RESEARCH COMMUNICATION

The endocrine pancreas of the Cape fur seal, *Arctocephalus pusillus* (Schreber, 1776): an immunocytochemical study

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ABSTRACT

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The indirect peroxidase method was employed to study the endocrine pancreas of the Cape fur seal. Immunoreactivity to insulin was confined to the cores of the islets and the insulin cells were more abundant than the other endocrine cell types, which occurred mainly in the mantles of the islets. Of these, glucagon cells were the most numerous, followed by somatostatin and pancreatic polypeptide (PP) cells. The latter were observed in the mantles of the islets and scattered in the exocrine tissue of the duodenal lobe.

The marked variation in the shape and the distribution of the endocrine cells in the mantles of the islets seen in the pancreas of the seal, seems to be typical of carnivorous species like the cat and dog.

Keywords: *Arctocephalus pusillus,* Cape fur seal, endocrine pancreas, immunocytochemical, immunoreactivity, insulin

INTRODUCTION

Cape fur seals are distributed from Algoa Bay to the Namibian coast and are concentrated on small, rocky islets which remain dry during high tide (Skinner & Smithers 1990). They are strictly carnivorous (Rand 1959) and their diet consists mainly of small schooling fish. Shaughnessy & Payne (1979) reported that the commercial fishing industry is responsible for the high mortality of these seals. Very little is known about their physiology (W.H. Oosthuizen, personal communication 1996), and this investigation is an effort to contribute to the knowledge of the physiology of this species.

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MATERIALS AND METHODS

Tissue samples representing all the regions of the pancreas (fixed in Bouin's fluid) were obtained from three specimens from The Sea Fisheries Research Institute (Cape Town). The specimens were shot after having been injured in commercial fishing activities. Tissue was dehydrated in an ethanol series and embedded in paraffin wax. Sections (5 µm) were cut and floated on slides pre-treated with poly-L-lycine (Huang, Gibson, Facer, Gu & Polak 1983). Sections were dewaxed and hydrated through a series of ethanols and transferred to 0,05 M Tris-saline. To block endogenous peroxidase, the sections were treated with 0,3% hydrogen peroxide for 30 min and rinsed in Tris-saline.

To reduce non-specific staining, the sections were incubated with 10% normal swine serum (Burns 1979). The indirect peroxidase method (Sternberger 1979) was employed to identify immunoreactivity of bioactive peptides. The reaction sites were revealed by the method of Graham & Karnovsky (1966). Details of the primary antisera employed are listed in

Table 1. Some sections were counterstained with haematoxylin. The endocrine cells of 30 islets in each of four consecutive sections, each stained for a specific peptide, were counted and the relative abundance of each cell type estimated.

As positive controls, sections of tissues from dog and swine, known to contain the peptides, were employed. Negative controls consisted of substitution of non-immune rabbit serum for the primary antibody and pre-absorption of the diluted antibody with 10 nmol or at least 20 µg/mℓ of the homologous peptides.

RESULTS AND DISCUSSION

All the antisera demonstrated endocrine cells in the pancreas and they were all absorbed by their parent peptides.

In the head, the islets were bigger and less numerous than in the body and tail. The larger islets were ovoid or elongated, while the smaller ones were more or less round.

Insulin cells were confined to the cores of the islets, and the poles of these cells facing the capillaries were more intensely immunostained (Fig. 1). Insulin cells constituted the bulk of the islets, and a few small islets contained insulin cells only.

Glucagon cells occurred in the mantle of the islets, but isolated ones were seen in some cores. Occasionally those of the mantle were seen to dip into the core. Glucagon cells were more abundant in the islets of the head, and sometimes they formed a tail running into the exocrine pancreas (Fig. 2), cuffing a blood vessel.

Somatostatin cells seen in the mantles of the islets (Fig. 3), were less abundant than their glucagon counterparts. Their relative density was consistent throughout the pancreas, some cells were seen to have cytoplasmic processes and some were located in the parenchyma.

Pancreatic polypeptide cells occurred mainly in the region of the head, seen in both the islets (Fig. 4) and the exocrine tissue, but the cells gradually diminished towards the tail. PP cells in the mantles of the islets were less numerous than their somatostatin counterparts, often restricted to one pole only. PP and glucagon cells occurred in the ductular epithelium.

