

SUBMICROSCOPIC STRUCTURE OF BOVINE ARTICULAR CARTILAGE IN
PRENATAL AND EARLY POSTNATAL PERIOD

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Abstract

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The bovine joint cartilage was studied in 14 subjects of both sexes from the 36th week of prenatal development till the end of the third month after birth using transmission and scanning electron microscopes. Between weeks 36 and 39, pericellular and intercellular matrix differentiated in the intercellular substance of the superficial layer of the cartilage. In the middle layer the fibrillar component was mostly represented by collagen fibrils. In the deep layer of the cartilage, these fibrils surrounded the cells and coursed irregularly in the intercellular matrix. From birth till the end of the third month of the cellular component and the intercellular substance of the joint cartilage acquired all substantial features of a differentiated tissue. Especially the fibrillar component of the superficial cartilage layer showed a typical arrangement in layers of collagen fibrils deposited in parallel with the surface of the cartilage. The filaments crossed at nearly right angles. After the third month only quantitative changes occurred consisting in the increased amount of fibrillar component as compared to the adult cartilage. In scanning electron microscope the groups of chondrocytes were separated from each other and from their surroundings by indentations.

Submicroscopic structure, cattle, cartilage layers, hip joint.

From the developmental, microscopic and functional point of view the joint represents a specially differentiated cavity originating in the blastema ground skeleton of mesenchyma, which is perfectly adapted to its function in the organism locomotion. The joint is formed partly by articular cartilage which covers bone ends, 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 articular cavity. Synovial fluid is produced by synovial membrane playing thus important role in cartilage by securing the nutrition of joint cartilage cells and by

decreasing friction of loaded cartilage areas.

It is generally known that joint cartilage originates from mesenchyma in the course of skeleton development as a component of cartilage blastema of bone ground which is rebuilt to the 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 the joint cavity (Bonucci 1967; Scherft 1972; Thyberg 1973; Anderson and Sajdera 1976; Ali 1976; Felix and Fleisch 1976; Hanooka 1976). Condensation process of mesenchyma 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 the femur begins, and in the 8th week joint cavity appears. Also Stirling's observations suggest it (1982). 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 the 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 may be, even the first movements (Glennister 1976). Owing to the process of ossification obtain chondrocytes their characteristic place, according to Godman et al. (1960), Gould et al. (1974) and Levitt and 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. A much greater attention is given to the study of chondrogenesis by rabbits (Pug-Rosado 1981) or birds and to its influence when using tissue cultures Green (1971), Scheck et al. (1975), Lamar et al. (1980), Bekoff and Klagsbrun (1983), Sato and Urist (1984) as well as stating explicit characteristics considering differentiation of chondrocytes with non differentiated cells (citation Solusch et al. 1982; Buckwalter and Rosenberg; 1983). Some articles are dealing only with partial problems as regulation mechanisms in relation to bone-cartilage (Nijwede 1982) or with incidence of cilia (Wilsmann and Fletcher 1978; Vidinov and Vasilev 1985) eventually other types of cartilage (Silbermann and Frommer 1974; Sanzone and Reith 1976). We are left in this branch to results of our own study.

Material 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 a transmission and scanning electron microscope. Material was taken mostly in a slaughterhouse.

Samples from femur head in direction to large trochanter were collected, in the early development stage then 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.

Samples for scanning electron microscopy were collected from joint cartilage quite close to place where sections for scanning electron

microscopy were cut out. Their size reached at least 5 x 5 mm.

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 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 of already mentioned sizes were also taken for the study of joint cartilage surface in a scanning electron microscope and before fixation left 15 minutes either in 1% hyaluronidase solution of 0,1 mg/ml concentration at 20°C or washed 3 x 15 minutes in saline. 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 being washed 3 x 15 min. in saline, then fixed in 10% formol or glutaraldehyde for 10 to 14 days. After fixation the tissue was dehydrated by method of critical point and coated with gold on a Blazer's coating apparatus. The surface was studied by a Stereoscan Cambridge Scanning Microscope.

Ultrastructure of joint cartilage in the period of 36th to 39th week after fertilization

The joint cartilage in this period undergoes further differential changes, so that towards the end of pregnancy it resembles in a considerable measure by its characteristics an adult cartilage. Whereas cells of the superficial layer keep the appearance similar to the preceding stage of development (H o r k ý 1985), cells of the middle and deep layer have all characteristics of differentiated chondrocytes. Significant changes in the intercellular substance, first of all in the middle and deep layer, occurred.

Submicroscopic structure of chondroblasts of the superficial layer

Chondroblasts of the superficial layer are spindle-shaped elongated cells, placed in the intercellular substance in 1 - 2 layers above one another reaching size of 10 - 12 µm x 3 - 5 µm.

Nucleus

Nucleus is of rod-like elongated shape, its appearance does not differ from nuclei of chondroblasts of the superficial layer of joint cartilage in the earlier developmental stage (Fig. 1).

Cytoplasm

There is a rather small amount of cytoplasm; it is mostly gathered on both cell ends and in shape of a narrow rim surrounds nucleus (Fig. 1).

Granular endoplasmic reticulum occurs in the cytoplasm as few mostly short cisternae or vesicles, situated near the nucleus and partly in areas of larger accumulation of cytoplasm. Its spaces are filled by a synthesized material of granular or filamentous appearance of medium electron density (Fig. 1).

A granular endoplasmic reticulum is present as single smooth vacuoles partly near the cell membrane, partly also among cisternae of granular endoplasmic reticulum (Fig. 1).

Golgi complex occupies only a small part of the cytoplasm and its structures are very little distinct.

There is a small number of mitochondria reaching about 0,3 to 0,5 μm in size.

We did not prove any lysosomes in the cytoplasm of chondroblasts of the superficial layer.

Cell membrane covers variously formed projections of cytoplasm; they are few in number and project at irregular distances. Some of them are branching and therefore it is possible to find their transverse sections near the cell. Projections reach about 0,5 to 0,8 μm in size and do not enter the intercellular matrix. Cell membrane forms solitary pinocytotic vesicles.

We did not observe any supporting formations or cilia.

Glycogen was present in the form of typically few granules dispersed in the cytoplasm or forming small clusters (Fig. 1).

Cytoplasmic fibrillar structures are less conspicuous having shape of diffusely dispersed filaments near nucleus.

Submicroscopic structure of chondroblasts of the middle layer.

Chondroblasts of the middle layer (Fig. 2) are oval to irregularly triangular cells, mostly separately deposited in the intercellular substance, in pairs only sporadically. They reach size of 8 - 9 x 5 μm and already in this stage of development they have an appearance of adult chondrocytes.

Nucleus

Most cells have an oval nucleus reaching about 5 x 3 μm in size (Fig. 2,3).

Nuclear envelope is formed by two membranes which surround in between a narrow perinuclear area and on some places form nuclear pores. To the outer membrane ribosomes are attached. We observed the connection of the outer membrane with cisternae of granular endoplasmic reticulum only exceptionally.

Chromatin forms karyosomes mostly deposited at the inner membrane of nuclear envelope. Some karyosomes are situated irregularly on the section of nucleus (Fig. 2,3). Zonula nucleum limitans about 0,5 - 0,8 μm thick is situated between chromatin and nuclear envelope.

Nucleolus is often found as well as nucleoli with nucleolonema which we always observed (Fig. 2).

Cytoplasm

As to the amount and equipment of organelles the cytoplasm does not differ from the cytoplasm of chondroblasts of the middle layer of previous stage of development.

When compared with the previous layer granular

e n d o p l a s m i c r e t i c u l u m is present in a larger amount. It is arranged either as vesicles or rather numerous flattened cisternae, often deposited in several layers above one another. The ends of cisternae widen rod-shaped and through suppressing vesicles arise from them coated also by ribosomes (Fig. 2,3). Inside of all the structures synthesized material of usual appearance occurs.

A g r a n u l a r e n d o p l a s m i c r e t i c u l u m is represented by only quite sporadic smooth vesicles.

