IMMUNOHISTOCHEMICAL LOCALIZATION OF SOMATOSTATIN, INSULIN AND GLUCAGON IN THE PRINCIPAL ISLETS OF THE ANGLERFISH (LOPHIUS AMERICANUS) AND THE CHANNEL CATFISH (ICTALURUS PUNCTATA) (1) (2)

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ABSTRACT Somatostatin, insulin and glucagon were localized in the principal islets of the anglerfish (Lophius americanus) and the channel catfish (Ictalurus punctata) by means of the unlabeled antibody-peroxidase-antiperoxidase immunocytochemical method. Both species showed a similar ratio of positive cells 9:6:4 (insulin:somatostatin:glucagon), but the interrelations of the three cell types differed between species. The large number of somatostatin-positive cells may be indicative of an important role for this hormone in teleost physiology. The principal islets appear to be a good source of tissue for further work on somatostatin.

Since the initial description of somatostatin (SRIF) in the islets of Langerhans of rats and guinea pigs (Luft et al., '74), its presence has been reported in insular tissues of a number of mammalian and avian species (Dubois, '75). The islet cell type in which somatostatin is localized has been established as the delta or alpha$_1$ cell (Hökfelt et al., '75, Orci et al., '75). The description of somatostatin has not been extended to the principal islets of teleosts, even though species such as the channel catfish (Ictalurus punctata) have a high percentage of delta cells in their islet tissue (Brinn, '71). This study was undertaken to see whether these principal islets also contain somatostatin, as well as to extend the knowledge of somatostatin's phylogenetic distribution.

MATERIALS AND METHODS Guinea pig anti-SRIF (GP-ASRIF) was obtained using a method previously described (Elde et al., '75). Guinea pig anti-bovine-porcine-glucagon (GP-ABPG) was a gift from Doctor Stanley Erlandsen, Department of Anatomy, University of Minnesota.

Rabbit anti-anglerfish-insulin (R-AAI) was generated in response to subcutaneous injection of a keyhole limpet hemocyanin-insulin conjugate prepared in the following manner. Anglerfish insulin, 50.0 mg (Novo Lot #2366) dissolved in 5.0 ml of 0.02M HCl, was added to 150.0 mg of keyhole limpet hemocyanin (Calbiochem) dissolved in 5.0 ml of 0.05N phosphate-buffered saline (PBS), pH 7.2. The final pH of the mixture was adjusted to 7.3 with NaOH. While stirring, 50 µl of 5.6% glutaraldehyde (Ladd) was added, followed 30 min. later by an additonal 50 µl. The solution was stirred overnight at room temperature, after which 100.0 mg of glycine (Sigma) was added. The conjugate was then dialized against and diluted with PBS to a final concentration of 2.0 mg. of insulin per ml. Antibodies were obtained after 2 injections of a total of 2.0 mg conjugate per animal.

Principal islets from anglerfish and channel catfish were fixed by immersion in Bouin's fluid. Following paraffin embedding, the localization of somatostatin, insulin and glucagon was accomplished on 5-micron tissue sections using the unlabeled antibody-peroxidase-antiperoxidase technique of Sternberger et al. ('70) as modified for use with heterologous antisera by Erlandsen et al. ('75). The specificity of the antisera was tested on adjacent tissue sections. Controls included (1) the addition of an excess of appropriate antigen to the immune sera prior to their application to the tissue sections, (2) the use of non-immune guinea pig and rabbit sera as first antibody, and (3) the omission of the first antibody. The dilutions of antisera used were GP-ASRIF 1/100, GP-ABPG 1/50 and R-AAI 1/4000.

RESULTS Localization of all three hormones was accomplished in both the anglerfish and the catfish. Absorption controls verified the specificity of each antiserum for the specific hormone against which it was produced. Tissue sections on which the first antibody was omitted or non-immune serum was used showed no specific cellular staining. The anglerfish showed a most striking arrangement of cell types. There were large areas of somatostatin-positivity surrounded first by a rim of glucagon-positive cells and second by insulin-positive cells. The arrangement of the catfish islet was not as regular as that of the anglerfish. Somatostatin-positive cells were present in large numbers, but all three cells types were intermingled within the endocrine tissue. Preliminary quantitation using linear scanning (Carpenter et al., '62) revealed an approximate ratio of 9:6:4 (insulin:somatostatin:glucagon) in both animals.

