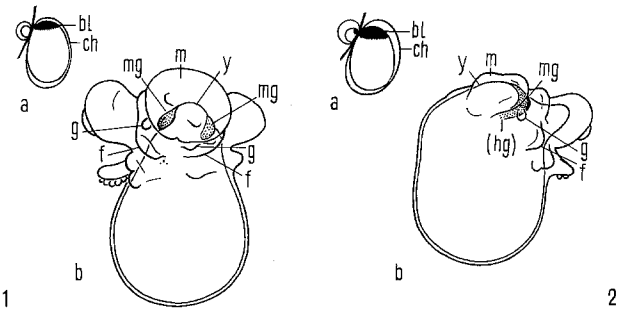

Fig. 1. Ventral view of a specimen ligated on the ventral side at stage 8–9 (a), preserved 6 days later (b). The hind-gut complex is lacking, the mid-gut parts are well developed on either side (reconstructed from cross sections).

Fig. 2. Ventral view of a specimen ligated ventrolaterally at stage 10 (a), preserved 6 days later (b). Mid- and hind-gut parts are lacking on the ligated side; the mid-gut rudiment is well developed on the other side and tapers off into the hind-gut area (reconstructed from cross sections).

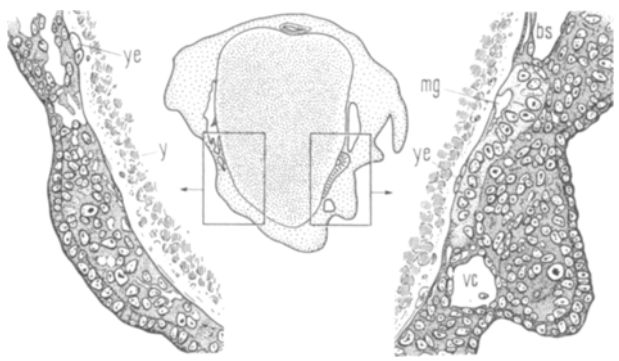

Fig. 3. Cross section of a specimen ligated ventrolaterally at stage 12–13, preserved 5 days later. On the ligated side, no trace of differentiation into an epithelium is visible; on the intact side, a distinct mid-gut epithelium is differentiated and begins to form a hepatic tube (camera lucida drawings of Epon section).
bl, blastodisc; bs, blood sinus; ch, chorion; f, funnel; g, gill; hg, hind-gut; m, mantle; mg, mid-gut; vc, vena cava limb; y, yolk; ye, yolk epithelium nuclei.

sectioned in thicknesses below 1 μ on an ultramicrotome or 6 μ on a regular microtome. The sections were stained with Azure Blue or Masson's Trichrome. The reconstructions shown in Figures 1 and 2 were made from drawings (camera lucida) of 6 μ cross-sections[7].

Results and discussion. Histological analysis of 10 specimens that had developed well after ligation showed that elimination of the ventral cortex in the presumptive hind-gut area inhibits the formation of the intestine and ink sac only while the rudiments of the actual mid-gut (stomach, caecum, hepatopancreas) form on either side (Figure 1). Removal of a lateral part of the cortex leads to the formation of an incomplete rudiment lacking the parts corresponding to the ligated area (Figures 2 and 3). In the live embryo, the presence of the respective parts of the rudiment appears in the constriction of the yolk which is absent at the site of the ligation.

If the original rudiment were a small one, restricted to a medioventral spot as KORSCHELT described it, the cortex area by which it is determined would have to be as small. Elimination of this ventral induction area would inhibit the entire mid- and hind-gut formation, whereas removal of the more lateral parts of the cortex would not primarily affect the formation of a complete mid- and hind-gut complex[8].

Zusammenfassung. Die Abschnürung von Teilen des ungefurchten Eicortex in der prospektiven Mittel- und Enddarmregion bei *Loligo pealei* führt zum Fehlen der entsprechenden Darmteile in späteren Stadien. Dies zeigt, dass die zusammenhängenden Anlagen des Mittel- und Enddarmkomplexes von Anfang an in Form eines lateral weit ausladenden Epithelstreifens vorliegen.