G o l g i c o m p l e x is a distinct structure of the cytoplasm of chondroblasts of the middle layer (Fig. 2,3). It occupies a rather large part of the cytoplasm and in some cases is composed of a larger number of single Golgi fields. Vesicles of different size separate from dictyosomes, some of them gradually changing to transport vacuoles containing rather dense fibrillar or granular substance (Fig. 2,3).

M i t o c h o n d r i a are few in number. Being usually of oval shape they reach about 0,5 - 0,6 μm in size (Fig. 2).

L y s o s o m e s are rare in the cytoplasm of chondroblasts of this layer. Provided we found them they were mostly multivesicular corpuscles (Fig. 3).

We did not prove any **c e n t r i o l e s** or **c i l i a** in this layer.

C e l l m e m b r a n e. The cytoplasm on the whole surface makes short wide projections covered by cell membrane. Their length does not reach more than 0,8 μm and they are usually ending at the intercellular matrix (Fig. 9). Pinocytotic vesicles are only sporadic.

G l y c o g e n occurs in the same amount and arrangement as in the previous layer (Fig. 2,3).

C y t o p l a s m i c f i b r i l l a r f o r m a t i o n s occur in a small amount as thin bundles of filaments either near nucleus and Golgi complex or near the cell membrane (Fig. 2,3).

S u b m i c r o s c o p i c s t r u c t u r e o f c h o n d r o b l a s t s o f t h e d e e p l a y e r

Chondroblasts of the deep layer are cells of round half-moon-like or elongated, sometimes triangular shape reaching size of about 8 x 4-5 μm . They are deposited in the intercellular substance of the deep layer of joint cartilage either in groups or in short columns (Fig. 4).

N u c l e u s

In comparison with chondroblasts of the middle layer is nucleus smaller. Its appearance and structure does not differ from nuclei of cells of the previous layer.

C y t o p l a s m

In relation to nucleus is the cytoplasm large and as to organelles, does not differ from chondroblasts of the deep layer of the previous stage.

G r a n u l a r e n d o p l a s m i c r e t i c u l u m is in a great majority formed of dilated capsules, only a smaller part has appearance of narrow flattened cisternae. Between both forms numerous interconnections are formed so that individual layers are connected with one another (Fig. 4). Widened cisternae and vacuoles separate cytoplasm

into small areas, and membranes of reticulum lean then close on other organelles, for example mitochondria (Fig. 4).

A granular endoplasmic reticulum is arranged in the same way as in the previous layer.

Golgi complex is in contrast to the cells of the middle layer little distinct. It is usually formed by one dictyosome and mostly by small capsules (Fig. 4).

Mitochondria have the same structure as in the middle layer, only quite often we encounter their elongated forms (Fig. 4).

Lysosomes occur in the cytoplasm of chondroblasts of this layer rather often. They appear as dark corpuscles reaching size of 0,3 - 0,5 μm (Fig. 4).

Centrioles and cilia were not observed.

Cell membrane. Cytoplasm on the whole periphery of cells forms numerous short projections covered by cell membrane. Only exceptionally projections of 0,5 - 0,6 μm occur, found mostly in areas where the intercellular matrix approaches the cell membrane (Fig. 4). Should the cells be of elongated or irregularly star-like shape, the cell membrane is arranged in the same way as by chondroblasts of the deep layer of the previous stage.

Glycogen is a regular component in the cytoplasm of cells of the deep layer (Fig. 4). Small clusters of its granules are irregularly dispersed in the cytoplasm among other organelles.

Cytoplasmic fibrillar structures are a very discrete part of the cytoplasm of chondroblasts of this layer. When observed, they did not differ by their arrangement and deposition from cells of the middle layer.

Arrangement of the intercellular substance of the joint cartilage

In the period of 36th to 39th week after fertilization, the largest changes occurred in the composition of the intercellular substance in the middle and deep layer, whereas the superficial layer changed only slightly.

In contrast to the previous developmental period, in the upper layer specialized spaces (pericellular matrix) around the cells arise. It is mostly formed by ground amorphous substance and aperiodic fibrils. Thus formed space is well developed especially in areas which incline to the surface of cartilage, whereas in the direction to deeper layers near cell membrane typical collagen fibrils penetrate (Fig. 1). The proper peripheral layer is build in the same way as in the previous stage.

In the middle layer there comes to a considerable increase of fibrillar component (Fig. 2,3) represented both by typical collagen fibrils and a small amount of aperiodic fibrils. The pericellular matrix is already distinctly formed around greater part of the cellular surface, and, as a rule, collagen fibrils get in contact with the cell membrane only on one pole of the cell. Collagen fibrils in the intercellular matrix are irregularly arranged and we can find them on elongated as well as transverse sections.

The area of pericellular matrix is most distinctly differentiated in the deep layer (Fig. 4) where the attached areas between column-like deposited chondroblasts are filled by this matrix, whereas on the averted areas this district is narrow or even missing, so that the intercellular matrix analogically to the middle layer adjoins with the cell membrane of chondroblasts of the deep layer. In the intercellular matrix, the fibrillar component forms an irregular and dense mesh-work.

Appearance of joint cartilage in scanning electron microscope

Appearance of the bovine joint cartilage in the 8th to 10th week of prenatal development is similar to human cartilage in the same period (H o r k ý 1984). We quoted first changes during the 20th to 23th week, when its surface appearance begins to form slightly differently (H o r k ý 1985).

The surface of the joint cartilage is rather uneven in this period. Several elevations of elongated spindle-like shape are prominent above its surface. In some cases the pairs of elevations are surrounded by furrows. Formations resembling the cipher 8 or rarely even in groups of 4 occur, separated from their surroundings by a common furrow.

The process of "immersing" and separating of individual elevations or of their pairs from the surrounding area continues and is important during the 36th to 39th week of development (Fig. 5). One can see that numerous elevations with a distinct spindle-like shape in the previous stage are becoming round and their amount decreases. At the same time they draw nearer to each other forming areas of accumulation of elevations. Among such districts areas without prominences with a fine wrinkled surface may be found. Surrounding furrows are deep and elevations are placed in thus formed grooves (Fig. 6).

Submicroscopic structures of bovine joint cartilage in early postnatal period

In the second part, we deal with the description differentiation changes in the period, when the joint cartilage of postnatal period resembles in main features the cartilage of adult individuals. Comparison of our results reveals that in cattle it occurs at about 3 months of age. The joint cartilage in this period the appearance of differentiated tissue which can be, according to the appearance of cells and the intercellular substance, divided into three layers. Cells of all the layers have already signs of typical chondrocytes; also the arrangement of intercellular substance resembles that of the adult cartilage.

Submicroscopic structure of chondrocytes of the superficial layer

Chondrocytes of the superficial layer are spindle-shaped elongated cells, usually deposited in two rows above one another and oriented in parallel with the surface of cartilage. They reach size up to $12 \times 2-3 \mu\text{m}$.

Nucleus

The nucleus is of a very elongated rod-like shape, only sporadically oval (Fig. 7).

Nuclear envelope is formed by two membranes which make little numerous nuclear pores. The perinuclear space is narrow, the interstage of membrane envelope to structure of granular endoplasmic reticulum was not observed. Nuclear envelope is smooth, only on poles of nuclear is projecting against karyoplasm into wide shallow invaginations (Fig. 7).

C h r o m a t i n is partly condensed into karyosomes deposited at the inner membrane of nuclear envelope, partly is forming one large cluster partitioning nucleus (Fig 7). Zonula nucleum limitans is distinctly formed in a shape of strip of middle osmiophilic fine granulated substance between chromatin and inner membrane of nuclear envelope, reaching size of $0,1 \mu\text{m}$.

N u c l e o l u s. We observed nucleolus rarely on sections of nucleus of chondrocytes of the superficial layer.

C y t o p l a s m

There is a larger amount of cytoplasm in comparison with chondroblasts of the superficial layer of previous stage, containing also more organelles.

G r a n u l a r e n d o p l a s m i c r e t i c u l u m is formed by numerous mostly flattened cisternae, in some cases deposited above one another (Fig. 7,8). In some chondrocytes dilated structures of reticulum, resembling vacuoles prevail. Flattened cisternae as well as vacuoles are filled with synthesized material of common appearance.

