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SUBMICROSCOPIC STRUCTURE OF THE HUMAN JOINT CARTILAGE

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Abstract

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Using a transmission and scanning electron microscope the joint cartilage was studied in 43 persons of the age from 5 to 75 years. As far as possible the present study gives a finished survey of the ultrastructure of chondrocytes of the superficial, middle and deep layers of the articular cartilage, arrangement of the intercellular substance; presented are basic data on the formation of the surface of the cartilage with regard to the age of the object. Special attention is given to cartilage intercellular substance which – from the point of view of electron microscopy – has been studied only little. The components of intercellular substance are arranged in such a way that we can distinguish the specialized areas near the chondrocytes as the pericellular matrix on the one hand, and the other intercellular substance, the intercellular matrix, on the other. The surface of the cartilage is covered with a specialized layer designated as lamina splendens. Results of the study proper are confronted with published data not only with regard to human joint cartilage but data on these structures have been studied also in lower mammals as much knowledge holds generally true for this type of tissue. The study summarizes the known facts about joint cartilage and provides a necessary basis for judging the degrees of changes occurring during some of their injuries.

Ultrastructure, hip joint cartilage, chondrocytes, intercellular matrix, SEM.

A number of authors dealt with the description of submicroscopic structure of joint cartilage; principal studies dealing with human cartilage were published by Zelander (1959), Godman et al. (1960), Fawcett (1966a), Meachim (1967), Stockwell (1967a), Silberger (1968), Weiss et al. (1968). Brower and Hsu (1969), Ghadially and Roy (1969), Meachim (1969), Meachim and Roy (1969), Hirohata and Morimoto (1971), Stockwell and Meachim (1973), Serafini-Fracassini and Smith (1974), and others. The present study gives the most possible finished survey about the ultrastructure of both the chondrocytes of the joint cartilage and the intercellular substance; given are also basic data on the formation of its surface in dependence on the age of the object. Results of our study are confronted with published data not only with regard to human joint cartilage but also to data on lower mammals as much knowledge holds generally true for this type of tissue. This fact has been proved in studies published by Barnett et al. (1961, 1963), Silberger et al. (1961), Davies et al. (1962), Palfrey and Davies (1966), Wassilev (1972), Stockwell (1971b, c), Meachim and Stockwell (1973), and others. The importance of the present study lies in the fact that it summarizes present known facts about this tissue and provides a necessary basis for estimating and comparing the degrees of changes occurring with some diseases of this basic part of the joint.

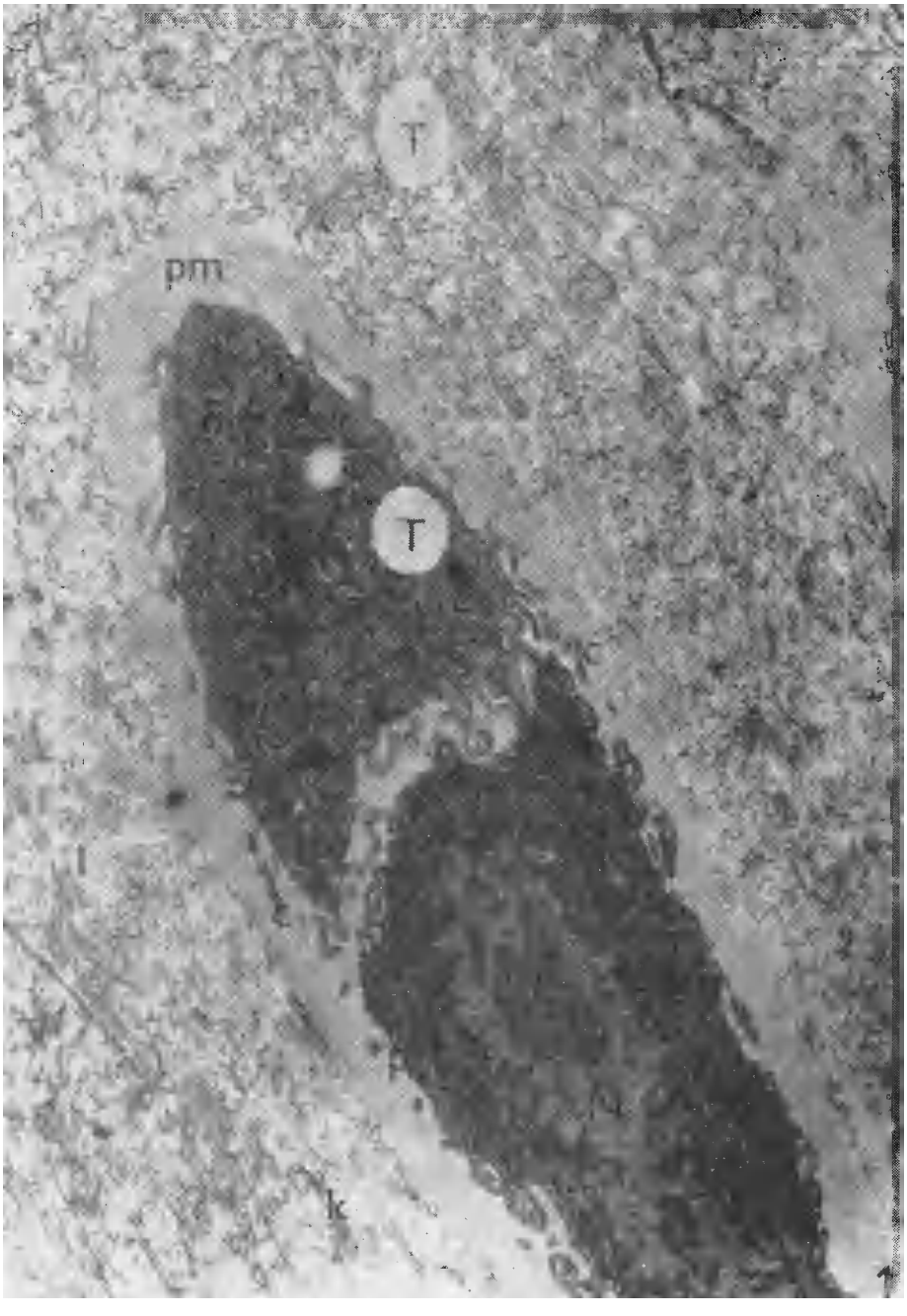


Fig. 1. Pairs of chondrocytes of the superficial layer of the cartilage.
Nucleus (N), zonula nucleum limitans (Z), nucleolus (n), granular endoplasmic reticulum (E), lysosomes (L), mitochondria (M), Golgi complex (G), centriole (C), lipid droplet (T), ribosomes (R), cytoplasmic filaments (f), processes of the cellular membrane (c), pinocytotic vesicles (p), pericellular matrix (pm), intercellular matrix (I), collagen fibres (K). × 25 000.

Materials and Methods

In studies of the ultrastructure of the joint cartilage samples were taken of the tissue from the hip joint of 43 patients 5 to 75 years either with total endoprostheses or also with old amputations due to other, mostly neoplastic diseases, not localized in the joint.

Tissue strips 1 × 1 × 2 mm in size were fixed in 3 % glutaraldehyde at pH 7.4, then decalcified in 0.1 M EDTA with 4 % glutaraldehyde at pH 7.4. The samples were postfixed with 1 % OSO_4 according to Millonig in a phosphate buffer at pH 7.4 and the blocks were embedded into Durcupan ACM, or Epon-Araldit. Sections were made on a Reichert OMU 3 ultramicrotome, they were stained with uranylacetate and lead citrate and studied with Tesla DS 613 and Tesla BS 500 electron microscopes.

