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THE ULTRASTRUCTURE OF ARTICULAR CARTILAGE IN THE PRENATAL PIG

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Abstract

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The articular cartilage of the femoral articulation was studied in 14 porcine foetuses of both sexes at 41, 61 and 94 days after fertilization. The structure of articular chondroblasts and intercellular matter enabled us to distinguish three zones as early as at 41 days. The surface zone contained cells at a low degree of differentiation in structure resembling fibroblasts. The medium zone showed thick bundles of collagen microfibrils penetrating through the cell membrane into the intercellular matter. At 61 days both the chondroblast cytoplasm and the cartilage surface of the medium zone had vesicles, several μm in size, bounded by a single membrane. At 91 days after fertilization the cytoplasm of chondroblasts found at the border between the surface and the medium zones revealed collagen microfibrils running through cell membranes. The fibrils showed signs of periodicity.

The surface layer of the cartilage in the period under study was made up largerly of aperiodic fibrils. In some regions below the surface bundles of collagen microfibrils ran parallel to the surface of the articular cartilage. They later became involved into the chondrosynovial membrane.

Ultrastructure, porcine articular cartilage, development

In the course of articular cartilage development both chondrocytes and intercellular matter are subject to differentiation processes which, in the prenatal period, lead to the formation of articular cartilage followed by further changes in the two components in the neonatal period. The changes in the developing articular cartilage are referred to as the maturation process and are determined genetically, endocrinologically, by nutritional conditions (Silberger and Silberger 1941; Silberger et al. 1961) and, particularly in the postnatal period, by endogenous environmental factors (Ghadially 1978, 1981, 1982; Perrin et al. 1978). Changes in morphology of articular cartilage are associated, to a major extent, with age (except for species-related changes). Such changes have been reported for instance in adult mice (Silberger et al. 1976), rabbits (Barnett et al. 1963; Davies et al.

1962), dogs (Lust et al. 1972; Lust and Sherman 1973; Wiltberger and Lust 1975), cattle (Horký 1983, 1984) and man (Ghadially and Roy 1969; Ghadially 1978, 1982; Horký 1980) and to some extent in pigs (Grondalen 1974c, f; Nakano et al. 1979a, b). However, only the work by Bhatnagar et al. (1981) has been concerned with the ultrastructure of porcine articular cartilage in the postnatal period. Therefore, we focused our attention on the ultrastructure of the articular cartilage in the pig during the prenatal period with the objective to supply the information still missing in this field.

Materials and Methods

Samples of articular cartilage were collected from 14 porcine foetuses of both sexes at 41, 61 and 94 days after fertilization to be studied by light, transmission and scanning electron microscopy. They were taken from the femoral head in the direction of the greater trochanter or, at early developmental stages, the whole head was used.

For transmission electron microscopy, the specimens were further dissected to obtain strips measuring 1 by 3 mm. These were fixed immediately in glutaraldehyde solution (300 mmol/l or 400 mmol/l) in phosphate buffer at pH 7.4 in 4 consecutive baths for 30 min each. This was followed by fixation with a 40 mmol/l solution of OsO_4 in phosphate buffer at pH 7.4 for 60 min. Dehydration, immersion and embedding in Durcupan ACM was performed in a standard manner. Ultrathin sections were cut with an Ultracut Reichert ultramicrotome and stained with either lead citrate alone or uranyl acetate and lead citrate. The sections were studied and photographed with a Tesla BS 500 electron microscope. From the embedded material, semi-thin sections were made, stained with 1 % methylene blue and Azure II, and examined under the light microscope.

Results

Ultrastr ucture of articular cartilage at 41 days after fertilization

At this period the articular cartilage was differentiated to such an extent that submicroscopic signs, particularly the state of chondroblasts, permitted recognition of three layers referred to as the surface, the medium and the deep zones.

Submicroscopic structure of surface zone chondroblasts

The chondroblasts at this stage of their development were almost uniform in appearance, spindle-shaped and up to 16 by 9 μm in size (Fig. 1). They lay parallel to the surface of the surface of the cartilage, usually in two layers.

Nucleus

The shape of nuclei was either oval (Fig. 1) or elongated, corresponding well to the shape of the cell.

The nuclear envelope had two membranes with rather shallow impressions. The perinuclear space along the most part of the nucleus circumference was narrow; dilations were only rarely found. Also endoplasmic reticulum cisternae were seldom seen to arise from the outer nuclear membrane. Nuclear pores, too, were few in number (Fig. 1). The outer membrane bore ribosomes which were more frequent at areas of dilated perinuclear space. At this developmental stage the zonula nucleum limitans was present as a very thin, often incomplete strip of medium osmiophilic granulated material attached to the inner nuclear membrane.

On a section through the nucleus, chromatin was diffusely distributed. Small karyosomes were attached to the nuclear envelope (Fig. 1).

Nucleoli were often found in the nuclei of chondroblasts. Usually only one nucleolus of reticular type was seen, some chondroblasts had 2 nucleoli. Occasionally also other forms were observed, such as ring-shaped nucleoli with thick envelopes of perinuclear chromatin (Fig. 1).

Cytoplasm

At this stage the cytoplasm of surface zone chondroblasts contained low amounts of cellular organelles and cytoplasmic inclusions (Fig. 1).

The granular endoplasmic reticulum consisted of short flattened cisternae or vesicles (Fig. 1). The cisternae, which were not dilated, were filled with light substance of net-like appearance. Numerous ribosomes were attached to both the cisternae and the vesicles. Among cisternae there appeared small and larger vesicles with dark, finely granulated content. They were 0.1–0.6 μm in size, enveloped in a smooth membrane (Fig. 1); they showed all characteristics of transport vacuoles.

The agranular endoplasmic reticulum was seen only occasionally. If present, it consisted of small smooth vesicles most probably originating from the Golgi complex. Vesicles found near the cell surface may have been derived from cell membrane invaginations (Fig. 1).

The Golgi complex was not a distinct organelle in the cytoplasm of surface zone chondroblasts. If seen, it took up only a minute region of the cytoplasm. It had a typical structure with cisternae-derived vesicles containing granulated medium osmiophilic material. These vesicles were often found close to the cell membrane and among cisternae of the granular endoplasmic reticulum, functioning as transport vacuoles.

Mitochondria were scarce. They attained sizes of 0.2–1.6 μm and their cristae had the usual pattern. They were, however, only few in number. Mitochondrial bodies were not observed.

Most of the ribosomes were attached to the cisternae of the granular endoplasmic reticulum, some were diffusely distributed in the cytoplasm. Polyribosomes could also be seen.

Lysosomes, centrioles and microtubules were not observed in chondroblasts at this stage of development.

Cell membrane. The cytoplasm of the chondroblasts situated near the cartilage surface occasionally formed short processes covered with the cell membrane (Fig. 1). These were 0.5–0.8 μm long and penetrated into the surrounding intercellular matter. Apart from them, the cell surfaces were smooth. Pinocytotic vesicles derived from the cell membrane were found only infrequently.

Desmosomes, other supportive structure or cilia were not observed in the cartilage specimens at this stage of development.

Glycogen was seen in the chondroblasts cytoplasm regularly but only in small amounts. It presented as clusters of beta-granules placed among cell organelles (Fig. 1).

Lipid droplets were found on rare occasions. Cytoplasmic fibrillar structures were seen even less frequently. When they did occur, they looked like bundles of filaments 10 μm wide, usually situated near the cell membrane.

Submicroscopic structure of medium zone chondroblasts

Compared to the surface zone cells, the medium layer chondroblasts were oval in shape and reached a size of 18 by 12 μm. In the intercellular matter they were found as single cells.

Nucleus

The nucleus was round or oval, 10 by 8 μm large. It was enveloped in two membranes separated by a narrow perinuclear space. Nuclear envelope invaginations were only very shallow. No connections between the outer nuclear membrane and the cisternae of the granular endoplasmic reticulum were observed.

Chromatin in the sectioned nucleus was accumulated into small karyosomes distributed diffusely or situated close to the inner nuclear membrane. Attached to this membrane was the zonula nucleum limitans the appearance and arrangement of which were similar to those found in the surface zone (Fig. 3). Nuclear pores were few in occurrence.

The nucleolus, in contrast to the surface zone cells, was a rare finding. If seen, it was a nucleolus of reticular type.

Cytoplasm

The medium zone chondroblasts had an increased number of cellular organelles and inclusions in their cytoplasm.

The granular endoplasmic reticulum consisted of longer and more dilated cisternae than was seen in the surface zone chondroblasts. The cisternae were filled with medium osmiophilic, finely granulated material (Fig. 3). The reticular structures were seen close to the nucleus and organelles and at the cell periphery.

The agranular endoplasmic reticulum showed no differences in either the amount or the appearance from that in the surface zone chondroblasts.

The Golgi complex was not too prominent. If it occurred, it had the usual structure. Its cisternae released vesicles of two kinds: small- and medium-sized vesicles (0.1–0.3 μm) contained homogeneous or finely granulated material; vesicles 0.5 μm long and large were filled with finely filamentous, electron-dense material (Fig. 3). The vesicles were bounded by smooth membranes. They made groups in the cytoplasm but could also be found as single vesicles. They were typical transport vacuoles.

Mitochondria had the usual structure and occurred in two forms. One corresponded to that of the mitochondria seen in the surface zone chondroblasts, the other were mitochondria doubled in size. These, in most cases, had impaired cristae and clear matrix (Fig. 3).

Ribosomes occurred in greater numbers than in the surface zone chondroblasts. This applied particularly to free ribosomes but the cisternae of the granular endoplasmic reticulum, too were endowed with numerous ribosomes.

Lysosomes, microtubules or centrioles were not observed in the cytoplasm of medium zone chondroblasts.

Cell membrane. The cytoplasm was covered with the cell membrane which sent out few short processes into the surrounding intercellular matter. Supporting structures or cilia were not observed.

Lipid droplets were found quite frequently. They were seen mostly near cytoplasmic inclusions: their size was 1 μm and more (Figs 3, 4).

Glycogen was a regular finding in the cytoplasm of medium zone chondroblasts.

