

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.  $\times 36,000$ . Inset:  $\times 74,000$ .

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<sup>15</sup>. We suggest, as a working hypothesis, 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<sup>16</sup>.

*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 «emio-citosi».

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<sup>15</sup> A. G. E. PEARSE, J. Histochem. Cytochem. 17, 303 (1969).

<sup>16</sup> 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 intra-islet and extra-islet argyrophilic cells (IAC and EAC). Both types of cell were considered to be of the same nature (preenterochromaffin cells: ERSPAMER<sup>1</sup>; A cells: FERNER<sup>2</sup>) until HAMPERL<sup>3</sup>, using the Bodian silver method instead of the Gros-Schultze one, found that in dog, unlike in man, only EAC were stained. HAMPERL<sup>3</sup> 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<sup>4</sup> presented a modification of Davenport's alcoholic silver method, which gave positive results also in canine IAC<sup>5,6</sup>.

At present argyrophilia takes an important place in islet cytology, as it is considered a specific property of D cells<sup>6,7</sup> and is employed to demonstrate the D cell origin of Zollinger-Ellison cells<sup>8</sup>. 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  $\mu$  thick paraffin sections of Bouin fixed pancreas from 20 mongrel dogs. Sections stained with Bodian's method (according to McMANUS and MOWRY<sup>9</sup>) 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

<sup>1</sup> V. ERSPAMER, Z. Anat. 107, 574 (1937).

<sup>2</sup> H. FERNER, *Das Inselsystem des Pankreas* (G. Thieme, Stuttgart 1952).

<sup>3</sup> H. HAMPERL, Virchows Arch. 321, 482 (1952).

<sup>4</sup> C. HELLERSTRÖM and B. HELLMAN, Acta Endocrin. 35, 518 (1960).

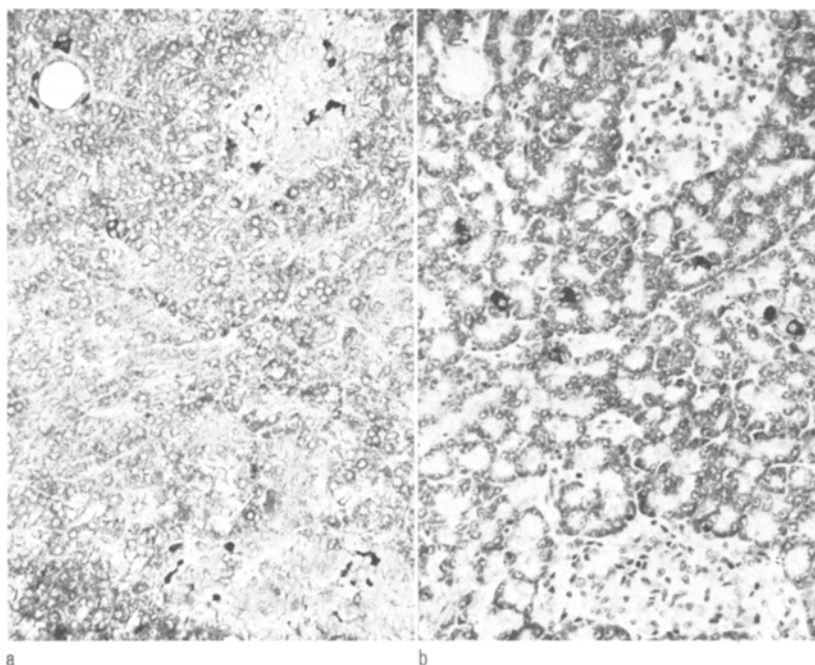
<sup>5</sup> C. CAVALLERO and E. SOLCIA, Acta Diabet. lat. 7, 5 (1964).

<sup>6</sup> T. FUJITA, Arch. histol. jap. 29, 1 (1968).

<sup>7</sup> A. EPPLER, Ergänz. Zool. Anz. 27, 461 (1964).

