

Distribution of serotonin-immunoreactive cells in the mouse pancreas during development

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Summary. The distribution and time of appearance of 5HT-storing cells were studied in samples from the pancreas of mice embryos from 7 to 19 days of gestation. Additionally, 1- and 15-day-old newborns and adult specimens were also examined.

Serotonin-immunoreactive cells appeared for the first time in the dorsal pancreatic primordium at 10 days of gestation and increased rapidly in number through E15. From this age, the cellular number diminished in the exocrine pancreatic parenchyma, although an increase of endocrine cells in Wirsung's duct can be detected. At day 15 of extrauterine life, we could only observe some cells in the surface epithelium of pancreatic duct of Wirsung. Islet immunoreactive cells could only be detected in adult animals.

Key words: 5-HT-storing cells, Endocrine cells, Pancreas, Ontogeny, Immunocytochemistry

Introduction

The physiological role of serotonin in pancreas is not well established (Gylfe, 1978; Koevary, 1983b). Although some histofluorescent (Cegrell, 1968), immunocytochemical (Koevary, 1983a) and silver staining (Grimelius and Wilander, 1980) studies have detected the presence of 5-HT cells in the mammalian pancreas, little is known about their ontogeny in this organ. Our study was performed in order to establish the appearance sequence of the serotonergic structures in the mouse pancreas using a sensitive immunocytochemical technique.

Materials and methods

Female albino mice were mated overnight with males in single cages. If vaginal plug was found on the following

morning, the female was considered at day 0.5 of pregnancy, since mating occurred at midnight. In our colony, birth usually occurred at day 19 of pregnancy. Fetuses from 7 to 19 days of gestation were used and, additionally, 1- and 15-day-old newborns and adult specimens were also examined.

Two hours before the sacrifice, the females were treated with an intraperitoneal injection of L-tryptophane (100 mg/kg) and 60 minutes before the sacrifice with a similar dose of pargiline. The fetuses were removed from ether-anesthetized pregnant mice. In our study, four females of each time of gestation were sacrificed and we removed four fetuses from each female, one for conventional histological study and three for immunocytochemical analysis.

In young fetuses (7 to 12 days of gestation), the whole fetal body was immersed six hours in Bouin's fixative with acetic acid at 4° C. In older animals, the pancreas was removed with the duodenum, immersed in the same fixative and dissected into small pieces. After dehydration and embedding in paraffin, the sections were cut in 6-8 µm thickness. The sections were dehydrated and immunostained with 5-HT antiserum. Immunocytochemistry and immunocytochemical staining for 5-HT was performed with the unlabelled antibody enzyme method using PAP complex as described by Sternberger et al. (1970). Prior to incubation with the antiserum, the sections were exposed to 3% H₂O₂ in methanol for 30 min. in order to eliminate the endogenous peroxidase activity. The sections were treated with normal swine serum diluted 1:20 in 0.05 M TRIS-HCl buffer, pH 7.6 for 30 min. Then, sections were incubated in a moist chamber at 4° C for 24 h. with the primary antiserum, raised in rabbit (MN 55082, Immunonuclear Corp.) diluted 1:1000 in 0.05 M TRIS-HCl buffer, pH 7.6. After rinsing in the same buffer, sections were incubated sequentially with 1:50 diluted antiserum against rabbit IgG raised in swine (Dako, Z-196) for one hour at room temperature and pancreatic duct at its juxtaduodenal zone (Fig. 5d).

establish the specificity of immunostaining, adjacent sections were incubated with control sera and processed in parallel with experimental sections. The control sera consisted of the diluted 5-HT antiserum pretreated overnight with an excess of 5-HT (10 µg/ml of diluted antiserum).

The peroxidase activity was demonstrated by exposure of the sections to a fresh solution of 3,3'-diaminobenzidine tetrahydrochloride (Sigma) (60 mgr/100 ml) and hydrogen peroxide (0.1%) in 0.05 M TRIS-HCl buffer, pH 7.6 under microscopic control.

The sections were counterstained with Mayer's haematoxiline, dehydrated, cleared in xylene and mounted with DePeX (Serva).

Results

By the immunocytochemical staining for 5-HT with Sternberger's unlabelled antibody enzyme method, we observed the presence of 5-HT storing cells in the pancreatic tissue of mice embryos from 10 day of gestation. The relative frequency of the immunoreactive cells in the various pancreatic zones was subjectively graded into different categories (Fig. 1).

