

ULTRASTRUCTURE OF BOVINE ARTICULAR CARTILAGE BETWEEN WEEKS  
8 AND 23 OF PRENATAL DEVELOPMENT

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The ultrastructure of bovine articular cartilage of the hip joint was studied in individuals between weeks 8 to 23 of prenatal development by both transmission and scanning electron microscopes. The joint cartilage of fetuses 8 to 10 weeks after fertilization was a tissue rich in cells in which superficial, middle and deep layers were differentiated according to their submicroscopic features. Chondrocytes of the superficial layer were less differentiated. Cells of the middle layer were of oval shapes with larger numbers of organelles and cilia. In the deep layer chondroblasts were present in groups. Centrioles were often encountered. The intercellular substance on the surface was 3-4  $\mu\text{m}$  thick, composed mostly of aperiodic fibrils; single collagen fibrils were found 1  $\mu\text{m}$  under the surface. Between weeks 16 and 23, among the cells of the superficial layer transient cell types with prominent Golgi complex and centrioles appeared. Chondroblasts of the middle layer did not differ from the adult ones. Intercellular substance was 0.2-0.5  $\mu\text{m}$  thick. Aperiodic fibrils formed bundles on the surface as well as in the deep intercellular substance.

Ultrastructure, bovine articular cartilage, development.

From the developmental, microscopic and functional point of view the joint represents a specially differentiated cavity originating in the blastema ground skeleton of mesenchyme, which is perfectly adapted to its function on body locomotion. The joint is formed partly by articular cartilage which covers cartilage ends of the bones partly by cartilage cavity cushioned by synovial membrane connected to the outer layer of cartilage capsule. Its integral part then is the synovial fluid which fills the joint cavity. Synovial fluid is produced by synovial membrane fulfilling thus important tasks in cartilage by securing the nourishing of joint cartilage cells and by decreasing friction of loaded cartilage areas.

It is generally known that joint cartilage originates from mesenchyme in the course of skeleton development as a component of cartilage blastema of bone ground which is rebuilt to final bone in the course of ossification. While the whole preformed bone ground is being gradually destructed, this process does not affect the joint cartilage retaining it in the direction to joint cavity (Bonucci 1967; Scherft 1972; Thyberg 1973; Ali 1976; Anderson and Sajdera 1976; Felix and Fleisch 1976; Hanaoka 1976). Condensation process of mesenchyme in blastema takes place in the early period of embryogenesis so that according to Gardner and O'Rahilly (1968) already in stage 18 according to Streeter (that is about 6th week after ovulation) chondrification of femur begins and in 8th week joint cavity appears. Also Stingl's (1982) observations suggest it. During this process vessels of perichondrium grow into the cartilage ground (Haines 1933; Hurrell 1934; Levene 1964; Lufti 1970; Stockwell 1971a; Agraval et al. 1984) not intruding the area of the future joint cartilage (Gray and Gardner 1969; Gardner and Gray 1970) and disappear about the 10th week. Before the joint cavity is formed a larger amount of small cavities appears in mesenchyme in the contact area of future joint bone spaces. Its final formation helps also the earlier formed muscles and ligaments in the area of joint (Drachman and Sokoloff 1966) and possibly even the first movements (Glenister 1976). Owing to the process of ossification chondrocytes obtain their characteristic place (Godman et al. 1960; Gould et al. 1974; Levitt et Dorfman 1974). It should be stated of course that all mentioned articles deal with the development of joint cartilage only partly referring to generally known informations. Much greater attention is given to the study of chondrogenesis in rabbits (Puig-Rosado 1981) or birds and to its influence when using tissue cultures Green (1971), Scheck et al. (1975), Bekoff and Klagsbrun (1983), Sato and Urist (1984) as well as stating explicit characteristics considering differentiation of chondrocytes with non-differentiated cells (citation Solurich et al. 1982; Buckwalter and Rosenberg 1983). Some articles are dealing with only partial problems, as regulatory mechanisms in relation to bone-cartilage (Nijweide 1982) or with incidence of ciliae (Wilsman and Flechter 1978; Vidinov and Vasilev 1985), eventually other types of cartilage (Silbermann and Frommer 1974; Sanzone and Reith 1976). In this area we are left to results of our own study.

#### Materials and Methods

Samples of bovine joint cartilage were collected from 14 individuals of both sexes 8 to 40 weeks after fertilization for ultrastructure study in transmission and scanning electron microscopes. Material was taken mostly in a slaughterhouse.

Samples from femur head near to large trochanter were collected, or of the early developmental stage the whole head. Stripes of 1 x 1 x 3-5 mm were cut and divided into blocks of 1 x 1 x 2-3 mm, then processed in a standard way.

Ultrathin sections were cut on Ultracut Reichert microtome, then stained with lead citrate or uranyl acetate followed by lead citrate. These sections were viewed and photographed by a Tesla BS 500 electron microscope. From the embedded material also semithin sections for light microscopy were cut and stained with 1% methylene blue and Azure II.

Samples were also taken for study of joint cartilage surface in a scanning electron microscope and before fixation left for 15 minutes either in 1% hyaluronidase solution of 0.1 mg/ml concentration at 20° C or washed 3 x 15 min. in saline solution. This space of time is sufficient to remove rests of synovial fluid from the surface of joint cartilage without damaging it. When using hyaluronidase, samples were washed 3 x 15 min. in saline fluid, then fixed in 10% formol or glutaraldehyde for 10 to 14 days. After fixation tissue was dehydrated by method of critical point and coated with gold on a Balzer's coating apparatus. The surface was studied by a Stereoscan Cambridge Scanning Microscope.

## Results

### Ultrastructure of the joint cartilage between weeks 8 and 10 after fertilization

During this period the bovine joint cartilage has a character of a rich cellular tissue. Chondrocytes already may be seen and so, according to submicroscopic characters, the joint cartilage can be divided into superficial, middle and deep layer.

Submicroscopic structure of chondroblasts in the superficial layer

Chondroblasts of the superficial layer are spindle-shaped elongated cells, oriented by their longitudinal axis parallel with their cartilage surface. They reach a length of 20  $\mu\text{m}$  and more, thickness does not exceed 3 - 4  $\mu\text{m}$ . Cells are located in several (up to 6) layers and interconnected by their long cytoplasmic projections. They resemble fibroblasts (Fig. 1).

#### Nucleus

Nucleus is of elongated rod-like shape reaching to 8  $\mu\text{m}$  of length and is about 3  $\mu\text{m}$  thick. Exceptionally, nuclei of different shapes are to be seen (Fig. 2).

Nuclear envelope is formed by two membranes which cross each other in places of nuclear pores. The perinuclear area is usually narrow, only seldom about double its usual size. Zonula nucleum limitans forms a narrow rim at the inner nuclear envelope membrane (Fig. 2).

Chromatin is of greater part diffusely dispersed; a small part of nucleoli sections forms sporadic karyosomes which are attached to the inner nuclear envelope membrane.

Nucleoli are found rarely; should they be seen are these nucleoli of reticular type (Fig. 2).

#### Cytoplasm

Cytoplasm of the superficial chondroblasts layer is located according to shape of cells first of all on both ends of the cell,

forming only a narrow rim around nucleus.

Granular endoplasmic reticulum is formed by long flattened cisternae which intervenes with spindle-shaped narrowing cytoplasmic projections. Inner spaces are filled by granular material of medium electron density (Fig. 1).

Agranular endoplasmic reticulum has not been observed.

The Golgi complex is formed by only a small dictyosome consisting of some dilated cisternae with a small amount of vesicles.

Mitochondria are rather frequent of usually oval to elongated shape reaching size of 0.5 to 1  $\mu\text{m}$  and are closely connected to cisternae of the granular endoplasmic reticulum.

Lysosomes occur sporadically in the cytoplasm of chondroblasts of the superficial layer. Their shape is of either oval corpuscles of about 0,8  $\mu\text{m}$  or of elongated rod-shape corpuscles. They contain homogenous dark material in both cases.

The cell membrane. Cytoplasm of chondroblasts of the superficial layer projects on its spindle-shaped narrow ends into conspicuous long, often branching, protrusions, covered by cell membrane, which contact with prominences of adjoining cells. Strengthening formations are usually not formed.

Lipid droplets and glycogen were not found in the cytoplasm of chondroblasts of the superficial layer.

Cytoplasmic fibrillar structures were localized only in the uppermost layers. Filaments are usually places near nucleus forming small bundles (Fig. 2).

#### Submicroscopic structure of chondroblasts of the middle layer

In contrast to cells of the superficial layer are chondroblasts of the middle layer oval, only seldom longitudinal cells reaching size of about 12 x 7  $\mu\text{m}$ . They are usually singular in the intercellular substance, rarely in pairs.

#### Nucleus

Nucleus is of irregularly oval shape, size about 6 x 3 - 4  $\mu\text{m}$ .

Nuclear envelope is in contrast to cells of superficial layer not smooth but in some areas extends against karyoplasm into numerous shallow invaginations (Fig. 3).

Chromatin and nucleolus do not differ from those found in cells of the superficial layer.

#### Cytoplasm

In comparison with the previous layer there is a larger amount of cytoplasm and organelles.

Granular endoplasmic reticulum is formed by narrow cisternae, thickly covered by ribosomes and widely dilated vesicles containing a fine granular demi-osmiophilic material. Its structures are in close contact to Golgi complex.

Agranular endoplasmic reticulum is present as single smooth vacuoles sporadically located in cytoplasm. Provided they are smooth vesicles close to Golgi complex or large vacuoles with granular content, are they derivatives of Golgi complex and so-called transport vacuoles (Fig. 3).

Golgi complex is a very distinct structure of chondroblasts of the middle layer. It occupies its large part and is formed

by numerous Golgi fields arranged circularly (Fig. 3). From bulb-shaped widened cisternae of dictyosomes a large amount of small and large Golgi vesicles separates, some containing medium-dense particles. Large vesicles, already mentioned transport vacuoles, remove them from Golgi area closer to the cell surface.

Mitochondria are liable to shape changes; elongated to rod-like forms prevail, reaching several  $\mu\text{m}$  (Fig. 3).

