Histological and histochemical characteristics of the bovine notochord

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ABSTRACT In 20 bovine embryos and fetuses 6-65 mm long (crown-rump length) and 23 to 60-70 days old, the structure and localization of acid and neutral mucopolysaccharides and glycogen in their notochord were investigated. Also, the localization in the notochord was examined of the activity of alkaline and acid phosphatases, α-glycerophosphate-, glucose-6-phosphate-, isocitrate-, glutamate-, lactate- and succinic- dehydrogenases, and nicotinamide-adenine-dinucleotide- and nicotinamide-adenine-dinucleotide-phosphate- diaphorases. It was found that the bovine notochord begins decomposing at the end of embryonal and the beginning of fetal development (45-50 days old) and that in the fetus aged 55-65 days it no longer represents an unbroken cord of notochordal cells. Secretory activity of notochordal cells which produce the notochordal sheath starts very early (in 10 mm-long embryos), and interruptedly increases up to the end of the embryonal developmental period when regression appears at the beginning of the fetal period. These findings agree with findings in the human embryo where, however, they relate to earlier developmental periods.

KEY WORDS: *notochord, histology, histochemistry, bovine embryos*

Introduction

The notochord is a rod-shaped formation of cells which extends along the whole length of the embryo, representing the initial axial skeleton around which, at later stages, both ontogenetically and phylogenetically, the body of vertebrae and intervertebral discs are formed. The notochord plays an important role in the induction of the neural plate, chondrogenesis (Kosher and Lash, 1975), somite formation (Burges, 1983) and the differentiation of neuroblasts (Van Straaten *et al.*, 1985) but may also have a role in the appearance of certain tumors in humans (Foschini *et al.*, 1990), and inherited disease conditions in animals (Woodard and Montgomery, 1978).

There are many papers on the structural, histochemical and ultrastructural organization of the notochord in different species, including the lamprey (Manfredi-Romanini, 1956), frog (Leeson and Threadgold, 1960; Adams *et al.*, 1990), chick (Moog, 1944; Leeson *et al.*, 1961; Ruggeri, 1972), mouse and rat (Mulnard, 1955; Jurand, 1974), rabbit (Leeson and Leeson, 1958), pig (Williams, 1908), and man (Peacock, 1951; McKay *et al.*, 1955; Rossi and Reale, 1957; Trout *et al.*, 1982; Heaton and Turner, 1985; Murakami *et al.*, 1985; Galic *et al.*, 1986; Johnson *et al.*, 1986; Shinohara and Tanaka, 1988). However, to our knowledge only one short study (Gomercic and Gomercic, 1974) on the bovine embryo has been carried out.

The purpose of this work was to examine the structure of the notochord in cattle and to determine the localization in it of certain chemical substances, enzymes in particular, in order to compare the results with those obtained in other species and gain better insight into one of the basic structures of vertebrates from the comparative point of view.

Results

In the youngest examined embryo (6 mm) the notochord consisted of a thin band of spherically arranged cells, so that the transverse section showed a circle of 10-12 cells (Figs. 1 and 2). In this embryo the notochord was spread uninterruptedly along the whole length of the body. The cells were roundish to cubical, with large round to oval nuclei containing a small amount of cytoplasm. At the periphery of the notochord a very thin notochordal sheath with acid mucopolysaccharides could be observed (Fig. 2). In the notochordal cells of that

Abbreviations used in this paper: CR, crown-rump; H&E, hematoxylin and eosin; PAS, periodic acid-Schiff; α-GP-Dh, α-glycerophosphate-dehydrogenase; L-Dh, lactate-dehydrogenase; Gl-Dh, glutamate-dehydrogenase; NADP-Dph, nicotinamide-adenic-dinucleotide-phosphate-diaphorase.