It presented as large deposits of typical granules usually close to the cell membrane (Fig. 3).

Cytoplasmic filamentous structures typical in appearance were seen only in the cytoplasm of some chondroblasts. Apart from the chondroblasts described, this zone also showed chondroblasts of different appearances. These were oval or irregularly-shaped cells (Fig. 4) 10 by 5 μm in size.

Nucleus

The nucleus was oval or pear-shaped, 5 by 3 μm in size. The nuclear envelope was formed by two membranes with occasional perinuclear spaces in between them. The outer membrane densely covered with ribosomes continued with cisternae of the granular endoplasmic reticulum.

Chromatin formed small karyosomes apparent on sections through the nucleus which was, consequently, light. On some occasions chromatin produced a larger clusters at the inner nuclear membrane. The nucleolus was a rare finding.

Cytoplasm

The cytoplasm showed different characteristics in different parts of the same cell (Fig. 4).

The granular endoplasmic reticulum of widely dilated cisternae with fine filamentous content, large mitochondria with frequently impaired cristae, occasional lysosomes and a small Golgi complex were all found near the nucleus towards the cartilage surface.

Below the nucleus the cytoplasm contained few narrow cisternae of the granular endoplasmic reticulum (Figs 4, 5) and occasional, mostly impaired mitochondria (Fig. 5). Electron microscopic observations mainly revealed ribosomes linked into polysome chains or rosettes. The cytoplasm also contained large bundles of fibrils (Figs 4, 5). These bundles ran through the entire cytoplasm, via the cell membrane and ended in the perinuclear matrix (Figs 4, 5) as collagen microfibrils with signs of periodicity. Among the microfibrillar bundles in the cytoplasm there were transport vacuoles (Fig. 5).

Cell membrane. The cytoplasm formed large and broad processes in the area below the nucleus. The remaining surface showed only short protrusions not exceeding 0.5 μm in length.

Glycogen formed only small clusters of beta-granules usually found at the cell periphery (Fig. 5). Lipid droplets were not observed.

Submicroscopic structure of deep zone chondroblasts

Chondroblasts of the deep cartilage zone had irregular shapes and either lay in pairs in a common lacuna or were arranged in columns. Their sizes were in the range of 10–12 by 6–8 μm.

Nucleus

The nucleus had an irregular oval or pear-like shape and measured 4 by 7 μm.

The nuclear envelope showed a typical structure. It sent out occasional shallow broad invaginations into the karyoplasm. The perinuclear space was narrow. Connections between the outer membrane and the cisternae of the granular endoplasmic reticulum were seen on rare occasions.

Chromatin formed aggregates larger than seen in sections through nuclei of the

medium zone chondroblasts (Fig. 6). The zonula nucleum limitans vas similar in appearance to that found in nuclei of the two previously described zones.

Nucleoli were inconspicuous and infrequent. If seen, they were of reticular type.

Cytoplasm

Large amounts of cytoplasm were accumulated in the adjoining areas of the cells (Fig. 6).

The granular endoplasmic reticulum consisted of short cisternae, which were usually dilated, and often encircled mitochondria. Its inner spaces were filled with medium density material of net-like appearance (Fig. 6).

The agranular endoplasmic reticulum presented as occasional smooth vesicles found most frequently at the periphery of the cytoplasm.

The Golgi complex was well developed and spread over 1–2 fields in the area above the nucleus on the convex cell side (Fig. 6). Vesicles and vacuoles derived from the cisternae gradually attained the appearance of transport vacuoles (Fig. 6).

Sectioned mitochondria showed circular or elongated shapes up to 0.5 by 1.5 μm in size. Cristae were short, the matrix of most mitochondria was light. Mitochondria with impaired cristae were occasionally seen.

Lysosomes and centrioles were observed only exceptionally.

Cell membrane. The cytoplasm formed occasional short processes, no longer than 0.6 μm, so that cell surface showed mild undulations. Only in adjoining regions the cytoplasmic processes, short and broad, were more frequent. These processes were the point of contact between the pairs of cell in lacunae. None of these points of contact, however, was supported by junctional structures. The periphery of cytoplasm bore numerous pinocytotic vesicles.

The cytoplasm of deep zone chondroblasts contained inclusions only occasionally. Intracytoplasmic fibrillar structures, too, were rare finding.

Structure of intercellular matter of articular cartilage

The intercellular matter of articular cartilage at 41 days after fertilization was similar in structure in all three zones with the exception of the surface part adjoining the articular cavity. The fibrillar component of all three layers was based on aperiodic fibrils made up into a thin uneven network (Figs 1, 2, 3, 4, 5, 6). The interfibrillary matrix was in excess of the fibrils. Thin aperiodic fibrils were found in equal numbers in both the close vicinity of chondroblasts and the intercellular space so that it was possible to distinguish between the pericellular and the intercellular matrix (Figs 1, 5, 6). Thin collagen fibrils were seen only in the surface zone in parallel arrays about 2 μm under the surface. The surface itself had a different appearance. In some areas (Fig. 1) the marginal layer consisted of bundles, varying in both width and length, comprising aperiodic fibrils. Some of these, with signs of periodicity, continued into the amorphous substance of the surface zone. Apart from regions of this structure, there were areas with unmasked aperiodic fibrils extending from the surface zone into the articular cavity or areas covered with thin coats of amorphous substance. Remnants of these were still found over the regions covered with the bundles of aperiodic fibrils (Fig. 2).

Ultrastructure of articular cartilage at 61 days after fertilization

This period was characterized by major changes in the surface and the medium zones with respect to both chondroblasts and intercellular matter. Changes in the deep zone were not so marked.

Submicroscopic structure of surface zone chondroblasts

Chondroblasts of the surface zone were conspicuous cells with spindle-like or flattened shapes up to 15 by 3 μm in size (Fig. 7). They formed 3–5 layers in which some cells overlapped. The adjoining cells at the same level were connected by means of cytoplasmic processes. Towards the medium zone the cells shortened and acquired rounded shapes.

Nucleus

The nucleus followed in shape the cell's appearance. It was elongated and had a size of 10 by 2 μm.

The nuclear envelope had the usual structure; nuclear pores were scarce.

Chromatin was distributed diffusely in the karyoplasm or formed a continual lining around the nuclear envelope, which made the nuclei appear dark on sections (Fig. 7). The zonula nucleum limitans was quite narrow.

Cytoplasm

Compared to the nucleus, the cytoplasm occurred in low amounts and was accumulated at the apical parts of the cells. The nucleus was surrounded only by a narrow band of the cytoplasm free from organelles.

The granular endoplasmic reticulum consisted of occasional short cisternae fiilled with darker granular or fibrillar material (Fig. 7).

Agranular endoplasmic reticulum was characterized only by occasional smooth vacuoles. Since they were usually found at the cytoplasm periphery, they could be considered pinocytotic vesicles.

The Golgi complex was observed only on rare occasions. It had a typical structure and occupied only a minor area of the cytoplasm.

Ribosomes in the cytoplasm of surface zone chondroblasts occurred in great numbers. They linked into polyribosomes diffusely scattered on a section through the cell.

Mitochondria were small, uniform in appearance, having conspicuously dark matrix. They were found in a spindle-like protrusion on each pole of the cell.

Lysosomes, centrioles, cilia or intracytoplasmic fibrillar structures were not observed.

The cell membrane formed processes only very rarely, which gave the chondroblast surface a smooth appearence with occasional unevenness. In some cases the spindle--shaped cell poles sent out cytoplasmic projections connected with those of the neighbouring cells. No phenomena reminiscent of the macular adherens were observed.

Submicroscopic structure of medium zone chondroblasts

This zone comprised cells of two types: oval cells measuring 9 by 4 μm (Fig. 9) and irregular spindle-shaped cells 10 by 2–3 μm in size (Fig. 9). The two types were scattered irregularly throughout the zone.

Nucleus of oval cells

The nucleus of these cells (Fig. 8) did not differ in either appearance of structural arrangement from that of middle zone chondroblasts found in the articular cartilage of the previous developmental stage.

Nucleus of spindle-shaped cells

The nucleus of these cells (Fig. 9) had an irregular oval shape. The nuclear envelope of usual structure occasionally extended outward to from invaginations of varying width and depth in the karyoplasm.

Chromatin was aggregated into rather large karyosomes attached to the inner nuclear membrane in the form of broad dark lining.

Nucleoli of either a reticular or a compact type were a frequent finding.

Cytoplasm of oval cells

The granular endoplasmic reticulum consisted of short infrequent cisternae distributed at random among the other organelles. It contained granular or fibrillar material of medium electron density (Fig. 8).

The agranular endoplasmic reticulum presented as occasional smooth vesicles which were likely to originate from the Golgi complex cisternae.

The Golgi complex took up only a minor region of the cytoplasm. It was composed of a small dictyosome and a large amount of small vacuoles. Close to it were transport vacuoles (Fig. 8).

Mitochondria were mostly oval in shape with a typical structure. Some of their cristae were dilated.

Lysosomes were present in small amounts but regularly in the cytoplasm of this chondroblast type. They contained dark granulated material as well as material of various electron densities.

Cell membrane. The entire surface of the cytoplasm bore numerous cell membrane processes reaching up to 1 μm in length. Some were branched and projected into the surrounding intercellular matter. In regions between them the cell membrane produced pinocytotic vesicles (Fig. 8). Neither glycogen nor intracytoplasmic filaments were observed.

Cytoplasm of spindle-shaped cells

Compared to the other cell type the cytoplasm in these cells occurred in greater amounts. The nucleus was situated in an eccentric way near one of the cell poles (Fig. 9).

The granular endoplasmic reticulum was similar in appearance to that of the oval cells. Its cisternae, however, were more frequent and, in some regions, arranged in 2–3 parallel layers. Their content was darker than it was found in the oval cells.

Vesicles of the agranular endoplasmic reticulum were densely distributed in the cytoplasm.

The Golgi complex did not differ in either structure or distribution from the other cell type.