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[7] The author is indebted to Dr. J. M. ARNOLD for valuable advice in ligation technique, Epon embedding and thin sectioning.
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A Possible Role of Microtubules in the C Cells Secretory Mechanism

It is well known that Calcitonin is released following hypercalcemia[1] and that the same condition causes a discharge of the secretory granules of the C (parafollicular) cells of the thyroid[2]. Evidence has been presented suggesting that the secretory granules are the site of Calcitonin storage[3]. In in vitro organ culture, loss of Calcitonin (demonstrable with immunofluorescence) and degranulation of the dog thyroid C cells follows an increase of the calcium level of the culture medium[4,5].

It is not yet known how Calcitonin containing granules are released. We investigated the mechanism of secretion of C cells both in normal and cultured (stimulated by high calcium concentration of the medium) dog thyroid. We paid special attention to a possible role of microtubules in the mechanism of secretion.

Thyroids were obtained from 5 dogs and either a) directly fixed in 3% buffered glutaraldehyde, post-fixed in OsO_4 1%, dehydrated and embedded in Durcupan ACM Fluka; the stain was performed with uranyl acetate during dehydration and lead citrate on the sections; or b) cultured for 36 h at 37 °C in organ culture according to the technique previously described[4,5]. The calcium level in the culture medium (TC 199 Wellcome) was increased to 7.5 or 10.2 meq/l by adding $CaCl_2$. After

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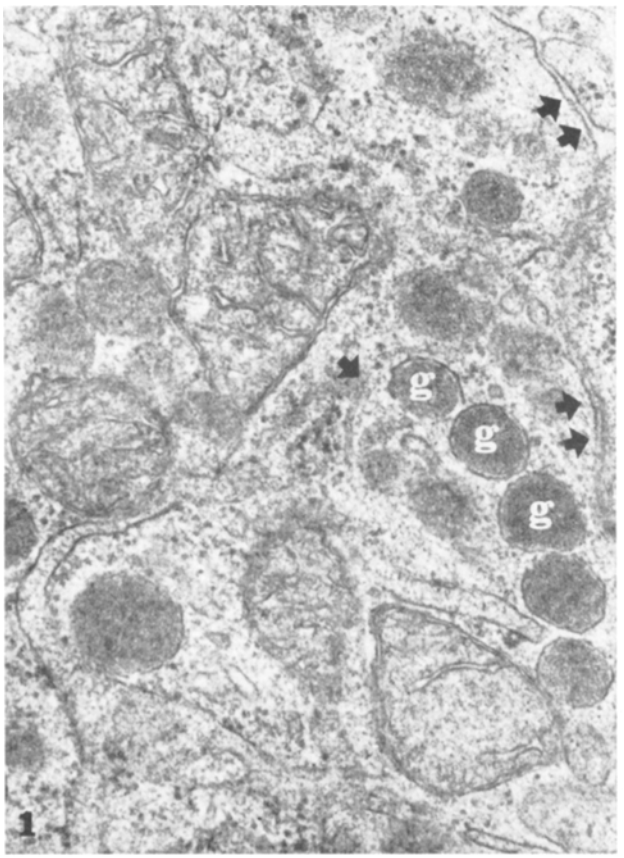

Fig. 1. Normal dog thyroid. C cell with microtubule (arrow) and secretory granules (g) near the plasma membrane (double arrows). ×42,000.