The first immunostained cells appeared at 10 days of incubation. They were sparsely distributed in the dorsal pancreatic primordium (Fig. 2a). No cells immunoreactive

to the antiserum were detected in the gastrointestinal tract at this stage. In the following days, stages 11, 12, 13 and 14, subjective assessment of the immunoreactive cells showed that, as a rule, the cells increased fairly steadily in number, and we observed immunoreactive cells with different morphological features: oval, round and triangular cells (Figs. 2b, 2c, 2d, 2e, 2f).

The first endocrine cells in the ventral pancreatic primordium appeared at 14 days of incubation, showing similar morphological features to those observed in the dorsal pancreas (Fig. 3a). Furthermore, at this stage we observed for the first time the appearance of immunoreactive cells in the surface epithelium of the Wirsung's duct (Figs. 3b, 3c). Also E14 was the earliest stage at which immunoreactive cells were found in the gastrointestinal tract; precisely in antrum and duodenum. At day 15 of gestation, we observed a drastic decrease in the serotonergic cellular population in the pancreatic parenchyma (Figs. 3d, 4a). On the contrary, the immunoreactivity in the main pancreatic duct was sharply increased, specially in the juxtaduodenal portion (Figs. 4b, 4c).

In embryos at day 16 of gestation, we observed the highest number of immunoreactive cells in the Wirsung duct (Fig. 4d).

From this stage until birth a progressive decrease of immunoreactive cells both in the pancreatic parenchyma

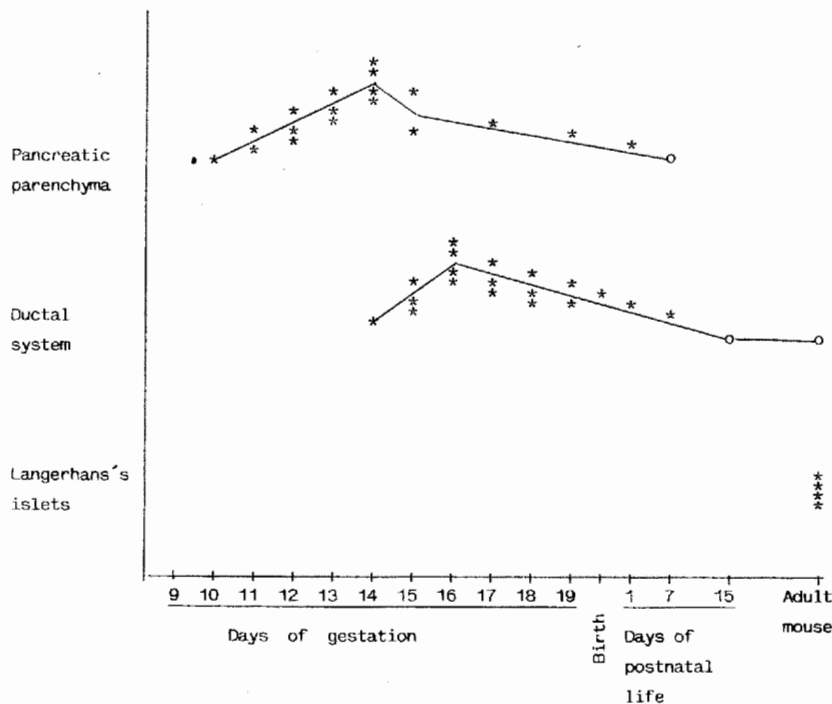


Fig. 1. Diagram to demonstrate the distribution and frequency of cells showing immunoreactivity for 5-HT in the pancreas of mice embryos. O: Occasional cells.

* Few cells but present in every specimen (all studied animals)