Most of the mitochondria had elongated forms with noticeably dark matrix. Apart from ribosomes bound to the membranes of the granular endoplasmic reticulum, the cytoplasm showed numerous free ribosomes sometimes present in the cytoplasmic projections.

Lysosomes were seen only on rare occasions. In an area under the nucleus we detected centrioles (Fig. 9).

The cytoplasm of these chondroblasts contained unusual bag-like bodies (Fig. 9). These were bounded by a double-membrane and their sizes were up to 3.5 by 2 μm. They were filled with finely granulated medium osmiophilic material which contained dark grains, 0.05–0.1 μm in diameter. Some grains had an envelope. Similar grains of larger sizes (2 by 1.5 to 3 by 1.8 μm) with filamentous content and a single membrane were also seen in close vicinity of these chondroblasts (Fig. 9) and near the surface of the articular cartilage (Fig. 10). The grains found near the cell membrane were in parts surrounded by cytoplasmic projections (Fig. 9). These observations were suggestive of phagocytosis.

Cell membrane. The cytoplasm of spindle-shaped cells, as compared with that of oval cells, formed only occasional processes so that the surface remained largerly smooth. Apart from ribosomes some of the broader processes showed intracytoplasmic filaments gathered into bundles (Fig. 9).

Submicroscopic structure of deep zone chondroblasts

Chondroblasts of the deep zone were oval to spindle-like cells, 8–11 by 3–4 μm size. Two to four cells were arranged in columns perpendicular to the cartilage surface (Fig. 11).

Nucleus

The shape of the nucleus followed that of the cell. The nuclear envelope had the usual structure. It sent out broad invaginations of varying depths against the karyoplasm. Connections between the outer nuclear membrane and cisternae of the granular endoplasmic reticulum were scarce.

The zonula nucleum limitans formed only a narrow band at the inner nuclear membrane.

Chromatin was aggregated into karyosomes deposited at the nuclear envelope. They could be detected only occasionally in the light karyoplasm of sectioned material. Nucleoli were not observed.

Cytoplasm

The deep zone chondroblasts exhibited less cytoplasm than those observed in the previous developmental stage.

The granular endoplasmic reticulum consisted of occasional short cisternae filled with dark material.

The agranular endoplasmic reticulum was well developed. It presented as smooth vesicles, 0.2 μm in diameter, which were most frequently found near the cell membrane (Fig. 11).

Mitochondria were infrequent and usually were elongated in shape. The cytoplasm contained a large amount of free ribosomes gathered into polysomes.

The Golgi complex was observed only on rare occasions. Lysosomes, transport vacuoles or centrioles were not seen.

Cell membrane. The cytoplasm formed processes only at sites of cell junction. The processes were in close contact but no supporting intercellular structures were seen. Cytoplasmic fibrillar structures were observed in the form of short bundles of dark filaments (Fig. 11).

Intercellular matter of articular cartilage

The surface of the articular cartilage consisted of a 2–3 μm thick layer of intercellular matter (Figs 7, 10, 12, 13) composed of aperiodic fibrils, collagen fibrils and the ground amorphous substance which was abundant. Compared to the previous stage (Fig. 2) the aperiodic fibrils occurred as short bundles found about 1 μm under the surface (Fig. 7) and as rather thick bundles several μm long seen close to the surface (Figs 10, 12). Apart from this set up, areas could be seen (Fig. 13) where the cartilage surface was covered with a meshwork of collagen fibrils some of which extended into the articular cavity. Under the surface in these areas, cell debris could generally be found. In the surface regions with bundles of aperiodic fibrils, close observation revealed that the fibrils embedded in the ground amorphous substance pursued an oblique course towards the articular cavity (Fig. 12).

In comparison with the previous developmental stage all the zones showed a multiplication of collagen fibrils which, in the first zone, were accumulated near the chondroblasts (Figs 7, 10). Some fibrils were attached to the cell membrane. The medium and the deep zones showed signs of differentiation of the intercellular matter into the pericellular and the intercellular matrix (Figs 9, 11).

Ultrastructure of articular cartilage at 94 days after fertilization

At this stage the differentiation of the articular cartilage had progressed so that in a number of aspects the cartilage resembled the tissue of an adult animal. This applied not only to the cells but also to the arrangement of intercellular matter. The differentiation of the cartilage into three zones was quite distinct.

Submicroscopic structure of surface zone chondroblasts

Chondroblasts of the surface zone were oval to spindle-like in shape and attained a size of about 10 by 3 μm. In the surface zone they were arranged in two parallel rows. Above them the intercellular matter made 1 μm or thicker layer (Fig. 14).

Nucleus

The nucleus was irregular in shape and attained a size of about 5 by 2 μm. The structure and the chromatin arrangement were similar to thouse found in nuclei of this zone at the previous stage (Fig. 14). Nucleoli were a rare finding.

Cytoplasm

The cytoplasm occurred at greater amounts as compared with the cells at 61 days of development; it also contained a larger number of organelles.

The granular endoplasmic reticulum formed short and often branched cisternae with many ribosomes. The cisternae were filled with medium density granular material.

The agranular endoplasmic reticulum was made up of smooth vesicles 0,1–0,2 μm large and occasionally up to 0,6 μm in diameter.

The Golgi complex consisted of a small dictyosome and a large number of small vesicles partly accumulated within the complex, partly scattered among the other organelles. The large vesicles gave rise to transport vacuoles with dark granular content.

Mitochondria had elogated shapes. They occurred in large numbers and their matrix was dark.

Lysosomes were present regularly (Fig. 14). They were seen close to the Golgi complex and at the periphery of the cytoplasm. They contained dark homogenous material.

Cell membrane. On the side facing deeper layers and on the poles of the cell the cytoplasm sent out short, sometimes branched processes which extended into the pericellular matrix, and thick long processes extending into the intercellular matrix (Fig. 14). Passing of collagen fibres through the cell membrane was most frequently seen at the foot of these processes, similarly to the chondroblasts of the medium zone at 41 days of development.

Glycogen was present only in the cytoplasm of cells lying close to the surface. It presented as small clusters of beta-granules scattered among organelles (Fig. 14). Lipid droplets were occasionally seen.

Cytoplasmic fibrillar structures were found as bundles of dark aperiodic filaments at the cell membrane near the extending processes.

Submicroscopic structure of medium zone chondroblasts

Chondroblasts of the medium zone were similar in appearance and ultrastructure to the oval cells found in the corresponding zone of the articular cartilage at 61 days after fertilization (Fig. 15). The only difference was that the granular endoplasmic reticulum had longer cisternae. The Golgi complex was spread over several fields and glycogen was present in the cytoplasm as small aggregates of beta-granules (Fig. 15).

Submicroscopic structure of deep zone chondroblasts

Chondroblasts of the deep zone at this stage of development were identical in their structure and arrangement to the chondroblasts of the deep zone 61 days after fertilization.

Intercellular matter of articular cartilage

The intercellular matter at this stage was arranged in a different way in each zone. The surface was made up to a thick tangle of thin collagen fibres embedded into the ground amorphous substance (Fig. 14). The whole surface zone was free from bundles of aperiodic filaments. In the vicinity of cells lying near the surface the distinction between the pericellular and the intercellular matrix was clear. This was not seen, however, in the surface zone chondroblasts located deeper under the surface (Fig. 14). Collagen fibrils often gathered into bunches were found close to the cell membrane, with some of them penetrating into the cytoplasm (Fig. 14). Collagen fibrils at the border of the medium zone were arranged parallel to the surface and formed a distinct, more or less continual band (Fig. 14). Collagen fibres in the medium zone appeared as a thin network with a large amount of the ground amorphous substance (Fig. 15). The pericellular matrix was present only in the vicinity of the cell membrane over an area facing the deep zone (Fig. 15). The intercellular matter in the deep zone had an arrangement similar to that found at 61 days of development (see Fig. 11).

Discussion

Previous studies on the ultrastructure of articular cartilage have been concerned largely with its description in human adults and small rodents (for review see Ghadially 1983; Horký 1980). The development of this specialized tissue, however,

has received much less attention (for review see Horký 1983, 1984, 1986, 1987). Information has been published on the structure of articular cartilage in the pig under physiological conditions by Perrin et al. (1978), Nakano et al. (1979), Wilsman et al. (1981) and Bhatnagar et al. (1981). Pathological changes in articular cartilage have been investigated by Grondalen (1974a, b, c, d, e, f), Grondalen and Grondalen (1974), Grondalen and Vangen (1974), Doige (1980), Nakano et al. (1982) and Denecke et al. (1985). In vitro studies (Burch and Lebowitz 1982; Farnum et al. 1984), the effects of nutrition, sex and hormones on the occurrence of cartilage lesions, and biochemical aspects (Simunek and Muir 1972a, b) have also been reported.

A question of general interest is the way in which chondroblasts are situated in the matrix, particularly in relation to the surface zone, during development. It is usually reported that the orientation of cells is conditioned by tensile forces imposed on the cartilage. This is one of the explanations but Gould et al. (1974) held an interesting view that this orientation was associated with the formation of intercellular matter. This is produced in large amounts in the area of the chondrification centre and compresses chondroblasts. Since during the prenatal development the tensile are limited to the tension produced by muscles, this assumption should be taken into consideration. Most probably the final arrangement of chondroblasts during the development involves the two factors which, according to their respective involvement, characteristically affect the course and arrangement of fibres and fibrils in the intercellular matter. This is, together with the amorphous substance, responsible for mechanical properties of the articular cartilage, while the chondroblasts play a key role in manufacturing and maintaining the matrix (Balazs et al. 1966; Imura 1984; Klamfeldt 1984).

The literature data (Haines 1933; Levene 1964; Brower and Hsu 1969; Lufti 1970; Stockwell 1971; Wolf 1975; Kincaid and Lindwall 1982; Agraval et al. 1984) as well as our observations involving cattle (Horký 1986) show the presence of blood vessels in the articular cartilage in the course of its development. In the pig, however, no blood vessels were found in the period under study. This may be due to the fact that our study included the period following the 41th day after fertilization and the literature data report the presence of blood vessels in this tissue at earlier stages.

