SOME FEATURES OF THE HISTOLOGIC STRUCTURE OF THE BOVINE FETAL PANCREAS

L. A. Kirsanova and V. N. Blyumkin

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The pancreas of ruminants is known to be a rich source of insulin [5]. In recent years interest in the bovine pancreas has been shown because of the possibility of using this material as a source for cultures of islet cells for experimental and clinical transplantation [1, 2, 6, 10]. It has been shown that to obtain such cultures it is best to use the fetal pancreas, because the relative number of islet cells and, in particular, of beta-cells in it is much greater than in the adult pancreas, and also because of the high ability of its epithelium to proliferate and differentiate [2, 6-8]. The histologic features of the bovine fetal pancreas at different periods of intrauterine development are inadequately reflected in the literature [7, 9, 12]. However, concrete ideas on the original histologic structure of the fetal pancreas are an essential prelude to the scientific programming of obtaining cultures of a particular type. The present investigation is devoted to some particular features of the histologic structure of the bovine fetal pancreas, which largely determine the character of cultures obtained from it.

EXPERIMENTAL METHOD

Altogether 110 pancreases from bovine fetuses at different periods of intrauterine development were subjected to histologic analysis. The fetuses varied in crown-rump length from 10 to 80 cm. The material was fixed in Bouin's mixture and embedded in paraffin wax. Sections 5-7 μ thick were stained with hematoxylin and eosin, with aldehyde-fuchsine by Gomori's method, and also by Mallory's method.

EXPERIMENTAL RESULTS

The study of material obtained from fetuses with a crown-rump length of 10-12 cm showed that at this stage unique differentiation of the epithelium of the primitive efferent ducts takes place in the bovine pancreas, and takes the form of the formation of growth buds, which are distal and lateral flask-shaped evaginations of the primitive ducts, formed in most cases by a single layer of epithelial cells; growth buds with a stratified structure also are found. Four types of epithelial cells can be distinguished in the composition of the growth buds: I) prismatic with basal-apical differentiation, not containing granules in the cytoplasm; II) without any marked differentiation into basal and apical parts, likewise not containing granules in the cytoplasm; III) prismatic or conical with a basal nucleus and with large eosinophilic zymogen granules, occupying the apical part of the cytoplasm (acinar cells); IV) with a central or eccentric position of the nucleus and with a few tiny aldehyde-fuchsine-positive granules in the cytoplasm (beta-cells). Growth buds containing different types of epithelial cells described above are thus "mosaic" in character. Terminal exocrine divisions (acini) develop from growth buds containing cells of types I and III, whereas so-called acino-insular complexes, consisting of typical acinar cells and groups of endocrine cells of different shapes and sizes adjacent to them, developed from growth buds containing all four types of epithelial cells. The topographic and quantitative relations between the endocrine and exocrine components of the acino-insular complexes may differ. Conventionally we distinguish varieties of acino-insular complexes of the bovine fetal pancreas in which: single endocrine cells are found in the form of individual "eruptions" in the epithelial lining of the acini (I); islet cells occupy a sector (segment) in the acinus (II); a group of endocrine cells is located outside the acinus, in close contact with its basement membrane; in this case the dimensions of the

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group of endocrine cells may be smaller than those of the acinus, equal to them, or they may be larger than the acinus (III), and also acino-insular complexes at the stage of differentiation of exocrine and endocrine parts (IV).

The number of acino-insular complexes in the bovine fetal pancreas increases with an increase in the period of intrauterine development, to reach, according to our findings, a maximum in fetuses with crown-rump length of 27-35 cm. The number of acino-insular complexes of type III increases in particular. Meanwhile the fraction of acino-insular complexes of types I and II gradually decreases. Growth of new acino-insular complexes takes place through mitotic division of the cells composing them.

During development of the fetus, in the course of morphogenesis of the acino-insular complexes, the endocrine parts of the complexes undergo a pinching off process, as a result of which they are converted into small pancreatic islets. According to our observations, this process is combined with growth of the capillaries into the newly formed microislets.

In fetuses 40-60 cm long the number of these separate pancreatic microislets increases sharply. Meanwhile there is a corresponding decrease in the number of acino-insular complexes of types I-III.

Pancreatic islets which have separated from the acino-insular complexes consist of a central zone, mainly occupied by beta-cells with characteristic abundant aldehyde-fuchsine-positive granulation in the cytoplasm, and a peripheral, thin mantle layer, occupied mainly by cells with small granules in their cytoplasm, staining with Orange G (A cells).

Some brief remarks about acino-insular complexes in the bovine fetal pancreas are given in a paper published by Sanches and von Lawzewitsch [12]. The results of the present investigation enable a more complete picture of these structures to be obtained. For instance, we regard the acino-insular complexes of the fetal pancreas as unique structures playing an important role in the morphogenesis of its endocrine and exocrine divisions. A matter of particular importance in this connection is the evaluation of the process of formation of pancreatic microislets. Besides this, our findings are of definite applied importance. In particular, they must be taken into consideration when methods of obtaining cultures of beta-cells from the bovine fetal pancreas at different periods of intrauterine development are to be chosen. There is evidence in the literature of the virtual impossibility of isolating islets from the fetal pancreas [11]. This can be clearly understood in the light of the data described above in the case of the pancreas of fetuses at the state of development when its endocrine tissue is almost entirely within the composition of acino-insular complexes. In such cases it would evidently be more rational to disperse the tissue of the bovine fetal pancreas with the aid of enzymes to the state of a monodisperse suspension in order to obtain monolayer cell cultures [1, 3, 4]. For instance, by combined treatment of the bovine fetal pancreas with collalytin and trypsin, followed by centrifugation and fractional sedimentation of the resuspended cells, monolayer cultures were obtained in which a high proportion of the foci of attachment and growth consisted of endocrine cells [1, 3].

The overwhelming majority of these cells were beta-cells. A detailed study of these cultures starting from the moment of attachment of the cells to the glass, showed that during the 1st day the acinar cells completely degenerated and were converted into coarsely granular debris. By the 2nd-3rd days the surface of the substrate was completely freed from debris whereas the endocrine cells completed the adhesion process, underwent mitotic division, and formed foci of growth. Experimental studies revealed the high insulin-producing capacity of these cultures [4]. Preparation of cultures containing a high percentage of beta-cells may be interpreted most satisfactorily in connection with our data, given above, on the histologic structure of the bovine fetal pancreas. In this case the fate of the cultures is evidently determined by adhesion of the endocrine cells to the glass and the inability of the acinar cells to adhere to the substrate, so that they die in the course of 1 or 2 days in vitro. Further investigations will help to determine the optimal times of intrauterine development of bovine fetuses from whose pancreas cultures with a high content of beta-cells are most likely to be obtained.

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