Lysosomes occur only sporadically in the cytoplasm of chondroblasts of the middle layer. Neither microtubules or centrioles were found.

The cell membrane. Cytoplasm projects from one place to another in short wide projections which also contain organelles. Their length does not reach more than 0.5  $\mu\text{m}$  intervening also the surrounding intercellular substance. Between some of the projections the cell membrane is falling down forming folds which, on cross sections can imitate structures of agranular endoplasmic reticulum or vacuoles of large dimensions (Fig. 3).

Cilia occur as so-called solitary cilia. They reach size about 2-2.5  $\mu\text{m}$  (Fig. 3).

Glycogen is a regular part of the cytoplasm of chondrocytes in the middle layer (Fig. 3). Its typical granules are mostly scattered between organelles, forming in some areas only linear formations or clusters of small dimensions (Fig. 3).

Cytoplasmic fibrillar structures occur in a very small amount. When found on sections they can be seen as bundles of typical intracytoplasmic filaments either close to nucleus or situated on cell periphery. We found in a few cases mitosis of chondroblasts in this layer (Fig. 4). Some chromosomes and fragments of nuclear envelope can be seen. These findings are exceptional, because either in human cartilage or in preceding layer mitosis has not been found.

Under superficial layer of intercellular substance of the middle layer blood capillaries occur (Fig. 1). Erythrocytes pass out of them loosely deposited among chondroblasts or contact them and may be phagocyted by chondroblasts (Fig. 5). This is also the most frequent evidence of phagocytic ability of chondroblasts.

#### Submicroscopic structure of chondroblasts in the deep layer

Chondroblasts of the deep layer are usually of circular, triangular or drop-like elongated shape reaching size of 10 - 12 x 8 - 10  $\mu\text{m}$ . They are deposited in the intercellular substance either in groups or in a column-like arrangement (Fig. 6).

#### Nucleus

The shape of nucleus is irregularly oval or slightly kidney-like reaching size about 5 x 6  $\mu\text{m}$  (Fig. 6).

The nuclear envelope is of usual structure. It projects into karyoplasma in wide shallow invaginations making some areas slightly waved. Perinuclear area is narrow. Seldom interconnection of outer membrane of nuclear envelope with cisternae of granular endoplasmic reticulum is encountered.

Chromatin by its arrangement does not differ from nuclei of chondroblasts in the middle layer.

Nucleoli are inexpressive and occur only sporadically.

### Cytoplasm

There is about the same amount of cytoplasm and organelles as in the middle layer of chondroblasts.

Granular endoplasmic reticulum is mostly formed of short and narrow cisternae with ribosomes which adhere thickly to them (Fig. 6). In some areas of the cytoplasm are reticular structures arranged in layers and are interconnected by transverse connections.

Agranular endoplasmic reticulum is similarly to chondroblasts of previous layers represented by single smooth vesicles and vacuoles mostly placed on periphery of the cytoplasm (Fig. 6).

The Golgi complex occupies only a small area in the cytoplasm of chondroblasts of this layer (Fig. 6). Its dictyosome is formed by a small number of smooth cisternae. Also small vesicles and Golgi vacuoles are few. No transport vacuoles were found.

Mitochondria were of uniform appearance in comparison with the previous layer. Small circular forms prevail while elongated or rod-like forms are only rare. Some have damaged cristae and clear matrix.

Lysosomes occur in the cytoplasm of chondroblasts of the deep layer only sporadically. When found they are of usual shape and size.

Centrioles occur mostly single; we never met pairs of them (Fig. 6).

The cell membrane. On the whole surface the cytoplasm makes numerous projections coated by cell membrane (Fig. 6). Some of these projections are short and wide, containing cell organelles, others are even some  $\mu\text{m}$  long and contain only ground cytoplasm. Long projections are often branching, reaching till intercellular matrix or touching projections of cells in the vicinity.

We did not prove any desmosomes and cilia in this layer.

Glycogen is found in the cytoplasm of chondroblasts of this layer in the same amount, and in the same way arranged as in the middle layer.

Cytoplasmic fibrillar structures are deposited as bundles of filaments mostly near nuclear surface or in the vicinity of centrioles (Fig. 6).

### Arrangement of intercellular substance of joint cartilage

Intercellular substance of bovine joint cartilage is in the period of weeks 8 to 10 after fertilization arranged differently in the superficial surface layer and in the rest of layers. The surface of joint cartilage (Fig. 2) is covered, practically in the whole extense, by massive layer of intercellular substance of 3 - 4  $\mu\text{m}$ , composed mostly of aperiodic fibrils and a thin mesh-work of collagen fibrils which are deposited in a less rich amorphous substance. The peripheral layer which is in close connection to the joint cavity is formed exclusively by aperiodic fibrils; about 1  $\mu\text{m}$  under the surface collagen fibrils course irregularly. A layer of about 1  $\mu\text{m}$  is placed under this zone, formed by collagen fibrils which cross one another and by a larger amount of amorphous substance. Close to cell membrane of the superficial chondroblasts leans a layer of about 0,5  $\mu\text{m}$  formed again by aperiodic fibrils (Fig. 2). Towards the depth of the surface layer amount of the filamentous component decreases

and that of the amorphous substance relatively increases. In the middle layer (Fig. 3) collagen fibrils are rare. Aperiodic fibrils situated close to the chondroblasts prevail so that the pericellular area is not distinctly formed. In the deep layer (Fig. 6) pericellular and intercellular matrix slightly differentiated at least on the peripheral parts of cells. Even septa begin to form in the vicinity of chondroblasts where collagen fibrils from the environmental matrix project.

#### Ultrastructure of the joint cartilage between weeks 16 and 23 after fertilization

During this period most changes take place in the upper layer, considering in the first place differentiation of filamentous component of the intercellular substance and formation of the peripheral layer of joint cartilage. Changes on chondroblasts lead to a further differentiation in such a way that especially cells of the middle layer acquire a character of adult chondrocytes, while in the deep layer these changes are not so conspicuous.

#### Submicroscopic structure of chondroblasts of the superficial layer

There are cells of two types in the superficial layer. Spindle-shaped cells of similar appearance as in the above mentioned stadium are deposited in 2 to 3 layers near the surface above one another (Fig. 7). Underneath, smaller elongated oval cells in 1 to 2 layers are located. Cells of the second type reach a size of  $12 - 13 \times 6 \mu\text{m}$  and form a transitional type between the upper and middle layer (Fig. 8). Since the uppermost cell layers correspond with their structure to chondroblasts of the superficial layer in the previous developmental period, we are now going to describe cells which are deposited on the periphery between the superficial and middle layer of chondroblasts.

#### Nucleus

The nucleus has an oval shape and reaches a size of  $8 \times 3 \mu\text{m}$ .

The nuclear envelope is of standard structure without unevenness covering nucleus. Nuclear pores are few in number (fig. 8).

Chromatin is diffusely dispersed on the nucleus section and only close to the membrane of the nuclear envelope forms a continuous rather thicker layer and 1 to 2 larger karyosomes located near the nuclear envelope. Zonula nucleum limitans makes a quite narrow rim between the nuclear envelope and chromatin.

Nucleolus is rare in the karyoplasm of chondroblasts in this layer; when found it is usually a nucleolus of a reticular type or ring-shaped nucleoli already observed from 2 to 3 on one section.

#### Cytoplasm

There is a larger amount of cytoplasm in comparison with the uppermost deposited chondroblasts. Besides organelles also cytoplasmic inclusions occur (Fig. 8).

Granular endoplasmic reticulum is formed by less numerous short flattened cisternae with ribosomes thickly attached to

their membrane (Fig. 8). Cisternae are irregularly located in the cytoplasm among other cell organelles. Provided we can follow their increase then it is evident that the inner space is filled by granular material of medium electron density (Fig. 8).

Agranular endoplasmic reticulum appears as 0,5  $\mu\text{m}$  vacuoles which occur mostly on the periphery of cytoplasm (Fig. 8).

The Golgi complex is distinct and occupies a rather large area. It is dispersed into several Golgi fields and is composed of small dictyosomes and a large amount of mostly small and medium Golgi vesicles. We did not find any transport vacuoles.

There is a small number of mitochondria. They are small and their oval forms with distinct dense matrix dominate in the section of cytoplasm.

Lysosomes occur only sporadically in the cytoplasm of chondroblasts of this zone. Their size is about 0.5 to 0.6  $\mu\text{m}$  and they contain homogenous highly dense material.

Centrioles are rather numerous. Similarly to the preceding developmental period they are solitary centrioles (Fig. 8).

The cell membrane. The cytoplasm is formed differently on side which turns to the surface and to middle layer. The cytoplasm of area which inclines to joint cavity is smooth and slightly waved whereas the cytoplasm in direction to the middle layer sends out projections of various length and width. Some of them are 1  $\mu\text{m}$  long and 0,1  $\mu\text{m}$  wide, other reach only half the length but double the width. Collagen fibrils are closely attached to the cell membrane. Pinocytic vesicles are few in number. We did not find any supporting formations of desmosome type or cilia.

Glycogen is a regular component of the cytoplasm of cells in this zone. Its small groups of granules occur dispersed in the cytoplasm, at one pole of the cell forming usually one larger cluster (Fig. 8).

Cytoplasmic filament structures occur in a small number as bundles of fine filaments usually near nucleus (Fig. 8).

#### Submicroscopic structure of chondroblasts of the middle layer

Chondroblasts of the middle layer are of oval (Fig. 9, 10) or irregularly triangular shape, deposited in the intercellular substance single or in pairs reaching size about 9-10 x 6  $\mu\text{m}$ .

#### Nucleus

Nuclei adapt their shape to that of cells, in sections having an irregular bulb-like or triangularly rounded shape (Fig. 9, 10). They are rather large reaching sizes to 6 x 4  $\mu\text{m}$ .

Nuclear envelope is formed by two membranes which transit into one another in places of nuclear pores and close the perinuclear area. Ribosomes are thickly attached to the outer membrane of nuclear envelope and this membrane forms in some places protrusions intruding cytoplasm, becoming so a part of the granular endoplasmic reticulum.