The surface zone chondroblasts in the prenatal period up to 94 days after fertilization were seen as less differentiated cells of mesenchymal origin. The immaturity of these elements was evidenced by a low number of cellular organelles and only occassional bundles of intracytoplasmic filaments (Ghadially 1983; Horký 1986). The zonula nucleum limitants, too, was incompletely formed (Horký, 1984, 1986, 1987) and its width changed with increasing age, which is in agreement with the findings of others (Oryschak et al. 1974; Ghadially et al. 1972).

The medium zone showed marked differentiation activity as early as at 41 days after fertilization. Chondroblasts of this zone differed from those of the surface and the deep zones in the larger amounts of cytoplasm, the marked granular endoplasmic reticulum, the well-developed Golgi complex and the transport vacuoles. These features are, according to Freeman (1973), typical of cells with an intense synthesis of intercellular matter. The importance of this zone for the metabolism and growth of the cartilage has been demonstrated by results of Stockwell and Meachim (1979) who studied the synthesis of proteoglycans and the level of mitosis. This fact is also supported by our observations of mitosis and particularly by our detection of the passage of bundles of collagen microfibrils with signs of periodicity across the cell membrane. The latter phenomenon has already been observed in our earlier

studies of the articular cartilage and the synovial membrane during the development in cattle (Horký 1984a, 1986). In the medium zone chondroblasts the presence of inclusions, such as glycogen and lipid droplets, is a typical feature (Ghadially 1983).

The intercellular matter, similarly to the cartilage cells, is subject to several quantitative and qualitative changes during the prenatal development. At the early stages the prevailing component is the amorphous substance with mainly aperiodic fibrils. For the joint function the surface zone is most important. In the period up to 94 days after fertilization, the fibrillar component bordering the articular cavity consists exclusively of aperiodic fibrils which may extend into the cavity. Only some regions show bundles of collagen microfibrils situated closely under and collagen fibrils at about 1 μm under the cartilage surface. The chondrosynovial membrane is not yet formed (Wolf 1969, 1975), which makes it easy for diffusion of substances from the articular cavity (Maroudas and Bullough 1968; Maroudas 1973, 1976). Owing to low amounts of collagen fibrils in the intercellular matter, neither the pericellular nor the intercellular matrix is produced. The first signs of their formation were observed in the medium and the deep zones at 61 days and in the surface zone at 94 days after fertilization.

Ultrastruktura kloubní chrupavky prasete v prenatálním období

Byla studována kloubní chrupavka kyčelního kloubu 14 jedinců prasete obojího pohlaví ve stáří 41, 61 a 94 dnů po oplození. Již v období 41 dnů můžeme na základě stavby chondroblastů a mezibuněčné hmoty rozdělit chrupavku do tří vrstev. V povrchové vrstvě jsou obsaženy buňky málo diferencované, podobné svou stavbou např. fibroblastům. Ve střední vrstvě jsme pozorovali výskyt mohutných svazků kolagenních mikrofibril, které prostupují buněčnou membránou do mezibuněčné hmoty. V období 61. dne jsme současně v cytoplasmě chondroblastů střední vrstvy a na povrchu kloubní chrupavky pozorovali váčkovité útvary velikosti několika μm, ohraničené jednoduchou membránou s identickým obsahem. 94 dní po oplození se v cytoplasmě chondrocytů na rozhraní povrchové a střední vrstvy objevují kolagenní mikrofibrily pronikající buněčnou membránou. Tyto fibrily již mají naznačenou periodicitu.

Na povrchu chrupavky v námi sledovaném období vývoje je hraniční vrstva tvořena převážně aperiodickými fibrilami. V některých úsecích se blízko pod povrchem vyskytují svazky kolagenních mikrofibril uložené paralelně s povrchem kloubní chrupavky. Stávají se součástí chondrosynoviální membrány.

Ультраструктура суставного хряща свиньи в пренатальный период

Изучали суставной хрящ тазобедренного сустава 14 особей свиней обоих полов в возрасте 41, 61 и 94 суток после оплодотворения. Уже на 41 сутки на основе строения хондробластов и межклеточной массы можно хрящ разделить на три слоя. В поверхностном слое содержатся не особо дифференцированные клетки, похожие по своему строению, например, на фибробласты. В среднем слое наблюдали наличие мощных пучков коллагенных микрофибрилл, проникающих клеточной мембраной в межклеточную массу. На 61 сутки наблюдались одновременно в цитоплазме хондробластов среднего слоя и на поверхности хряща пузырные образования величиной в несколько мкм, ограниченные простой

мембраной с идентичным содержанием. 94 сутки после оплодотворения в цитоплазме хондроцитов на пределе между поверхностным и средним слоями появляются клейдающие микрофибриллы, проникающие клеточной мембраной. Данные фибриллы отличаются уже признаками периодичности.

На поверхности хряща в исследуемый нами период развития предельный слой состоит преимущественно из апериодических фибрилл. На некоторых участках вблизи под поверхностью встречаются пучки клейдающих микрофибрилл, расположенные параллельно поверхности суставного хряща. Они становятся составной частью хондросиновиальной мембраны.