For scanning electron microscopy the 5 × 5 mm blocks were fixed with formol or glutaraldehyde, dehydrated with alcohol and coated with gold on a Balzers coating apparatus. The samples were studied using a Stereoscan Cambridge scanning microscope.

Results and Discussion

Submicroscopic structure of chondrocytes

On the basis of results of studies using a light and electron miccroscope it is evident that chondrocytes are, in principle, arranged into three layers: superficial, middle and deep (Palfrey and Davies 1966; Meachim 1967; Weiss et al. 1968; Meachim 1969 and others).

Superficial layer

The chondrocytes of the superficial layer are spindle-shaped elongated cells, of a size of 5 × 10—12 μm, deposited in a longitudinal axis approximately parallel to the surface of the cartilage. According to Gould et al. (1974) this orientation is connected with the formation of the intercellular substance where spiral-shaped formations begin to form in the chondrification centre and the chondrocytes on the periphery of the cartilage are compressed by the produced intercellular substance. Another factor which undoubtedly contributes to the deposition of the chondrocytes of the superficial layer is the adaptation to pressure affecting the chondrocytes from the outside and adapting to these stimulations. From the mechanical point of view is this arrangement the most convenient for cells as was stated by Clarke (1971 a, b; 1974) and Zimny and Redler (1972). The shape of the chondrocytes on the section is most frequently elliptic or arched in an S-shape. They are deposited in capsules of the basic substance mostly solitary; only sporadically do they occur in pairs (Fig. 1) as was also quoted by Crelin (1957), Zelander (1958), Fawcett (1966a), Meachim (1967), Stockwell (1967a), Silberger (1968), Weiss et al. (1968), Ghadially and Roy (1969), Meachim and Roy (1069), Hirohata and Morimoto (1971), Stockwell (19 (1971c), Stockwell and Meachim (1973), Meachim and Stockwell (1973).

a) Nucleus

The chondrocytes of the superficial layer of an adult joint cartilage contain one ovoid or irregularly lobular nucleus (Fig. 1).

The nuclear envelope is formed by two membranes. Ribosomes attach rather sporadically onto the external membrane of the nuclear envelope. The external nucleus membrane passes in places into the membranes of the granular endoplasmic reticulum (Davies et al. 1962; Barnett et al. 1963; Palfrey and Davies 1963; Meachim 1967; Silberger 1968;Toner and Carr 1968; Weiss

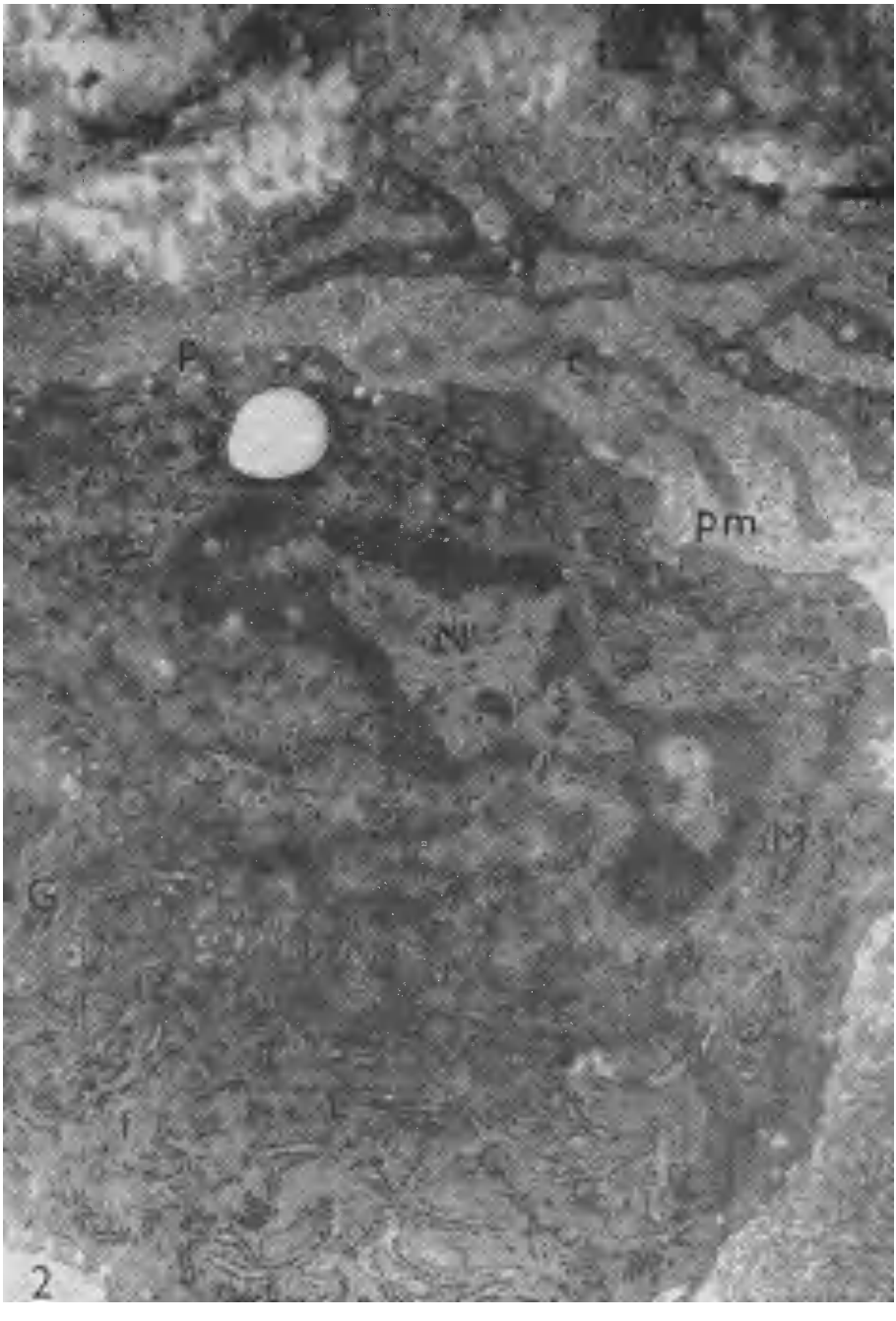


Fig. 2. Part of the chondrocytes of the middle layer of the normal cartilage.
Nucleus (N), zonula nucleum limitans (z), granular endoplasmic reticulum (E), Golgi complex (G), mitochondria (M), ribosomes (R), glycogen (g), cytoplasmic filaments (f), cytoplasmic processes (c), pinocytotic vesicles (p), pericellular matrix (pm), × 12 900.