TABLE 1 Primary antisera used

Antisera	Code	Dilution	Source
Glucagon	138	1:1 000	J.M. Polak: London
Insulin	2384	1:1 000	J.M. Polak: London
Pancreatic polypeptide (bovine)	068A	1:1 000	Dr S.A. Woolfe-Coote: Tygerberg
Somatostatin	744	1:1 000	J.M. Polak: London

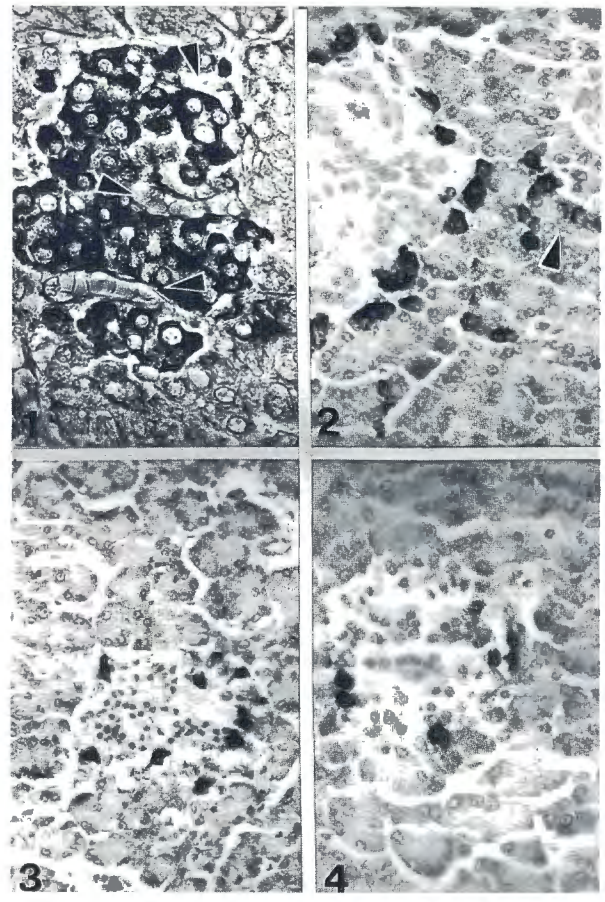

FIG. 1 A small islet in the splenic region immunoreactive to insulin. The insulin cells that abut on the capillaries (arrow heads) have more immunoreactive material on the side of the capillary

900x Indirect peroxidase and phase contrast microscopy

FIG. 2 A portion of an islet in the splenic region showing endocrine cells immunoreactive to glucagon in the mantle. These cells form a tail running into the exocrine pancreas (arrow head)

600x Indirect peroxidase and counterstained with haematoxylin

FIG. 3 Somatostatin immunoreactive cells scattered in the mantle of an islet in the splenic lobe

400x Indirect peroxidase and counterstained with haematoxylin

FIG. 4 Cells immunostained for pancreatic polypeptide in the mantle of a small islet in the head of the pancreas

600x Indirect peroxidase and counterstained with haematocylin

In general, the endocrine pancreas of the fur seal is comparable to that of other mammals (Samols, Bonner-Weir & Weir 1986). In contrast to some mammals (Kohrent, Fält, Witt, Ziegler & Ziegler 1988; Ravazolla, Effendic, Ostenson, Tatemoto, Hutton & Orci 1988), islets of the fur seals were considerably heterogeneous in shape, as in the cat (Furuzawa, Ohmori & Watanabe 1992), dog (Redecker, Seipelt, Jörns, Bargsten & Grube 1992) and slender mongoose, *Glerella sanguinea* (unpublished findings). However, the micro-architecture resembles the situation in the dog (Redecker *et al.* 1992) more closely than that in the cat (Furuzawa *et al.* 1992).

Glucagon cells were found mainly in the mantles, but were more numerous in the cores of some islets of the dog (Redecker *et al.* 1992) than in those of the fur seal. Furthermore, they were preferentially located at a single pole of some islets, in both these species (Redecker *et al.* 1992). In the fur seal this seems to be of physiological significance, if judged from their juxtaposition to blood vessels, and one is tempted to regard it as a route for the quick mobilization of glucagon.

Insulin cells occupying the cores of the islets in the seal, and also constituting their bulk, are typically mammalian (Samols *et al.* 1986). Polarity within the insulin cells (Bonner-Weir 1988), where more secretory vesicles were located in the pole of the cells that abut the capillary, confirms our findings.

The distribution of somatostatin cells in the mantles of the seal islets is comparable to the mammalian pancreas in general, but the long cytoplasmic processes seen in guinea-pigs were not observed in the seal or the dog (Larson 1985); nevertheless they may implicate a paracrine function (Grube 1984; Larson 1985).

The distribution and abundance of PP cells in the pancreas of the seal is in agreement with the situation in domesticated carnivores (Redecker *et al.* 1992; Furuzawa *et al.* 1992), and particularly in the dog (Redecker *et al.* 1992) in which the PP cells were often confined to one pole only. Isolated PP cells seen in the exocrine pancreas of the seal and other mammals (Hazelwood 1980) are in agreement with their influence on the parenchymal cells (Shiratori, Lee, Chang, Jo, Coy & Chey 1988). Endocrine cells in the ductular epithelium of the seal pancreas are typically mammalian (Dorn, Lorenz & Koch 1977).

The juxtaposition of the intra-islet cell types in relation to blood flow in the islet is under investigation (Epple & Brinn 1987). This may lead to a better understanding of the intra-islet regulation (Samols, Stagner, Ewart & Marks 1988; Samols & Stagner 1990). Our findings, together with those of Redecker *et al.* (1992) on the dog, confirm the opinion of Baetens, Malaisse-Lagae, Perrelet & Orci (1979) that the understanding of intra-islet regulation is complicated by the heterogeneity of individual islets.