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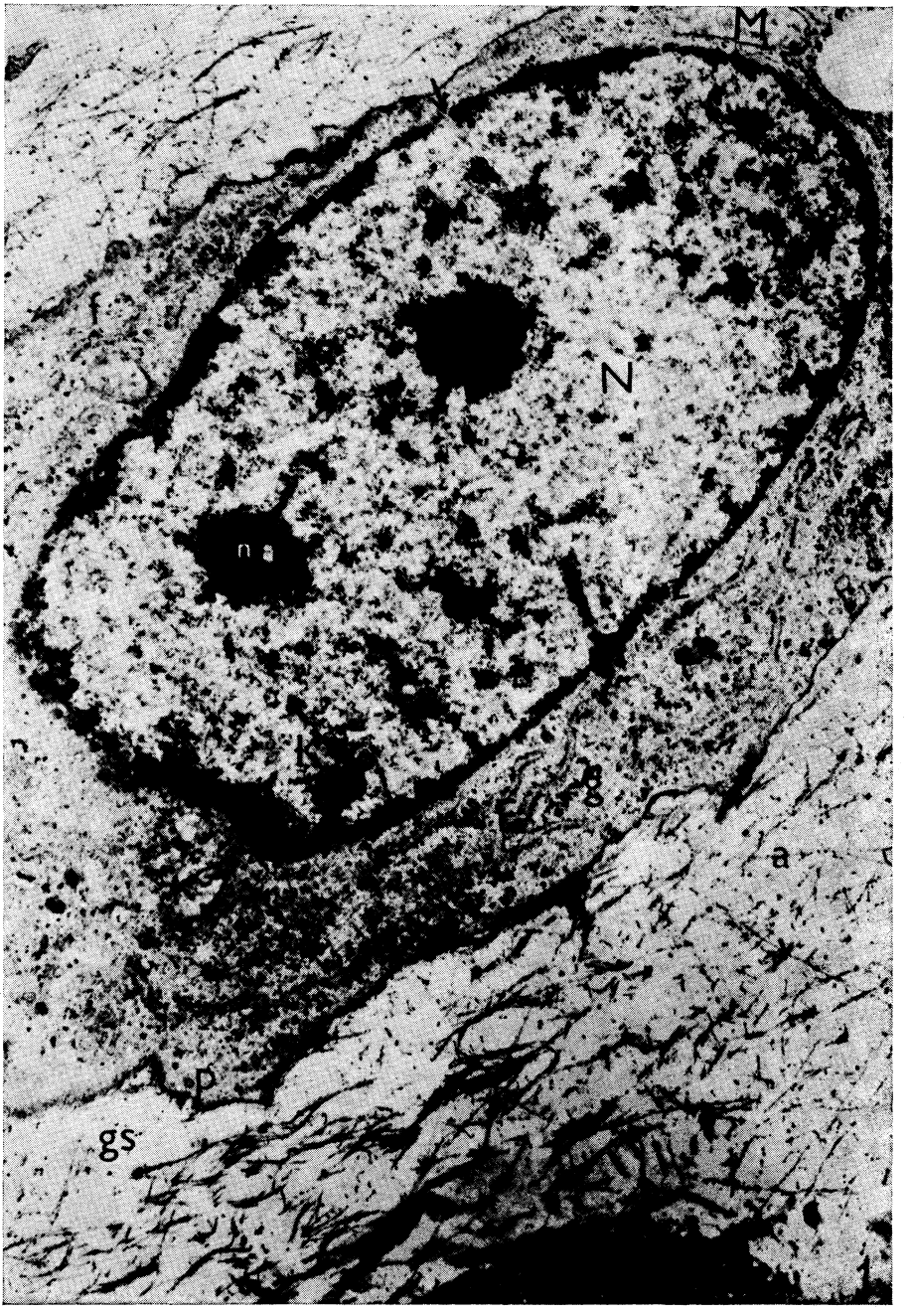
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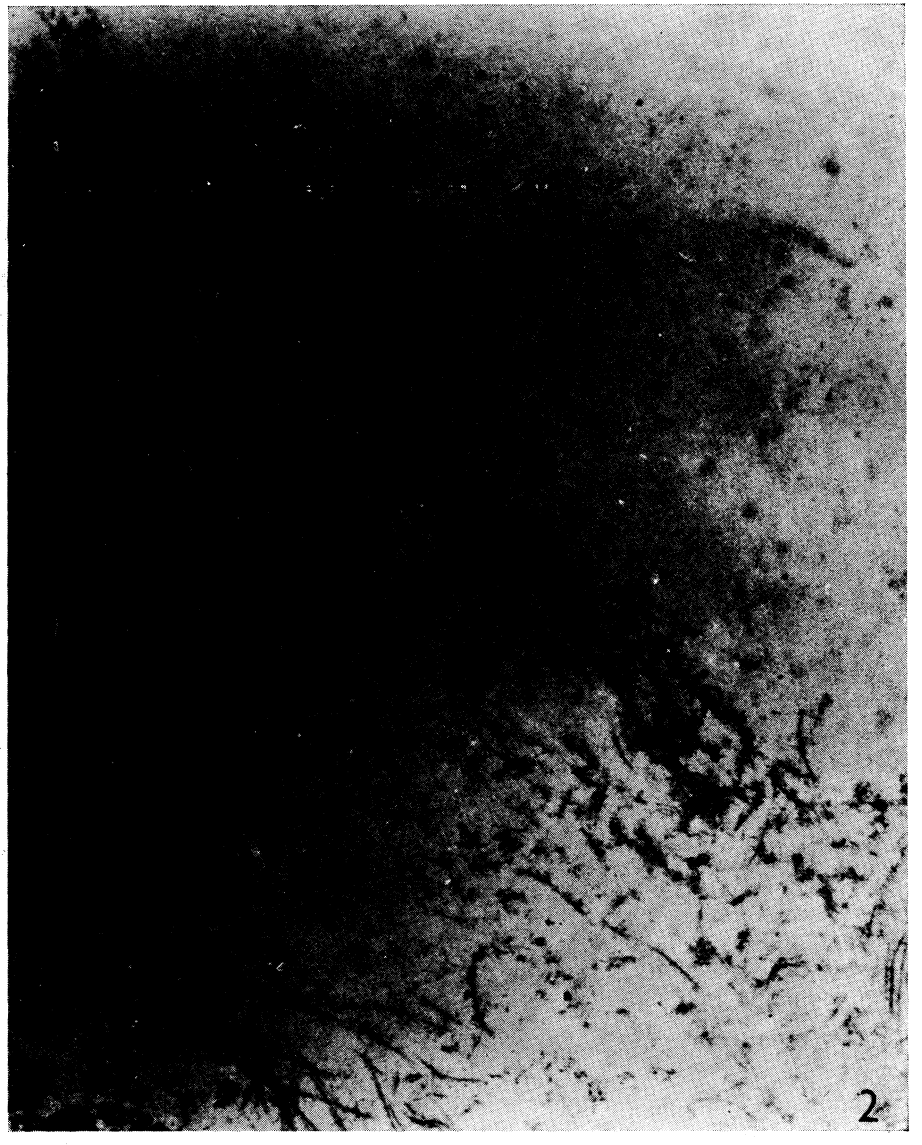
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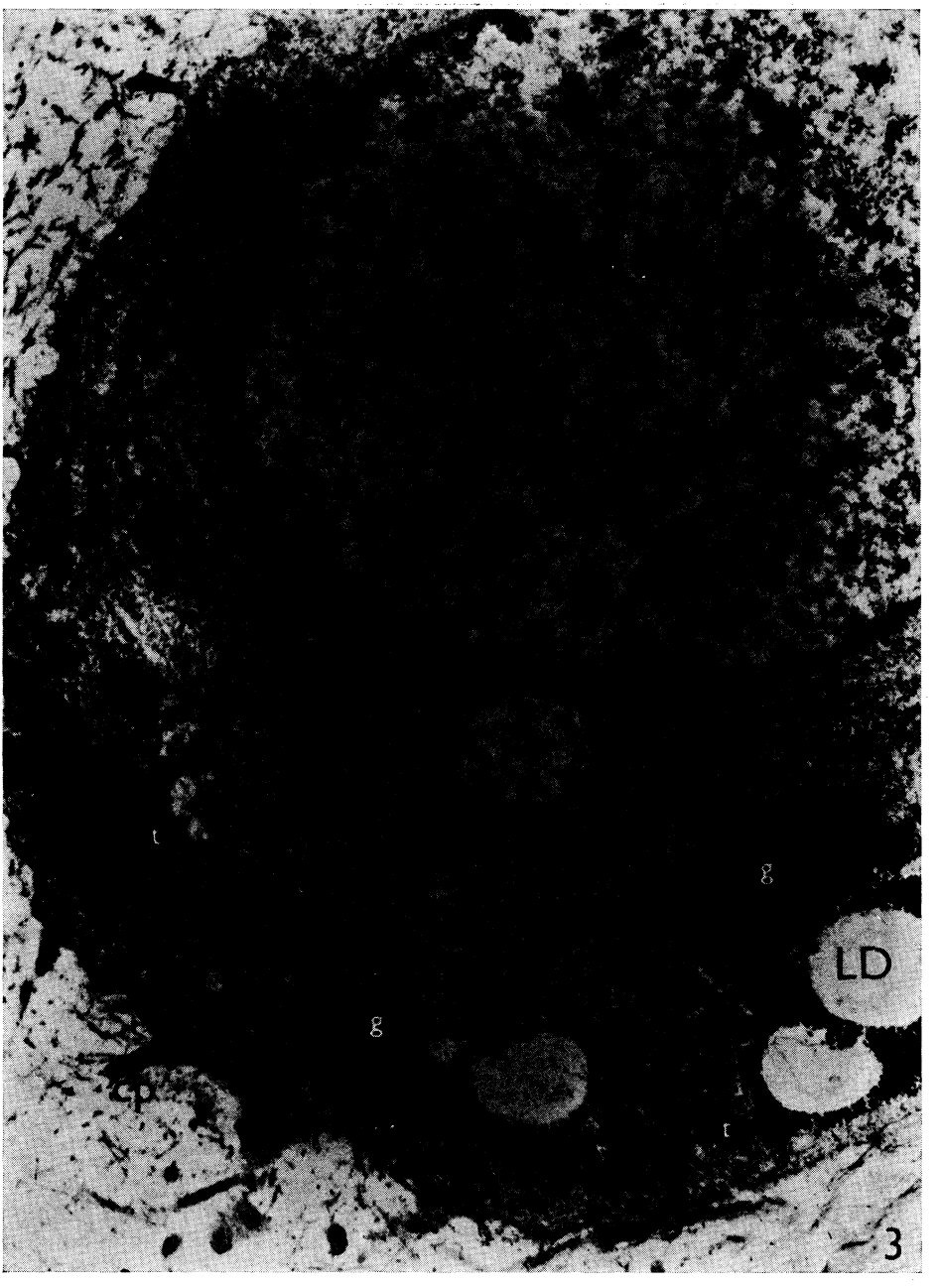
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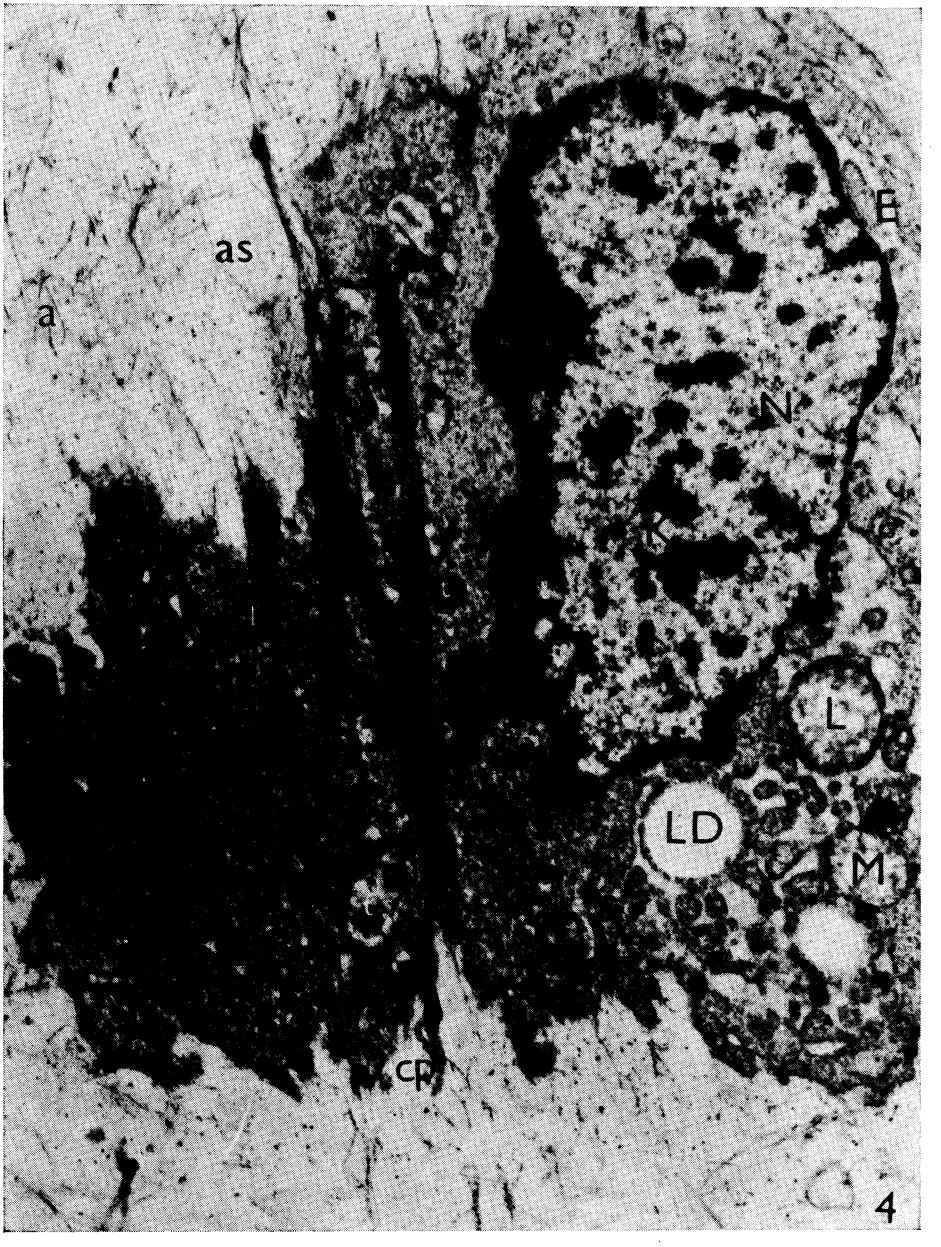
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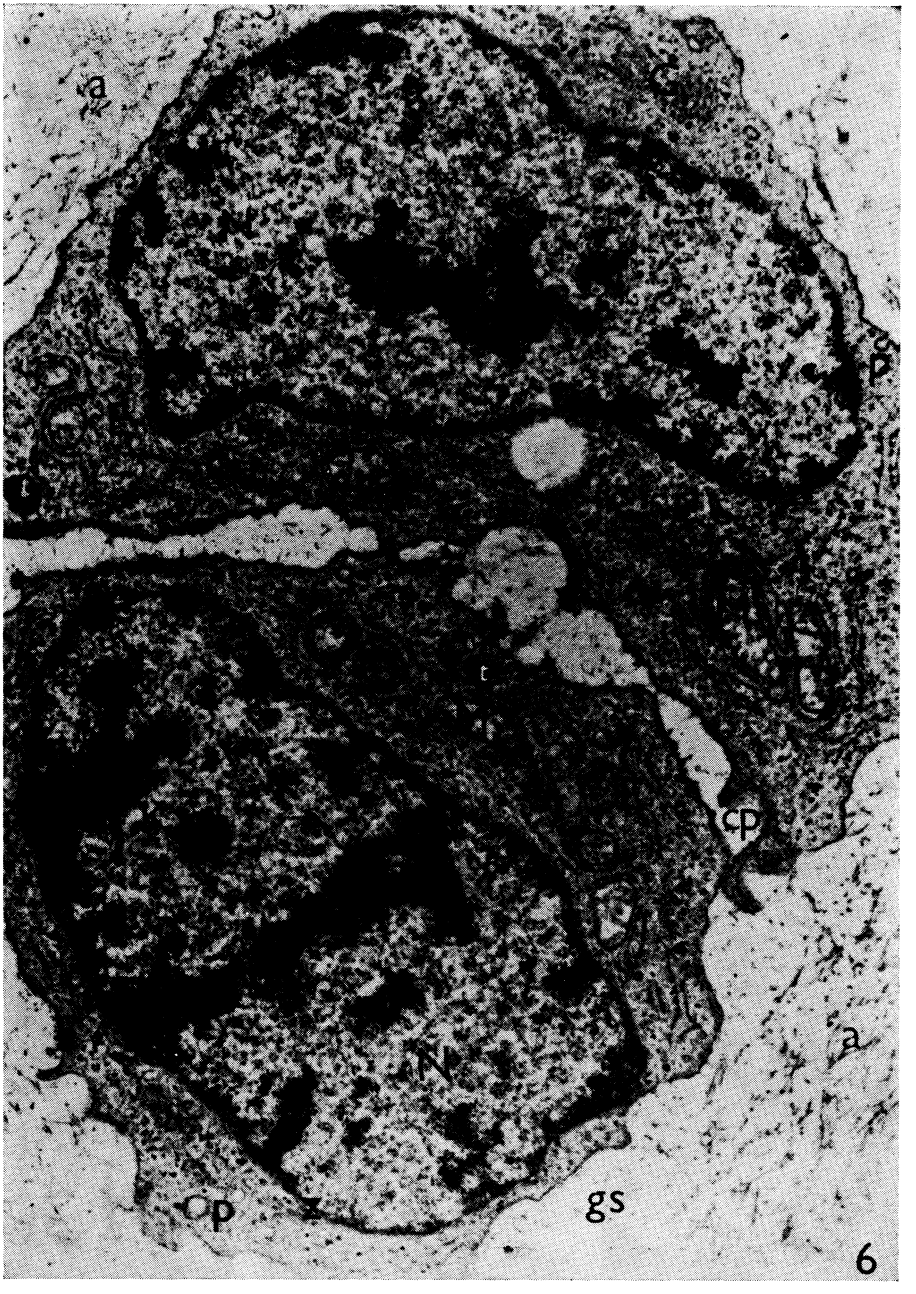
