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Development of the endocrine pancreas during larval phases of *Rana temporaria*

An immunocytochemical and ultrastructural study

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Summary. The pancreatic endocrine component was studied at different stages of development in the tadpoles of *Rana temporaria*. The material was embedded in Epon, and serial semithin and thin sections were made in order to correlate ultrastructural features and tinctorial traits of the endocrine cells. Serial semithin sections were also stained with the peroxidase-antiperoxidase immunocytochemical method and with silver impregnations for argyrophilia and argentaffinity. In early larvae (legless tadpoles), A and B cells are present. Both can be found within ducts and exocrine tissue or, more frequently, in cellular clusters among the ducts and acini. These primitive islets are solid structures, surrounded but not penetrated by capillaries. Mitoses were observed in A and B cells. In the following phase (tadpoles with hindlegs), D and pancreatic polypeptide-immunoreactive cells are also present, as well as numerous endocrine cells scattered among exocrine tissue. There is also a change in the vascular-insular pattern: capillaries not only surround but also penetrate the endocrine group. The structure of the endocrine pancreas in older tadpoles is similar. Tinctorial traits and ultrastructural features of endocrine cells are described, and the origin of primitive islets is discussed.

Key words: Pancreas, endocrine – Larval development – Serial thin/semithin sections – Immunocytochemistry – *Rana temporaria* (Anura)

Four major hormonal peptides are usually present in the pancreatic endocrine component of vertebrates: insulin, somatostatin, glucagon, and pancreatic polypeptide (PP). In Amphibia, A, B, and D cells were first described with conventional light- and/or electron-microscopic techniques. Immunocytochemical techniques in Anura confirmed the presence of insulin, glucagon (Lange et al. 1975), and somatostatin cells (Falkmer et al. 1978; Hacker et al. 1983) and revealed the existence of PP cells (Kaung 1979). Other immunocytochemical studies (Kaung and Elde 1980; Tomita and Pollock 1981; El-Salhy et al. 1982) also described the four pancreatic peptides. Only one of these papers (Tomita and Pollock 1981) describes the morphology of four different secretory granules by electron microscopy.

Most of the papers on the amphibian endocrine pancreas have dealt with adult animals. In contrast, the embryonic development of the endocrine pancreas has received little attention. The purpose of the present paper is to describe the embryonic development of the pancreatic endocrine cells in larvae of *Rana temporaria*, a species whose pancreas has already been studied in our laboratory in adult frogs (Díaz de Rada et al. 1986). As shall be made clear, a very detailed morphological study has been performed in the tadpoles through the observation of serial semithin and thin sections of the developing pancreas.

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Materials and methods

Forty specimens of *Rana temporaria* in various larval (posthatching) stages were used. Material was collected in February and March and reared to the desired stages in the laboratory. Tadpoles were maintained at 18° C in continuously renewed rain water. They were fed ad libitum with boiled spinach and commercial fish food.

There exists great variability in the terminology to describe larval development in Anura (Shumway 1940; Taylor and Kollros 1946; Witschi 1956; Gosner 1960; Etkin 1964; Houillon 1973; Dodd and Dodd 1976). In this study the animals were staged according to Houillon (1973), who defines four phases in *R. temporaria* development: I (embryos and early posthatching, nonfeeding tadpoles with external gills), II (free-swimming tadpoles with internal gills showing slow development of hindlegs), III (tadpoles with hindlegs), and IV (tadpoles with forelegs). The resorption of the tail leads then to a newly metamorphosed frog. In laboratory conditions, this process takes 2–3 months to occur.

Small pieces of dissected pancreas from phase-II, phase-III, and phase-IV tadpoles were used. Forty specimens (15, 15, and 10 of each phase, respectively) were studied. Different fixation procedures were carried out in order to apply diverse techniques. Part