THE ULTRASTRUCTURE OF SYNOVIAL MEMBRANE IN THE PRENATAL PIG

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Abstract


The synovial membrane from 6 prenatal pigs of both sexes at 57 days after fertilization was studied. Tissue samples for examination by light and electron microscopy were collected in all instances from the capsule of the hip joint.

The synovial membrane under study consisted largely of intercellular matter which included cells at early stages of differentiation. These, however, could be distinguished on the basis of their ultrastructure into A and B types. Apart from these clear-cut types, some intermediate types, particularly among B cells, were observed; they were designated types I and II. They differed in the character of their granular endoplasmic reticulum and by the absence of secretory granules while the other characteristics of B cells were preserved. Intracytoplasmic filaments were not demonstrated in any type of the cells; desmosomes connecting the cells were not developed and the basal membrane was missing.

The fibrillar component of intercellular matter was represented mainly by aperiodic filaments. Collagen fibrils were few in number and infrequently formed small bundles running in various directions. Occasional collagen fibrils protruded into the articular cavity in the areas of the synovial membrane not covered by flat protrusions of synovialocytes. Collagen fibrils passing through the cell membrane of B cells were detected. In their vicinity in the ground amorphous matter, bundles of aperiodic filaments were observed.

Synovial membrane plays a very important role in functioning of the joint in both physiological and pathological conditions and thus investigations into its submicroscopic structure and the ultrastructure of synovialocytes in particular have been carried out in various mammalian and avian species (Barland et al. 1962; Davies and Palfrey 1966; Krey and Cohen 1973; Watanabe et al. 1974; Wyllie et al. 1964; Fell et al. 1976; Bozdech and Horn 1970; Cutlip and Cheville 1973; Horký et al. 1975; Ghadially 1982; Horký, 1981; 1984; Linck and Porte 1978; Okada et al. 1981; Wassilev 1972; 1974; 1975) and others. All these observations have led to the classification of synovial cells into two categories seen nearly in all mammalian species so far studied: i) cells resembling histiocytes (A cells, M, macrophage-like, cells) whose phagocytic capacity has been demonstrated earlier (Ball et al. 1964; Cochrane et al. 1965) and ii) cells with well-developed granular endoplasmic reticulum probably secreting proteins (B cells or F, fibroblast-like, cells or S, secretory, cells) and were first described by Barland et al. (1962) and then by Wyllie et al. (1964), Krey and Cohen (1973), Johansson and Rejnö (1976), Okada et al. (1981), Graabæk (1984; 1985) and others.

It was surprising to find that the ultrastructure of synovial membrane in the pig received only little attention (Fell et al. 1976). Therefore we decided to make a thorough study of the ultrastructure of synovial membrane during the ontogenic development of this mammalian species that would include all its components, since the other authors have usually been concerned with a mere description of the cell types involved.
Materials and Methods

Porcine synovial membrane was obtained from 6 pigs of both sexes at 57 days after fertilization. In all cases the tissue was collected from the articular capsule of the femur for light and electron microscopy. Samples of synovial membrane with some subsynovial tissue were carefully separated into strips (1 by 1 by 2–3 mm) in a drop of fixation liquid. The strips were immediately fixed in glutaraldehyde (300 mmol/l) in 0.1 M phosphate buffer at pH 7.4 in 60 min and 180 min baths and then rinsed in three fresh baths of 0.1 M phosphate buffer at pH 7.4. This was followed by fixation carried out for 15 and 45 min in OsO₄ (40 mmol/l) in phosphate buffer at pH 7.4. The samples were dehydrated in a graded acetone series completed with two 30 min baths of absolute acetone. Immersion was performed in a routine manner and the tissue was embedded in Durcupan ACM. Polymerization took place in an oven at 60 °C for three days. Ultrathin sections were cut with an Ultracut Reichert ultramicrotome, stained with lead citrate according to Reynolds or with 1 % uranyl acetate followed by lead citrate. The sections were observed and photographed with a Tesla BS 500 electron microscope. Semithin sections for light microscopy were made from the same embedded material stained with 1 % methylene blue and Azure II.

Results

Submicroscopic Structure of the Cells of Prenatal Synovial Membrane

Porcine synovial membrane at this stage of development was composed of synovial matrix involving small numbers of synovialocytes. Cells were sparsely distributed in two or three parallel layers. According to the cell shape, nucleus appearance and amount and occurrence of cytoplasmic organelles, two types of cells could be distinguished.