* Moderate number of immunoreactive cells

* High number of immunoreactive cells

* Highest number of detected cells

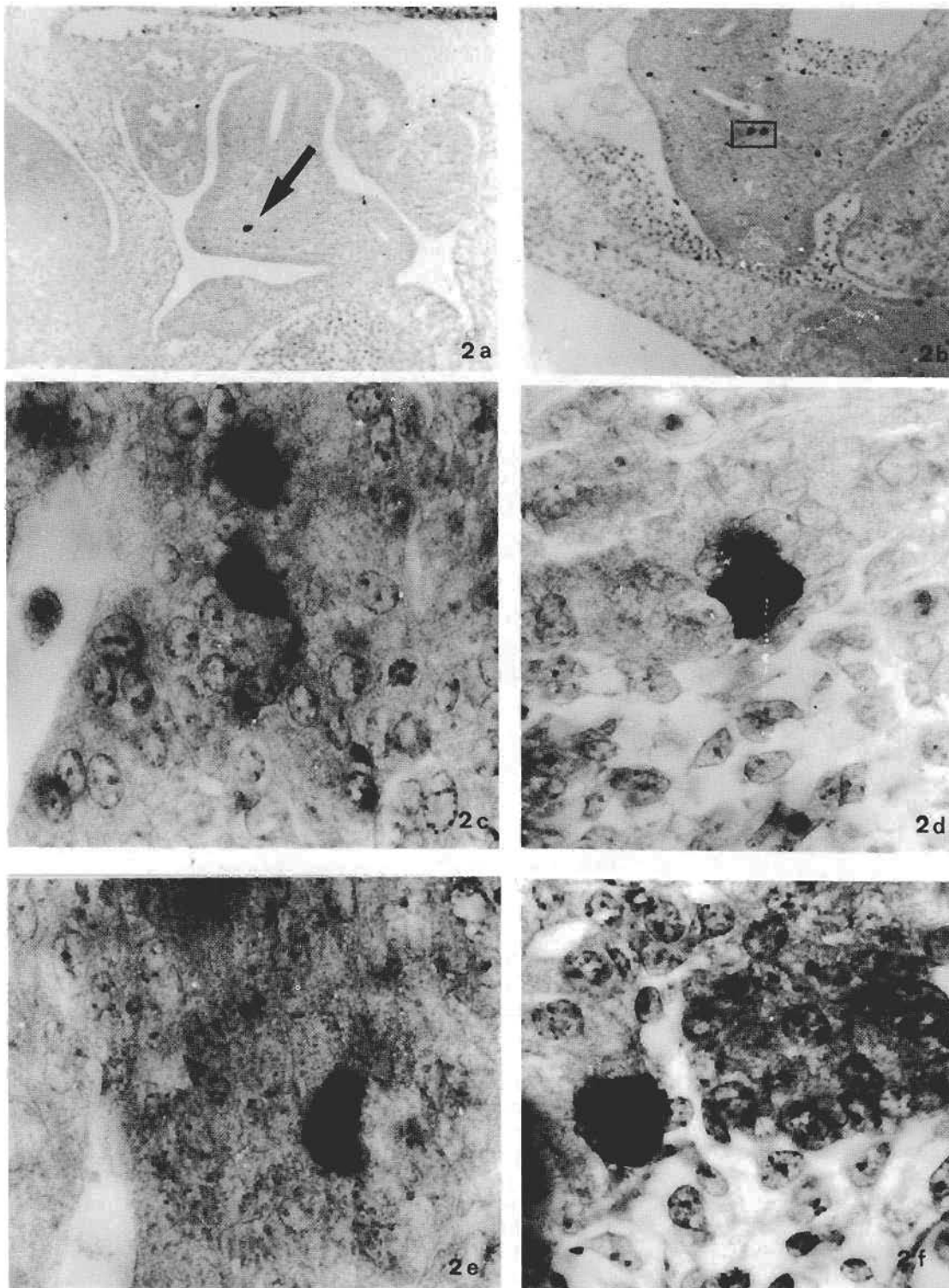


Fig. 2. Pancreatic tissue showing the early appearance of immunoreactive 5-HT cells.

- a)** The dorsal pancreatic primordium at 10 days of gestation. An immunoreactive cell can be observed (arrow). $\times 125$.
- b)** Serotonergic immunoreactivity in the pancreas of an embryo at 11 days of gestation. $\times 125$.
- c)** High magnification of the portion enclosed by a rectangle in Fig. 2b. $\times 1,250$.
- d)** Triangular immunoreactive cell in the dorsal pancreas of an embryo at 12 days of gestation. $\times 1,250$.
- e)** Oval serotonergic cell in the dorsal pancreas of an embryo at 12 days of gestation. $\times 1,250$.
- f)** Round immunoreactive cell in the dorsal pancreas of an embryo at 13 days of gestation. $\times 1,250$.

and the pancreatic duct could be detected and the immunoreactivity was concentrated fundamentally in the juxtaduodenal portion of the Wirsung's duct (Fig. 5a). In newborns the serotonergic immunoreactivity was very scarce (Fig. 5b) and in 7-day-old mice 5-HT somas were only rarely found in the pancreas. Occasionally

some cells were detected in the ductal system (Fig. 5c). In animals at day 15 of extrauterine life, the pancreatic serotonergic immunoreactivity was practically absent. We could only observe some opened cells, with a bottle or triangular shape in the surface epithelium of the pancreatic duct at its juxtaduodenal zone (Fig. 5d).