We conclude that the endocrine pancreas of the Cape fur seal has specific features found in terrestrial carnivores, that may be linked to diet and physiology.

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REFERENCES

BAETENS, D., MALAISSE-LAGAE, F., PERRELET, A. & ORCI, I. 1979. Endocrine pancreas: Three-dimensional reconstruction shows two types of islets of Langerhans. *Science,* 206: 1323–1325.

BONNER-WEIR, S. 1988. Morphological evidence for pancreatic polarity of f3-cells within islets of Langerhans. *Diabetes,* 37:616–621.

BURNS, J. 1979. Immunohistological methods and their application in the routine laboratory. *Recent Advances in Histopathology,* 10:337–349.

DORN, V.A., LORENZ, D. & KOCH, G. 1977. Immunohistochemischer nachweis von Insulin und Glucagon im Gangepithel des Pancreas. *Acta Histochemica,* 58:364–367.

EPPLE, A. & BRINN, J.E. 1987. Comparative physiology of pancreatic islets. Berlin: Springer-Verlag: 58.

FURUZAWA, Y., OHMORI, Y. & WATANABE, T. 1992. Immunohistochemical morphometry of pancreatic islets in the cat. *Journal of Veterinary Medical Science,* 54:1165–1173.

GRAHAM, R.C. (Jr.) & KARNOVSKY, M.J. 1966. The early stages of absorption of injected horseradish peroxidase in proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. *Journal of Histochemistry and Cytochemistry,* 14:291–302.

GRUBE, G. 1984. Somatostatin (D) cells in the endocrine pancreas of mammals: Endocrine or paracrine elements? *Frontiers in Hormonal Research,* 12:41–43.

HAZELWOOD, R.L. 1980. The avian gastro-enteric-pancreatic system: structure and function, in *Avian endocrinology,* edited by A. Epple & M. Stetson. New York: Academic Press: 231–250.

HUANG, W.M., GIBSON, S.J., FACER, P., GU, J. & POLAK, M.J. 1983. Improved section adhesion for immunocytochemistry using high molecular weight polymers of lysine as a slide coating. *Histochemistry,* 77:275–279.

KOHNERT, K-D., FÄLT, K., WITT, S., ZIEGLER, B. & ZIEGLER, M. 1988. Identification of monoclonal antibodies to pancreatic islet cells by immunoperoxidase staining. *Acta Histochemica,* 83:159–165.

LARSON, L-I. 1985. Distribution and morphology of somatostatin cells. *Advances in Experimental Medical Biology,* 188:383–402.

RAND, R.W. 1959. The Cape fur seal *(Arctocephalus pusillus).* Distribution, abundance and feeding habits off the south-western coast of the Cape Province. *Investigational Report: Division of Sea Fisheries*, 34:1–75.

RAVAZOLLA, M., EFFENDIC, S., OSTENSON, C.G., TATEMOTO, K., HUTTON, J.C. & ORCI, L. 1988. Localization of pancreastatin immunoreactivity in porcine endocrine cells. *Endocrinology,* 123:227–229.

REDECKER, P., SEIPELT, A., JÖRNS, A., BARGSTEN, G. & GRUBE, D. 1992. The microanatomy of canine islets of Langerhans: implications for intra-islet regulation. *Anatomy and embryology*, 185:131–141.

SAMOLS, E., BONNER-WEIR, S. & WEIR, G.C. 1986. Intra-islet insulin-glucagon-somatostatin relationships. *Clinics in Endocrinology and Metabolism*, 15:33–58.

SAMOLS, E., STAGNER, J.I., EWART, R.B.L. & MARKS, V. 1988. The order of islet microvascular cellular perfusion is B à A à D in the perfused rat pancreas. *Journal of Clinical Investigation*, 82:350–353.

SAMOLS, E. & STAGNER, J.I. 1990. Islet somatostatin–microvascular, paracrine, and pulsatile regulation. *Metabolism*, 39: 55–60.

SHAUGHNESSY, P.D. & PAYNE, A.I.L. 1979. Incidental mortality of Cape fur seals during trawl fishing activities in South African waters. *Fisheries Bulletin of South Africa*, 12:20–25.

SHIRATORI, K., LEE, K.Y., CHANG, T-M., JO, Y.H., COY, D.H. & CHEY, W.Y. 1988. Role of pancreatic polypeptide in the regulation of pancreatic exocrine secretion in dogs. *American Journal of Physiology*, 255:G535–G541.

SKINNER, J.C. & SMITHERS, R.H.N. 1990. *The mammals of the southern African subregion,* 1st ed. Pretoria: University of Pretoria: 523–526.

STERNBERGER, L.A. 1979. *Immunocytochemistry,* 2nd ed. New York: Wiley & Sons: 104–169.