Before we present here the results of investigation of each cell type we wish to state that at this stage of development we did not find clear and fully differentiated types known in adults of other mammalian species and designated A (M) and B (F, S) cells (see Introduction). In spite of it we followed this classification for the sake of clarity. Apart from the two types mentioned we also observed intermediate types.

Ultrastructure of A cells

Type A cells were found in larger numbers than type B cells in the synovial membrane of the prenatal pig. They were situated close to the membrane surface, frequently 5 μm below the surface (Fig. 1), less frequently right on the surface (Fig. 2), see Plates XI. and XII. at the end of the volume.

Nucleus

It was quite large (about 5 by 10 μm) and had an irregular oval shape. The nuclear envelope extended against the karyoplasm forming deep invaginations (Figs 1, 2). The perinuclear space was considerably dilated and continued with cisternae of granular endoplasmic reticulum. The zonula nucleum limitans formed an indistinct band about 0.1 μm thick (Figs 1, 2). Nuclear chromatin made up a strip, varying in width, along the inner membrane of the nuclear envelope and also a few karyosomes seen on the section through the nucleus (Figs 2, 1). A nucleolus of reticular type was always present.
Cytoplasm

The granular endoplasmic reticulum of A cells was seen as few flat cisternae situated close to the nucleus. Inner spaces were filled with medium osmiophilic material of mesh-like appearance (Fig. 2). Occasional cisternae of the endoplasmic reticulum were dilated (Fig. 2).

The agranular endoplasmic reticulum presented as an accumulation of numerous small vesicles and larger vacuoles near the Golgi complex from which they most probably originated (Fig. 2). Some of the vesicles and vacuoles were filled with medium osmiophilic dark material, which was granular or homogeneous in appearance, suggesting their role as transport vacuoles.

The Golgi complex was not very distinct and occupied only a minor area in the cytoplasm of A cells (Fig. 2). Derived from it were large vacuoles (up to 0.5—0.8 \( \mu \text{m} \)) found near the cell membrane (Fig. 2).

Mitochondria were round in shape, 0.5 by 0.8—1.0 \( \mu \text{m} \) in size. Their structure had the usual character and their amounts were low in the cytoplasm of A cells.

Ribosomes were largely associated with the membranes of granular endoplasmic reticulum. Apart from it they were also observed scattered in the cytoplasm as single bodies, groups of four or as rosettes.

Lysosomes were present in the cytoplasm of A cells only very rarely. Centrioles were not found.

Cell membrane. The cytoplasm of A cells extended into short projections, up to 0.8—1.0 \( \mu \text{m} \), covered with cell membrane and into flat, broad cytoplasmic protrusions several tens of \( \mu \text{m} \) wide. These protrusions were localized on the surface of the synovial membrane which they covered in part (Figs 1, 2). Pinocytotic vesicles were few in numbers.

Lipid droplets and glycogen were not observed in the cytoplasm of A cells. Cytoplasmic fibrous structures were represented by filaments 3—5 nm thick, occurring frequently as small bundles near the nucleus.

Ultrastructure of intermediate cell types

These cells were distributed among differentiated A cells in the second and third layers of synovialocytes in rather large numbers (Figs 3, 4). Their respective ultrastructures were markedly different allowing us to distinguish between cells with conspicuous A cell characteristics (type I) and cells with prominent signs of B cells (type II), see Plates XIII. and XIV. at the end of the volume.

Ultrastructure of type I cells

These cells were spindle-shaped and attained sizes 12 by 6—8 \( \mu \text{m} \).

Nucleus

In accordance with the cell shape it was spindle- to rod-like, 10 by 3 \( \mu \text{m} \) large. The nuclear envelope occasionally extended against the karyoplasm in broad invaginations. The perinuclear space was found to increase twice in some areas. The outer nuclear membrane was rich in ribosomes (Fig. 3). Nuclear chromatin was arranged similarly to that of nuclei in A cells (Fig. 2). The occurrence of two nucleoli of reticular type was quite frequent. Areas of perichromatin granules were also seen (Fig. 3)
Cytoplasm
The cytoplasm of type I cells contained markedly more cellular organelles than differentiated A cells.