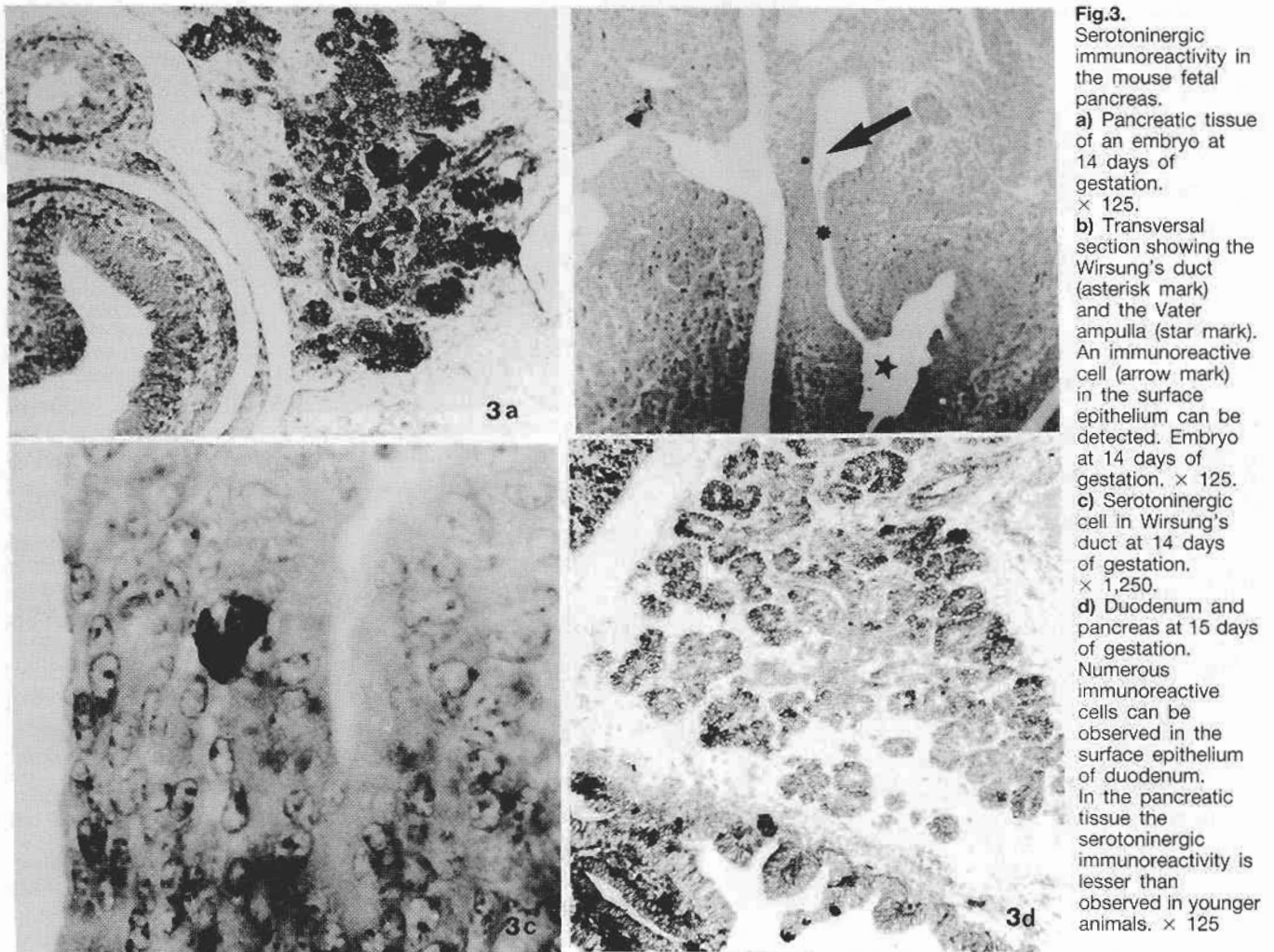


Fig.3. Serotonergic immunoreactivity in the mouse fetal pancreas.
a) Pancreatic tissue of an embryo at 14 days of gestation. $\times 125$.
b) Transversal section showing the Wirsung's duct (asterisk mark) and the Vater ampulla (star mark). An immunoreactive cell (arrow mark) in the surface epithelium can be detected. Embryo at 14 days of gestation. $\times 125$.
c) Serotonergic cell in Wirsung's duct at 14 days of gestation. $\times 1,250$.
d) Duodenum and pancreas at 15 days of gestation. Numerous immunoreactive cells can be observed in the surface epithelium of duodenum. In the pancreatic tissue the serotonergic immunoreactivity is lesser than observed in younger animals. $\times 125$

Islet serotonergic cells could only be detected in adult animals. In these specimens the cellular population in the Langerhans islets was very numerous (Figs. 5e, 5f).

In addition some positive cells, very scarcely distributed, were found in the ductal system, fundamentally in the juxtaduodenal portion of the some nerve cell bodies were observed in close contact to pancreatic vessels in the adult pancreas main pancreatic duct.

Discussion

Since Cegrell et al. (1968) undertook a survey of biogenic monoamines in the mammalian pancreas, further information has accumulated on 5-HT-storing cells and serotonergic fibres in adult animals (Kyosola, 1978; Koevary, 1983a), although Inokuchi et al. (1982) and Watanabe et al. (1984) did not observe by immunocytochemistry any positive serotonergic structure in the rat and chicken pancreas.