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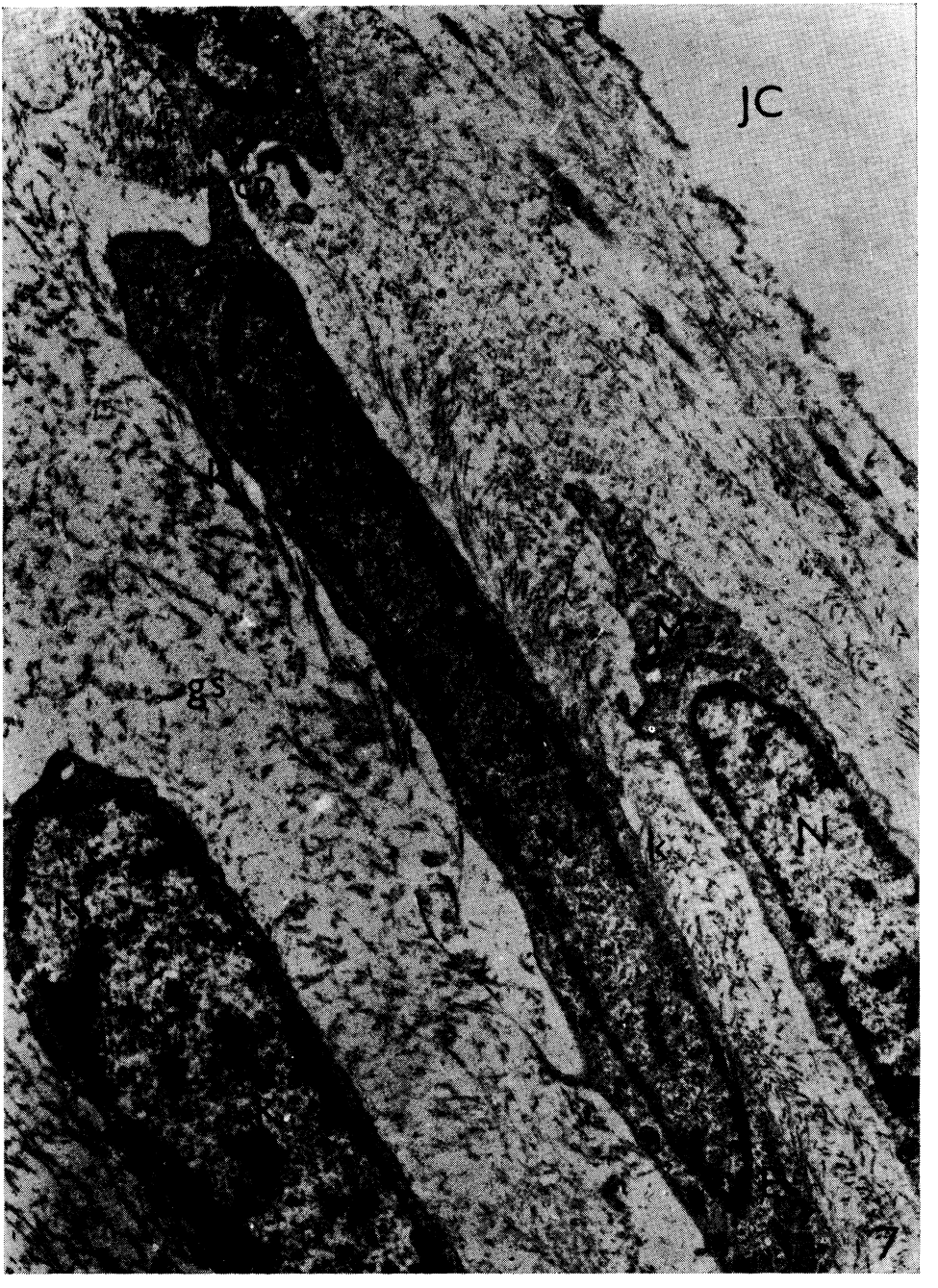
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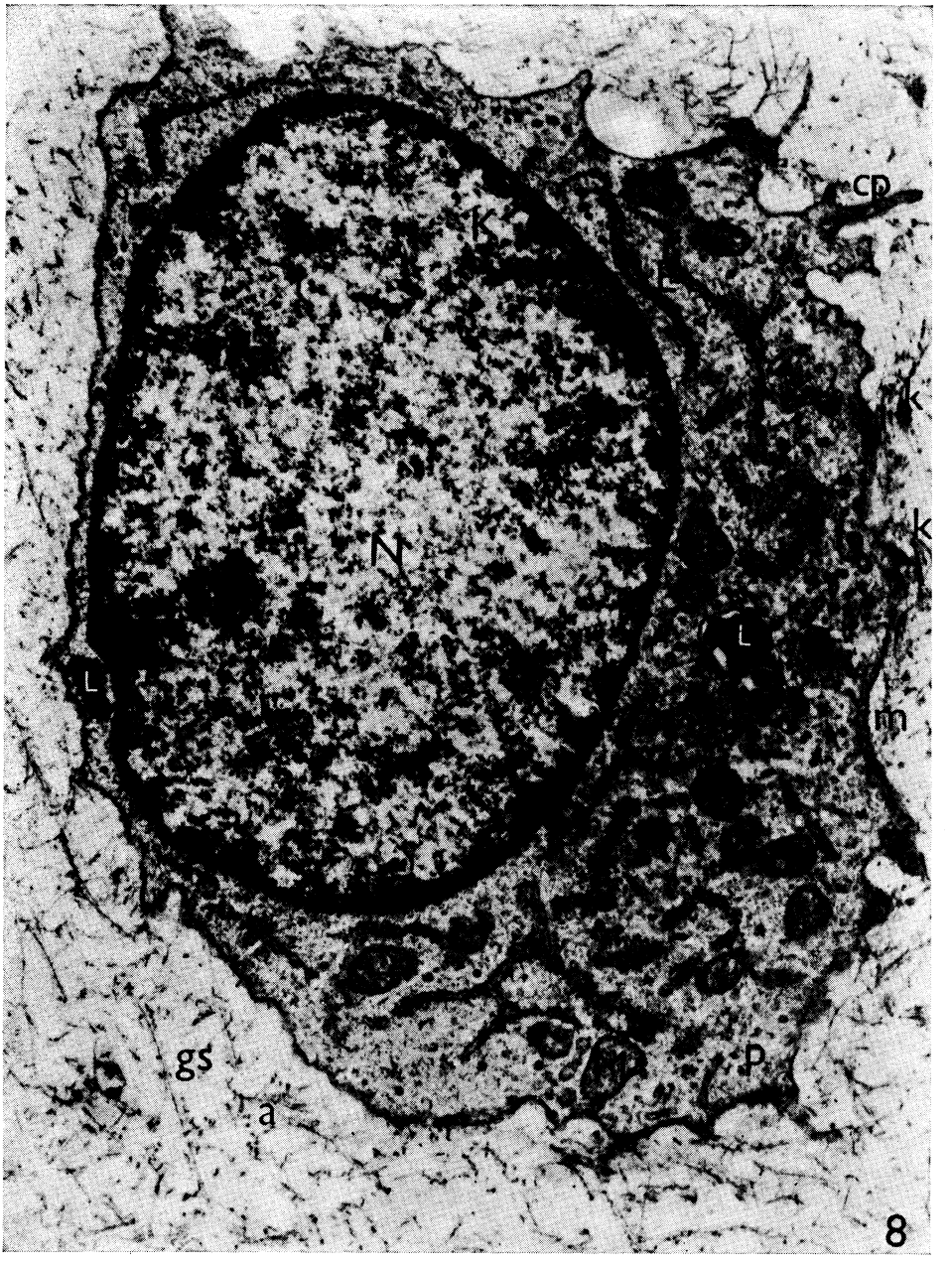
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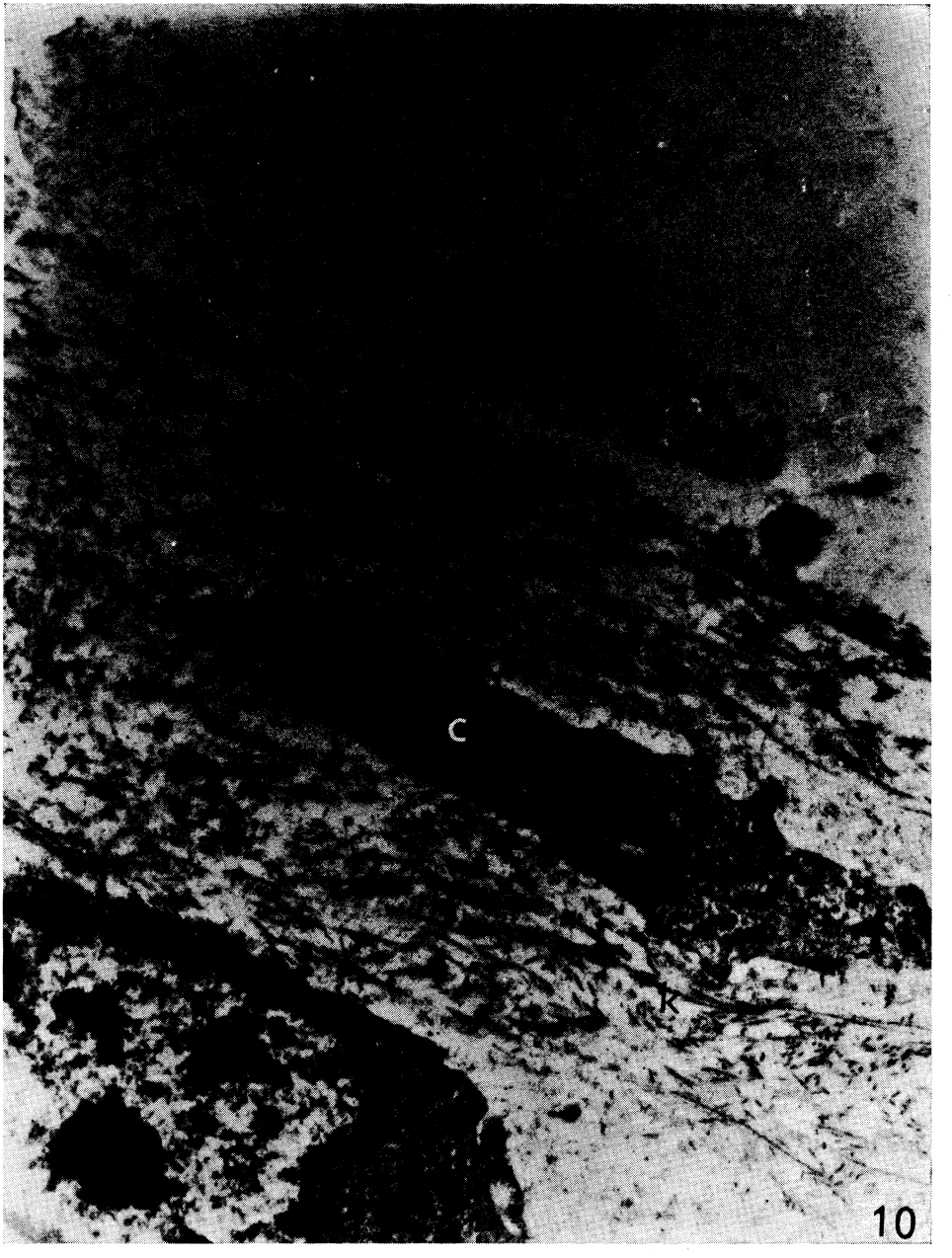
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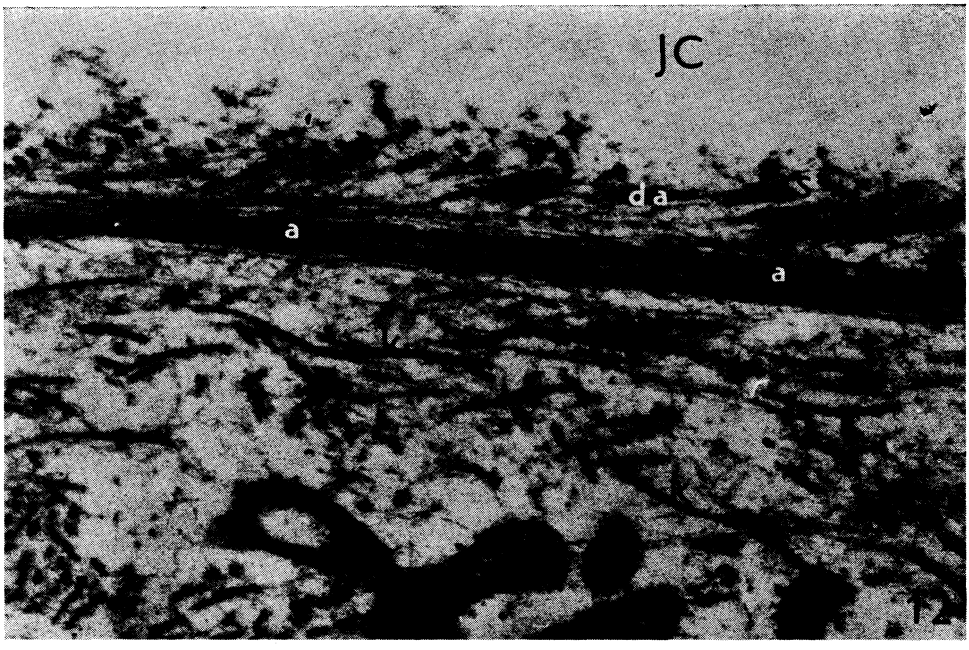
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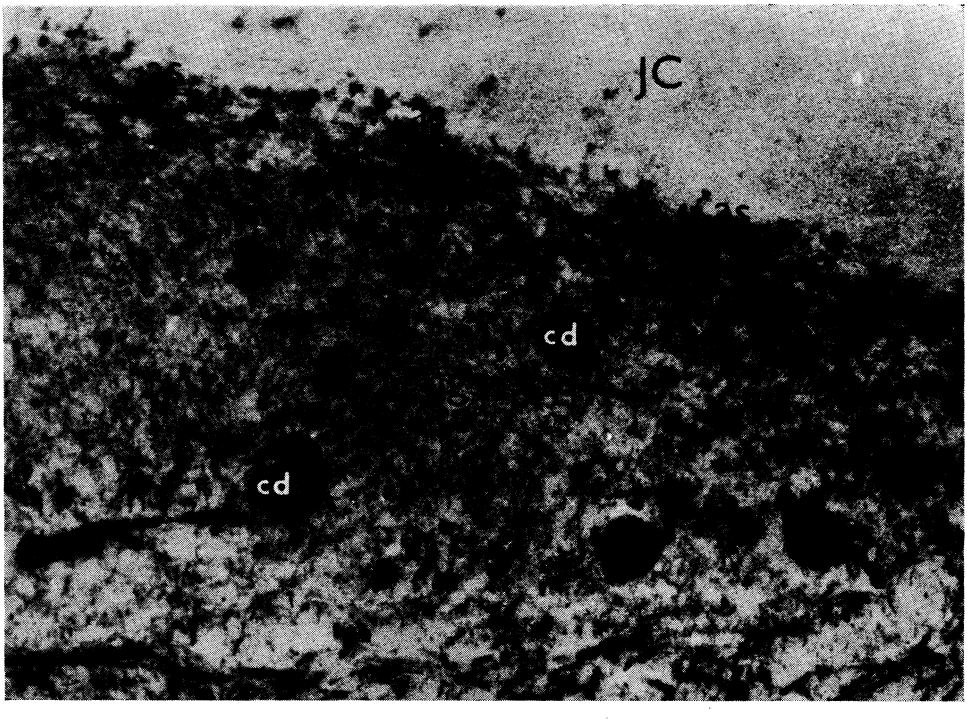
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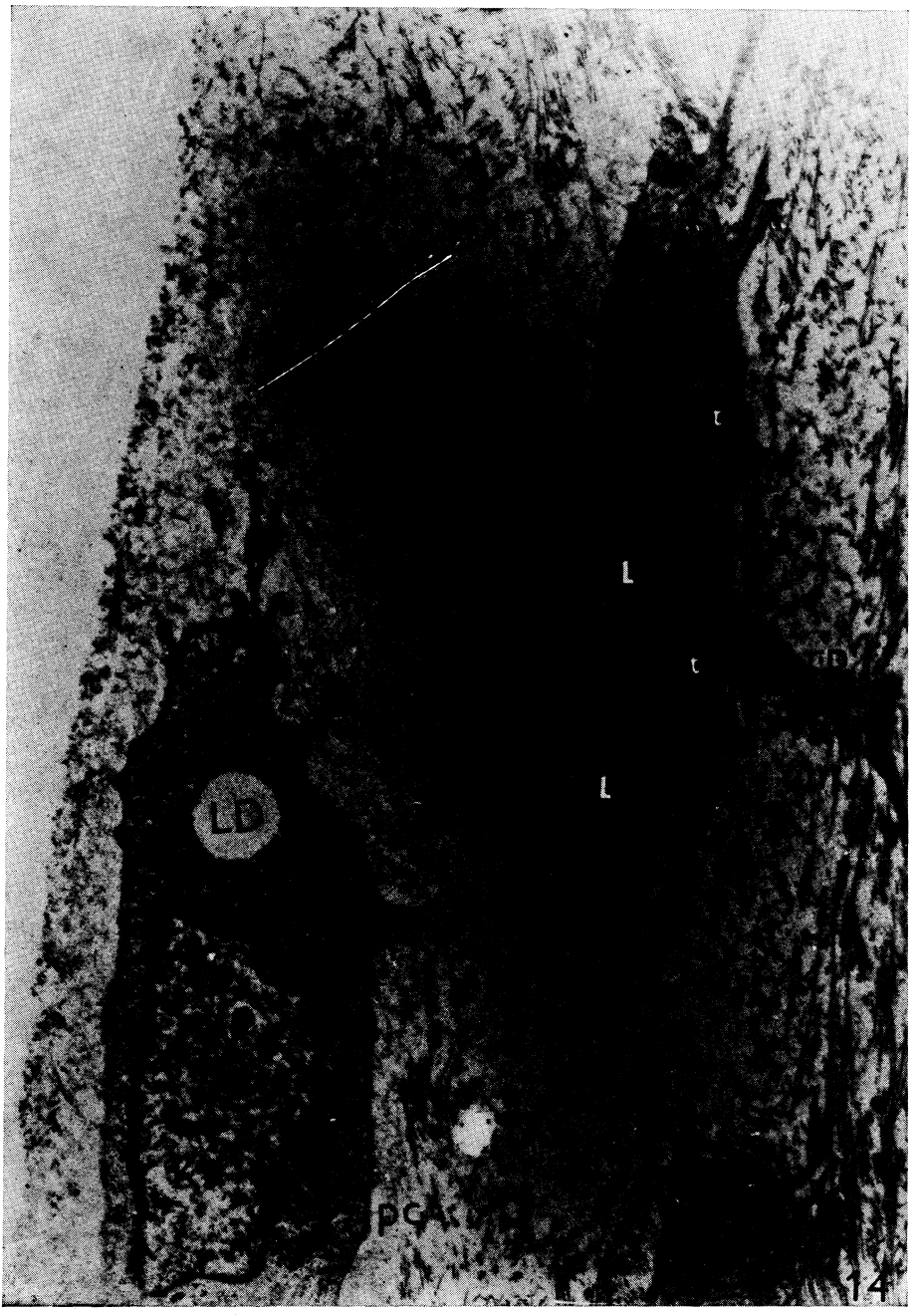
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Fig. 1. A chondroblast of the articular cartilage surface zone at 41 days after fertilization. Nucleus (N), nucleolus (n), nuclear pore (→), zonula nucleum limitans (z), karyosomes (K). Granular endoplasmic reticulum (E), transport vacuoles (t), mitochondria (M), pinocytotic vesicles (p), glycogen (g), intracytoplasmic filaments (f). Aperiodic fibrils (a), ground amorphous substance (gs). Magnification: × 12.500