The granular cytoplasmic reticulum formed only occasional and slightly dilated cisternae situated near the periphery of cytoplasm.

The agranular endoplasmic reticulum was unusually well developed (Fig. 3). The cytoplasm included conspicuously large amounts of smooth vesicles and vacuoles ranging in size from 0.02 to 1.2 μm. Some of the vesicles seemed empty, a great proportion of them was filled with material of varying electron density. In respect to their position some could be considered pinocytotic vesicles, some transport vacuoles (Fig. 3).

The Golgi complex was difficult to discern among the abundance of vacuolar structures.

Mitochondria did not differ either in number, size or structure from those in the cytoplasm of A cells.

Ribosomes were either associated with the membranes of agranular endoplasmic reticulum or free, occurring in clusters near invaginations of the nuclear envelope (Fig. 3).

Lysosomes were a regular feature in the cytoplasm of type I cells. They were 0.1—0.2 μm in size and contained material that was either electron dark, quite homogeneous or of varying electron density (Fig. 3).

Cell membrane. The cytoplasm of type I cells had almost smooth surface which occasionally extended into short projections and frequently sank forming pinocytotic vesicles and larger vacuoles.

Lipid droplets and glycogen, similarly to A cells, were not seen. Cytoplasmic fibrous structures were gathered into bundles situated as in A cells (Fig. 3).

Ultrastructure of type II cells

These cells did not differ from type I cells in size but marked differences could be found particularly in the arrangement of cytoplasm. They were reminiscent of type B differentiated synovialocytes and as such were considered their predecessors (Figs 4, 5).

Nucleus
In type II cells the nucleus was not different in size or component arrangement from nuclei of type I cells.

Cytoplasm
Type II cells contained a larger amount of cytoplasm and a greater number of organelles than type I cells.

The granular endoplasmic reticulum occurred in two forms. In some cells it presented as broad flat cisternae arranged in parallel rows which occupied a considerably large part of the cytoplasm (Fig. 4). In other cells it formed broadly dilated cisternae. In both instances the spaces in the reticulum were filled with mesh-like, medium electron density material (Fig. 5). In both cell types interconnections of cisternae could be observed.

The agranular endoplasmic reticulum was seen only on rare occasions as small smooth vesicles. These were most likely derived from the Golgi complex and remained in its vicinity, or presented as few smooth vacuoles occurring near the cell membrane (Fig. 4, 5).
The Golgi complex was large and occupied a big area of the cytoplasm. Close to it were vesicles with dark content (Fig. 4). The same vesicles could be followed from the Golgi area to the cell membrane, which placed them in the category of transport vacuoles (Fig. 4).

Mitochondria were numerous with rich cristae; their size did not differ from that of type A cells (Fig. 4).

Ribosomes were mainly associated with cisternae of the granular endoplasmic reticulum. Apart from these, numerous polyribosomes seen as rosettes were among mitochondria and small transport vacuoles (Fig. 4).

Lysosomes occurred in the cytoplasm of type II cells only on rare occasions. If detected, they appeared as dark homogenous particles 0.1 µm in size.

Cell membrane. The cytoplasm of intermediate type II cells occasionally produced short, broad projections 0.8 µm long or it formed wide, shallow folds. Pinocytotic vesicles were few in number (Fig. 4). In some cells penetration of collagen fibrils through the membrane was observed (Fig. 5). This occurred almost always in cells with broadly dilated cisternae of the granular endoplasmic reticulum.

Neither lipid droplets, glycogen or cytoplasmic filaments were observed.

Ultrastructure of synovial matrix

This term is usually applied to intercellular matter of the synovial membrane which consists of the ground fibrillar and the ground amorphous substances. This component has generally received little attention in the literature.

The ground fibrillar matter is composed of two types of fibrous structures. One is formed by typical collagen fibrils 60—100 nm wide and several µm long which branches only occasionally and shows periodicity characteristic of collagen fibres, i.e., of about 64 nm. The other structure comprises aperiodic fibres 50 nm wide and about 0.1 µm or more long. Fibrous structures of this appearance have been reported by Ghadially and Roy (1966), Roy and Ghadially (1967), Wassilev (1972; 1975), Krey and Cohen (1973), Ghadially (1983) and Horký (1984) in the synovial matrix of various mammalian species.