Less attention has, however, been paid to the embryonic mammalian pancreas. Before the establishment that 5-HT was the substance synthesized by the EC-

cells, various studies were performed in order to see the distribution and time of appearance of these cells in the digestive tract. Simard and Campenhout (1923) observed EC cells for the first time at day 13 of incubation in avian embryos, and Monesi (1960) observed them from day 14 of incubation. More recently, the results are also contradictory. Andrew (1976) observed EC cells at day 16 in avian embryos. Walter (1979) with the Mason-Fontana method did not observe any EC cells in the avian proventriculus from day 8 of incubation until 4 days posthatching and Renda et al. (1980), by the Gibb's diazo-reaction, described them from day 13 of incubation in the chicken duodenum.

In human fetuses, Falck and Owman (1965) by FIF, reported some fluorescent cells in the duodenum at 13-14 weeks of gestation and Moxley and Trier (1977) at 10-12 weeks. However, the ontogeny of serotonergic structures in the mammalian pancreas is practically unknown. Our study shows several new findings in the chronological development of pancreatic 5-HT cells. The population of pancreatic serotonergic cells appears to arise early in the development.

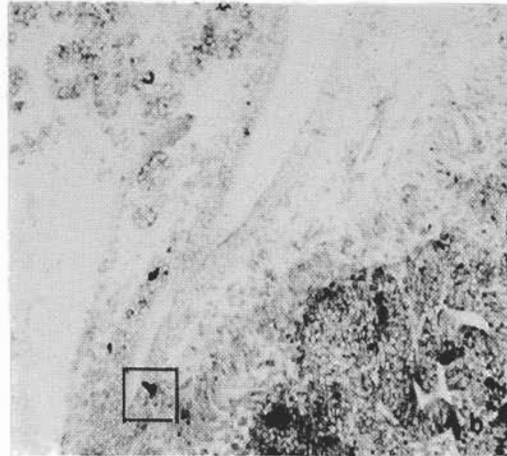
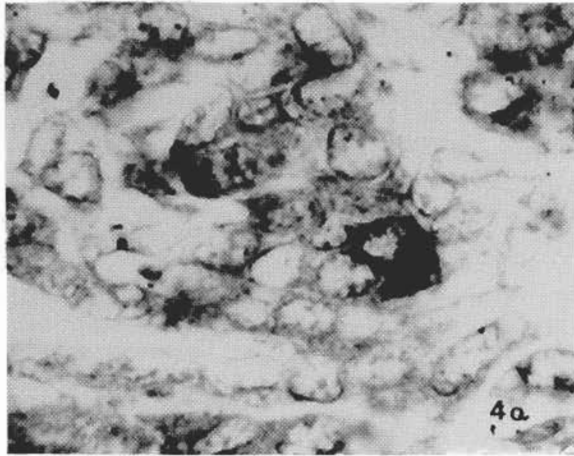


Fig. 4. Pancreatic serotonergic immunoreactivity at different days of gestation.

a) Immunoreactive cell in the pancreatic parenchyma at 15 days of gestation. $\times 1,250$.