Fig. 2. Part of the articular cartilage surface at 41 days after fertilization. Bundles of aperiodic fibrils (a) at the border of the articular cavity (JC). Some of them show signs of periodicity (fp) and extend into the articular cavity. Remnants of amorphous matter above the bundles of fibrils (as). Magnification: × 18.000

Fig. 3. Part of a chondroblast of the cartilage medium zone at 41 days after fertilization.Nucleus (N), karyosomes (K), zonula nucleum limitans (z), cisternae of the granular endoplasmic reticulum (E), transport vacuoles (t), mitochondria (M). Cytoplasmic processes (cp), lipid droplets (LD), glycogen deposits (g). Magnification: × 16.000

Fig. 4. A chondroblast of the articular cartilage medium zone at 41 days after fertilization. Nucleus (N), karyosomes (K). Cisternae of the granular endoplasmic reticulum (E), mitochondria (M), transport vacuoles (t), lysosomes (L), polyribosomes (R). Bundles of intracytoplasmic filaments (f) penetrating into the surrounding ground amorphous substance (as). Lipid droplets (LD), cytoplasmic processes (cp). Aperiodic fibrils (a) in the intercellular matter. Magnification: × 12.000

Fig. 5. Part of nucleus and cytoplasm of a chondroblast of the medium cartilage zone at 41 days after fertilization. Nucleus (N), karyosomes (K). Dilated cisternae of the agranular endoplasmic reticulum (E), mitochondria (M), lysosomes (L), transport vacuoles (t), polyribosomes (R), bundles of intracytoplasmic filaments (f) which extend into the ground amorphous substance (gs). Collagen microfibrils (cm), cytoplasmic processes (cp). Magnification: × 28.000

Fig. 6. A pair of chondroblasts in the deep zone of the articular cartilage at 41 days after fertilization. Nucleus (N), karyosomes (K), zonula nucleum limitans (z). Granular endoplasmic reticulum (E), mitochondria (M), Golgi complex (G), transport vacuoles (t), cytoplasmic processes (cp), pinocytotic vesicles (p). Ground amorphous substance (gs) with aperiodic fibrils (a). Magnification: × 12.000

Fig. 7. Surface zone of the articular cartilage at 61 days after fertilization. Chondroblast nuclei (N), karyosomes (K), zonula nucleum limitans (z). Granular endoplasmic reticulum (E), agranular endoplasmic reticulum (A), Golgi complex (G), mitochondria (M). Ground amorphous matter (gs) with bundles of aperiodic fibrils (a) and collagen fibrils (k) frequently found close to the cell membrane. Cytoplasmic processes (cp). Magnification: × 12.000

Fig. 8. A chondroblast of the medium zone of the articular cartilage at 61 days after fertilization Nucleus (N), karyosomes (K), zonula nucleum limitans (z). Short cisternae of the granular endoplasmic reticulum (E), numerous vesicles of the Golgi complex (G), mitochondria (M), lysosomes (L). Cytoplasmic processes (cp), pinocytotic vesicles (p). Ground amorphous substance (gs) with aperiodic fibrils (a) and collagen microfibrils (k) close to the cell membrane (cm). Magnification: × 16.000

Fig. 9. Parts of nucleus and cytoplasm of chondroblasts in the medium zone of the articular cartilage at 61 days after fertilization. Nucleus (N) with large karyosomes (K). Cisternae of the granular endoplasmic reticulum with dark content (E), numerous vesicles of the agranular endoplasmic reticulum (A), numerous minute vesicles of the Golgi complex (G). Mitochondria with dark matrix (M), centriole (c). Large vacuoles with granular and net-like content (V) found both in the cytoplasm and at the cell membrane. Cytoplasmic processes (cp), filament bundles in the processes (f). Ground substance (gs) with collagen microfibrils (k). Magnification: × 16.000

Fig. 10. Part of the articular cartilage surface at 61 days after fertilization. Articular cavity (JC) lined with large vesicles containing net-like substance (V). Intercellular matter with chondroblasts (C), short bundles of aperiodic filaments (f) and collagen fibrils (k) embedded in the ground amorphous substance (gs). Magnification: × 16.200

Fig. 11. Columns of chondroblasts in the deep zone of the articular cartilage at 61 days after fertilization. Nuclei (N), karyosomes (K), zonula nucleum limitans (z). Occasional cisternae of the granular endoplasmic reticulum (E) with dark material, vesicles of the agranular endoplasmic reticulum (A), mitochondria (M), polyribosomes (R). Cytoplasmic processes (cp). The intercellular matter differentiated into the pericellular (pc) and the intercellular (im) matrix. Magnification: × 16.000

Fig. 12. Part of the articular cartilage surface at 61 days after fertilization. The region adjacent to the articular cavity (JC) is covered with thick bundles of aperiodic fibrils (a). Towards the articular cavity, these sent out single filaments either unmasked (da) or coated with amorphous matter (as). Underneath thin networks of collagen fibrils can be seen (k). Magnification: × 28.000

Fig. 13. Part of the articular cartilage surface at 61 days after fertilization. Surface networks of collagen fibrils (k) are embedded in amorphous matter (as) which covers the cartilage facing the cavity (JC). Some fibrils are unmasked. Deeper parts of the ground amorphous matter (gs) show cellular detritus (cd). Magnification: × 18.000

Fig. 14. Surface layer of the articular cartilage at 94 days after fertilization. Chondroblast nucleus (N), minute karyosomes (K). Branched cisternae of the granular endoplasmic reticulum (E), vesicles of the agranular endoplasmic reticulum (A), transport vacuoles (t), lysosomes (L), mitochondria (M). Cytoplasmic processes (cp) extending into the intercellular matrix (im). Collagen fibrils and bundles of aperiodic filaments passing across the cell membrane (→). Small deposits of glycogen in surface cells (g), lipid droplets (LD). Intercellular matter is distinguished into pericellular (pc) and intercellular (im) matrix with abundance of collagen fibrils (k). Magnification: × 11.200

Fig. 15. A chondroblast of the medium zone of the articular cartilage at 94 days after fertilization. Nucleus (N), nucleolus (n), karyosomes (K), zonula nucleum limitans (z). Granular endoplasmic reticulum (E), Golgi complex (G) extending over several fields, mitochondria (M), lysosomes (L), transport vacuoles (t), glycogen (g), small bundles of aperiodic filaments (a). Cytoplasmic processes (cp) running into the perinuclear matrix (pm), intercellular matrix (im) with networks of collagen fibrils (k). Magnification: × 12.000

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