Immunohistochemical Identification of Pituitary Hormone Producing Cells in the Sockeye Salmon (Oncorhynchus nerka, Walbaum)

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Summary. Antisera to six mammalian pituitary hormones and a synthetic corticotrophin (Synacthen) were prepared and tested for specificity. The gamma-globulin fractions of the antisera were conjugated with fluorescein isothiocyanate and the labelled antibodies were isolated by column chromatography.

Fresh-frozen sections of pituitary glands of adult migratory sockeye salmon (Oncorhynchus nerka) were incubated with the antibody solutions and examined with a fluorescence microscope. Anti-ACTH and anti-Synacthen appeared to be bound by the epsilon cells, whereas anti-prolactin reacted with the eta cells. Anti-GH was bound to the acidophil cells of the proximal pars distalis. Anti-LH reacted with some of the basophil cells of the proximal pars distalis. Antibodies to FSH or TSH failed to react.

Key-Words: Pituitary—Salmon—Hormones—Immunohistochemistry.

The application of immunohistochemical techniques in localizing pituitary hormones was first reported by Marshall (1951), who was able to identify adrenocorticotropic hormone (ACTH) producing cells in the hog pituitary gland. Since then, several investigators successfully used this method in localizing a number of hormones in the pituitary glands of a variety of mammals. Thus, corticotrophs were also identified by Leznoff et al. (1962), Pearse and van Noorden (1963), McGarry et al. (1964), Hachmeister and Kracht (1965), Kracht et al. (1965, 1966), and Brozman (1967). Direct evidence regarding the cellular site of prolactin production was presented by Emmart et al. (1963, 1965), Shiino and Rennels (1966), Nayak et al. (1968), and Stokes and Boda (1968). Moreover, Emmart and co-workers showed that antibodies to ovine prolactin are capable of binding a prolactin-like fraction prepared from pituitary glands of two teleost fishes: carp and pollack (Emmart et al., 1966). They demonstrated, furthermore, that the same antibodies, conjugated to fluorescein isothiocyanate, are specifically bound to the cells of the

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rostral pars distalis in the teleosts *Fundulus heteroclitus* (Emmart *et al*., 1966; Emmart and Mossakowski, 1967) and *Carassius auratus* (Emmart, 1969). Growth hormone (GH) has been localized in certain pituitary acidophils of mammals (Leznoff *et al*., 1960; Pearse and van Noorden, 1963; Parker *et al*., 1965; Shiino and Rennels, 1966; Nayak *et al*., 1968; Stokes and Boda, 1968; Mason *et al*., 1969). Further, Hayashida (1969) has shown by double gel diffusion technique that antibodies to rat GH are capable of binding a substance in pituitary extracts from various tetrapods. Using a radioimmunoassay, these antibodies were also shown to cross react with pituitary extracts of the lungfish, sturgeon, paddlefish, mackerel and coho salmon (Hayashida, 1969; Hayashida and Lagios, 1969).

There is less immunohistochemical evidence for the location of glycoprotein hormones. Midgley (1963, 1966), Pomerantz and Simmons (1968), and Monroe and Midgley (1969) demonstrated the cellular site of luteinizing hormone (LH) in the human, cow, and rat respectively. Follicle stimulating hormone (FSH) cells were identified in man (Midgley, 1964) and thyroid stimulating hormone (TSH) cells in man (Brozman, 1967) and the rat (Nakane, 1968). In summary, antibodies to pituitary hormones of various mammals cross react with the same hormones in other species of this class, as well as with non-mammalian pituitary hormones.

This investigation concerns the identification of hormone-producing cells in the pituitary gland of sockeye salmon.

**Materials and Methods**

*Collection of Pituitary Glands.* Sockeye salmon belonging to the “Chilko race” were collected along their migratory route. The fish were captured by a variety of methods: troller, reef net, gill net, dip net, and beach sein. Scale samples were taken to verify that all fish were of the same race. The fish collected were of both sexes, in different stages of sexual maturity, and were from both sea water and fresh water. A total of 46 fish was collected. Twenty-six pituitary glands were immediately frozen in isopentane cooled in liquid nitrogen and kept frozen in sealed vials. The remainder were fixed in Bouin Hollande-Sublimate (B.H.S.; Herlant, 1956). In addition, pituitary homogenates were prepared from 100 glands of sexually mature chinook salmon (*Oncorhynchus tshawytscha*) collected at the Qualicum River, Vancouver Island, B. C. These glands were frozen on dry ice.

*Hormone Preparations.* The following hormones were used as antigens: porcine ACTH (Sigma, 140 IU/mg), Synacthen (Giba, β2-24-corticotrophin), ovine prolactin (Sigma, 17 IU/mg), ovine prolactin (NIH-P-S8, 28 IU/mg), ovine GH (NIH-GH-S9, 1.09 IU/mg), ovine LH (NIH-LH-S13, 0.93 IU/mg), ovine FSH (NIH-FSH-S5, 1.42 IU/mg), bovine TSH (Sigma, 1.0 IU/mg), and ovine TSH (NIH-TSH-S5, 1.44 IU/mg). To prepare salmon pituitary homogenates, glands were homogenized in a 13 × 100 glass homogenizer. The brei was centrifuged and the supernatant was removed for immunochemical analysis.

*Immunization Procedure.* Ten groups of four New Zealand white rabbits were used. Each of nine groups was injected with one of the above antigens and four rabbits were used as un.injected controls. Antigens were dissolved in 0.15 M NaCl in concentrations of 0.83 mg/ml (ACTH), 0.31 mg/ml (Synacthen), 2.50 mg/ml (Prolactin), and 1.0 mg/ml (GH, LH, FSH, and TSH). Prior to injection each solution was mixed with an equal volume of Freund's complete adjuvant (Hyland Lab.) Injections were given intramuscularly or subcutaneously once every week. Three days after the fourth injection the rabbits were bled from the ear marginal vein and the antibody titer was estimated. When the titer was sufficiently high (usually 7 weeks after the first injection), larger blood samples of approximately 30 ml were taken. Bleeding was performed once a week between injections. Ten days after the last injection most rabbits were chloroformed and exsanguinated. Some were kept for another 7 months after

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