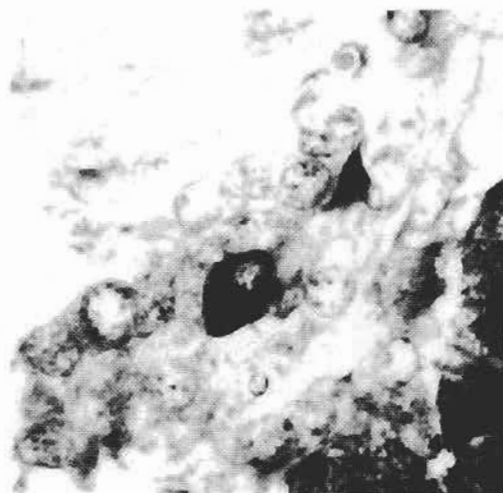
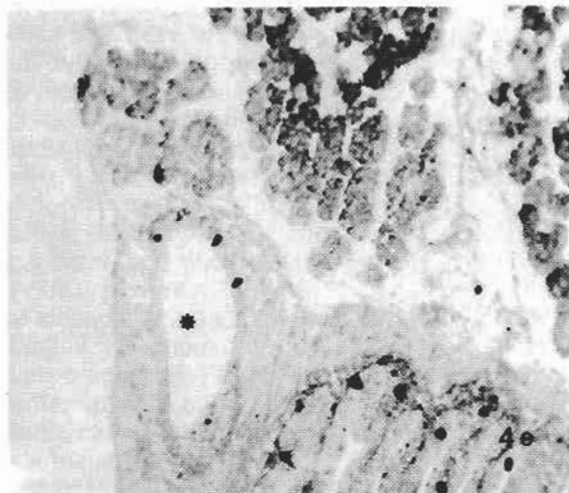
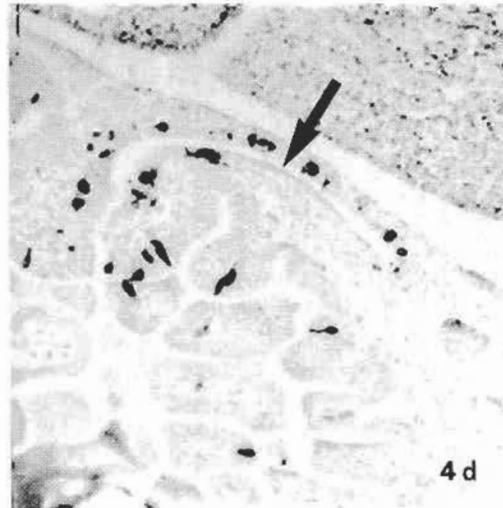
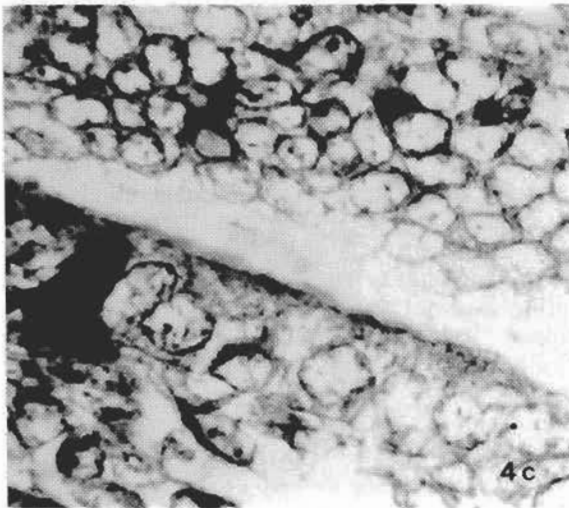
b) Serotonergic immunoreactivity in Wirsung's duct at 15 days of gestation. $\times 125$.

c) High magnification of the enclosed portion in Fig. 4b. $\times 1,250$.

d) Vater's ampulla and longitudinal section of the Wirsung's duct (arrow) at 16 days of gestation. Numerous immunopositive cells in the Wirsung's duct and duodenum can be detected. $\times 125$.

e) The Wirsung's (asterisk mark) at 17 days of gestation. Some immunoreactive cells in the duct's epithelium can be observed. $\times 125$.

f) Oval serotonergic cell, with an excentric nucleus, in the pancreatic parenchyma at 17 days of gestation. $\times 1,250$.