et al. 1968; Meachim 1969; Ghadially and Roy 1969) even though these connections in adult joint cartilage are much less frequent than in cartilage in the period of development as was demonstrated by Godman et al. (1960), Barnett et al. (1961), Brower and Hsu (1969), Meachim (1969) in humans, Silberger et al. (1961), Wassilev (1972), Oakes and Handley (1974) and others on experimental material. Both on the cross and tangential sections of the nucleus not very numerous pores of the nucleus envelope are evident. On the cross sections their shape is rounded, their size ranges on average from 50 to 75 nm. Nuclear chromatin on the ultrathin section of the chondrocyte nucleus is generally arranged into karyosomes located by the nuclear membrane and into several smaller clusters outside the nuclear envelope (Meachim 1969; Weiss et al. 1968). A typical structure regularly occurring in the karyoplasm of the chondrocytes of the superficial layer is the so called zonula nucleum limitans (Fig.1). It is evident on the nucleus sections as a layer of finely granulated, moderately osmiophilic substance attached to the internal membrane of the nuclear envelope. Its thickness in the nuclei of chondrocytes of human joint cartilage ranges between 20—40 nm. It was first described in various cells of invertebrates (Pappas 1956; Beams et al. 1957; Cogeshall and Fawcett 1964) and then in vertebrates (Fawcett 1966b; Patrizi and Poger 1967; Schubert 1967; Ghadially et al. 1972, 1974; Oryschak et al. 1974). It is interesting that neither Zelander (1959), Davies et al. (1962), Barnett et al. (1963), Meachim (1967), Palfrey and Davies (1966), Silberger (1968), Weiss et al. (1968), Ghadially and Roy (1969), Hirohata and Morimoto (1971), Meachim and Stockwell (1973), Stockwell and Meachim (1973) nor any other authors presented this layer when describing the ultrastructure of chondrocytes even though it is demonstrable in chondrocyte nuclei of both human cartilage and cartilage of lower mammals. This structure is not unchangeable and in the chondrocytes of various age is unevenly differentiated. As proved by Oryschak et al. (1974) on rabbit chondrocytes, it is evident immediately after birth as a very thin strip 4.6 nm wide; however, at the age of 4 months its thickness reaches 23 nm. This author found similar conditions in dog, cow and horse. Similarly Ghadially et al. (1972) proved that during the process of reparation of a serious defect of the car-cartilage during early proliferation of cells the zonula nucleum limitans is thinner than in older cells. It thus follows that this structure is not a static formation of fixed dimensions in a given cellular type and that it is subjected to changes in the course of various physiological and pathological conditions of the cell. However, its origin and function are as yet unknown.

The nucleolus of the chondrocytes of the superficial layer is rather large (Fig. 1). Providing it is found on the nucleus section it is always a nucleolus of a reticular type. The fact that it is present only seldom is documented in literature in such a way that only some authors mention the nucleolus (Davies et al. 1962; Fawcett 1966a; Toner and Carr 1968; Ghadially and Roy 1969), whereas Barnett et al. (1963), Palfrey and Davies 1(966), Silbereger (1968), Weiss et al. (1968), Hirohata and Morimoto (1971) and others do not mention it in their studies.

b) Cytoplasm

A characteristic feature of the cytoplasm of chondrocytes of the superficial layer of the joint cartilage is a relatively large amount of cell organelles, whereas cytoplasmic inclusions (lipid droplets and glycogen) occur only to a limited extent.

α) Cell organelles

Granular endoplasmic reticulum in the cytoplasm of chondrocytes of this layer is arranged on the one hand in the form of short flattened cisternae and in the form of vesicles on the other hand. The cisternae are more or less disarranged and are deposited in the basic cytoplasm sporadically between the mitochondria (Fig. 1). Very rarely can we observe their linkage with the external membrane of the nuclear envelope. The internal spaces of the granular endoplasmic reticulum are nearly always filled up with a medium osmiophilic, homogeneous material. Our investigations agree with data published by Zelander (1959), Godman et al. (1960), Silberger et. al. (1961), Davies et al. (1962), Fawcett (1966a), Meachim (1967), Schubert (1967), Silberger (1968), Ghadially and Roy (1969), Meachim and Stockwell (1973), and Stockwell and Meachim 1(973), in contradistinction to Barnett et al. (1963), Palfey and Davies (1966) and mainly Weiss et al. (1968) and Hirohata and Morimoto (1971), who found in their material either only small amounts of granular endoplasmic reticulum, or they describe its cisternae as very dilated. We cannot agree especially with the investigations of Weiss et al. (1968) who distinguished two types of cells in the superficial zone of the joint cartilage according the amount of granular endoplasmic reticulum and also according to the appearance and arangement of chromatin in the nucleus. It is evidently the same phenomenon as described by Hirohata and Morimoto (1971), i. e. the beginning degeneration of chondrocytes of the superficial layer which could be manifested in the initial stage by these characters.

Agranular endoplasmic reticulum appears in the cytoplasm of chondrocytes of the superficial layer only very exceptionally. In our material the agranular endoplasmic reticulum was observed in the form of small vesicles scattered in the cytoplasm. It is possible that they originate from cisternae of the granular endoplasmic reticulum; this is generally accepted as one of the possibilities of their origin.

The Golgi complex on the sections of the chondrocytes of the superficial layer of the joint cartilage can be found deposited in the cytoplasm in one zone — Golgi apparatus — which is usually localized nearer to the surface of the cell (Fig. 1). In the region of the Golgi complex proteins accumulate, synthesized on the level of the granular endoplasmic reticulum, namely precursors of collagen (protocollagen, tropocollagen and proline residua) and are transferred either into the cytoplasm where their aggregation takes place, or extracellularly in which the vacuoles of the Golgi complex play an important role (Sheldon and Kimball 1962; Revel and Hay 1963; Cox et al. 1976; Ghadially and Roy 1969; Meachim and Stockwell 1973 and others).

Mitochondria do not differ in their structure from mitochondria of other somatic cells. On the sections they are rounded or only slightly elongated. Their length does not exceed 1 μm, on the cross section their diameter is about 0.5 μm. The internal membrane protrudes in scarce cristae which are leaf-shaped (Fig. 1). In some cases among the mitochondria of a usual appearance there appear also small amounts of mitochondria with a strikingly light matrix and slightly damaged cristae. They are probably disintegrating mitochondria as was also stated by Kaplan and Meyer (1959), Meachim et al. (1965), Weiss and Mirrow (1972), Ghadially et al. (1970), Mitchell and Shepard (1970), Dearden et al. (1974), Gritzka et al. (1974), Refior (1974), Rother and Rödel (1975), Hanaoka (1976), Podrushniak and Cerkasov (1976), Silberger et al.

(1976), and others especially on the basis of investigations of these organelles in ageing cartilage and initial forms of arthroses.

Ribosomes in the cytoplasm of chondrocytes of the superficial layer are mostly bound to membrane of the granular endoplasmic reticulum. Only a small amount of them is freely deposited in the cytoplasm in the form of monosomes; polysomes can be found only very sporadically. This fact could be the cause of them being generally omitted in literature.

Lysosomes occur in small amounts but regularly in the cytoplasm of the chondrocytes. On the surface they are enclosed by a simple membrane and they reach a dimension of 0.5—0.8 μm. Their content is nearly in all cases electron dense and is formed by a material of a homogenous appearance (Fig. 1). The opinion of most authors is that they are secondary lysosomes but in their studies they usually designate them as electron dense corpuscles (Zelander 1959; Silberger et al. 1961; Davies et al. 1966; Meachim 1967; Silberger 1968; Ghadially and Roy 1969; Meachim and Stockwell 1973; Stockwell and Meachim 1973). It was Chrisman et al. (1962) and Chrisman (1969) who dealt with the role of lysosomes in chondrocytes and who, besides the usual enzymes, proved cathepsin in them. The function of lysosomes in chondrocytes under experimental conditions was studied by Riede (1974), Oryschak and Ghadially (1974), Meikle (1975), and Oryschak and Ghadially (1976) who instilled various substances into the joint cavity (e. g. gold salt, Thorotrast, etc.) and proved their accumulation in the lysosomes.