A g r a n u l a r e n d o p l a s m i c r e t i c u l u m occurs only sporadically. Some of the large vacuoles, enveloped by a smooth membrane are formed by folds of the nuclear membrane.

G o l g i c o m p l e x is strongly developed and laid out into several fields. From its dictyosomes a number of mostly small Golgi vacuoles (Fig 7,8) separate, as well as single vacuoles of about $0,5 \mu\text{m}$ with dense granulated substance, which represent transport vacuoles.

M i t o c h o n d r i a reach the size of $0,5 - 1 \mu\text{m}$, in comparison with the previous stage we often observed their elongated forms (Fig. 8). Their matrix was often cleared.

L y s o s o m e s as well as centrioles and cilia were not observed in chondrocytes of this layer.

C e l l m e m b r a n e. The cytoplasm is not arranged in the same manner on the whole surface. On the area leaning to the surface of cartilage it is more or less smooth or only slightly waved (Fig. 7,8). Towards the middle layer it makes rather numerous often winding up and differently long projections reaching the intercellular substance, where their transverse or oblique cuts can be seen (Fig. 8). Collagen fibrils are surrounded by projections that nearly close them (Fig. 8).

G l y c o g e n is regularly present in the cytoplasm of chondrocytes of this layer in form of sporadic small clusters of granules, deposited irregularly among organelles (Fig. 7,8).

C y t o p l a s m i c f i b r i l l a r s t r u c t u r e s are forming strong bundles and occupy a great part of the cytoplasm on the periphery of the cell. We observed them near nucleus as fine bundles of fibrils (Fig. 7,8).

S u b m i c r o s c o p i c s t r u c t u r e o f c h o n d r o c y t e s o f t h e m i d d l e l a y e r

Chondrocytes of the middle layer are oval to triangularly rounded cells reaching a size of $8-9 \times 6 \mu\text{m}$. They are deposited in the intercellular substance single or in pairs.

N u c l e u s

In most cases is the nucleus rounded or slightly oval on section. Its size, appearance and structure does not differ from nuclei of chondrocytes of the middle layer of previous stage or of those of an adult cartilage (Fig. 9).

C y t o p l a s m

Chondrocytes contain a larger amount of cytoplasm as against fetal cells. The cytoplasm is accumulated at one pole and the nucleus at the opposite pole of the cell (Fig. 9).

Granular endoplasmic reticulum is formed by numerous wide dilated cisternae interconnected with one another and by vesicles which separate themselves from bulb-shaped widened cisternae (Fig. 9). All the inner spaces of reticulum are filled by synthesized material.

Agranular endoplasmic reticulum occurs only sporadically as single vacuoles, mostly deposited near the cell membrane.

Golgi complex is well developed and in the cytoplasm of chondrocytes of this layer occupies a large portion of the cytoplasm (Fig. 9). From its cisternae a large amount of vacuoles of different sizes including transport vacuoles reaching up to $1\ \mu\text{m}$ (Fig. 9) arise through splitting.

Two sizes of mitochondria were observed: a smaller one (about $0,5 - 0,6\ \mu\text{m}$) with usual arrangement of cristae and appearance of matrix, and a larger one ($1,5 - 1,8\ \mu\text{m}$) with damaged cristae and clear matrix (Fig. 9). It seems that in the second case the mitochondria were disintegrating.

Lysosomes occur in the cytoplasm of chondrocytes of this layer only sporadically as well as centrioles.

Cell membrane. The cytoplasm on the whole surface forms projection covered by cell membrane. Their length does not exceed more than $1\ \mu\text{m}$, width $0,4\ \mu\text{m}$. Cytoplasmic projection bridge a somewhat narrow space of pericellular matrix ending on at the border of pericellular and intercellular matrix (Fig. 9). In some areas collagen fibrils attach closely to the cell membrane.

Glycogen occurs in the cytoplasm of chondrocytes of the middle layer in a larger amount than in cells of the superficial layer (Fig. 9). Its granules form small clusters irregularly dispersed in the cytoplasm and near the pole of the cell usually one larger area also, where a part of glycogen can be washed out (Fig. 9).

Cytoplasmic fibrillar structures form bundles of filaments, which can be observed on the elongated as well as transverse section often near the cell membrane (Fig. 9).

S u b m i c r o s c o p i c s t r u c t u r e o f c h o n d r o c y t e s o f t h e d e e p l a y e r

Chondrocytes of the deep layer are mostly cells of irregular prismatic shape size $6 \times 6\ \mu\text{m}$, placed in the intercellular substance usually in pairs in one capsule (Fig. 10).

N u c l e u s

The nucleus is of irregular shape (about $5 \times 3 \mu\text{m}$).

N u c l e a r e n v e l o p e is of standard structure, partly nearly smooth, at places forming wide shallow indentations (Fig. 10).

N u c l e o l u s is small, of reticular type, surrounded by perinuclear chromatin (Fig. 10).

C y t o p l a s m

The cytoplasm is arranged in the same manner and contains organelles in the same amount as the cytoplasm of chondroblasts of the deep layer of joint cartilage in the previous developmental period. The difference at this stage (Fig. 10) is the striking occurrence of intracytoplasmic filaments, at this stage of development which occupy rather large areas of the cytoplasm and in form of thick bundles surround the nucleus.

A r r a n g e m e n t o f i n t e r c e l l u l a r s u b s t a n c e

The intercellular substance of joint cartilage of 3 months old individual differs from the adult cartilage first of all by its thickness and arrangement of fibrillar components of the superficial layer. The peripheral layer proper is covered by a nearly continuous chondral membrane of the same appearance as is the human cartilage. Under the chondral membrane (Fig. 8) is an almost $1 \mu\text{m}$ thick layer of intercellular substance, where the fibrillar component is represented by a mesh-work of mainly collagen fibrils and already at this stage one can follow the deposition of fibrils crossing each other at right angles as a basis of future layer arrangement of the fibrillar component of the superficial layer in an adult cartilage. The pericellular matrix is distinctly formed predominantly on the side which turns to the joint cavity whereas this area on the opposite side of chondrocytes is not formed and the collagen fibrils are in close contact with the cell membrane (Fig. 8).

In the middle layer is the pericellular matrix formed on the whole periphery of chondrocytes (Fig. 9). Only exceptionally collagen fibrils besides aperiodic fibrils reach into this area. In the surrounding intercellular matrix collagen fibrils form a distinctly thicker layer (about $1 - 1,5 \mu\text{m}$ wide) which surrounds the chondrocyte. Behind this area, the collagen fibrils are mesh-work-arranged (Fig. 9).

In the deep layer pairs of chondrocytes are placed in one common capsule (Fig. 10) and between the cells no septum is formed. Pericellular matrix is very distinct and is composed of aperiodic fibrils and amorphous substance. It reaches a size of $1 - 1,5 \mu\text{m}$; its part are also sections through the cytoplasmic projections of chondrocytes. Collagen fibrils of the intercellular matrix surround lacunae of chondrocytes. In this layer, $2 \mu\text{m}$ thick, collagen fibrils are arranged in parallel with its surface (Fig. 10). Behind this layer bundles of collagen fibrils arranged irregularly in the amorphous layer can be seen.

Appearance of joint cartilage in scanning electron microscope

The surface of joint cartilage 3rd month after birth differs only little from the adult cartilage and is formed in two manners. From place to place (Fig. 11,12) wide shallow grooves are formed, on their bottom elevations equal to those of the previous stage are situated. These round to slightly elongated formations are surrounded by a rather deep incision and a mound from the near-by prominences (Fig. 12). Between thus formed districts large areas occur, the surface of which is wrinkled by fine and coarse crest-like lines coursing in parallel (Fig. 11,12). The fine crests may reach also into the area of elevations surrounding humps as well as onto their surface.