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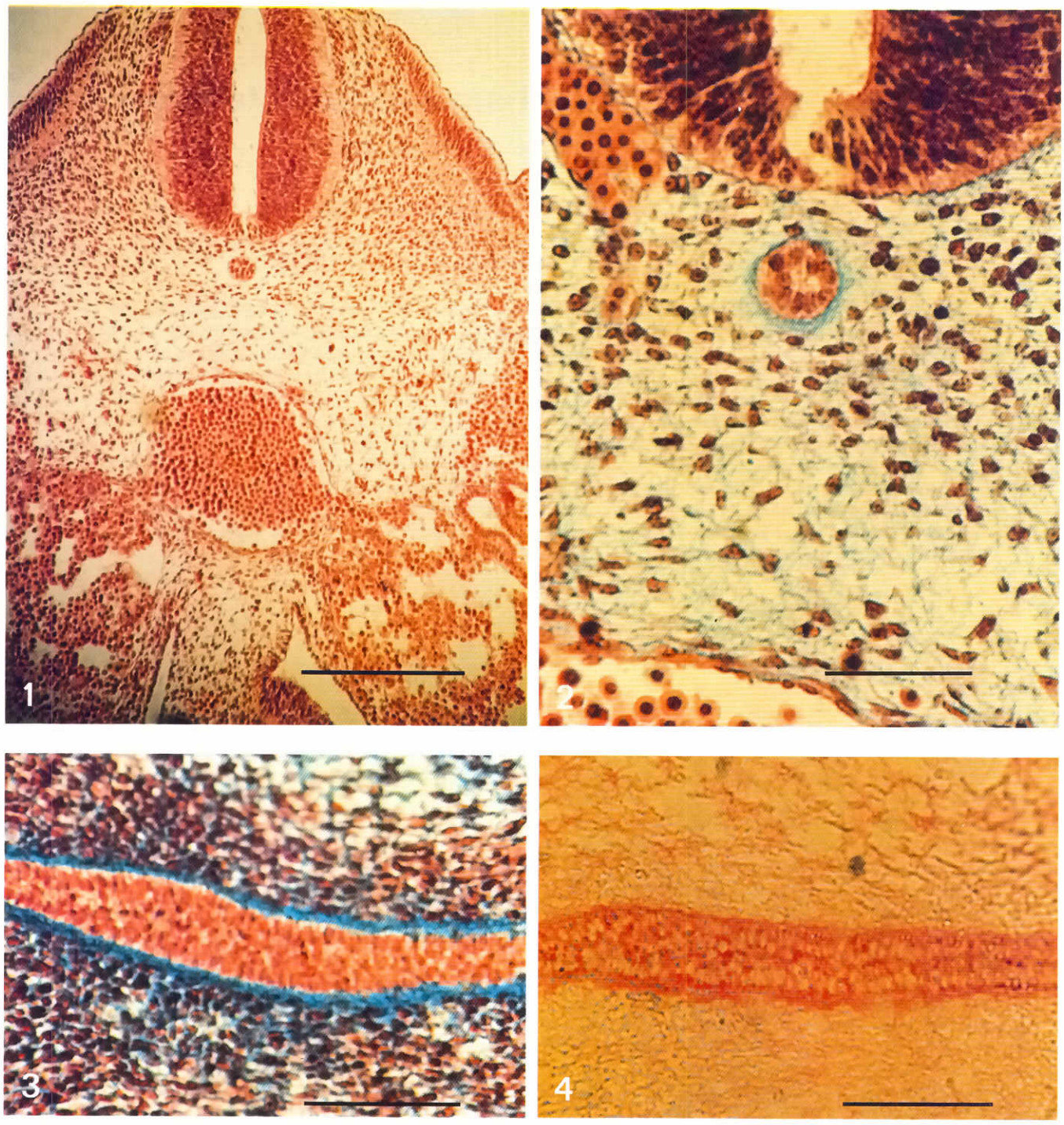
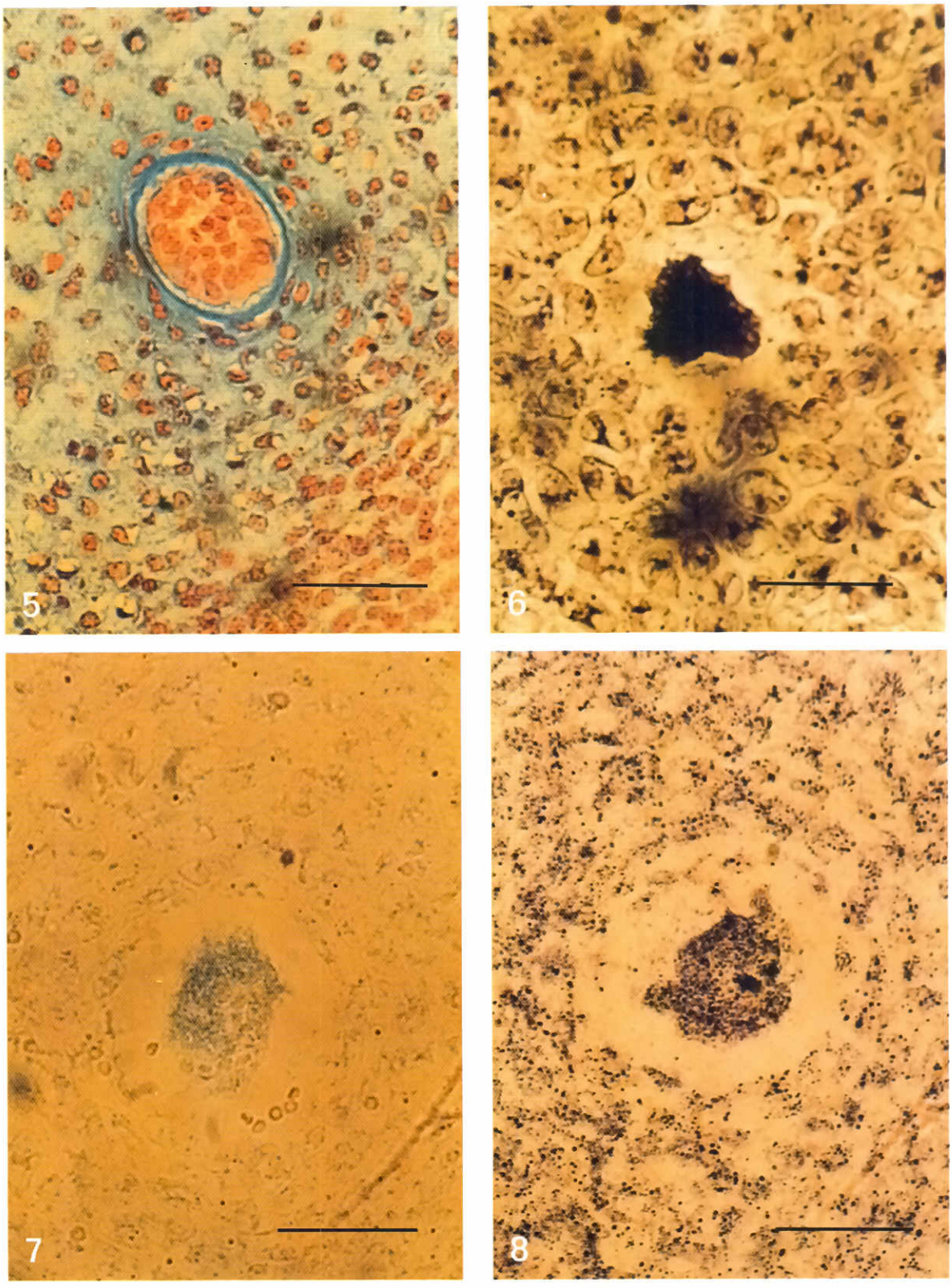