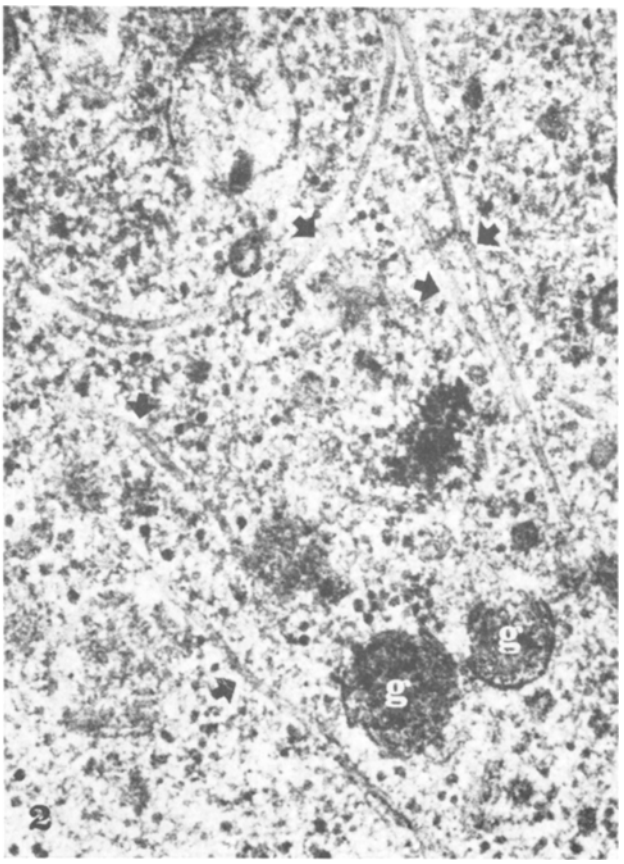

Fig. 2. Cultured dog thyroid. C cell with numerous microtubules (arrows) and some secretory granules (g). ×52,000.

culture the tissue blocks were processed as in a). Control cultures with 2.4 and 3.7 meq/l calcium level were obtained as previously described[4,5].

In the normal dog thyroid, C cells showed their typical pattern as described by others[6]. The microtubules were located near the Golgi area and in the peripheral areas of the cells (Figure 1). Similar findings have been described in the C cells of the rat[7], guinea-pig, tree-shrew thyroid and avian ultimobranchial body[8]. In the C cells of dog thyroid cultured in TC 199 with high calcium level, the secretory granules were few in number and mainly located at the periphery of the cell. In this part of the cell numerous microtubules were present; their length and aggregation varied from section to section (Figure 2). Some of them were in close relationship to the plasma membrane or to the secretory granules (Figure 3). Piling up of secretory granules in proximity of infoldings of the plasma membrane could occasionally be seen (Figure 3). Similar aspects have been found by others in cultured pancreatic A[9], and B cells[10,11], and referred to emiocytotic mechanism of secretion of hormone-containing granules.

The function of microtubules is still debated. It has been suggested that they are a sort of cytoskeleton with contractile capacity and serve to the transport of suspended solids[12]. In the B cells of the pancreas, LACY et al.[11] have observed microtubules 'linking the sac around the beta granules to the plasma membrane'. This cytoskeleton 'would provide an internal pathway of the granules within the cells'. Microtubules seem therefore to play an important role in the mechanism of emiocytosis which has been suggested to explain the secretory mechanism of some endocrine cells[9–11,13], which is however denied by others[14].

Apart from the difficulty of drawing dynamic data from morphological pictures, it is not easy in electron microscopy to decide whether 2 structures are just adjacent or actually fused. If the topographical relationship between secretory granules and microtubules represents a linkage, then our findings would support the view that microtubules have a role in the secretion

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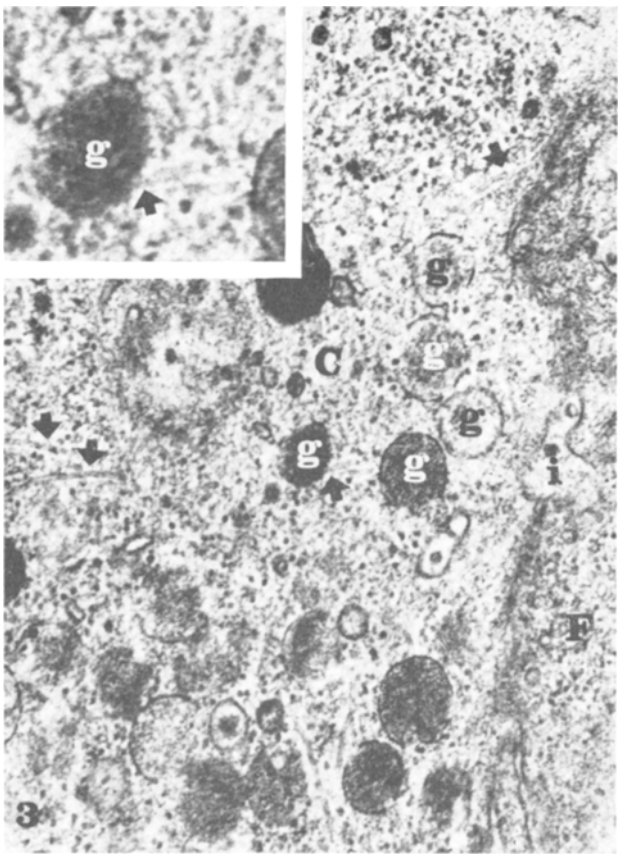