The ground amorphous matter, which includes the fibrous component, consists mainly of the protein-hyaluronic acid complex and sulphonated mucopolysaccharides. This composition is similar to the amorphous matter of other types of connective tissue. Electron micrographs show these two substances as finely granulated, medium osmiophilic matter filling the spaces between collagen fibres. It has been demonstrated that both the protein-hyaluronic acid complex and mucopolysaccharides pass into synovial fluid and probably take part in lubrication of the joint.

The arrangement of intercellular matter and particularly of ground fibrous matter changes from the surface towards deeper zones of the synovial membrane in relation to the amount and distribution of synovial cells.

The intercellular matrix in the period under study was composed largely of ground amorphous matter with few collagen fibrils which occasionally formed small bunches. The fibrils were detected in cross, longitudinal and oblique sections (Figs 1, 2). At this stage of development collagen fibrils showed apriodicity. In the areas of the synovial membrane where projections of synocialocytes did not cover its whole surface, synovial matrix got in direct contact with the articular cavity. In such cases occasional collagen fibrils protruded into the arti-
cular cavity. In some areas the surface of synovial membrane was overlaid with a thickened layer of amorphous matter (Fig. 1).

In the vicinity of cells containing broadly dilated cisternae of granular endoplasmic reticulum, the ground amorphous matter showed bunches of aperiodic filaments (Fig. 6).

Discussion

The submicroscopic structure of synovial membrane of adult animals of various mammalian species has been investigated by a number of authors who found out that its characteristics are very similar in all species so far studied (Langer and Huth 1960; Barland et al. 1962; Ghadially and Roy 1966; Bozdech and Horn 1970; Cutlip and Cheville 1973; Fell et al. 1976; Krey et al. 1976; Linck and Porte 1978; Okada et al. 1981; Horký 1981, 1984) with the exception of the rat in which Roy and Ghadially (1967) and Wassilev (1972, 1973, 1975) in particular reported the synovial membrane with marked aggregation of cells and, at the same time, reduction of synovial matrix.

The synovial membrane in the pig has been studied by Fell et al. (1976) who has been interested in its structure in vivo and the behaviour of synovialocytes in vitro. The prenatal development of synovial membrane has not yet been investigated so that our present results can be compared, to some extent, only with our previous studies of the prenatal synovial membrane in cattle (Horký 1984).

A characteristic feature of the porcine synovial membrane reported in this paper was a mixed population of cells already differentiated (A cells) and intermediate type cells. A clear distinction between A and B cells (Barland et al. 1962; Horký 1981, 1984; Ghadially 1983) was not possible at that stage of development since B cells did not show secretory granules in the cytoplasm even though their structure and the presence of cellular organelles made some of them similar to differentiated cells. The secretory granules were described both in adult animals (Wyllie et al. 1964; Ghadially and Roy 1969, Linck and Porte 1978; Graa bacteria 1984, 1985) and in the prenatal period (Horký 1984). They have been given various names and also the views on their origin, composition and function have varied. Linck and Porte (1978) and particularly Okada et al. (1981) and Graa bacteria (1985) have demonstrated that a major role in the origin of secretory granules is played by the granular endoplasmic reticulum and the Golgi complex, i.e. the cellular organelles specifically involved in the process of synthesis and possible extrusion of a secretion. Okada et al. (1981) and Graa bacteria (1985) provided evidence suggesting that the secretory granules of B cells did not contain acid phosphatase but had mucopolysaccharides and glycoproteins bound to a protein carrier which they demonstrated by protein digestion. However, secretion of the granules into synovial fluid has not been consistently proved and their involvement in the process of friction reduction in the joint still remains a presumption, even though Graa bacteria (1985) is of a different opinion.

The results of our studies concerning the synovial membrane in the prenatal pig together with those of Linck and Porte (1987), Okada et al. (1981) and our earlier work (Horký et al. 1975) support the concept of possible transformation from A cells to B cells. In the porcine synovial membrane under study the intermediate cells were observed frequently as a predominant type among differen-
tiated cells. As for the occurrence of intermediate types in adults, our view is in agreement with that of Linck and Porte (1978) who suggest that this phenomenon is indicative of the functional flexibility of the synovial membrane but does not mean the loss of cell functioning. It should also be kept in mind that there are differences in the submicroscopic structure of the synovial membrane in relation to the mammalian species, as was demonstrated by comparing the structures of synovial membranes in cattle and sheep (Cutlip and Cheville 1973; Horký 1984).