Figs. 1-4. Bovine embryo. (1) *6 mm CR length, transverse section. H & E. Scale bar 200 µm.* **(2)** *6 mm CR length, transverse section. Alcian blue and H & E. Scale bar 50 µm.* **(3)** *9 mm CR length, median section. Alcian blue and H & E. Scale bar 50 µm.* **(4)** *9 mm CR length, median section. PAS-reaction. Scale bar 50 µm.*

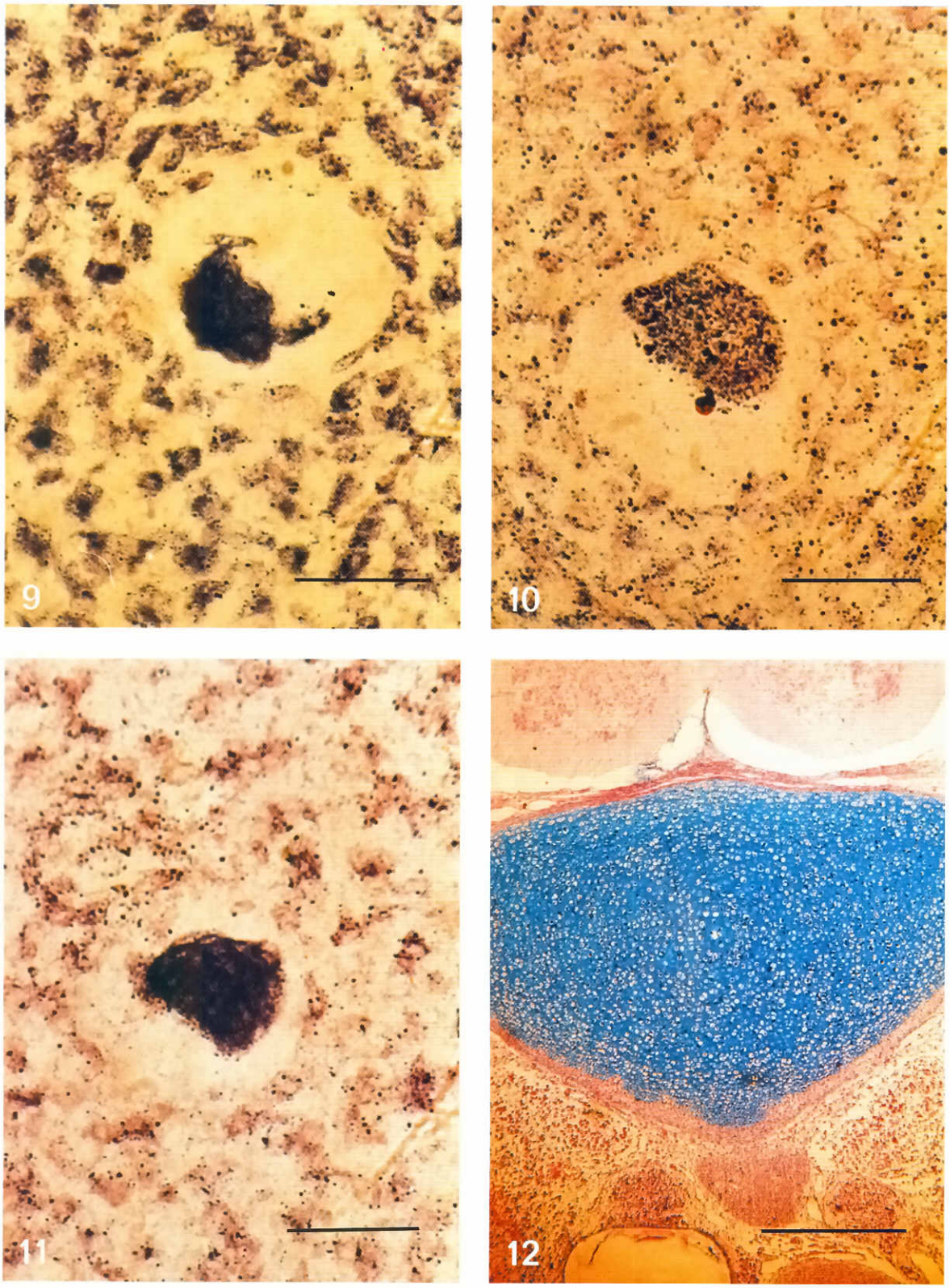
developmental stage there were no glycogen nor neutral mucopolysaccharides. Nor were they present in the notochordal sheath (Figs. 1 and 2).

