. Among non-argentaffin cells, R cells showed the strongest argyrophil reactivity and, on occasion, reacted even to S-M I. ECL cells failed to react to S-M II but reacted faintly to S-M III. These findings differ from those reported by Capella, Solcia and Snell (1973) who, however, used Sevier-Munger's method as block staining, whereas they are almost consistent with those by Hakanson et al. (1971a) applying Grimelius's method on ultra-thin sections from the rat. It was reported by a research group of Pavia that G cells were non-reactive to Sevier-Munger's method but reactive to Grimelius' method (Vassallo, Capella and Solcia, 1971a, b). The present investigation, however, showed that, when Sevier-Munger's method was used, existence of two types of G cells became manifest in the same specimen. In one type of G cells, most granules reacted to Sevier-Munger's method, but in the other, all granules failed to react even to the S-M III method. This difference in silver reactivity suggests that G cells identified as a single type by conventional electron microscopy can be classified into at least two types or two phases of a chemical or functional cycle. At all events, it would be inadequate simply to declare that G cells are non-reactive to Sevier-Munger's method.

It is widely accepted that the argentaffin reaction of the endocrine cell granules is due to the occurrence of biogenic monoamines. Hakanson et al. (1971b) demonstrated that the granules of non-argentaffin cells of the rat became argentaffin after

<table>
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<th>Cell types</th>
<th>argentaffin (M-H)*</th>
<th>argyrophil (S-M)**</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>EC</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>G</td>
<td>-</td>
<td>+ &amp; -</td>
</tr>
<tr>
<td>Tumor</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ECL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-like</td>
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* M–H: Masson-Hamperl’s reaction ** S–M: Sevier-Munger’s reaction

Hakanson et al., 1971a).
the cells were loaded with L-DOPA. In the case of the murine ECL cells and mastomys carcinoid cells, histamine is known to be contained in the granules, which will be discussed below.

**Mastomys gastric tumors**

It has been proven that carcinoid tumors of the mastomys stomach arose almost exclusively from the fundic mucosa (SOGA et al., 1969a) and it has been demonstrated not only by the light but also by the electron microscope that the cells of carcinoid tumors show a non-argentaffin argyrophil reaction. Though, under the electron microscope, not all granules in the tumor cells fixed with glutaraldehyde-osmium tetroxide were reactive to SEVIER-MUNGER’s method, materials fixed in formaldehyde unequivocally showed that all the carcinoid cell granules were intensely argyrophil in this method (Fig. 19).

While the majority of the carcinoid cells resembled the normal ECL cells in their fine structure, a few cells were reminiscent of the R cells. The possibility that R cells are also a parent cell of the mastomys gastric carcinoids may be taken into consideration, although they reacted more intensely to SEVIER-MUNGER’s method than neoplastic cells. The fact that the pleomorphic granules generally considered to be specific to EC cells are on occasion recognized in mastomys gastric carcinoids may suggest some problems: If they were defined as EC cells, the argentaffinity would not necessarily be a criterion of EC cells on the one hand and EC cells could be transformed possibly from certain argyrophil and non-argentaffin cells in mastomys carcinoids on the other; if they were not, the general morphologic criteria for EC cells would not be applicable to the neoplastic cells now in question. Our preference is the latter postulation and the presence of “pleomorphic non-argentaffin granules” in mastomys carcinoids seems, in particular, to indicate that the granules are different from EC cell granules.

Carcinoid tumors have been defined by SOGA (1973) as neoplasms originating from the endocrine and related cells of the primitive gut system and characterized by PEARSE as members of “apudoma” family derived from his APUD concept (PEARSE and POLAK, 1974; PEARSE, POLAK and HEATH, 1974). On the basis of localization, morphology and silver reactivity of normal endocrine and neoplastic cells, ECL and/or R cells were considered to be candidates for the possible parent cells of the mastomys carcinoid cells.

Histamine has been localized in the ECL cells of the rat and mouse by HAKANSON et al. (1971b). On the other hand, HOSODA et al. (1970) revealed that the carcinoid cells of mastomys contained much histamine and explained how they may cause hyperacidity and ulcers in this animals. These findings seem to provide strong support to our view that the ECL cell is the source cell of the mastomys carcinoid. It is of interest now to elucidate whether the R cell, which we regard as another progenitor of the mastomys carcinoid might also produce histamine.

Polypeptide hormones which should be produced, together with the aminic products, by the ECL, R and carcinoid cells must be studied, in order to clarify the identity of the cells as well as to account for the pathogenic significance of this peculiar neoplasm more precisely.
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References