Microtubules were described for the first time by Behnke (1964) in various cells of vertebrates and Palfrey and Davies (1966) proved them in chondrocytes of rabbits. In human cartilage they were described by Weiss et al. (1968). Their function in chondrocytes is not exactly known. Ehrlich and Bornstein (1972) are of the opinion that they can contribute to transcellular distribution of procollagen. In our material they were not found.

Centrioles (Fig. 1) occur in chondrocytes only very sporadically (Ghadially and Roy 1969; Scherft and Daems 1967; Scheck et al. 1975). The rare occurrence of these organelles is most probably connected with the fact that the differentiated chondrocytes separate under normal conditions only very exceptionally (Crelin 1957; Chrisman 1969). Their activity increases e. g. during reparative processes (Chrisman 1969; Ghadially et al. 1971) when in the place reparation of the defect mitoses can be observed.

β) cytoplasmic inclusions

Lipid droplets are a regular component of the cytoplasm of chondrocytes of the superficial layer. In accordance with the author (Fig. 1) they were described by Barnett et al. (1961), Davies et al. (1962), Barnett et al. (1963), Stockwell (1966), Collins et al. (1965), Stockwell (1967b). Ghadially et al. (1965) found lipids also outside the cells both in the superficial and in the deep layer. They presume that they can originate either from the synovial liquid or that they are extruded from the cytoplasm of the chondrocytes into the intercellular substance.

Glycogen in the cytoplasm of chondrocytes in the superficial layer occurs in a small amount and is present in the form of individual granules. Our investigations about the amount, shape and localization of glycogen granules corresponds with data presented by Ghadially and Roy (1969), Meachim and Roy (1967), Meachim and Stockwell (1973), whereas, for example, Weiss et al. (1968)

and Hirohata and Morimoto (1971) did not record their occurrence in chondrocytes of this layer.

Cytoplasmic fibrillar structures are evident on ultrathin sections of chondrocytes of the superficial layer of the joint cartilage as bundles of fine filaments deposited in the ground cytoplasm. They are not enclosed from the surrouding cytoplasm by a membrane and in the largest amount they are usually deposited in a perinuclear area (Fig. 1). Their size ranges between 9—12 nm, they are closely clustered and they run more or less parallelly. On the longitudinal sections no periodicity is evident. Fibrils of this kind were described earlier both in chondrocytes of various species of lower mammals (Davies et al. 1962; Barnett et al. 1963; Palfrey and Davies 1966) and in cells of the human cartilage (Collins et al. 1965, Ghadially et al. 1965; Meachim et al. 1965; Fawcett 1966a; Meachim 1967; Meachim and Roy 1967, 1969; Silberger 1968; Weiss et al. 1968; Hirohata and Morimoto 1971; Freeman 1973; Meachim and Stockwell 1973; Kostovič-Knežević and Svajger 1975) and others. In connection with the occurrence of cytoplasmic fibrillar structures it is necessary to state that morphologically similar fibrillar material occurs extracellularly in the close vicinity of chondrocytes. It is part of their capsule and is formed by less distinct fibrils the arrangement of which is irregular and they are arranged more scarcely. It is probable that the same as in rabbit chondrocytes where their appearance and localization are the same these filaments are synthesized in the spaces of the granular endoplasmic reticulum and transported into the spaces of Golgi complex where they were also proved by Sheldon and Kimball (1962). Their formation can be increased also experimentally as was shown, for example, by Silberger et al. (1965) on chondrocytes of joint cartilage of mice after administering estrogen. In the studies of Revel and Hay (1963) with labelled proline and of Goldberg and Green (1964) it was proved that it is tropocollagen which initially accumulated in the cells and later extruded into the intercellular space where it polymerized into typical collagen fibres (Freeman 1973).

γ) Cell surface

The cytoplasm of chondrocytes of the superficial layer over the whole periphery protrudes in short cytoplasmic processes of various distances apart. On the longitudinal sections they are 0.5—1 μm long, their width does not exceed 0.7 μm. They afflict the pericellular matrix, only exceptionally do they penetrate into the intercellular substance (Fig. 1).

The cell surface of chondrocytes of this layer formed in the mentioned way in lower mammals was described by Silberger et al. (1961), Davies et al. (1962), Barnett et al. (1963), Palfrey and Davies (1966), Brower and Hsu (1969), Stockwell (1971c), Stockwell and Meachim (1973), in human joint cartilage by Collins et al. (1965), Meachim et al. (1965), Ghadially et al. (1965), Stockwell (1967a), Silberger (1968), Weiss et al. (1968), Ghadially and Roy (1969), Meachim (1969), Meachim and Roy (1969), Hirohata and Morimoto (1971), Meachim and Stockwell (1973). On the basis of the author's own material it can be stated that his investigations are in accordance with the conclusions of Weiss et al. (1968) and Ghadially and Roy (1969) only in the arrangement of the chondrocyte area turned towards the surface of the cartilage. On the area turned towards the middle layer the authors quoted found a much larger amount of cytoplasmic processes as compared with the surface area. No