We have no notice about the presence of pancreatic endocrine cells in stages as early as day 10 of gestation. Fujii (1977) describes the first endocrine elements in rat pancreas at day 11 of gestation (glucagon-like immunoreactivity), Yoshinari and Daikoku (1982) in the same species observe them from day 12 of gestation,

Sundler et al. (1983) reported the presence of insulin, glucagon, somatostatin and PP-like immunoreactivities in the porcine dorsal pancreatic primordium from week 4 of gestation and in human fetuses such an event has been described at week 9 of gestation. However, the only reference to 5-HT development in pancreas is reported by

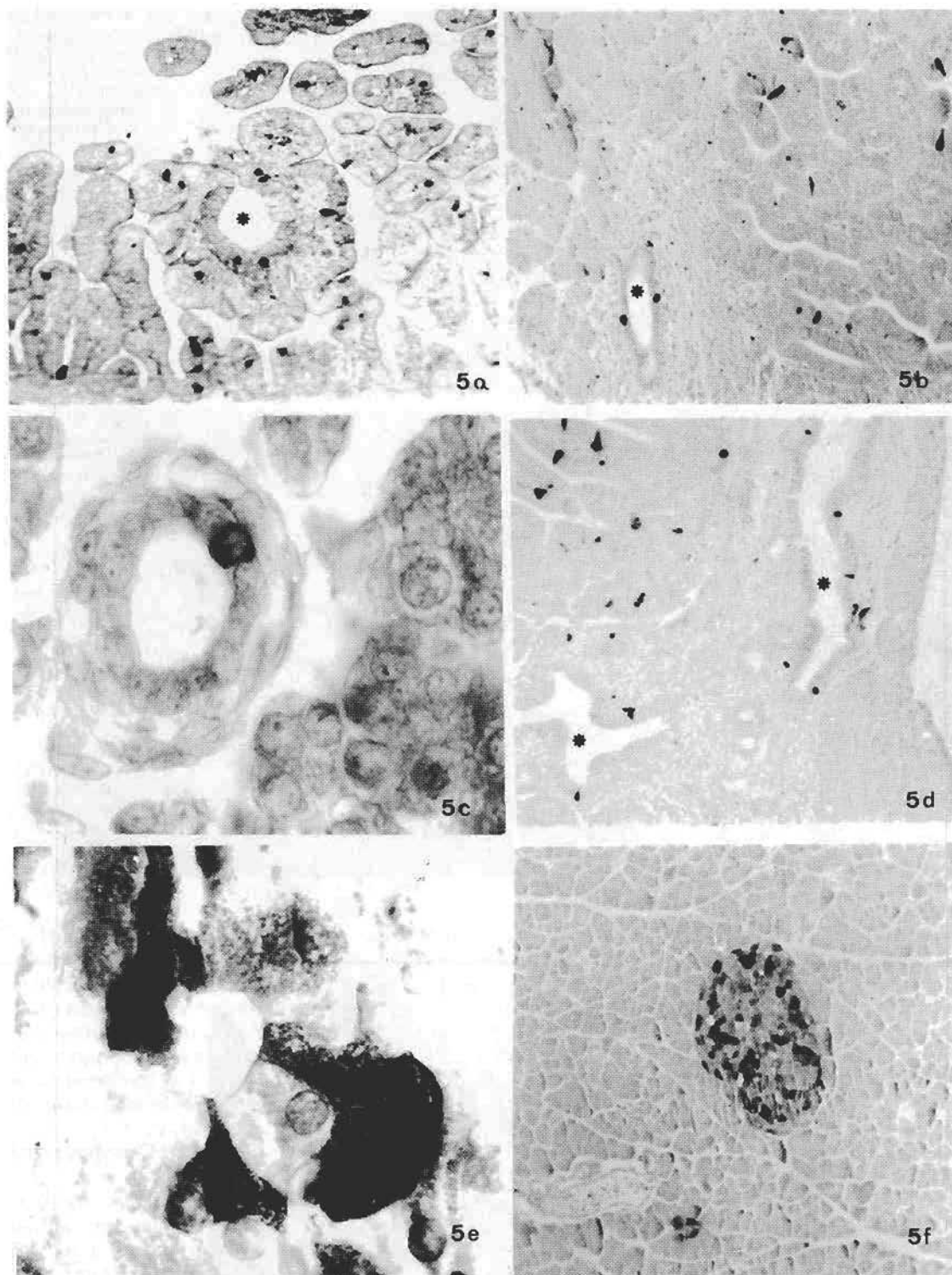


Fig. 5. Serotonergic immunoreactivity in the latest days of gestation and after birth.

a) Vater's ampulla (asterisk mark) at 18 days of gestation. Some immunoreactive cells can be detected. The immunoreactive cells in the duodenum are very numerous. $\times 125$.

b) Two immunoreactive cells in the Wirsung's duct of an animal at two days of postnatal life. $\times 125$.

c) Transverse section of a secondary pancreatic duct in a mouse at 7 days of postnatal life. $\times 1,250$.

d) Some immunopositive cells in Wirsung's duct and Vater's ampulla (asterisk). Mouse at 15 days of postnatal life. $\times 125$.

e) Immunoreactive cells in a pancreatic islet of an adult mouse. $\times 1,250$.

f) Serotonergic immunoreactivity in a Langerhans's islet of an adult mouse. $\times 125$

Koevary et al. (1983b). These authors, using autoradiography on rat fetuses from day 18 of gestation, describe the presence of cells and fibres that can take up the radioactive-labelled indolamine. We can distinguish two periods in the developmental pattern of those immunoreactive cells. A first period, from day 10 to 14 of

gestation, at which a progressive increase in number is detected and a second period from day 15 of gestation, at which the immunoreactive population decreased progressively.

Regarding the appearance of 5-HT cells on day 10 of gestation, we assume that these cells are the first

pancreatic endocrine elements, appearing even before other classical endocrine cells. Furthermore, a notable feature relating to the ontogeny of 5-HT cells is that those in the gut appear later in development (from day 14 of gestation) whereas the pancreatic cells differentiate much earlier. Similar to other endocrine cells, the 5-HT storing cells are also firstly located in the dorsal pancreatic primordium.