Chromatin does not differ by its arrangement from previous stage.

Nucleolus is rare. In one case we regarded a formation reminding of micronucleolus.



### Cytoplasm

There is a larger amount of granular endoplasmic reticulum when compared with the cytoplasm of chondroblasts of the previous zone. It is formed by numerous flattened or dilated cisternae of various length connected to one another by transverse connections (Fig. 9, 10). These dilated parts are filled with granular material of usual appearance which also may be seen in smooth vacuoles near cells surface (Fig. 9).

Agranular endoplasmic reticulum (Fig. 9) is represented either by a few small or by some 0,5 to 0,8  $\mu\text{m}$  large smooth vacuoles located either near Golgi complex or on the periphery of cytoplasm.

The Golgi complex is similarly as in chondroblasts of the middle layer of previous developmental stage formed very rich and in the cytoplasm spreads into several fields. Cisternae of dictyosomes are often widely dilated (Fig. 9) or club-like widened on the ends where from a large amount of fine vesicles separates deposited close to dictyosome. Some of the vesicles are filled with granular material and appear at the periphery of cytoplasm near cell membrane (Fig. 10).

Mitochondria (Fig. 9) are rather numerous, with usual structure and reach size of 0,3 to 0,6  $\mu\text{m}$ . Mitochondria of elongated shape are few in number.

Lysosomes occur sporadically, mostly as multivesicular corpuscles (Fig. 9).

Centrioles were often found in the cytoplasm of chondroblasts of this layer (Fig. 9, 10).

The cell membrane. The cytoplasm extends on the whole surface into rather short and wide projections, where also cytoplasmic organelles may be found (Fig. 10). As a rule just in these parts of the cell membrane we found penetration of collagen fibrils into the intercellular area (Fig. 9). Some projections turn bow-like thus imitating vacuoles. In single cases the cell membrane forms deep folds into which e. g. cilia may protrude (Fig. 10). Number of pinocytotic vacuoles is small.

Glycogen is present regularly but in a small amount either as single granules or small clusters (Fig. 9), which are irregularly scattered among cell organelles.

Cytoplasmic fibrillar structures occur similarly to the previous layer as fine bundles of filaments either on periphery of the cytoplasm near cell membrane or near nucleus (Fig. 9).

### Submicroscopic structure of chondroblasts in the deep layer

Chondroblasts of the deep layer of joint cartilage are in this developmental stage rather variable in shape. They can have a triangularly rounded column-like elongated or irregular star-like shape (Fig. 11, 12). They form either groups in the intercellular substance of the deep layer or are linearly arranged in short columns vertical to the surface of cartilage. They reach size of 8 to 9 x 4 - 5  $\mu\text{m}$ .

### Nucleus

Similar to previous layer the nucleus adapts to the cells shape and by its appearance and structure does not differ from nuclei of chondroblasts of the middle layer (Fig. 11, 12).

## Cytoplasm

Compared with the middle layer there is a smaller amount of cytoplasm which is, for instance, accumulated near cell pole or is shifting to the massive projection (Fig. 12) whereas around nucleus only a narrow rim is formed.

Granular endoplasmic reticulum is represented by long flattened cisternae oriented in parallel with nuclear surface reaching into the wide projections of the cytoplasm. On the cell periphery its cisternae dilated (Fig. 11), the inner spaces of which are filled with granular or filamentous material of medium electron density (Fig. 12).

Agranular endoplasmic reticulum has not been stated in the cytoplasm of chondroblasts of this layer. Provided some smooth vacuoles occurred, these were large vacuoles of Golgi complex of cross sections through invaginations of the cell membrane.

The Golgi complex occupies usually a small area. It is formed by only one dictyosome, from its cisternae small and large vacuoles containing filamentous material are separating (Fig. 11). Large vacuoles are distributed on periphery of the cytoplasm as transport vacuoles (Fig. 11, 12).

Mitochondria are of usual structure and size, similar in numbers as in the middle layer.

Lysosomes are regularly found in the cytoplasm of chondrocytes of the deep layer, here most often observed as multivesicular corpuscles (Fig. 12).

Centrioles occur in smaller number than in cells of the middle layer.

The cell membrane. As mentioned above, chondrocytes of the deep layer are of various shapes and consequently their cytoplasm is arranged in a different way. Its projections covered by cell membrane withdraw in irregular distances, often make branches reaching the length of some tenths of micrometre up to 3 to 5  $\mu\text{m}$  and these long projections are usually in contrast with projections of cells in the vicinity. Whereas long and mighty projections contain also cell organelles, in the short and thin projections organelles are missing. Analogically to cells of the middle layer we found penetration of collagen fibrils through the cell membrane (Fig. 12). We did not find any supporting formations in places of mutual contact of cells.

Cilia occur rather often being of the same shape as in the middle layer (Fig. 12).

Glycogen has the same appearance and arrangement as in the cytoplasm of chondroblasts of the previous layer (Fig. 11); the same concerns also cytoplasmic filamentous structures.

## Arrangement of the intercellular substance in the joint cartilage

The intercellular substance of bovine joint cartilage changes most in the superficial layer during the period of 16th to 23rd week after fertilization (Fig. 7), whereas in the remaining layers these changes are less conspicuous (Fig. 9, 11).

The fibrillar component of the intercellular substance is in the superficial layer formed by typical collagen fibrils partly arranged into bundles, passing in parallel with the surface of the joint cartilage and cells, these fibrils run oblique to the cell membrane of chondroblasts of the superficial layer. In

the direction to joint cavity chondroblasts are covered by about 0,6 to 0,8  $\mu\text{m}$  thick layer of intercellular substance, the border proper being formed by about 0,2 to 0,5  $\mu\text{m}$  thick layer where fine aperiodic fibrils, forming in some areas thick bundles predominate (Fig. 7). There is a rather large amount of amorphous substance in this peripheral layer, where aperiodic as well as some collagen fibrils are deposited. The intercellular substance of the superficial layer is not differentiated into pericellular and intercellular matrix. In the direction to middle layer the amount of collagen fibrils decreases (Fig. 8) and the number of aperiodic fibrils increases. They occur mostly near the cells so that a higher "halo" arises in their vicinity indicating differentiation of pericellular and intercellular matrix.

In the middle layer (Fig. 9, 10) the only filamentous components are aperiodic fibrils and at the same time the amount of amorphous component increases so that the intercellular substance has a conspicuously thin texture.

In the deep layer, as in the previous developmental stage, appear besides aperiodic fibrils also typical collagen fibrils (Fig. 11, 12). Their amount, however, is much smaller than in the superficial layer. In the rich amorphous ground substance aperiodic filaments occur exclusively round the column-like cells so that especially with small magnification the dividing line between the pericellular and intercellular matrix may be distinctly seen.

#### Appearance of the joint cartilage in a scanning electron microscope

The bovine joint cartilage in 8 to 10th week of prenatal development has an appearance of very cellular tissue (Fig. 13, 14). We found first changes in the period of 8th to 10th week, when appearance of its surface is beginning to form rather differently.

The surface of the joint cartilage in this period is rather uneven (Fig. 15). Several elevations of elongated spindle-like shape are seen above the surface. In some cases there are pairs surrounded by a depression (Fig. 16), so that formations of the figure 8-appearance, in rare cases even groups of four of these elevations, separated by common grooves occur.

#### Discussion

During our study of the ultrastructure of bovine joint cartilage we stated the fact that no sufficient attention has been given to this problem in any known investigations (Horký 1983). There is a similar situation in the development of joint cartilage in this animal species as there are only few articles published which would deal with the morphology of joint cartilage during the period of development. But in no case has been described its development on the sumicroscopic level in a more or less continuous line since formation of the joint cavity (that is about the 8th week after ovulation) till birth, when its structure corresponds - except for certain quantitative signs - to the adult joint structure. From this point of view our investigations are original. Studies concerning this problem have been published by Cameron and Robinson (1958),

who only compared matrix formation of epiphyseal and articular cartilage. Brower and Hsu (1969) and Stockwell (1971) observed vessel supply of the joint cartilage and possibility of diffusion of material from cartilage canals to the intercellular substance in different species of mammals, or thickness of the joint cartilage and density of chondrocytes (Stockwell 1971a). We therefore compare results of our study with findings on the joint cartilage also by other species of mammals including man as far as they have general validity.

A striking sign of chondroblasts of the superficial layer during the developmental period is the way of their deposition. It is commonly declared that this orientation is conditioned by pressure forces which affect the cartilage. This is one of the possible reasons, but Gould et al. (1974) presented an interesting statement according to which this orientation is in connection with the formation of intercellular substance when chondroblasts are compressed by this substance which is produced in the area of chondrification centre. Since during the prenatal development pressure forces are confined only to the pressure of developing muscles, this point of view cannot be omitted although no justification is found for the fact why the mentioned pressure of intercellular substance does not manifest itself on chondroblasts of the subsuperficial layers. It is most probable that in the final arrangement of chondrocytes in the developmental period both factors participate together and according to these forces changes process and arrangement of filaments of the intercellular substance, which together with amorphous component are the bearers of mechanical qualities of joint cartilage, whereas chondrocytes play the main role in their synthesis (Balasz et al. 1966; Imura 1984; Klamfeldt 1984). This arrangement behold chondrocytes of the superficial layer also in maturity.

During the prenatal period the chondroblasts of the superficial layer appear as of less differentiated cells originating from mesenchyme as for example in fibroblast in other tissues. They keep this appearance practically to the 3rd month after post partum when their cytoplasm differs very little from chondrocytes of an adult cartilage. Their shape changes and first of all in the cytoplasm appear organelles in an amount which is characteristic for differentiated cells and being of specific structures there are intracytoplasmatic filaments of vimentin type (Ghadially 1983).

Zonula nucleum limitans is formed (Horký 1984b) during the developmental process in nucleus, its thickness changing according to literary statements with increasing age.