Discussion

When studying the ultrastructure of bovine joint cartilage we stated already before (H o r k ý 1983) that in the world literature no sufficient attention had been to this problem. There is a similar situation as to the development of joint cartilage of this animal species, for only a few articles were published dealing with the morphology of joint cartilage during development. But in no case was described its development on submicroscopic level in a more or less continuous periods since the stage when joint cavity is being formed (that is about the 8th week after ovulation) till the time after birth, when its structure, except for certain quantitative signs, agrees on the whole with the structure of an adult cartilage. From this point of view our findings are original. Articles dealing with this problem were published by C a m é r o n and R o b i n s o n (1958) who but only compared matrix formation of epiphyseal and articular cartilage. B r o w e r and H s u (1969) and S t o c k w e l l (1971) observed vessel supply of joint cartilage and diffusion possibility of substances from the cartilage canals into the intercellular substance in different mammalian species, or thickness of joint cartilage and density of chondrocytes (S t o c k w e l l 1971 a). We are therefore going to confront results of our own study with the findings on joint cartilage in other animals including man as far as they should have common validity.

A conspicuous sign of chondroblasts of the superficial layer during its period of development is the manner of their deposition. It is generally reported that this orientation is conditioned by pressure forces which affect the cartilage. This is one of possible reasons, but G o u l d et al. (1974) pronounced an interesting statement, according to which this orientation is a product of the formation of intercellular substance. Chondroblasts are thus compressed by this substance produced in the area of chondrification centre. While during the prenatal development the pressure forces are restricted only to pressure development by muscles, one cannot omit this point of view even if no cause is found why the stated pressure of the intercellular substance does not make itself on felt chondroblasts of the undersuperficial layers. It is probable that on the final arrangement of chondrocytes in the period of development both factors participate together and because of these forces also the course and arrangement of filaments of the intercellular substance changes, which together with the amorphous component are the bearers of mechanical properties of joint cartilage, whereas chondrocytes play the key role in their synthesis (B a l a s z et al. 1966; I m u r a 1984; K l a m f e l d t

1984). Chondrocytes of the superficial layer behold this arrangement also in adult age.

During the prenatal period chondroblasts of the superficial layer have appearance of little differentiated cells originating from mesenchyma for example fibroblasts. They keep this appearance practically until the 3rd month post partum, when their cytoplasm differs only very little from chondrocytes of adult cartilage. They change their shape, but first of all organelles occur in the cytoplasm in an amount which is characteristic for differentiated cells and being of specific structure are these intracytoplasmic filaments of vimentin type (G h a d i a l l y 1983).

Zonula nucleum limitans is formed (H o r k ý 1984 b) in the nucleus during the whole period of development; its thickness, in agreement with literary data (O r y s c h a k et al. 1974) changes with increasing age.

In the middle layer differentiation of chondroblasts occurs already in a very early period, in our material from the 8th week of development (H o r k ý 1985). Chondroblasts of this layer differ during the whole period from cells of the superficial and deep layer by a greater amount of cytoplasm, a distinct granular endoplasmic reticulum, Golgi complex, a greater amount of elongated mitochondria and by a frequent occurrence of transport vacuoles. These signs are, according to F r e e m a n (1973) typical for chondrocytes with an increased synthesis of components of the intercellular substance. Also data from S t o c k w e l l and M e a c h i m (1979), who followed the level of synthesis of proteoglycans as well as mitosis, agree with the observation that this layer is, from the metabolic point of view, important also for growth of the cartilage. We also succeeded to observe mitosis in our material; its finding in a cartilage is generally infrequent (G h a d i a l l y 1983). Similarly to S t o c k w e l l (1971 a), our material from the 8th to 10th week revealed in the intercellular substance of the middle layer blood capillaries and erythrocytes deposited partly among chondroblasts, partly phagocytized. In the later period capillaries did not occur in the joint cartilage, which agrees with observations of G a r d n e r and G r a y (1970).

In the later period of development (16th to 23th week after fertilization) appear in the superficial layer besides chondroblasts also temporary types of cells which we reported already before (H o r k ý 1983, 1985), which have already some characteristic typical of chondroblasts of the middle layer. It is first of all the conspicuous Golgi complex and often occurring centrioles. Chondroblasts of the middle layer do not differ by their appearance from the previous stage. The fact, that these are cells very active from the point of view of production of the intercellular substance is clear from our finding of penetration of collagen fibrils through the cell membrane into the intercellular space. We found this phenomenon only in a developing synovial membrane (H o r k ý 1984 a); it was not described in chondrocytes.

Differentiation changes of chondroblasts as well as of the intercellular substance are distinctly reflexing in the period of 36th to 39th week after fertilization. Whereas cells of the superficial layer behold appearance of less differentiation chondroblasts, they do not much differ in the middle and deep layer from adult chondrocytes (H o r k ý 1983).

The intercellular substance similarly to cells of joint cartilage undergoes a series of quantitative and qualitative changes during the prenatal period of development. It is formed in the early periods by an abundant amorphous substance with prevailing aperiodic fibrils. From the standpoint of joint function the main role plays the superficial layer. During the period of 8th to 10th week of development the fibrillar component on the periphery of joint cartilage with the cavity of joint is composed only of aperiodic fibrils; about

1 μm under the surface sporadic collagen fibrils appear. Chondrosynovial membrane is not formed (W o l f 1969, 1975) so that diffusion of substance from the joint cavity is easy (M a r o u d a s and B u l l o u g h 1968; M a r o u d a s 1973) and 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 joint cartilage between 16th to 23rd week after fertilization, when on the periphery with joint cavity sections of 0,2 - 0,5 μm thick, formed of bundles of fine filaments, begin to shape (H o r k ý 1985). Thus formed surface can also be found during the 36th to 39th week after fertilization (H o r k ý 1983). In this period also the pericellular and intercellular matrix differs in all layers of the cartilage.

Whereas cells of the middle and deep layer reached already during the prenatal period characteristic signs of differentiated cells, in the superficial layer begin these changes the 3rd month post partum. On the surface of the cartilage the peripheral layer is formed by a nearly continuous chondral membrane of typical appearance of adult cartilage (H o r k ý 1983). In the superficial layer collagen fibrils begin to arrange themselves in layers which cross in a characteristic way, although their size does not reach the size of layers of the adult cartilage (H o r k ý 1983, 1984 b). Its final definitive formation, consisting only of quantitative changes occur in the following period. It can be stated that during the 3rd month of postnatal life the bovine joint cartilage acquires all characteristic features of the adult tissue.

Submikroskopická struktura kloubní chrupavky skotu v prenatálním a časném postnatálním období

Transmisním a restrovacím elektronovým mikroskopem byla studována kloubní chrupavka 14 jedinců skotu obojího pohlaví v období 36. týdne po oplození až 3 měs. po narození. V období 36. - 39. týdne po oplození dochází v mezibuněčné hmotě povrchové vrstvy chrupavky k rozlišení na pericelulární a intercelulární matrix. Ve střední vrstvě je vláknitá složka reprezentována převážně kolagenními fibrilami. Tyto fibrily ve vrstvě hluboké jednak opřádají buňky, jednak probíhají v intercelulární matrix neuspořádaně. V období od narození do 3. měsíce získává kloubní chrupavka všechny podstatné znaky diferencované tkáně jak ve složce buněčné, tak v mezibuněčné hmotě. Zejména vláknitá složka povrchové vrstvy má typické uspořádání v podobě vrstev kolagenních fibril, uložených paralelně s povrchem. Vlákna se v jednotlivých vrstvách kříží téměř v pravých úhlech. Ve srovnání s dospělou chrupavkou dochází po 3. měsíci p.p. pouze ke kvantitativním změnám, které spočívají ve zmožení vláknité složky. V rastrovacím elektronovém mikroskopu se v období 3. měs. po narození objevují skupiny chondrocytů v mělkých širokých prohlubních; jednotlivé chondrocyty jsou navzájem i od okolí ohraničeny zářezy.