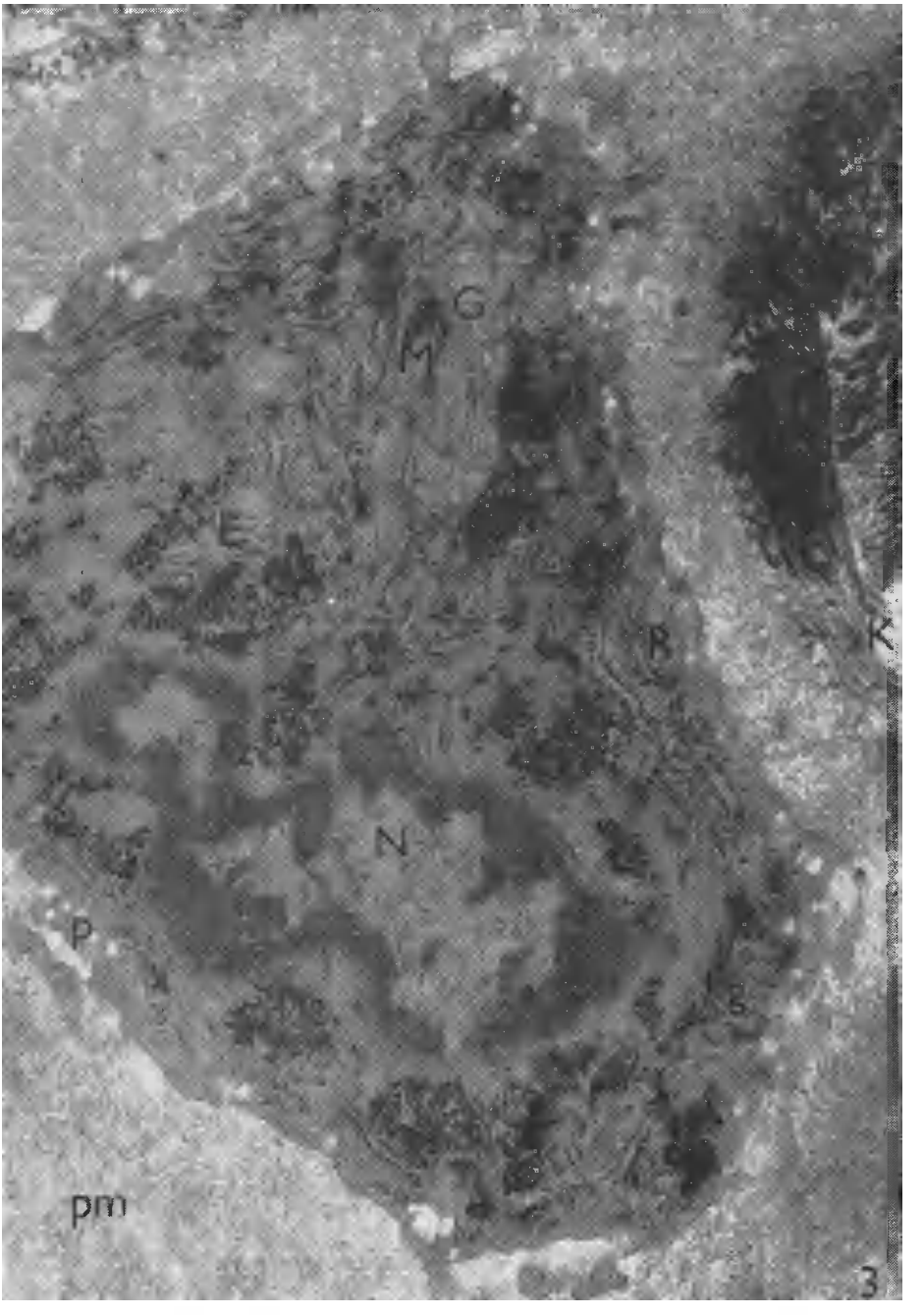


Fig. 3. Chondrocyte of the middle layer of a normal cartilage.
Nucleus (N), zonula nucleum limitans (z), granular endoplasmic reticulum (E), Golgi complex (G), mitochondria (M), ribosomes (R), glycogen (g), cytoplasmic filaments (f), pinocytotic vesicles (p), collagen fibres (K), pericellular matric (pm). × 25 600.

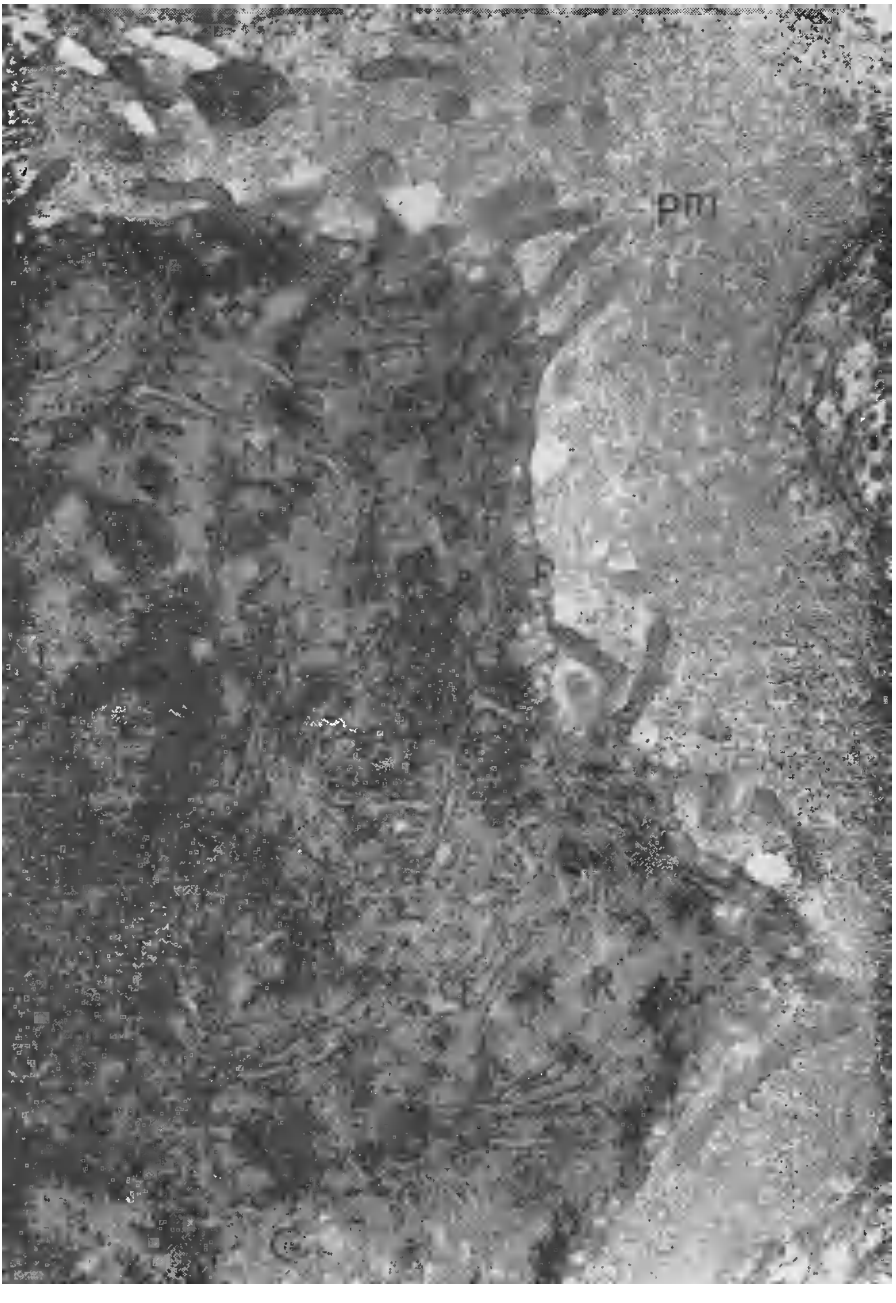


Fig. 4. Part of the nucleus and cytoplasm of a chondrocyte of the middle layer of a normal cartilage. Nucleus (N), zonula nucleum limitans (z), granular endoplasmic reticulum (E), Golgi complex (G), mitochondria (M), ribosomes (R), glycogen (g), cytoplasmic filaments (f), cytoplasmic processes (c), pinocytotic vesicles (p), thick collagen fibres with periodicity (K), pericellular matrix (pm). × 12 600.

difference were found by the author in the number and arrangement of opposite areas. The author's findings are in accordance with those of the other authors in the occurrence of pinocytotic vesicles which occur in variable numbers near the cell surface between the cytoplasmic processes, even though such a high frequency of occurrence cannot be confirmed as was given by Palfrey and Davies (1966) in chondrocytes of the superficial layer of the joint cartilage of the rabbit where these vesicles were found in regular distance of 120 nm and length as much as 2 μm. This phenomenon is probably connected with the actual state of cellular metabolism.

Strengthening formations of a desmosome type between the chondrocytes of the superficial layer were observed in lower mammals only (Palfrey and Davies 1966; Scheck et al. 1975) in the period immediately after birth in the dividing chondrocytes. In mature cartilage, both in lower mammals and in humans, they were not observed even if the chondrocytes were deposited in the close vicinity in a common envelope and their processes adjoined each other. Neither were they found in the author's material.

Cilia have been found only in the epiphyseal cartilage of mice embryos (Scherft and Daems 1967) together with basal corpuscles. Their fine structure, however, differed from the typical cilia in a defect of the microtubular apparatus. The above authors' opinion is, that even when this structure is based in the chondrocytes, its differentiation does not reach such a degree as in other types of cells. In adult human cartilage they have not yet been found.