Fig. 3. Cultured dog thyroid. Piling up of secretory granules (g) near an infolding of plasma membrane (i). Microtubules (small arrows). A microtubule is intimately associated to a secretory granule (inset). C, C cell; F, Follicular cell. × 36,000. Inset: × 74,000.

of C cells. They would act in a way similar to that shown in the B cells of the pancreas. Once at the periphery of the cell, the granules might well be expelled by a mechanism of emiocytosis.

The C cells, together with the pancreatic A and B cells, and many other polypeptide-secreting endocrine cells of the APUD series are already known to have many properties in common[15]. We suggest, as a working hypotesis, that all these endocrine cells could possess a common way of secreting the hormone-containing granules. Microtubules might well be involved in such a mechanism[16].

Riassunto. Le modalità di secrezione dei granuli contenenti Calcitonina da parte delle cellule C sono state studiate in tiroidi di cane in condizioni normali e in coltura organotipica con alto tenore di calcio. È stata notata la presenza di numerosi microtubuli alla periferia delle cellule e alcune immagini suggeriscono un attacco dei microtubuli ai granuli secretori. Viene prospettato che i microtubuli abbiano importanza nel meccanismo di secrezione delle cellule C e forse delle cellule della serie APUD in generale, e che questo possa essere del tipo «emiocitosi».

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[16] We are deeply indebted to Prof. A. G. E. Pearse, of the Royal Post-Graduate Medical School of London for encouragement and to Prof. F. Mollo for his criticism. We acknowledge Dr. R. Navone for supplying the thyroid cultures.

Argyrophilic Cells of the Dog Pancreas Following Different Silver Stains

It is well known that pancreas contains both intrainsular and extrainsular argyrophilic cells (IAC and EAC). Both types of cell were considered to be of the same nature (preenterochromaffin cells: Erspamer[1]; A cells: Ferner[2]) until Hamperl[3], using the Bodian silver method instead of the Gros-Schultze one, found that in dog, unlike in man, only EAC were stained. Hamperl[3] also pointed out that alcoholic fixation, which destroyed argyrophilia of human IAC, did not suppress argyrophilia of EAC and suggested that EAC may be related to gastrointestinal argyrophilic cells more than to IAC. In 1960 Hellerström and Hellman[4] presented a modification of Davenport's alcoholic silver method, which gave positive results also in canine IAC[5,6].

At present argyrophilia takes an important place in islet cytology, as it is considered a specific property of D cells[6,7] and is employed to demonstrate the D cell origin of Zollinger-Ellison cells[8]. Bodian's and Davenport's modified methods are the most popular silver stains used.

For these reasons we have performed a comparative study between these 2 methods, using 4 μ thick paraffin sections of Bouin fixed pancreas from 20 mongrel dogs. Sections stained with Bodian's method (according to McManus and Mowry[9]) were sometimes counterstained with Kernechtrot and haematoxylin.

Argyrophilic cells were found within the islets of Langerhans, within the exocrine acini, in the connective tissue surrounding the exocrine ducts and among the cells of ductal epithelia. No difference was found between the tail and the uncinate process. IAC were easily stained by Davenport's modified method, but were unstained after Bodian's impregnation. Numerous deeply blackened intraacinar cells were observed following Bodian's method, while very few cells appeared to be Davenport-argyrophilic. Both intra- and peri-ductal argyrophilic cells appeared to be more numerous after Davenport's than after Bodian's stain. Using serial sections stained respectively with the 2 methods, the cells which reacted

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