Субмикроскопическая структура суставного хряща крупного рогатого скота в утробный и послеродовый период

Трансмисионным и расровым электронным микроскопом изучали суставный хрящ крупного рогатого скота в период 36 недель после оплодотворения до 3 месяцев после рождения. Отбор материа-

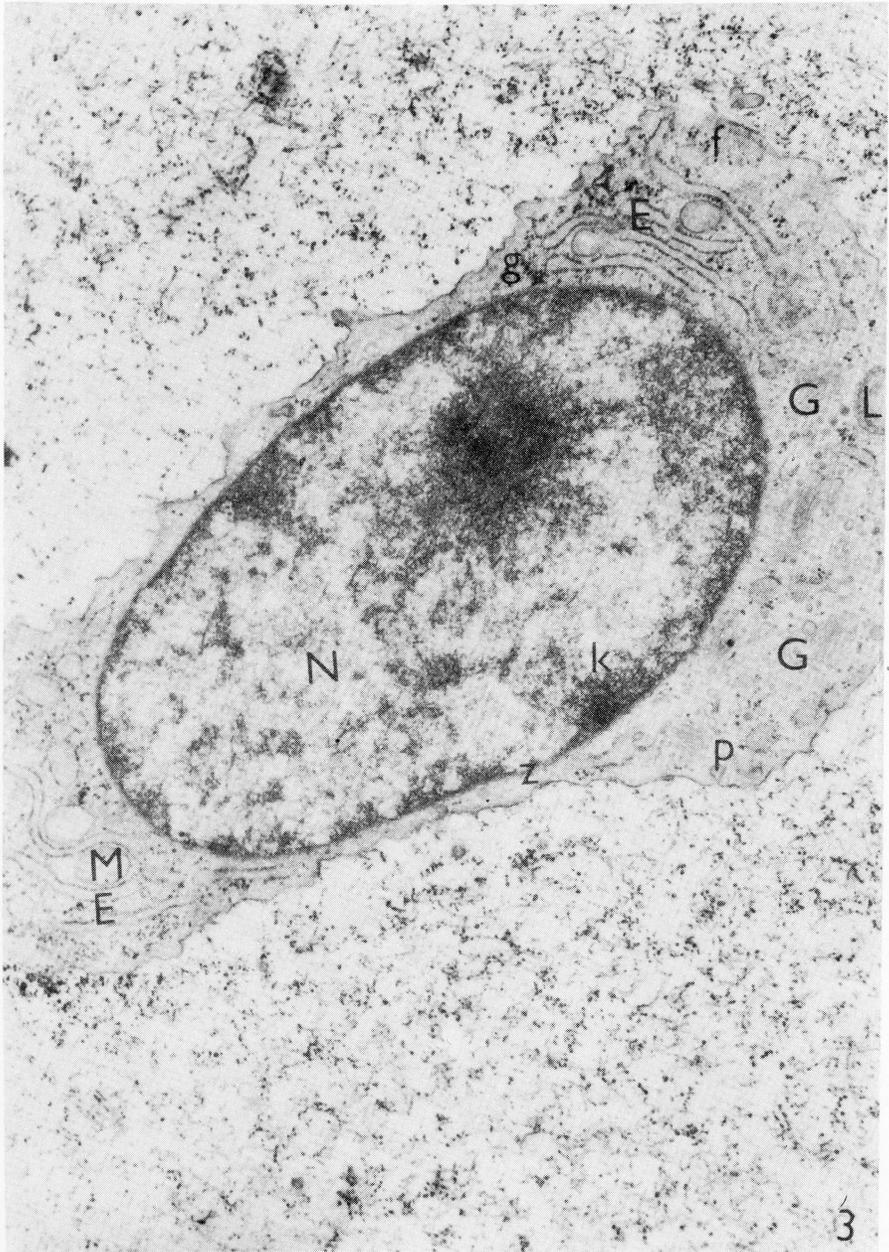
ла проводили с головки тазобедренного сустава и обрабатывали обычным способом. В период 36 - 39 недели после оплодотворения в межклеточном поверхностном слое наступает различие перичеллюлярных и интерчеллюлярных ростковых слоев. Волокнистая часть среднего слоя представлена преимущественно коллагеновыми фибриллами. Данные фибриллы в глубоком слое окружают клетки, а также в беспорядке проходят в интерчеллюлярном ростковом слое. В период со дня пождения до 3 месяцев суставный хрящ приобретает все существенные признаки дифференцированной ткани не только в клеточной части, но и в межклеточной массе. В особенности волокнистая составляющая поверхностного слоя отличается характерным слоистым расположением клейдающих фибрилл, находящихся параллельно с поверхностью. Направление волокон в отдельных перекрещивается почти под прямым углом. В последующий период происходят по сравнению с созревшим хрящом лишь количественные изменения, заключающиеся в умножении волокнистой компоненты. В растровом электронном микроскопе с 3 месяца после рождения появляются группы хондроцитов в мелких и широких углублениях. Отдельные хондроциты взаимно и от окружения ограничены надрезками.