Middle layer

Cells in this layer are mostly rounded and their size is approximately 15—20 μm. Chondrocytes are deposited in capsules single or in pairs. In the present study mostly single deposited chondrocytes in well formed capsules (Figs. 2, 3, 4) were observed. In this respect the author's findings are in accordance with the data of Meachim and Roy (1967, 1969), Ghadially and Roy (1969), whereas, for example, Weiss et al. (1968) observed especially pairs of chondrocytes in this layer.

a) Nucleus

On ultrathin sections the nucleus is of an irregularly oval shape, or is of a triangle-like rounded shape (Figs. 2—5). The same as in chondrocytes of the superficial layer the outer and inner membranes join and form nuclear pores. The perinuclear space contoured by the inner and outer membranes of the nuclear cover is very narrow in the majority of cases and only rarely expands. The zonula nucleum limitans which is of the same appearance and thickness as in the nuclei of the superficial layer adheres to the inner membrane (Figs. 2—5). Thus the zonula nucleum limitans separates the nuclear chromatin from the inner membrane of the nuclear envelope.

In the nuclei of chondrocytes of the middle layer chromatin is arranged similarly as in the preceding layer, however, it forms larger karyosomes pressing onto the zonula nucleum limitans, whereas the remaining area of the nucleus (on the section) is light (Figs. 2, 3). Chromatin arranged in such a way was observed by Meachim and Roy (1967, 1969), Weiss et al. (1968), Ghadially and Roy (1969), Meachim (1969) in chondrocytes of the middle layer of human cartilage and by Barnett et al. (1961, 1963), Silberger et al. (1961), Davies et al. (1962), Palfrey and Davies (1966) and others in the joint cartilage of rabbits and mice.

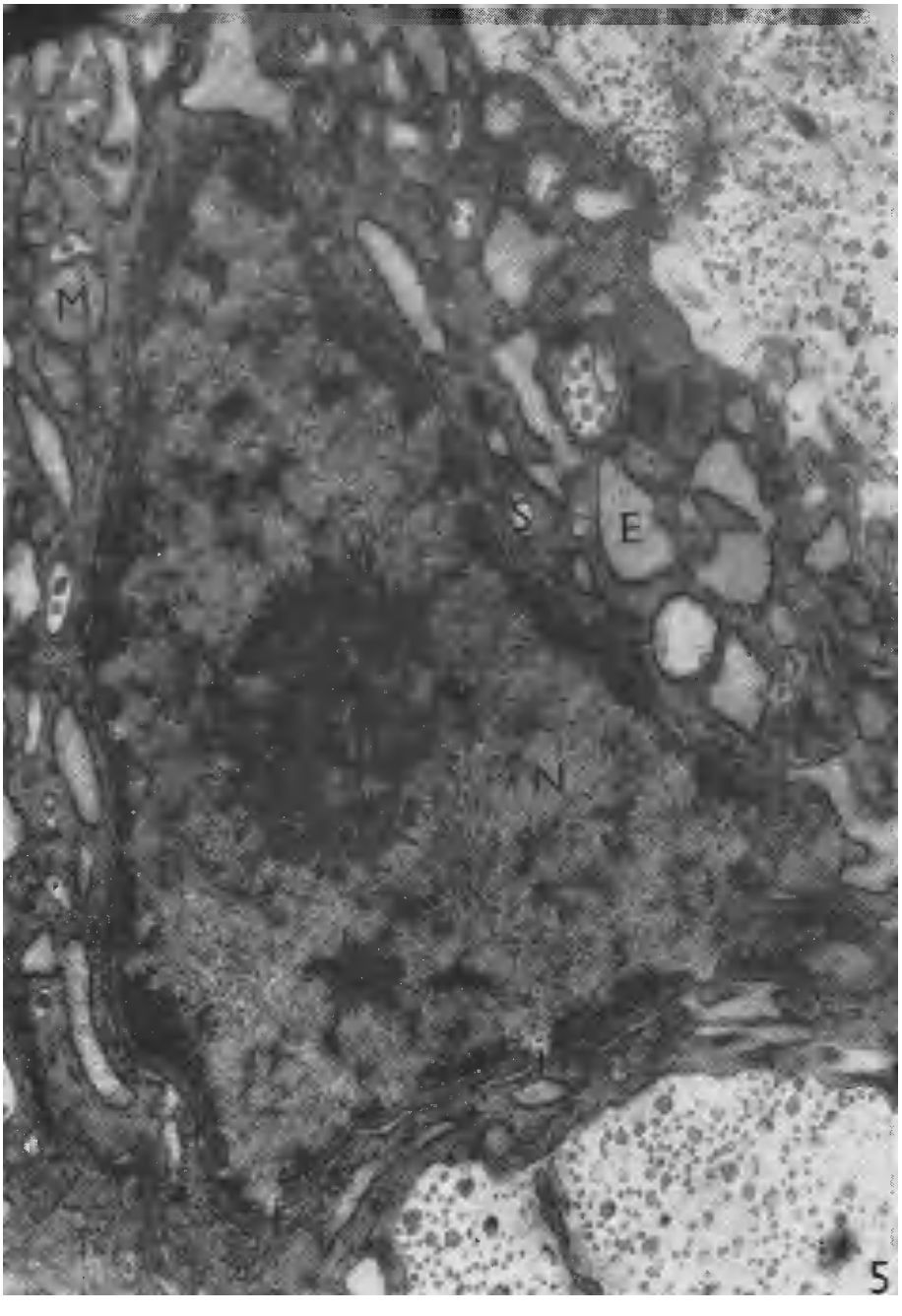


Fig. 5. Part of the nucleus and cytoplasm of a chondrocyte of the middle layer of a normal cartilage. Nucleus (N), nucleolus (n), zonula nucleum limitans (z), nuclear pores (j), dilated cisternae of the granular endoplasmic reticulum (E), agranular endoplasmic reticulum (S), mitochondria (m). × 26 400.

The nucleolus was found on the section of the nucleus of chondrocytes of the middle layer more frequently than in nuclei of cells of the superficial layer (Fig. 5). In all the cases the cross section was observed of one nucleolus of the reticular type the size of which was often as much as 2 μm.

b) Cytoplasm

In comparison with chondrocytes of the superficial layer the cytoplasm on the sections occupies a larger area (estimated semiquantitatively); in the cytoplasm there is a larger number of cellular organelles and an accumulation of inclusions occurs, especially of glycogen.

α) Cell organelles

Granular endoplasmic reticulum is arranged in two ways in the cytoplasm of chondrocytes of the middle layer. In the first case it has the shape of relatively short flattened cisternae some of which are widened in a stick-like way at the ends (Figs. 2, 3, 4). In most cases they are simple, their ramification or interconnection with the neighbouring cisternae can be observed only sporadically. In the other case they occur in the cytoplasm of the cisternae of the granular endoplasmic reticulum considerably dilated and they are filled up with a moderately osmiophilic, finely granulated material (Fig. 5). Such an arrangement of the granular endoplasmic reticulum was observed especially in the cytoplasm of chondrocytes in the space between the middle and deep layers.

Agranular endoplasmic reticulum occurs only very sporadically in the cytoplasm of chondrocytes of the middle layer of the joint cartilage, similarly as in the superficial layer. In no case were vesicles of the smooth endoplasmic reticulum found to occur in larger numbers near the deposited glycogen. These observations are in accordance with data found also in the cartilage of lower mammals (Barnett et al. 1962; Palfrey and Davies 1966; Meachim 1967; Ghadially and Roy 1969).