In the middle layer there comes to differentiation of chondroblasts already in a very early period, according to our material from 8th week of development. Chondroblasts of this layer differ in the course of the whole period from cells of the superficial and deep layer by a larger amount of cytoplasm, by distinct granular endoplasmic reticulum, Golgi complex and by a larger amount of elongated mitochondria and rather frequent transport vacuoles. These characteristics are, according to Freeman (1973), typical for chondrocytes with graded synthesis of components of the intercellular substance. Also data from Stockwell and Meachim (1979), who followed the level of synthesis of proteoglycans as well as mitosis agree that from the metabolic point

of view is this layer also important for the growth of cartilage. In our own material we succeeded also to follow mitosis which is generally infrequent in a cartilage (Ghadially 1983). Analogically to Stockwell (1971a) we found in our material from 8th to 10th week in the intercellular substance of the middle layer blood capillaries and erythrocytes deposited partly between chondroblasts partly phagocyted. In the later period capillaries did not occur in the joint cartilage which corresponds with data of Gardner and Gray (1970).

In the later developmental period (16 to 23rd week after fertilization) in the superficial layer besides chondroblasts also temporary types of cells we referred to already before (Horký 1983), which have the same characteristics typical for chondroblasts of the middle layer. It is first of all the typical Golgi complex and frequently occurring centrioles. The appearance of chondroblasts of the middle layer does not substantially differ from the previous stage. We stated penetration of collagen fibrils through the cellular membrane into the intercellular area which shows that these are cells, from the point of view of production of the intercellular substance very active. This phenomenon has been stated only in a developing synovial membrane (Horký 1984a), not in chondrocytes.

Similar to cells of the joint cartilage also the intercellular substance undergoes several quantitative and qualitative changes during the prenatal development. In the early periods it is formed by abundant amorphous substance with prevalence of aperiodic fibrils. From the standpoint of joint function the superficial layer plays the main role. Between weeks 8 and 10 of development is the filamentous component on the periphery of joint cartilage with joint cavity formed exclusively by aperiodic fibrils. About 1  $\mu\text{m}$  under the surface may be single collagen fibrils found. Chondrosynovial membrane is not formed (Wolf 1969; 1975) so that diffusion of material from the joint cavity is easy (Maroudas and Bullough 1968; Maroudas 1973); thanks to the small amount of collagen fibrils the pericellular and intercellular matrix is not formed. We found the first signs of formation of the chondral membrane on the surface of cartilage from 16th to 23rd week after fertilization, when on the periphery with the joint cavity sections of 0,2 to 0,5  $\mu\text{m}$  thick, formed of bundles of fine filaments begin to grow.