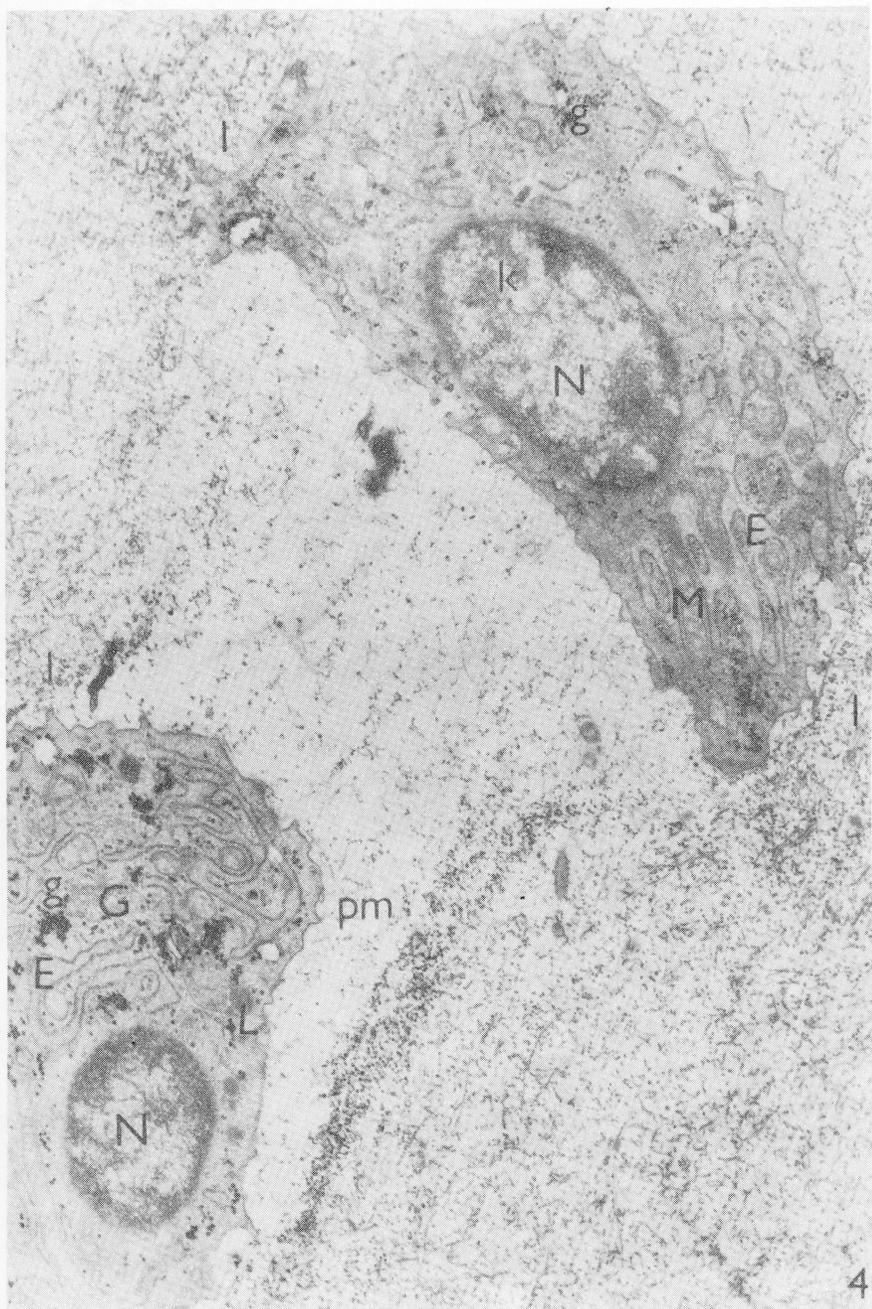
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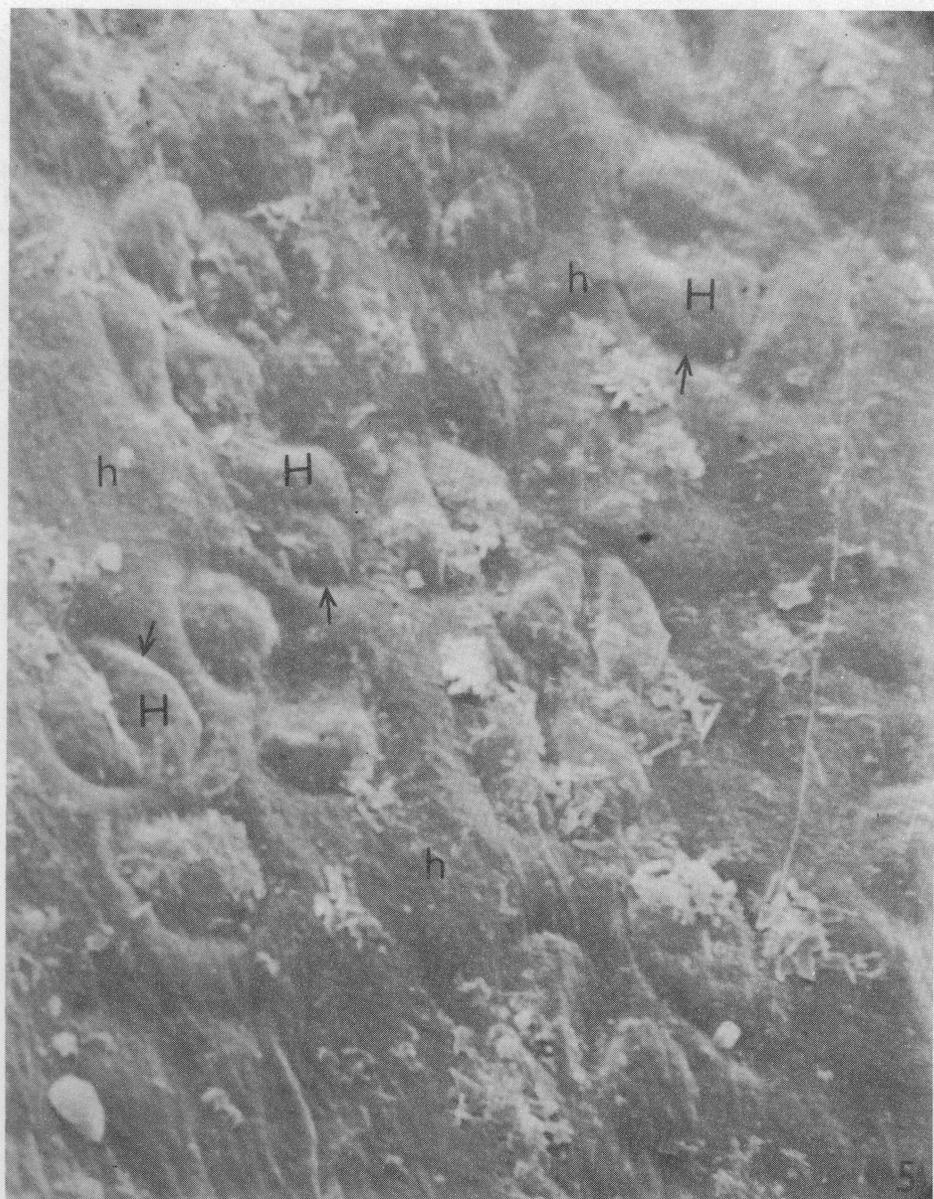
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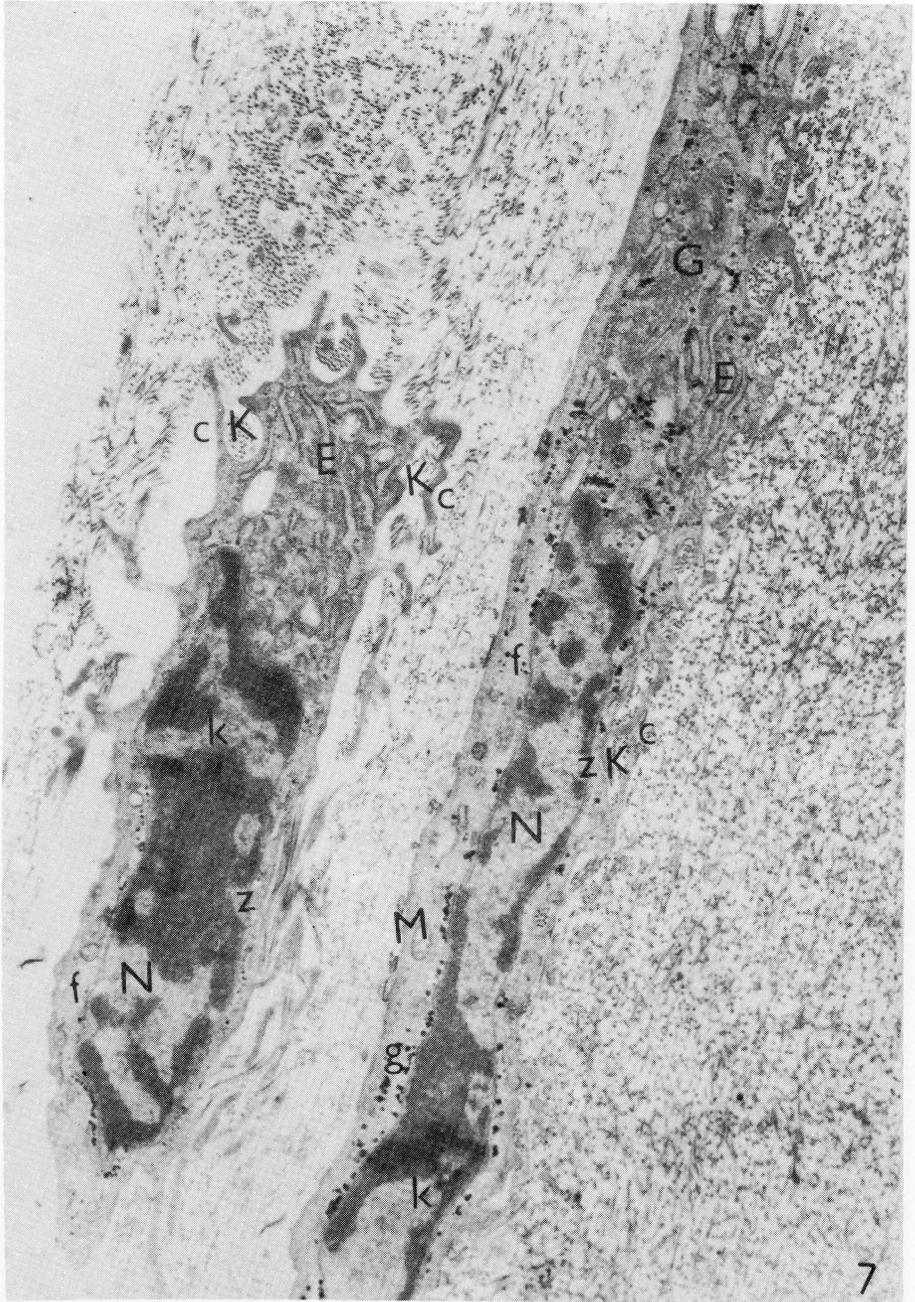