In older embryos (9 mm), the number of cells in the notochord section is somewhat increased, as is the amount of cytoplasm in the cells which have a small amount of glycogen (Fig. 4). The notochordal sheath is a little thicker, containing a larger amount of acid mucopolysaccharides and a very small amount of neutral mucopolysaccharides (Figs. 3, 4).

In even older embryos (14 and 19 mm) the number of cells



Figs. 5-8. Bovine embryo. (5) *14 mm CR length, transverse section. Alcian blue and H & E. Scale bar 50 μm.* **(6)** *29 mm CR length, transverse section. Sudan black B. Scale bar 50 μm.* **(7)** *29 mm CR length, transverse section. Non-specific esterases. Scale bar 50 μm.* **(8)** *35 mm CR length, transverse section. α-Gp-Dh. Scale bar 50 μm.*



Figs. 9-12. Bovine embryo. (9) *35 mm CR length, transverse section. L-Dh. Scale bar 50 µm.* **(10)** *35 mm CR length, transverse section. Gl-Dh. Scale bar 50 µm.* **(11)** *35 mm CR length, transverse section. NADP-Dph. Scale bar 50 µm.* **(12)** *65 mm CR length, transverse section. Alcian blue and H & E. Scale bar 500 µm.*

in the notochord continues to increase, the notochordal sheath becomes even thicker, containing a still larger amount of acid and neutral mucopolysaccharides (Fig. 5).

In embryos about 30 mm long, the notochordal cells start diminishing. By this time, the notochordal sheath has considerably thickened, containing a large amount of acid and a smaller amount of neutral mucopolysaccharides. At this developmental stage the amount of free lipids in the cells is very high, whereas in the notochordal sheath it is not present at all (Fig. 6).

In the oldest examined fetuses (55-65 mm long) the notochord is no longer an uninterrupted cord of cells, due to segmentation, and in some places both the notochord and its sheath have totally disappeared. A small canal without cells in the middle of the vertebral body partially remained (Fig. 12). In some segments, small agglomerations of cells are still visible, containing negligible amounts of glycogen and notochordal sheath with a large amount of acid mucopolysaccharides.

Activity of the enzymes examined (α-glycerophosphate-, glucose-6-phosphate-, isocitrate-, glutamate-, lactate- and succinic- dehydrogenases, and nicotinamide-adenine-dinucleotide- and nicotinamide-adenine-dinucleotide-phosphate diaphorases) in the notochordal cells is two-fold: markedly granular and mildly diffuse, while in the notochordal sheath it does not exist at all (Figs. 8-11). The most intense activity of these enzymes comes at the stages of development when embryos reach a length of 14 to 35 mm. A more pronounced enzymatic activity (both granular and diffuse) is observed in α-glycerophosphate-, glucose-6-phosphate-, glutamate-, lactate- dehydrogenases and nicotinamide-adenine-dinucleotide- and nicotinamide-adenine-dinucleotide-phosphate- diaphorases, while the activities of isocitrate- and succinic- dehydrogenases proved considerably weaker. Activity of non-specific esterases is observed in the notochordal cells, especially in the period relating to an embryonic length of 14-35 mm (Fig. 7), while the activities of alkaline and acid phosphatases exist neither in the notochordal cells nor in the notochordal sheath.