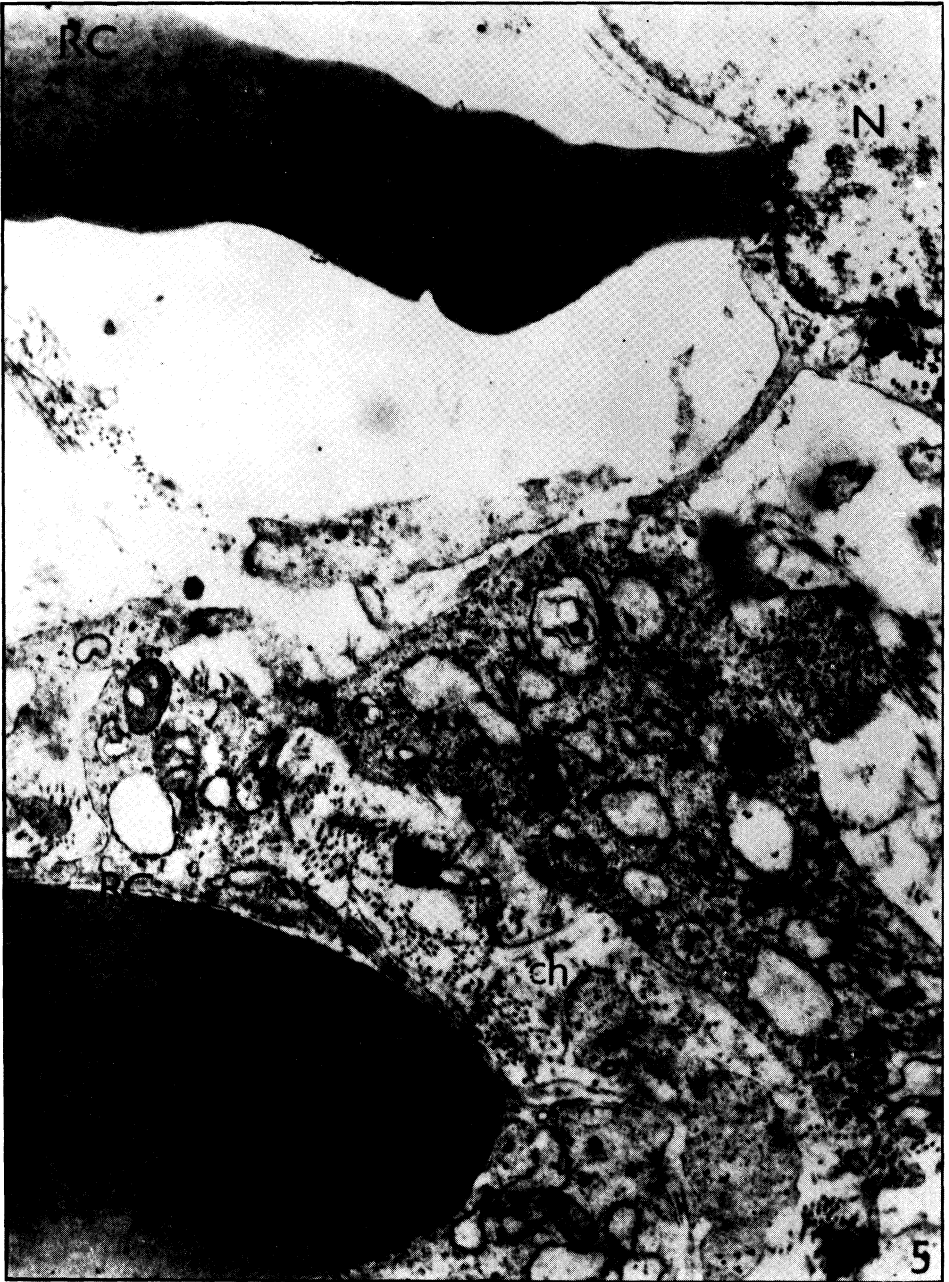
JC

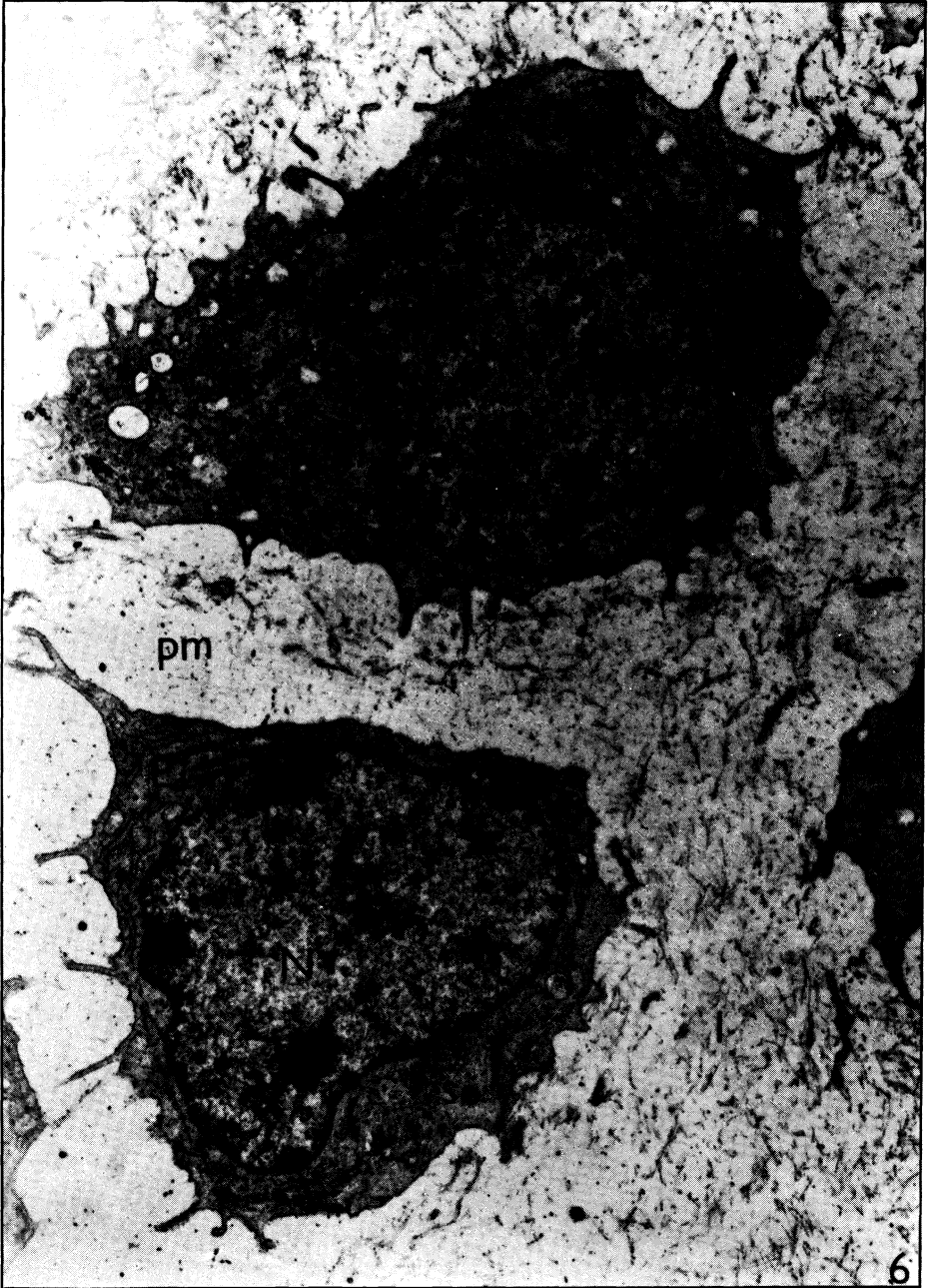


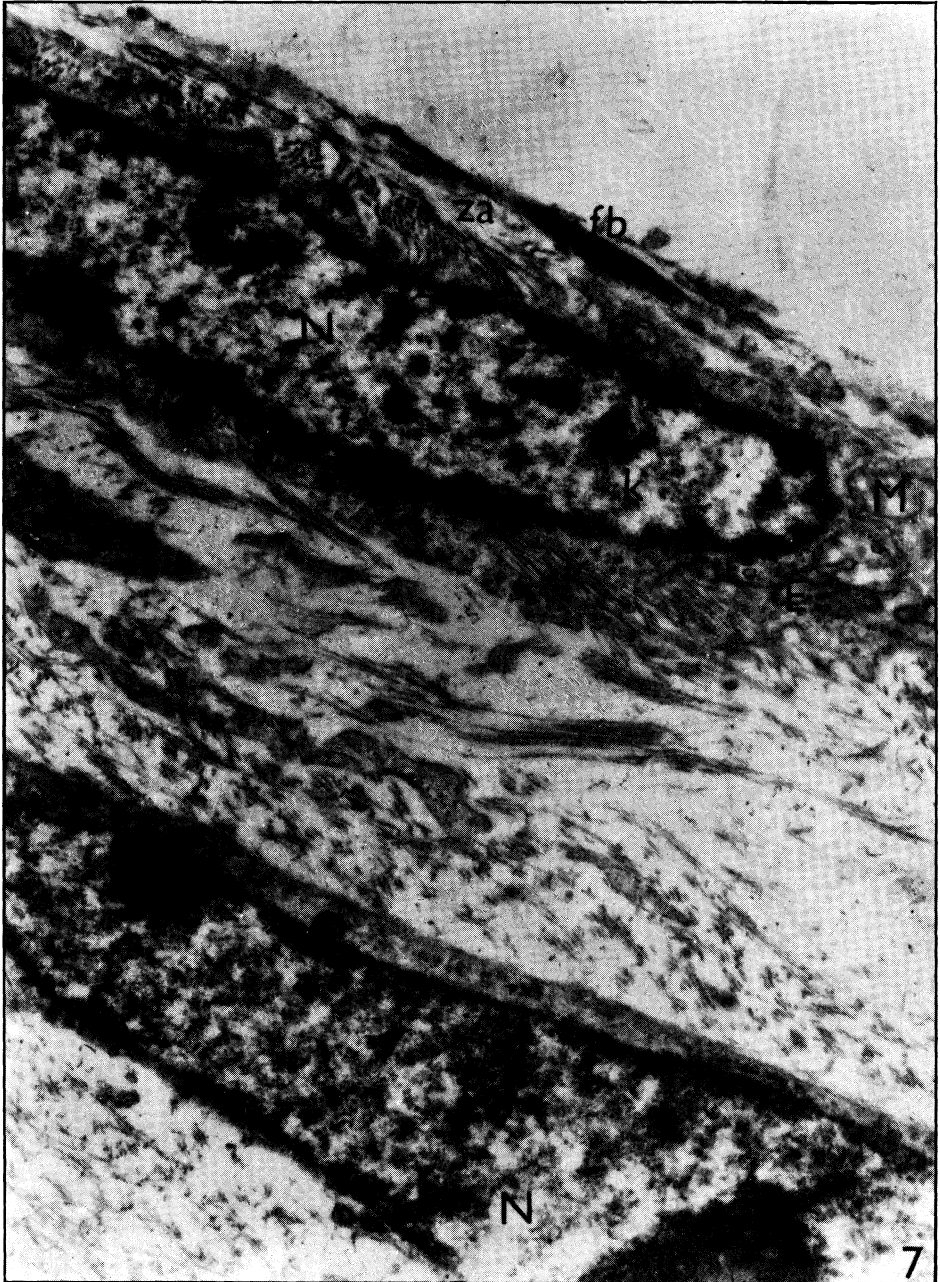




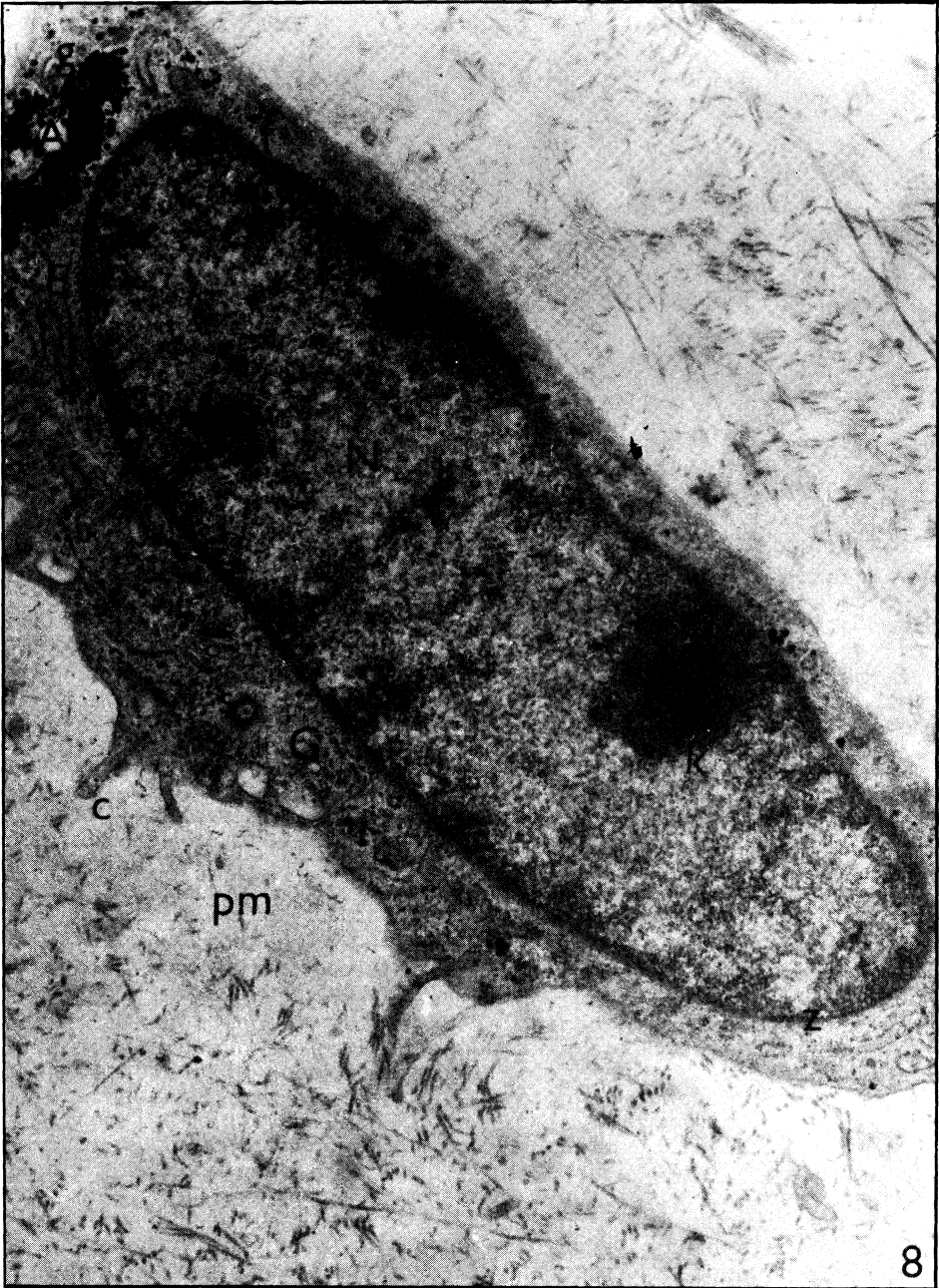