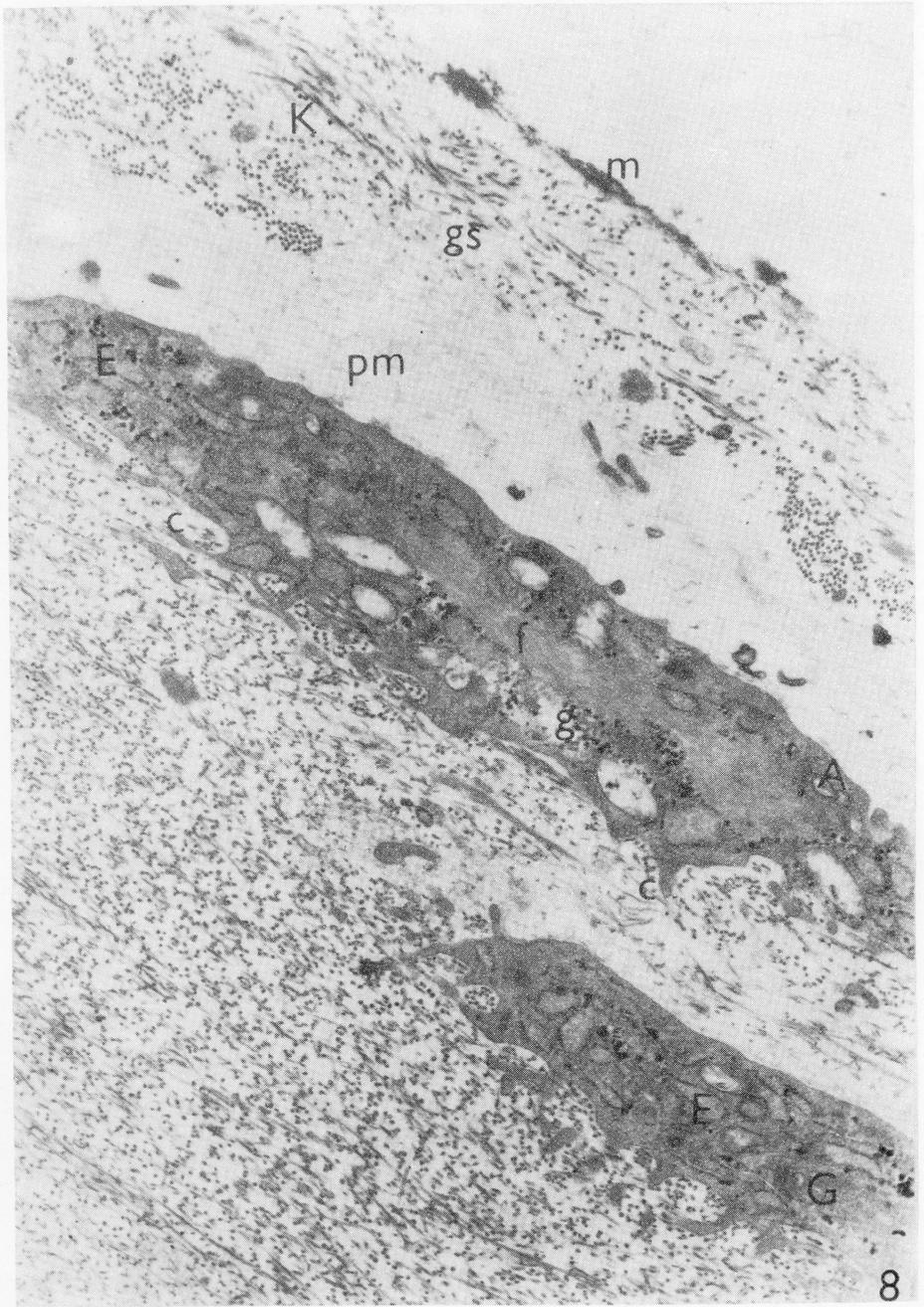


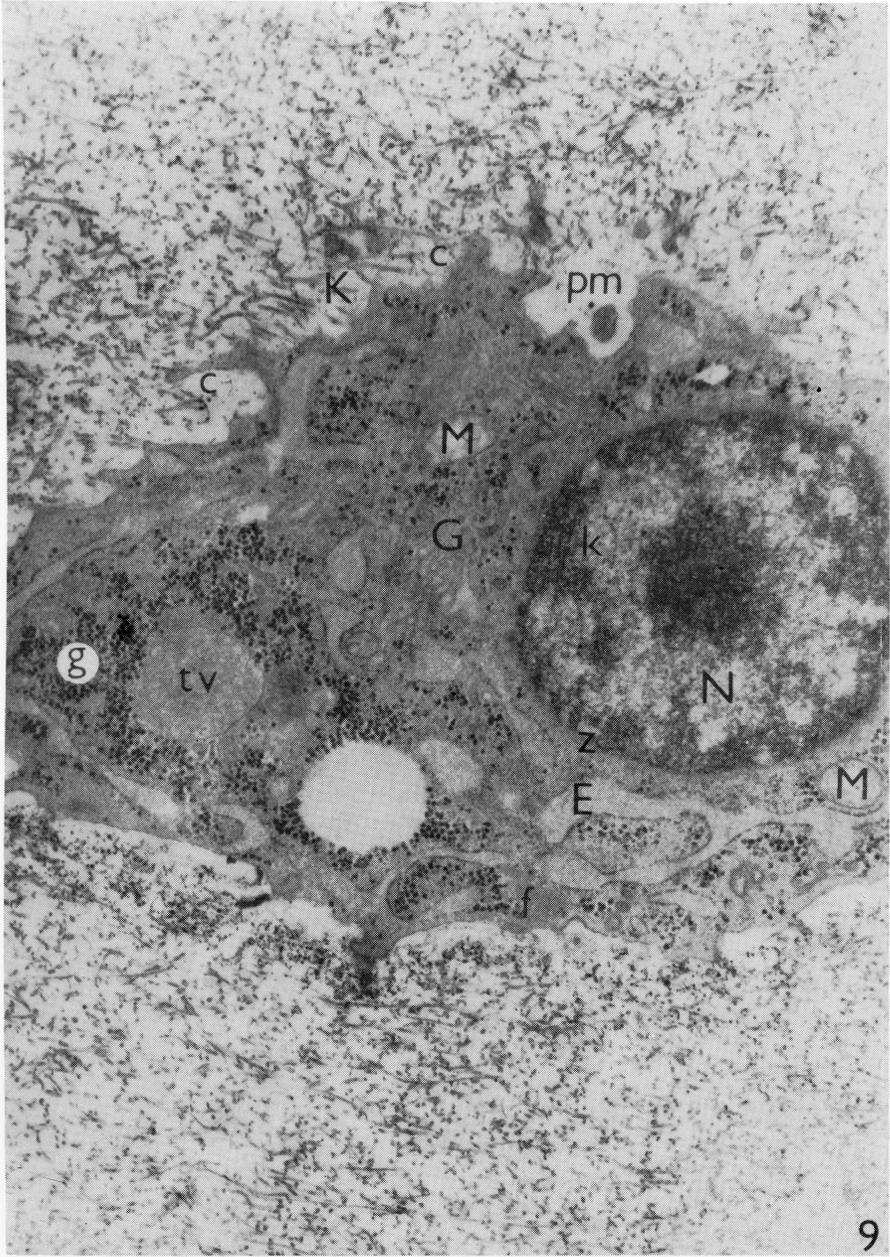


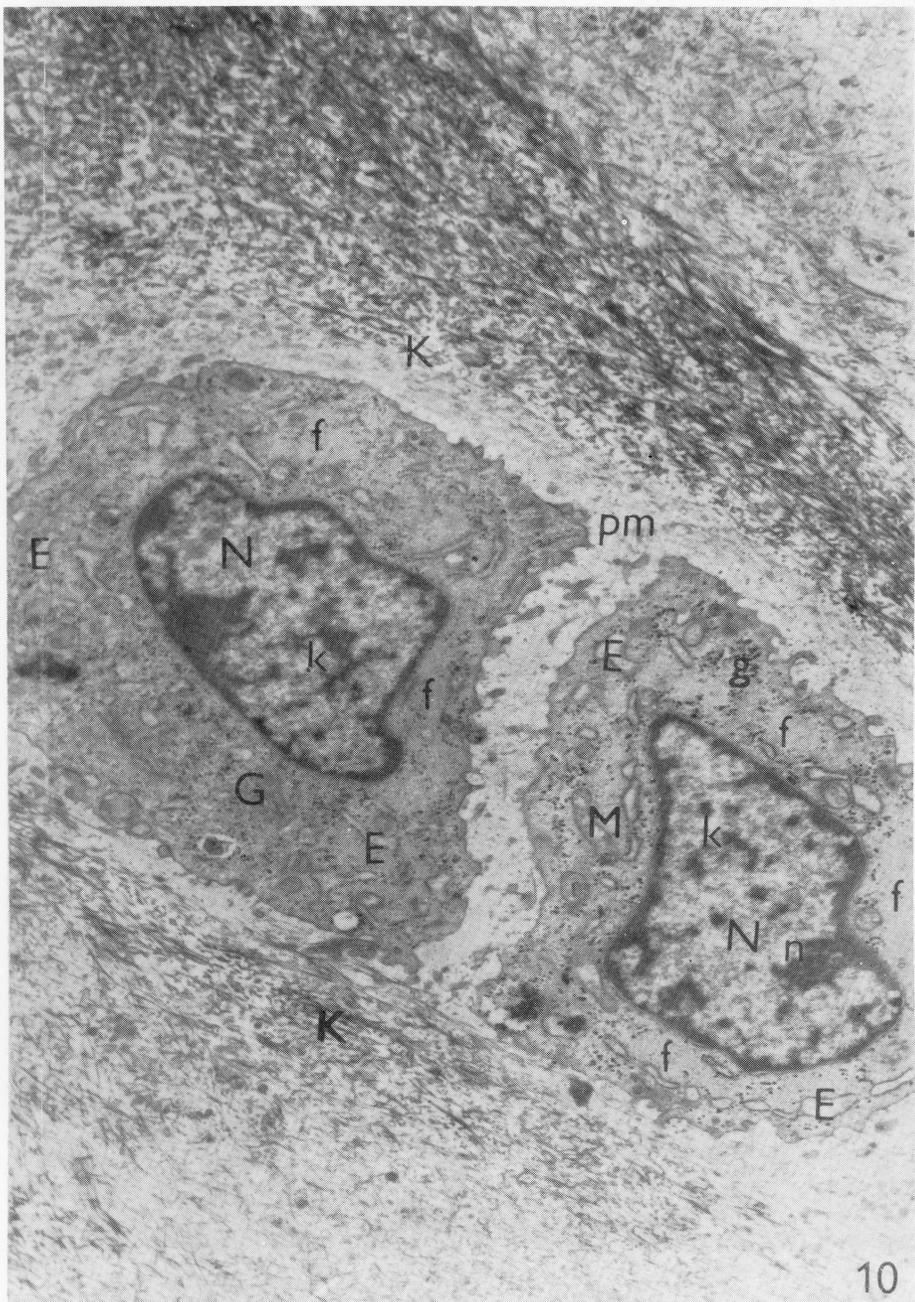
















I.

- Fig. 1. Part of the superficial layer of bovine joint cartilage 36th to 39th week after fertilization. Nucleus of chondroblast (N), nucleolus (n), karyosomes (k) and a thin zonula nucleum limitans (z). Granular endoplasmic reticulum (E), agranular endoplasmic reticulum (A), granules of glycogen (g), intracytoplasmic filaments in fine bundles (f). On the surface of cartilage bundles of fine fibrillar material are found (fb) and in the ground amorphous substance are besides aperiodic also collagen fibrils (K) projecting to the cell membrane. Magnification, x 21.400.
- Fig. 2. Chondroblast of the middle layer of bovine joint cartilage 36th to 39th week after fertilization. Nucleus (N), karyosomes (k), nucleolus of reticular type (n), zonula nucleum limitans (z). Dilated cisternae of granular endoplasmic reticulum (E) with synthesized material, the distinct Golgi complex (G), mitochondria (M), lysosomes (L), small clusters of glycogen (g), discrete bundles of filaments (f). Short projections of the cytoplasm (c) end close to intercellular matrix (I), where also collagen fibrils may be found (K). Magnification, x 20.000.
- Fig. 3. Chondroblast of the middle layer of bovine joint cartilage 36th to 39th week after fertilization. Nucleus (N) with karyosomes (k) and a thin zonula nucleum limitans (z). Cisternae of granular endoplasmic reticulum (E), a distinct Golgi complex (G), mitochondria (M), lysosomes (L), glycogen (g), intracytoplasmic filaments (f), pinocytotic vesicles (p). The cell membrane and the intercellular substance show the same arrangement as in previous figure. Magnification, x 16.000.
- Fig. 4. Pairs of chondroblasts of the deep layer of bovine joint cartilage 36th to 39th week after fertilization. Nucleus (N) with karyosomes (k). Dilated cisternae of the granular endoplasmic reticulum (E), mitochondria (M), an inexpressive Golgi complex (G), lysosomes (L), numerous small clusters of glycogen (g). The intercellular substance is differentiated to pericellular (pm) and intercellular matrix which is in contact with a part of the cell surface (I). Magnification, x 24.000.
- Fig. 5. Appearance of bovine joint cartilage 36th to 39th week after fertilization. Groups of rounded elevations are formed (H), only slightly elevating over the surface. Surrounding incision (→) is deeper than in the previous stage and areas between the groups are widening (h). Scanning electron microscope. Magnification, x 2.200.
- Fig. 6. Detail of the surface of bovine joint cartilage 36th to 39th week after fertilization. Groups of elevations (N) rimmed by an incision (→). Light waved surface on spaces between groups and on the elevations. Scanning electron microscope. Magnification, x 4.200.

