CYTOLOGIC OBSERVATIONS ON THE PANCREATIC ISLETS WITH REFERENCE TO SOME ENDOCRINE-LIKE CELLS OF THE GASTROINTESTINAL MUCOSA*

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With 15 Figures in the Text

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Summary. Some findings obtained from cytological studies on the pancreatic islet of man and different animal species by applying several appropriate methods are reported. Three islet cell types were found to be simultaneously demonstrated by toluidine blue after methylation and saponification of tissue sections; metachromasia appeared to be in some way a constant finding of argyrophil D-cells which, at least in duck and man, displayed also a weak PAS-positivity. No one of the three insular cell types was found to show all the morphologic patterns of enterochromaffine cells, nor enterochromaffine cells showed all the patterns of insular cells. The existence of possible morphologic relationships between insular A-cells and some endocrine-like cells in fundic mucosa and, on the other hand, between argyrophil D-cells and some cells in antro-pyloric mucosa of the stomach is emphasized.

Recent investigations with new technical approaches on the cytology of pancreatic islets have thrown more light upon this intriguing field of research. In particular, helpful results have been obtained by employing Davenport’s silver impregnation, metachromatic stain with basic dyes and dark-field microscopy of cryostatic sections (Manocchio, 1960; Petersson et al., 1962, Hellman et al., 1962). We have described in previous works on islet cytology our findings with these methods (Solcia, 1962b; Cavallero and Solcia, 1964a; Solcia and Sampietro, 1965).

In the past it has been suggested that possibly morphological relationships exist between insular cells and some endocrine-like cells of the gastro-intestinal mucosa: particular attention has been given to argentaffine (enterochromaffine) and argyrophil cells (Erspamer, 1937 and 1939; Ferner, 1952; Hamperl, 1952; Fodden, 1953; Korf and Le Compte, 1955; Dina and Mancini, 1955) as well as to the so called “X cells” of the gastric mucosa (Davis, 1954). Moreover, biochemical and physiological studies have shown several endocrine activities in the gastro-intestinal tract, which appear to be in some way similar to those of pancreatic islet cells. In this connection, it should be remembered that an hyperglycemic and glycogenolitic factor physiologically identified as glucagon has been extracted from the fundic mucosa by Sutherland and De Duve (1948) and by Makman and Sutherland (1964); on the contrary an hypoglycemic activity has been found in duodenal mucosa by Laughton and Macallum (1932), McIntyre et al. (1964) and Dupré (1964). True carcinoids arising in the pancreas have been observed (Dengler, 1959; Gepts et al., 1960; Waldenström, 1961; Peart et al., 1963; van der Sluys Veer et al., 1964) and intestinal carcinoids associated with hypoglycemic (Kahr, 1956; Kähler and Heilmeyer, 1961) or hyperglycemic

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symptoms (Weisberg and Schaeffer, 1952) have been also described. Further it has been reported that both insular (Zollinger and Ellison, 1955) and gastro-duodenal endocrine tumours (Oberhelman et al., 1961; Goodrick and Dockerty, 1963) can be associated with Zollinger-Ellison’s syndrome.

In the present work further studies have been dedicated to the morphological differentiation of insular cells. Moreover, these cells have been compared with some endocrine-like cells of gastro-intestinal mucosa; in particular, the relationships between enterochromaffine and islet cells have been discussed and attention has been given to the pyloric and fundic mucosa of the stomach considered as possible sites of production and secretion of hormonally active substances.

Material and Methods

Tissue samples from pancreas, stomach (both of fundic and antral regions), duodenum and ileum were removed immediately after killing from monkey (Papio hamadryas, Macacus rhesus), horse, dog, rabbit, guinea-pig, rat and duck; surgical specimens of human pancreas and gastro-intestinal tract including appendix were also examined.

The material was partly fixed in 10% neutral formol for one week or in Bouin’s fluid with 5% or 2% acetic acid, partly frozen in isopentane cooled in a bath of dry ice and alcohol. Paraffin sections of fixed tissues were silver-impregnated by employing a modified Davenport’s method (Hellerström and Hellman, 1960) or stained in aqueous solutions of toluidine blue at pH 5 and pH 4. Before toluidine blue staining, some sections were methylated for 60–90 min in acidified methyl alcohol according to Fisher and Lillie (1954) and then saponified in 1% KOH in 80% ethyl alcohol according to Lillie (1958) for 20 min as suggested by Manocchio (1964). The Masson-Hamperl, Schmorl and diazonium reactions following Pearse (1960) were also applied to the paraffine sections for the demonstration of enterochromaffine cells. Generally, it should be noted that Davenport’s method and toluidine blue stains give the best results on Bouin-fixed sections; on the contrary, the reactions for enterochromaffine cells are best performed on formol-fixed tissues.

In some instances, when indicated, conventional stains such as Heidenhain’s iron hematoxylin, aldehyde-fuchsin with or without counterstaining with Harris’s hematoxylin, phloxine, xylidine ponceau or with a mixture of orange G and light green, were used on paraffin sections. The Hotchkiss-McManus periodic acid-Schiff reaction, acetylation in acetic anhydride-pyridine and desacetylation in KOH were performed according to McManus and Mowry (1960). Finally, frozen samples were cut in a cryostat and the sections examined under dark-field microscopy as previously indicated (Solcia, 1962a).

Reserpine (Serpasil, CIBA) was given intraperitoneally to four adult dogs, 1 to 5 mg/kg daily for 2 days and synthalin A to two dogs, 10 mg/kg daily for two days; reserpin-treated animals were killed 12 hours and synthalin-treated animals 20 hours after the last injection. In both cases pancreatic and gastro-intestinal material was studied as above.

Results

Pancreatic islets

In accordance with previous observations, the pancreatic islets of all species examined showed, at dark-field microscopy, highly luminous silver-white granules contained in glucagon-producing A-cells and, by employing Davenport’s method, a particular type of silver impregnated cell which was identified as D-cells. In some species, for instance in man, monkey and dog, with toluidine blue at pH 5 — but not at pH 4 — a clear-cut red metachromasia was found in D-cells; this finding was less evident in duck and rabbit, and practically negative in rat and guinea-pig. As a rule, A-cells did not electively stain with toluidine blue, with the exception of the duck where A-cells showed a weak violet-red colour.