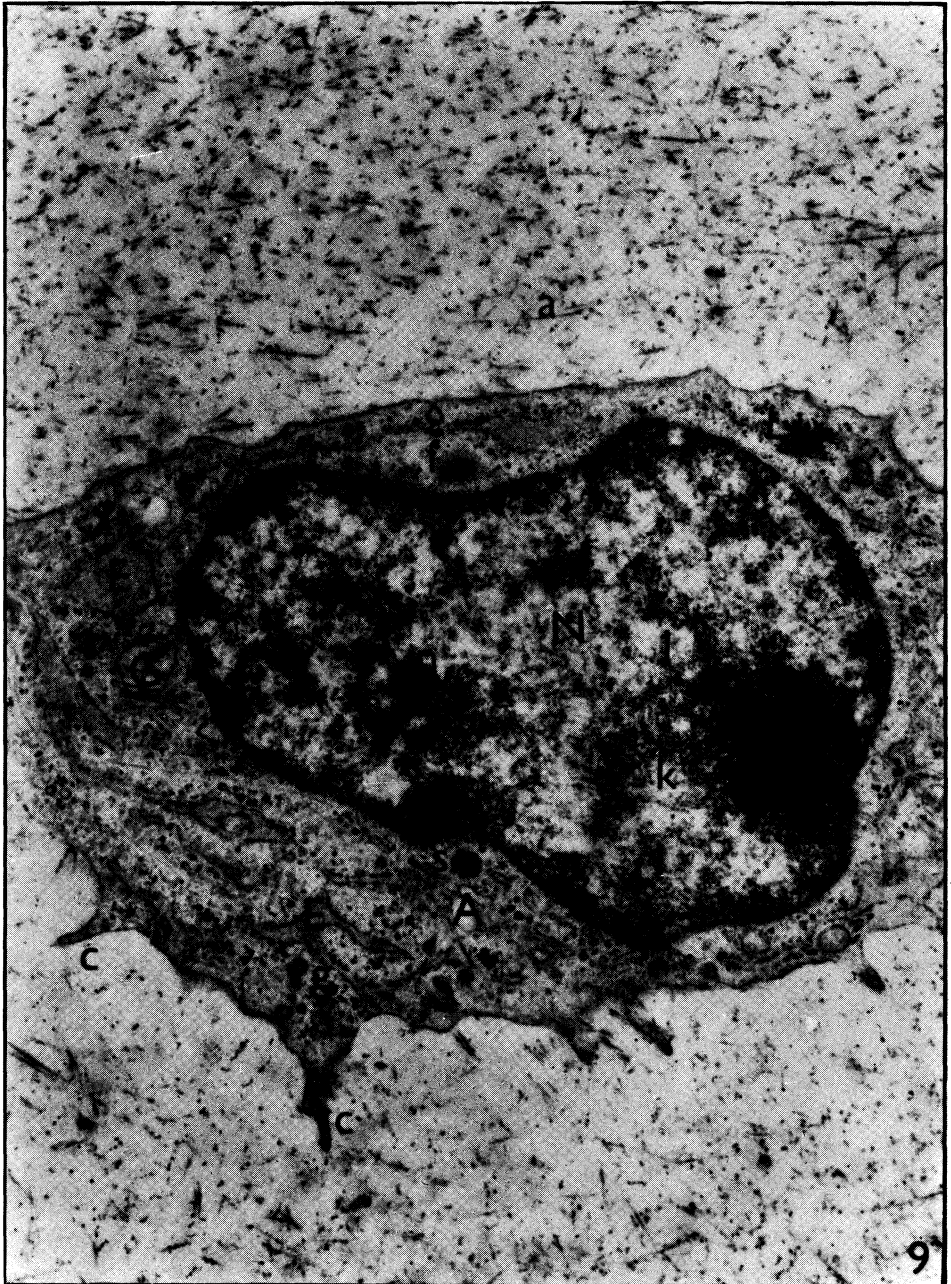


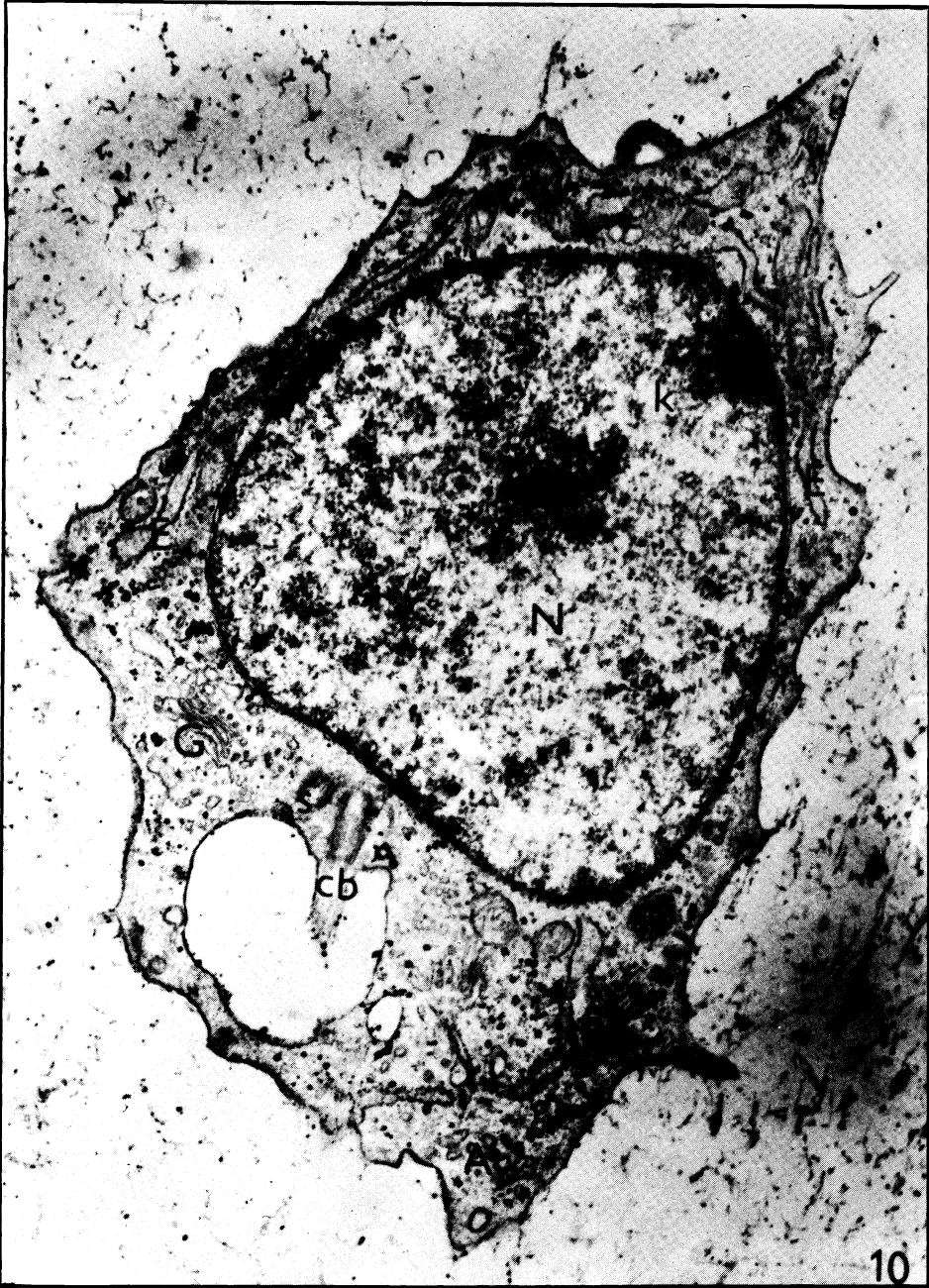


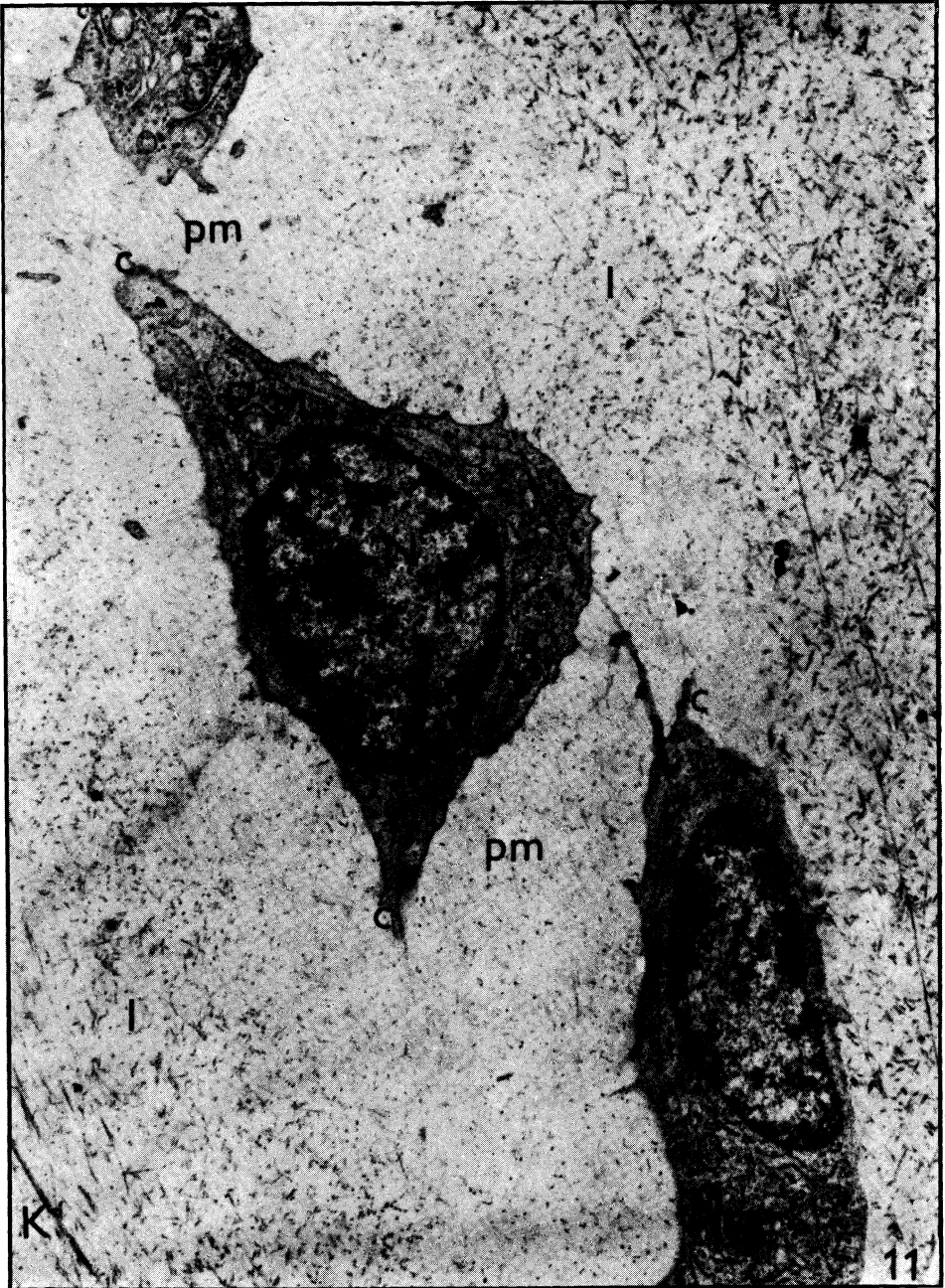