Fig. 7. The superficial layer of bovine joint cartilage 3 months after birth. Nuclei of chondrocytes (N) with large karyosomes (k), zonula nucleus limitans (z). Numerous cisternae of granular endoplasmic reticulum (E), Golgi complex (G), mitochondria (M) with a clear matrix, here and there granules of glycogen (g), bundles of intracytoplasmic filaments (f). Collagen fibrils (K) are deposited near the cell membrane between projections of the cytoplasm (c). Magnification, x 6.000.

Fig. 8. The superficial layer of bovine joint cartilage 3 months after birth. In the cytoplasm of chondrocytes dilated cisternae of granular endoplasmic reticulum (E), agranular endoplasmic reticulum (A), Golgi complex (G), glycogen (g), bundles of intracytoplasmic filaments (f) occupy a large area. On the surface of cartilage the chondral membrane (m) and in the ground amorphous substance (gs) collagen fibrils cross under right angles. Pericellular matrix (pm) is formed in direction to the surface. Cytoplasm on the upturned side runs into projections (c) reaching intercellular matrix. Magnification, x 6.000.

Fig. 9. Part of the middle layer of bovine joint cartilage 3 months after birth. Nucleus of chondrocytes (N), karyosomes (k), zonula nucleus limitans (z). Wide dilated cisternae of granular endoplasmic reticulum (E), Golgi complex (G) is deposited into several fields, transport vacuoles (tv), mitochondria (M). Glycogen (g) forms large clusters, bundles of intracytoplasmic filaments (f). Pericellular matrix (pm) is narrow, intercellular collagen fibrils in matrix (K) in contact with projections of the cytoplasm (c). Magnification, x 16.000.

Fig. 10. The deep layer of bovine joint cartilage 3 months after birth. Pairs of chondrocytes in a common lacuna (pm). Nuclei (N), karyosomes (k), nucleolus (n). Cisternae of granular endoplasmic reticulum (E), mitochondria (M), Golgi complex (G), glycogen (g), thick bundles of intracytoplasmic filaments (f). Collagen fibrils (K) are concentrically arranged round pericellular matrix. Magnification, x 6.000.

Fig. 11. Appearance of bovine joint cartilage 3 months after birth. The surface of cartilage is sinking in shape of wide shallow grooves (h) and on their bottom groups of elevations (H) are deposited, rimmed by incisions (→) separating them from the surrounding. Light wavy surface on areas of the surrounding groups and on the surface of elevations. Scanning electron microscope. Magnification, x 2.100.

Fig. 12. Appearance of bovine joint cartilage 3 months after birth. Groups of chondrocytes (H) in the shallow depressions on bottom of the grooves. Wavy surface (l) also on the bottom of grooves on the surface of chondrocytes. Scanning electron microscope. Magnification, x 2.100.

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