Golgi complex is regularly present in the cytoplasm of chondrocytes of the middle layer and it occupies a relatively large area on the section (Figs. 2, 3). It is localized on the periphery of the cytoplasm in the vicinity of the cellular membrane and its structures were also found perinuclearly. It is interesting that in the case of perinuclear localization the Golgi complex was not so strikingly developed as in the case of peripheral localization. The results of the present study generally agree with observations concerning this subject published by Silberger (1968), Weiss et al. (1968), Meachim (1969), Meachim and Stockwell (1973), Stockwell and Meachim (1973) and others on the ultrastructure of chondrocytes of human joint cartilage and also with studies dealing with the cartilage of lower mammals (Barnett et al. 1961, 1963; Ghadially and Roy 1961; Davies et al. 1962; Palfrey and Davies 1966; Meachim and Roy 1967).

Mitochondria are less frequent as compared with the cytoplasm of chondrocytes of the superficial layer, especially as far as the cells from the area between the middle and deep layers are concerned (Fig. 5). It is very difficult to confront the results of the present study with literature data because e.g. Weiss et al. (1968) are lapidary in expressing the number of mitochondria, the same as other authors (Meachim and Roy 1967; Meachim 1969; Meachim and Stockwell 1973; Stockwell and Meachim 1973) only state that the mitochondria in the middle layer of the joint cartilage are numerous. According to the author's

observations they are deposited irregularly in the cytoplasm and no topographic relationship was proved to other organelles or inclusions.
Ribosomes, similarly as in the cytoplasm of chondrocytes of the superficial layer, are also in this case in their majority bound to the membranes of the granular endoplasmic reticulum. Only a small amount is deposited freely in the cytoplasm and polysomes occur sporadically.
Lysosomes occur in the cytoplasm of chondrocytes of the middle layer only rarely. Provided they are found they appear as electron dense corpuscles, contoured by a smooth membrane which is difficult to demonstrate, of a size of 0.3—1 μm (Fig. 5). That it is a rarely occurring organelles can also be followed from the fact that e.g. Weiss et al. (1968) did not find it in the cytoplasm of cells of the middle layer of the cartilage.
Microtubules and centrioles were not found in the cytoplasm of this layer.

β) cytoplasmic inclusions

Lipid droplets occur in the cytoplasm of chondrocytes of this layer in lower amounts than in cells of the superficial layer (Fig. 2). They are contoured by a simple membrane and they contain homogenous, light material. On the sections they are 1—2 μm on average. Lipid inclusions of the same appearance and size were found both in experimental material (Barnett et al. 1961; Davies et al. 1962; Silberger et al. 1964; Stockwell 1967b; Ghadially and Roy 1969), and in humans (Collins et al. 1965; Ghadially et al. 1965; Weiss et al. 1968; Dearden 1974; Scheck et al. 1975).
Glycogen is a very striking structure of the cytoplasm of chondrocytes of the middle layer. As compared with the superficial layer the amount is strikingly large (Figs. 2, 3, 4). In the cytoplasm it is deposited in the form of granules of a size of 40 nm which are accumulated and form large deposits. In this respect the results of the present study fully correspond with the investigations of Barnett et al. (1961, 1963), Davies et al. (1962), Silberger et al. (1964), Stockwel (1967b), Weiss et al. (1968), Ghadially and Roy (1969), Meachim (1969), Dearden et al. (1974), Scheck et al. (1975), and others.
Cytoplasmic fibrillar structures have the same appearance and localization as in chondrocytes of the superficial layer. The largest amount is deposited perinuclearly (Figs. 2, 3) where they form a more or less continuous envelope; also, bundles of these filaments are deposited in the vicinity of the cell membrane. The author's observations fully correspond with the above quoted data; however, as far as the so called secretory vacuoles are concerned (Weiss et al. 1968) containing filamentous material and adjoined to the cell membrane, the author's results were negative.

γ) Cell surface

Chondrocytes of the middle layer are usually of a rounded, only rarely slightly elongated, shape on the section. Over the whole surface the cytoplasm protrudes in variously numerous processes covered by a cellular membrane (Figs. 2—4). On the longitudinal sections they reach a length of 1 μm and more, the width ranges from 0.5 to 1 μm. The processes are simple in the majority of cases, only sporadically do they ramify, and they often partition the chondrocyte capsule so that they reach the intercellular substance. Besides ribosomes no other cell organelles occur in the cytoplasm of the processes; however, the cellular membrane

sinks into numerous pinocytotic vesicles (Figs. 2—4). Chondrocytes of such an appearance and arrangement of cell surface in the middle layer of the joint cartilage were described by Barnett et al. (1963), Davies et al. (1962), Silberger et al. (1964), Palfrey and Davies (1966), Meachim (1969), Weiss et al. (1968), Dearden et al. (1974) and others.
Desmosomes and cilia, similarly as in chondrocytes of the superficial layer, were found neither in the middle layer of the joint cartilage and neither have they been described in literature.

Deep layer

Chondrocytes of the deep layer reach a size of about 7 × 10 μm and when compared with cells of both of the preceding layers they are more varied in shape. Some of them are of an oval or spindle-like elongated shape, but very often we can observe chondrocytes of an irregularly prism-like shape occurring predominantly in columns oriented vertically to the cartilage surface (Fig. 6). The cells are usually deposited in twos in one capsule. Between the neighbouring lacunae there is a thin layer of interfibrillar substance which separates the neighbouring closely connected groups of chondrocytes. The chondrocytes in one capsule do not contact each other closely either, they are generally separated by a layer of perinuclear matrix of different thickness. Such an arrangement of the cellular component in the deep layer of the joint cartilage is characteristic for all types of hitherto investigated mammals including man (Zelander 1959; Godman and Porter 1960; Barnett et al. 1961, 1963; Ghadially and Roy 1961; Silberger et al. 1961; Davies et al. 1962; Fawcett 1966a; Palfrey and Davies 1966; Meachim 1967, 1969; Schubert 1967; Stockwell 1966, 1967a; 1971b, c; Toner and Carr 1968; Silberger 1968; Weiss et al. 1968; Brower and Hsu 1969; Ghadially and Roy 1969, Meachim and Roy 1969; Ghadially et al. 1970; Mitchell and Shepard 1970; Hirohata and Morimoto 1971; Weiss and Mirrow 1972; Meachim and Stockwell 1973; Stockwell and Meachim 1973; Dearden et al. 1974; Refior 1974; Serafini-Fracassini and Smith 1974; Silberger and Hasler 1974, 1975; Földes 1975; Meachim and Fergie 1975; Hanaoka 1976; Schumacher 1976; Vignon et al. 1976), and others.

a) Nucleus

The nucleus of the chondrocytes of the deep layer is most fequently of an oval shape. The nuclear envelope is formed by two membrane units which mutually pass into each other in the place of nuclear pores. Ribosomes sporadically adjoin onto the outer membrane of the nuclear envelope. The perinuclear space is narrow. The zonula nucleum limitans of the same appearance and arrangement as in chondrocytes of the superficial and middle layers (Fig. 6) adheres to the inner membrane of the nuclear envelope over the whole periphery of the nucleus. Nuclear chromatin is arranged in the same way as in nuclei of chondrocytes of the superficial and middle layers.
The nucleolus occurs in the nucleus of chondrocytes of the deep layer only rarely. Provided it is found it is always a nucleolus of a reticular type.

b) Cytoplasm

When comparing with chondrocytes of the middle layer it is evident that cells in the deep layer contain smaller amounts of cytoplasm.