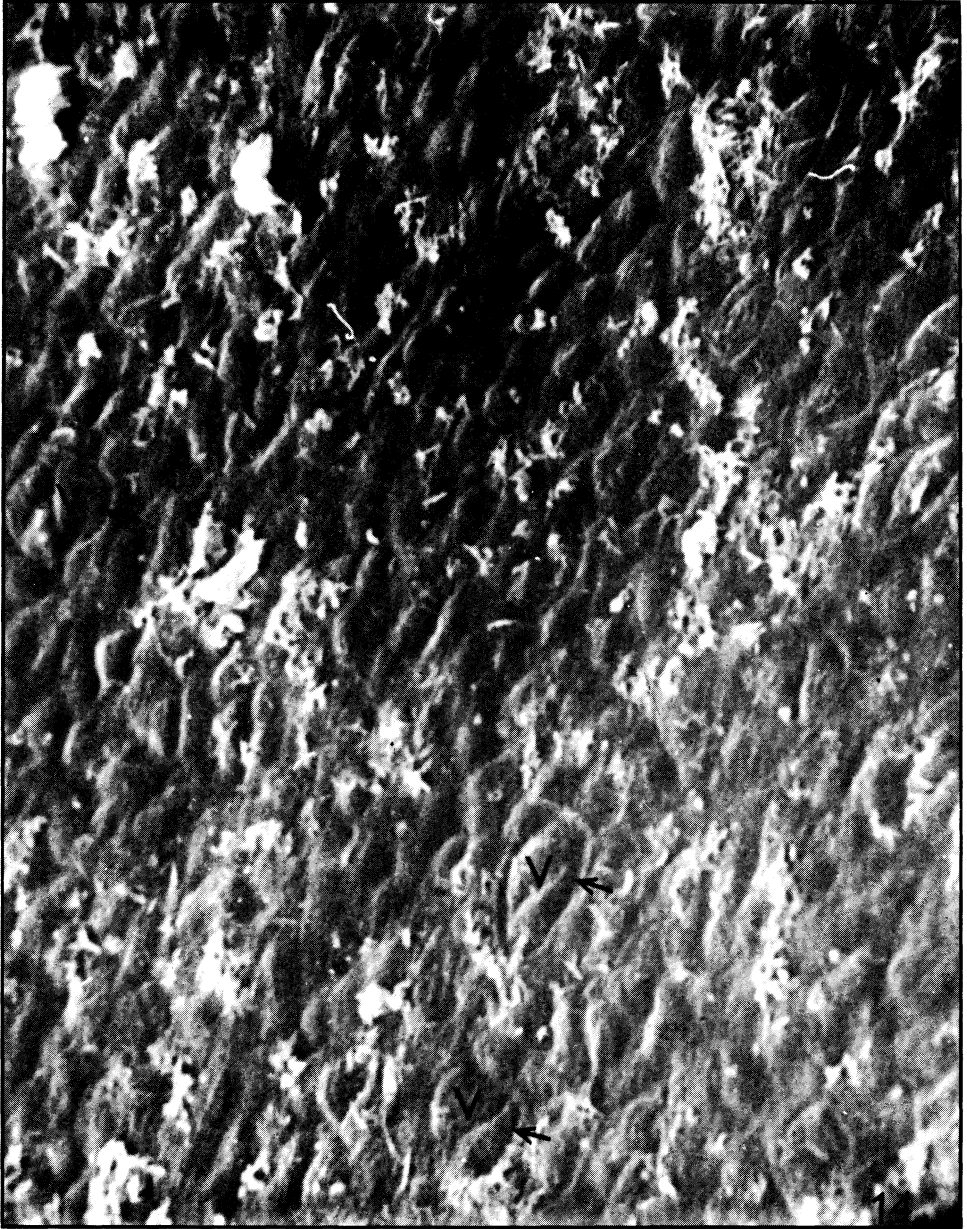


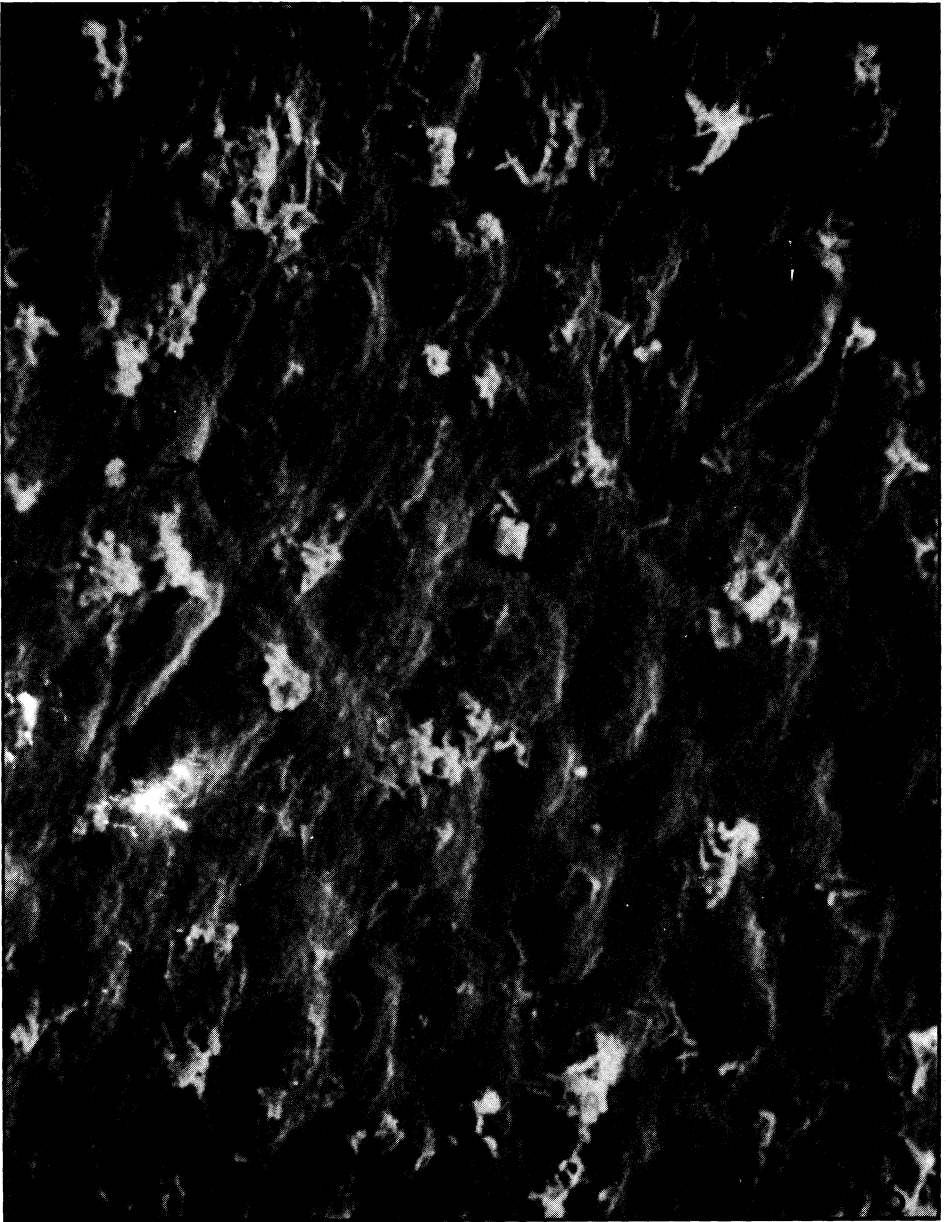




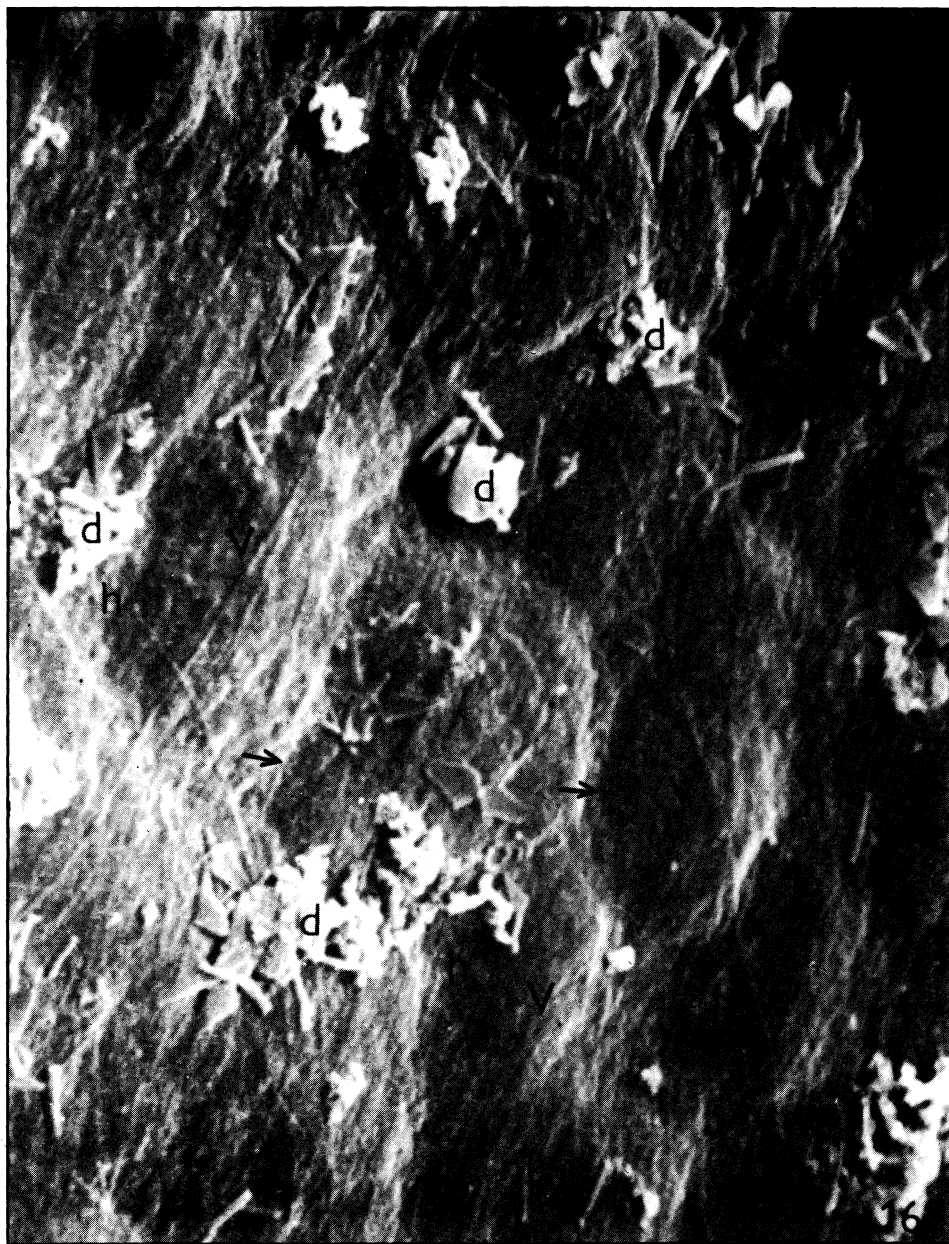












Legends to Figures 1 - 16.