Discussion

Since in the literature we could not find reports on the localization of the activity of oxydative enzymes (dehydrogenases and diaphorases) or other substances in the bovine notochord, we can compare our results only indirectly with similar findings in other species. Our observations have shown that in cattle the existence of the whole notochord coincides with the embryonal developmental stage, while at the end of this stage and the beginning of the fetal one (45-50 days) (Evans and Sack, 1973) the decomposition, or fragmentation, of the notochord occurs. A markedly granular activity of the enzymes examined in the bovine notochordal cells is most intense in the embryos between 14 and 35 mm long. As these enzymes are markers of mitochondria, it is justifiable to maintain that in this developmental period the bovine notochord has its most intense metabolic activity, *i.e.* the most intense secretory activity. According to our findings, the secretory activity of notochordal cells starts very early (in embryos about 10 mm long) and continues increasing uninterruptedly until the end of the embryonal developmental period. This can be concluded on the basis of the amount of acid mucopolysaccharides, which with age increases in this period, and also on the basis of the appearance of notochordal cells, which ever more visibly assume the appearance of metabolically active cells. In this period, at least on the basis of the activity of oxidative enzymes, the number of mitochondria could be considered to increase in the notochordal cells, as well as the activity of mitochondrial enzymes (dehydrogenases and diaphorases) and non-specific esterases. Our findings concerning the secretory activity of bovine notochordal cells are, in principle, in agreement with the results of similar investigations in other species, except as regards the human embryo, which essentially differs as far as the time of occurrence of these activities is concerned. In the 32-day human embryo, the notochordal cells are undifferentiated, with few intracellular organelles. The human notochord begins to have a secretory function in 34-35 day-old embryos (Shinohara and Tanaka, 1988). It could be said that the function and significance of the bovine notochord in the development of the individual are considerably earlier than in man, in whom they become of importance during a somewhat later ontogenetical development. In the human embryo aged 40-50 days, the notochord is at its peak of development and secretory function, whereas at that time the bovine notochord gradually enters into a functional and structural regression. Our findings about the absence of the activity of hydrolitic enzymes (alkaline and acid phosphatases) in the bovine notochord roughly correspond to those in the human notochord (McKay *et al.*, 1955; Rossi and Reale, 1957), except that we found a relatively strong activity of non-specific esterases in the bovine notochordal cells, which also suggests an increased metabolic and secretory activity of these cells.

Materials and Methods

This study was carried out in the embryos and fetuses of Croatian Simmental cattle *(Bos taurus)*. All embryos and fetuses were obtained from cows from the Zagreb slaughter-house. Immediately after slaughter and removal of uteri, the embryos and fetuses were measured (crown-rump length) and transported to the laboratory. Their age was determined according to the CR length (Evans and Sack, 1973). A total of 20 embryos and fetuses, 6-65 mm CR long and 23 to 60-70 days old, were examined.

Whole embryos and/or parts of fetuses were placed immediately into a fixative. The fixatives employed were 10% formalin, formol-calcium and -20°C. Most specimens were transversely cut, but some of them were sagittally and horizontally cut. Formalin-fixed specimens were embedded in paraffin, sectioned at 6 μm, and the following staining procedures were used: hematoxylin and eosin, Alcian blue, according to Mowry 1960; and periodic acid-Schiff reaction, according to McManus (both before and after amylase digestion) (Pearse, 1968; Romeis, 1968). Formol-calcium-fixed material was cut on a freezing microtome. The sections obtained were stained with Sudan black B, according to Lison 1934 (Romeis, 1968), and stained using the procedure for alkaline phosphatase, according to Burstone; and acid phosphatase, according to Barka and Anderson 1962 (Pearse, 1968); and the procedure for non-specific esterases, according to Gomori (Pearse, 1972). Deep freezing materials of fresh specimens cut on a freezing microtome for the demonstration of α-glycerophosphate- (α-Gp-Dh), glucose-6-phosphate-, isocitrate-, glutamate- (Gl-Dh), lactate- (L-Dh) and succinic- dehydrogenases, and nicotinamide-adenine-

dinucleotide- and nicotinamide-adenine-dinucleotide-phosphate- (NADP-Dph) diaphorases according to Hess *et al.*, 1958 and Nachlas *et al.*, 1957, 1958 (Culling, 1974).

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