<sup>8</sup> C. CAVALLERO, E. SOLCIA and R. SAMPIETRO, Gut 8, 172 (1967).

<sup>9</sup> J. F. A. McMANUS and R. W. MOWRY, *Staining Methods* (Hoeber, New York 1960).



Adjacent serial sections of dog pancreas stained respectively with Davenport's modified (a) and Bodian's counterstained with haematoxylin (b) methods. Argyrophilic cells stained by one method do not correspond to those stained by the other.  $\times 190$ .

with one method did not react with the other one. However, since we were not able to perform both stains simultaneously on the same section, we cannot exclude that a small number of argyrophilic cells may react with both methods.

Although the argyrophilic methods lack histochemical significance, our observations support the suggestion of HAMPERL<sup>3</sup> concerning a different nature of IAC and EAC or, at least, a portion of the latter. On the other hand, our results showing a different reaction of argyrophilic cells to different silver stains are in agreement with previous investigations from this laboratory<sup>10-12</sup>, concerning neoplastic islet cells in Zollinger-Ellison syndrome; these cells in 2 cases appeared to be argyrophilic only when stained with Bodian's method, whilst extra-tumoral islet cells exhibited strong argyrophilia also after Davenport's modified impregnation.

More definite investigations on the extension and the significance of discordances between different silver stains are required. It may be pointed out that dog pancreas, for the topographic distribution of different types of argyrophilic cells, may represent a useful experimental model.

**Riassunto.** L'esame comparativo dei risultati ottenuti dopo impregnazione argantica di sezioni di pancreas di cane con i metodi di Davenport (modificato da HELLERSTRÖM ed HELLMAN) e di Bodian ha rivelato notevoli diversità. L'indagine su sezioni seriate fa ritenere che le cellule argirofile nei confronti di un metodo non lo siano nei confronti dell'altro. I riflessi sulla citologia endocrina del pancreas di cane e sul significato delle differenti metodiche argentofile sono brevemente discussi.

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<sup>10</sup> A. TARDINI and C. BORDI, Arch. Vecchi 57, 1 (1968).

<sup>11</sup> A. PERACCHIA, P. BOBBIO and C. BORDI, Giorn. It. Chir. 24, 19 (1968).

<sup>12</sup> A. TARDINI, P. ANVERSA and C. BORDI, Am. J. clin. Path. 52, 25 (1969).

### Kälte als Auslöser einer Glykogen-Speicherung während der Oogenese von *Musca domestica*<sup>1</sup>

Während der Oogenese von *Musca domestica* wird Glykogen nach Abschluss der eigentlichen Wachstumsphase als letzter Reservestoff in der Eizelle gebildet<sup>2</sup>. Ein spätes Einsetzen der Glykogen-Synthese wurde ähnlich auch bei anderen Insekten<sup>3,4</sup> und Wirbeltieren<sup>5</sup> autoradiographisch ermittelt. Durch direkte Hemmung der Protein-Synthese<sup>6</sup> oder ihre indirekte Beeinflussung über eine Blockierung der RNS-Versorgung<sup>2</sup> mit spezifisch inhibierenden Antibiotica wird eine vorzeitige Glykogen-

Speicherung auf frühen und mittleren Oogenese-Stadien ausgelöst. Sowohl die Eiweiss-Synthese<sup>6</sup> wie die RNS-Zufuhr<sup>7</sup> können im *Musca*-Ovar auch durch Temperaturabsenkung gedrosselt werden. Es sollte daher geprüft werden, ob durch Kälteeinwirkung sich ebenfalls eine verfrühte Glykogen-Einlagerung in Gang setzen lässt.

*Musca domestica* wurde im Klimaraum bei 21 °C unter Standardbedingungen<sup>8</sup> gehalten, unter denen die ♀♀ 4 Tage nach der Imaginalhäutung das Oogenese-Stadium 3<sup>9</sup>