Fig. 1. The superficial layer of bovine joint cartilage from 8 to 10th week after fertilization. Chondrocytes with nuclei (N), karyosomes (k). Flattened cisternae of the granular endoplasmic reticulum, (E) with granular content, mitochondria (M), lysosomes (L). Capillaries are (bv) deposited under several layers of spindle-shaped elongated chondroblasts. Cytoplasm makes long projections (c) through which some cells get in contact. In the intercellular substance amorphous component prevails (za), only on the surface is the fibrillar component formed by a mesh-work of aperiodic fibrils (a). Magnification: 4.200 x.

Fig. 2. A part of the surface of bovine joint cartilage 8 to 10th week after fertilization. Nucleus of chondroblast (N), karyosomes (k), zonula nucleum limitans (z). Sporadic cisternae of granular endoplasmic reticulum (E), mitochondria with a clear matrix (M), lysosom (L), intracytoplasmic filaments (f). In the direction of joint cavity (JC) is the intercellular substance formed by an amorphous component (za), where numerous aperiodic fibrils (a) and crossing collagen fibrils (K) are placed. In direction to the deeper layers the intercellular substance has a thinner texture. Magnification: 16.000 x.

Fig. 3. The middle layer of bovine joint cartilage 8 to 10th week after fertilization. Nucleus of chondroblast (N) with karyosomes (k), zonula nucleum limitans is not distinct. Strong developed granular endoplasmic reticulum (E), Golgi complex (G), transport vacuoles (tv), mitochondria (M) have often a prolonged shape. The cytoplasm makes some short and wide projections (c) except for places where cilia (cb) occur. Fine bundles of intracytoplasmic filaments (f) are mostly placed on the cell periphery, among organelles are granules of glycogen irregularly dispersed (g). Magnification: 18.000 x.

Fig. 4. Mitosis of chondroblast of the middle layer of bovine joint cartilage of the same stage. Magnification: 12.000 x.

Fig. 5. Erythrocytes in the beginning and advanced phase of phagocytosis of chondroblasts of bovine joint cartilage middle layer 8 to 10th week after fertilization. Nucleus of chondroblast (N), its cytoplasm (ch), erythrocytes (RC). Magnification: 20.000 x.

Fig. 6. The deep layer of bovine joint cartilage 8th to 10th week after fertilization. Nucleus of chondroblast (N), minute karyosomes (k) attached to the membrane envelope. Several short cisternae of the granular endoplasmic reticulum (E), agranular endoplasmic reticulum (A), Golgi complex (G), mitochondria (M), centrioles (s), glycogen (g), intracytoplasmic filaments (f). The intercellular substance is partly differentiated on pericellular (pm) and intercellular matrix (I). Magnification: 8.000 x.

Fig. 7. A part of the superficial layer of bovine joint cartilage 16th to 23rd week after fertilization. Nuclei (N) of spindle-shaped elongated chondroblasts with karyosomes (k), zonula

nucleum limitans (z). Sporadic cisternae of granular endoplasmic reticulum (E), mitochondria (M), lysosomes (L). The intercellular substance on surface is formed by an amorphous component (za) and aperiodic fibrils, oriented into bundles (fb) and near cells by collagen fibrils (K), deposited into a richer amorphous substance. Magnification: 20.000 x.

Fig. 8. Chondroblast of the transitional zone of bovine joint cartilage 16th to 23rd week after fertilization. Nucleus (N) with diffusely arranged chromatin and karyosomes (k), thin zonula nucleum limitans (z). Cisternae of granular endoplasmic reticulum (E), agranular endoplasmic reticulum (A), structures of Golgi complex (G), centriol (s), glycogen (g), intracytoplasmic filaments (f). Projections of the cytoplasm are short (c) and reach into partly formed pericellular matrix (pm). Magnification: 12.000 x.

Fig. 9. Part of the middle layer of bovine joint cartilage 16th to 23rd week after fertilization. Nucleus of chondroblast (N), karyosomes (k), zonula nucleum limitans (z) is narrow. In karyoplasm a formation resembling micronucleus (n). Numerous flattened and dilated structures of granular endoplasmic reticulum (E), agranular endoplasmic reticulum (A). The Golgi complex (G) with dilated cisternae, lysosom (L), centriol (s), granules of glycogen (g) in small clusters, intracytoplasmic filaments (f). In the intercellular substance aperiodic fibrils (a) in a rich amorphous component. Magnification: 16.000 x.

Fig. 10. Chondroblast of the middle layer of bovine joint cartilage 16th to 23rd week after fertilization. Nucleus (N), karyosomes (k), zonula nucleum limitans (z). Granular endoplasmic reticulum (E), agranular endoplasmic reticulum (A), Golgi complex (G), transport vacuoles (tv), mitochondria (M), cilia (cb), centriol (s), small clusters of glycogen (g). The surface of the cell and the intercellular substance have the same appearance as in the previous figure. Magnification: 16.000 x.

Fig. 11. Part of the deep layer of bovine joint cartilage 16th to 23rd week after fertilization. Column-like arranged chondroblasts with nuclei (N), karyosomes (k). Granular endoplasmic reticulum (E), Golgi complex (G), mitochondria (M), glycogen (g). Spindle-like projections of cytoplasm (g) reach into the area of pericellular matrix (pm). In the intercellular matrix (I) collagen fibrils occur (K). Magnification: 9.000 x.

Fig. 12. Chondroblast of the deep layer of bovine joint cartilage 16th to 23rd week after fertilization. Nucleus (N), karyosomes (k), zonula nucleum limitans (z). Dilated cisternae of granular endoplasmic reticulum (E), vacuoles of the Golgi complex with granular material (V), lysosomes (L), cilia (cb), glycogen (g). Collagen fibrils penetrate the cell membrane. Magnification: 20.000 x.

Fig. 13. The surface appearance of bovine joint cartilage 20th to 23rd week after fertilization. Numerous elevations (V) placed

single are limited off the environment by differently deep incisions. Rests of the synovial fluid (d). Scanning electron microscopy. Magnification: 1.000 x.

Fig. 14. Appearance of the surface of bovine joint cartilage 20th to 23rd week after fertilization. Dominating elevations (V) form pairs (1) or groups of four (2), marked off the environment by a common incision. Between the groups smoother spaces with a light waved surface are formed (h). Scanning electron microscopy. Magnification: 1.000 x.

Fig. 15. Appearance of the surface of bovine joint cartilage 20th to 23rd week after fertilization. Above level of the cartilage dominate several elevations (V), deposited in shallow depressions rounded by distinct incisions (V). In this case chondrocytes are deposited mostly single. Scanning electron microscopy. Magnification: 6.000 x.

Fig. 16. A detail of the surface of bovine joint cartilage 20th to 23rd week after fertilization. Single chondrocytes (V) are covered by a fine waved surface layer. Depressions between chondrocytes (h) with rests of the synovial fluid (d). Elevations (V) are rounded by an depression (—). Scanning electron microscopy. Magnification: 12.000 x.

#### Ultrastruktura bovinní kloubní chrupavky v 8. - 23. týdnu prenatálního vývoje

Byla studována ultrastruktura kloubní chrupavky kyčelního kloubu skotu v období 8.-23. týdne prenatálního vývoje pomocí transmisní a rastrovací elektronové mikroskopie. V období 8.-10. týdne po oplození má kloubní chrupavka vzhled velmi buněčné tkáně, ve které již lze podle submikroskopických znaků odlišit vrstvu povrchovou, střední a hlubokou. Chondrocyty povrchové vrstvy mají vzhled málo diferencovaných elementů. Ve střední vrstvě mají buňky okrouhlý tvar a obsahují větší množství organel a cilie. V hluboké vrstvě jsou chondroblasty menších rozměrů ve skupinách. Z organel se často vyskytují centrioly. Mezibuněčná hmota je na povrchu 3- 4  $\mu\text{m}$  mohutná, složená převážně z aperiodických fibril, ojedinělé fibrily kolagenní se vyskytují 1  $\mu\text{m}$  pod povrchem. V období 16.-23. týdne se mezi buňkami povrchové vrstvy objevují typy přechodné s výrazným Golgiho komplexem a centrioly. Chondroblasty střední vrstvy se do značné míry podobají buňkám dospělým. Mezibuněčná hmota dosahuje tloušťky 0,2 - 0,5  $\mu\text{m}$ . Aperiodické fibrily vytvářejí svazky jednak na povrchu, ale i hlouběji v mezibuněčné hmotě.



Ультраструктура суставного хряща тазобедренного  
сустава крупного рогатого скота в период  
8 - 23 недели утробного развития

Изучали ультраструктуру суставного хряща тазобедренного сустава крупного рогатого скота в период 8-23 недели утробного развития с помощью трансмиссионной и пастровой электронной микроскопии. На 8 - 10 неделе после оплодотворения суставной хрящ приобретает вид клеточной ткани, в которой по субмикроскопическим признакам можно уже различить поверхностный, средний и глубокий слои. Вид хондроцитов поверхностного слоя - мало дифференцированных элементов. Клетки среднего слоя круглой формы, содержат большое количество органелл и циллий. Хондробласты в глубоком слое размером поменьше, группами. Из числа органелл часто встречаются центриоли. Толщина межклеточной массы на поверхности 3 - 4 мкм; она состоит преимущественно из аperiodических фибрилл, отдельные клейдающие фибриллы встречаются 1 мкм под поверхностью. В период 16 - 23 недели между клетками поверхностного слоя появляются переходные типы выразительным комплексом Гольджи и центриоли. Хондробласты среднего слоя значительно похожи на взрослые клетки. Межклеточный слой достигает толщины 0,2 - 0,5 мкм. Аperiodические фибриллы образуют не только на поверхности, но и глубже, в межклеточной массе, пучки.

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