Koevary et al. (1983b) detect in rat fetuses some nerve fibres that can take up the radioactive-labelled 5-HT. We have been unable to detect any immunoreactivity in nervous structures during the development. The discrepancy may reflect different specificity of the antisera used.

The exact significance of the 5-HT cells during development remains unknown. The exogenous administration of 5-HT provokes an inhibition of insulin release (Koevary, 1983a). Perhaps, an insulin-serotonin interaction could also occur in fetal stages and not only exogenous administration. Perhaps, the appearance of somatostatin cells at day 12 of gestation could influence the neighbouring 5-HT cells.

The decrease of the serotonergic population from day 14 of gestation could be perhaps related to the presence of other modulator systems. Somatostatin-like immunoreactivity can be observed from day 14 of gestation in rat fetuses. Somatostatin inhibits the release of insulin and glucagon and the development of the somatostatinergic system could replace the serotonergic system in regulating the endocrine pancreatic function during foetal life.

Concerning the 5-HT cells in the main pancreatic duct, there is no reference in the literature about their presence in mouse fetuses. Kyosola (1978) by FIF, described 5-HT cells with similar morphological features in the surface epithelium of the Wirsung duct in adult cats. In the cat, the indolamine evokes a bifasic response on the Oddi sphincter, but the significance of these cells in the fetuses is still not clear.

Serotonergic immunoreactivity in the Langerhans islets are only observed in adult animals and this finding also confirms those previously reported by other authors, using FIF. Cegrell (1968) reported the presence of 5-HT and dopamine in the A cells of the cat and guinea pig islets, and these results were confirmed by Kyosola (1978). However Inokuchi et al. (1982) in the rat and Watanabe et al. (1984) in the chicken, by FIF, were unable to demonstrate immunoreactive material in the pancreatic islets. This discrepancy probably reflects the different sensibility of the techniques. We observed some nerve cell bodies close to pancreatic vessels in the adult pancreas. This finding agrees with the previous report from Kyosola (1978), although we do not observe any immunoreactive nerve fibre.

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