The cells of prenatal porcine synovial membrane, in contrast to bovine tissue, did not show intracytoplasmic filaments, desmosomes or basal membrane. Similarly to the synovial membrane in the prenatal calf (Horký 1984) the penetration of collagen fibrils through the cell membrane of B cells was observed in the pig.

The composition of the synovial membrane surface in the period under study did not differ substantially from that of the foetal synovial membrane in cattle (Horký 1981, 1984) or from those in adult animals of other mammalian species.

Ultrastruktura synoviální membrány prasete v prenatálním období

Byla studována synoviální membrána 6 jedinců obojího pohlaví stáří 57 dní po oplození. Vzorky tkáně byly odebrány ve všech případech z pouzdra kyčelního kloubu pro účely světelné a elektronové mikroskopické histologie.


Ультраструктура синовиальной мембраны свиней
в пренатальный период

Проводили изучение синовиальной мембраны 6 особей обоего пола в возрасте 57 суток после оплодотворения. Образцы ткани во всех случаях были взяты из сумки тазобедренного сустава для целей светодой и электронно-микроскопической гистологии.

В данный период синовиальная мембрана состоит преимущественно из межклеточной массы. В нее вставлены малодифференцирован-
ные клетки, которые однако можно разделить на основе их ultraструктуры на типы A и В. Понимо упомянутых четко определенных типов нами в особенности между клетками В наблюдались промежуточные типы, обозначаемые нами типы I и II. Они явно отличаются распорядком гранулярной эндоэндоплазматической сети, а также отсутствием секреторных гранул. Остальные характеристики клеток В у них сохраняются. Ни в одном из типов клеток не были выявлены интрацитоплазматические нити; между клетками не образовались десмосомы и отсутствует также базальная мембрана.

Волокнистый компонент межклеточной массы представлен преимущественно апериодическими фибриллами. Коллагеновые фибриллы малочисленны и образуют иногда пучки, проходящие в разном направлении. Отдельные коллагеновые фибриллы выступают в полость сустава в частях синовиальной мембраны, перекрытых плоскими выступами синовиоцитов. Было установлено проникновение коллагенных фибрил клеточной мембраной клеток В. В их близости в основной аморфной массе встречаются пучки апериодических нитей.

References


Fig. 1: The synovial membrane surface is partly covered by protrusion of synovialocytes (cp), partly by the thickened synovial matrix (ms). A part of an A cell (A), bundles of collagen fibrils (CF) and cross sections through cell protrusions (cp) in the ground amorphous matter (za). x 25,000
Fig. 2: An A cell at the surface of the synovial membrane. Nucleus (N), nucleolus (n), karyosomes (K). Mitochondria (M), granular endoplasmic reticulum (E), Golgi complex (G). Collagen fibrils of synovial matrix (cf) protrude into the articular cavity (JC). x 21.000
Fig. 3: Intermediate type I cell. Elongated nucleus (N) with nucleoli (n). Small karyosomes (k), perichromatin granules (pg). Mitochondria (M), numerous vesicles of agranular endoplasmic reticulum (a), transport vacuoles (tv), lysosomes (L), short cisternae of granular endoplasmic reticulum (E). x 20,000.
Plate XIV.

Fig. 4: Intermediate type II cell. Parallel flat cisternae of granular endoplasmic reticulum (E), Golgi complex (G), mitochondria (M), transport vacuoles (tv), free ribosomes (r). x 22,000.

Fig. 5: Part of the cytoplasm in an intermediate type II cell. Markedly dilated cisternae of granular endoplasmic reticulum with medium osmiophilic filamentous content (E), pinocytotic vesicles (pv), free ribosomes (r). Collagen fibrils passing through the cell membrane (-----). x 24,000.

Fig. 6: Part of the cytoplasm in an intermediate type II cell. Broadly dilated cisternae of granular endoplasmic reticulum with filamentous content (E), mitochondria (M), free ribosomes (r). In intracellular matter there are collagen fibrils (cf) in bundles seen on cross and longitudinal sections and bundles of aperiodic fibrils (af). x 24,000.