DISCUSSION Hypothalamic somatostatin has been reported in a number of vertebrate species (Dubois et al., '74), but the description of somatostatin in the islets of Langerhans has been limited to mammals and birds. This study confirms its presence in the principal islets of two representative teleosts, and establishes its coexistence with insulin and glucagon over a wide phylogenetic range. The large number of somatostatin-positive cells in these two species is certainly of interest considering the generally low number of similar cells in mammalian islets. In teleosts, the physiologic roles of the principal islets, as well as of insulin and glucagon, have been difficult to elucidate. Though similarities do exist, most work in the field has served to point out the differences between insular function in fish as compared to mammals (Brinn, '73; Epple, '69). Since teleost principal islets appear to be regulated by factors differing from those which regulate islet function in higher forms, the presence of large amounts of somatostatin, known to influence insulin and

glucagon release, is intriguing. Somatostatin may be an intermediate between the primary regulating factor in fish and the final insulin or glucagon response. Somatostatin-cells may not only be involved in local regulation. Their presence in such large numbers (two-thirds as many as beta cells and one and one-half times the number of $alpha_2$ cells) may indicate an involvement in the homeostasis of the entire organism.

Somatostatin is known to inhibit the release of insulin and glucagon (Alberti et al., '73; Erich et al., '74), but nothing is known about regulation of its synthesis or release. The principal islets in teleosts, long used as a source of tissue for islet experimentation, now appear to be an excellent source of tissue for this type of work on somatostatin. Experimental systems using this tissue may well give insight into the complex interactions between the different cell types found in islet tissue.

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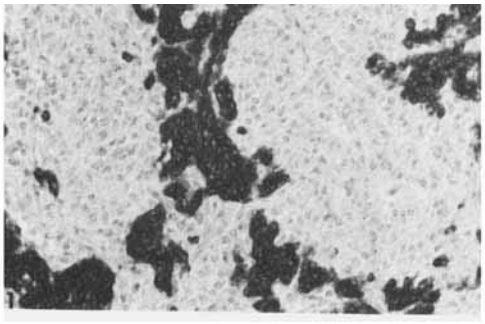
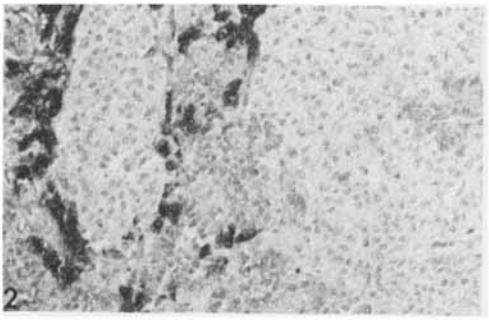
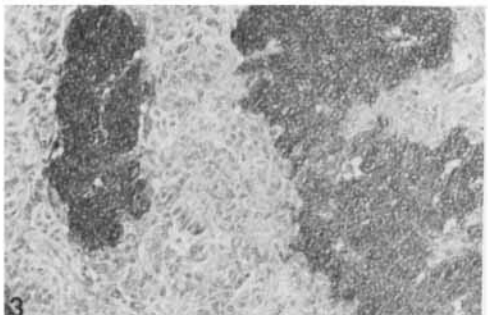
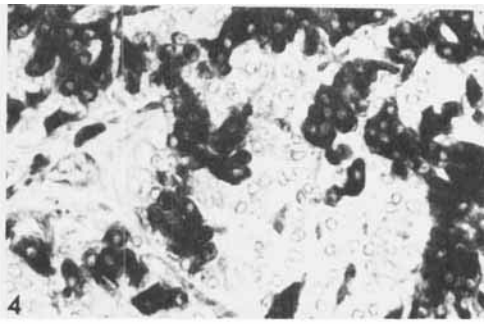
FIGURE LEGEND

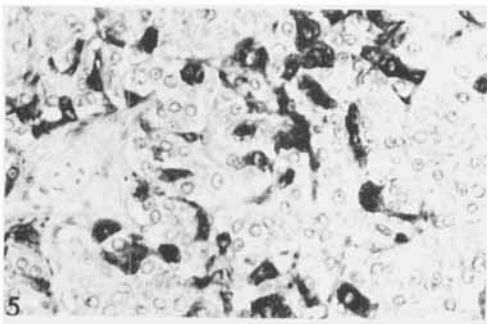
Serial sections through the principal islets of the anglerfish figures 1-3 (100X) and the catfish figures 4-6 (200X). Following specific hormonal localization, the sections were lightly counterstained with hematoxylin. Top row, insulin; middle row, glucagon; bottom row, somatostatin.

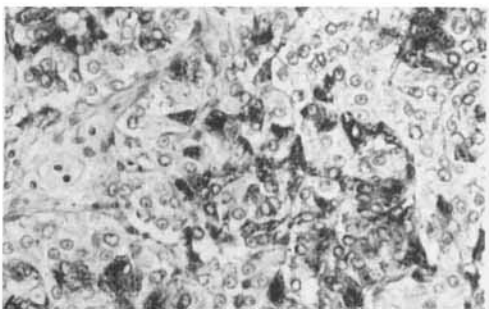












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