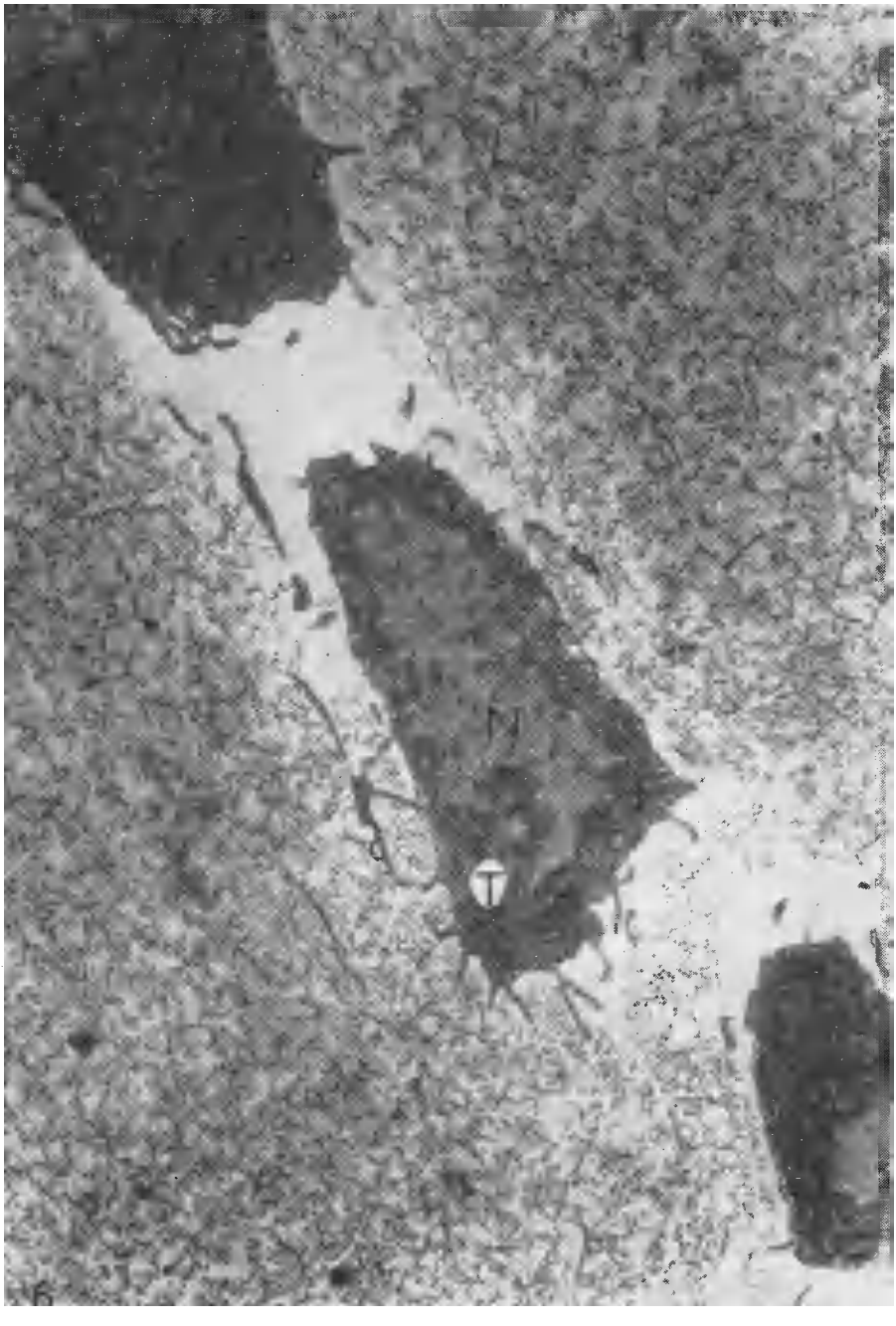


Fig. 6. Groups of chondrocytes of the deep layer of a normal cartilage.
Nucleus (N), zonula nucleum limitans (Z), granular endoplasmic reticulum (E), Golgi complex (G), mitochondria (M), lysosomes (L), lipid droplets (T), processes of the cytoplasm (c). × 11 400.

α) cell organelles

Granular endoplasmic reticulum occurs in the form of scarce cisternae and sacs, irregularly dispersed in the cytoplasm (Fig. 6). On electronograms it is deposited either perinuclearly or is accumulated on the periphery of the cell outside the region of the nucleus. The author's investigations on the occurrence of the granular endoplasmic reticulum in chondrocytes of the deep layer of the joint cartilage are in accordance with the findings of Zimny and Redler (1969), whereas Weiss et al. (1968) found only less developed organellae in cells of this layer. The results of the present study, as far as the occurrence and arrangement of the granular endoplasmic reticulum are concerned, to a certain extent support the opinion of Weiss et al. (1968) on the synthetic activity of chondrocytes of the individual layers of the cartilage. The author followed from data on the activity of chondrocytes of the given layers provided on the basis of autoradiography using $^{35}SO_4$ by Collins and Elliger (1960), Collins and Meachim (1961), Revel and Hay (1963). These authors proved that labelled material is, to the largest extent, incorporated into cells of the middle layer. This fact also corresponds with morphological findings mentioned above about the amount and arrangement of cell organellae.
Agranular endoplasmic reticulum occurs in the cytoplasm of chondrocytes of the deep layer only very sporadically.
The Golgi complex occupies only a small area in the cytoplasm of the chondrocytes of this layer and when compared with cells of the preceding layer it is developed to a much smaller extent (Fig. 6). The author's morphological investigations correspond with the present ideas about the function of this organellae in chondrocytes and its role in the process of secretion of collagen (Sheldon and Kimball 1962; Revel and Hay 1963; Goldberg and Green 1964; Cox et al. 1967; Freeman 1973).
Mitochondria do not differ from mitochondria of cells of the preceding layers in their structure and size. Striking is the relatively great density of their matrix (Fig. 6). In the cytoplasm of chondrocytes of this layer of the joint cartilage they are not frequent. The results of the author's investigations correspond with data published by Zimny and Redler (1969), whereas Weiss et al. (1968) observed a large number of mitochondria in the cytoplasm of chondrocytes of the deep layer.
Ribosomes in the cytoplasm of chondrocytes of this layer have the same arrangement and frequency of occurrence as in the cells of the other two layers.
Lysosomes occur only sporadically. As in the cytoplasm of cells of the other two layers, in the deep layer they have the appearance of dark corpuscles (Fig. 6), of a size of about 0.5 μm. Lysosomes of this appearance were also described by Riede (1974), Oryschak and Ghadially (1974), Meikle (1975), Oryschak and Ghadially (1976a).
Microtubules and centrioles did not occur in the cytoplasm of chondrocytes of the deep layer of the joint cartilage in the author's material and they are not stated in literature either.

β) cytoplasmic inclusions

Lipid droplets were observed in larger quantities only in the region near the calcifying zone. The largest of them reach as much as 1.5 μm and are contoured by a distinct smooth membrane. A similar occurrence of lipid droplets is not usual, in literature (Weiss et al. 1968; Zimny and Redler 1969) only a sporadic

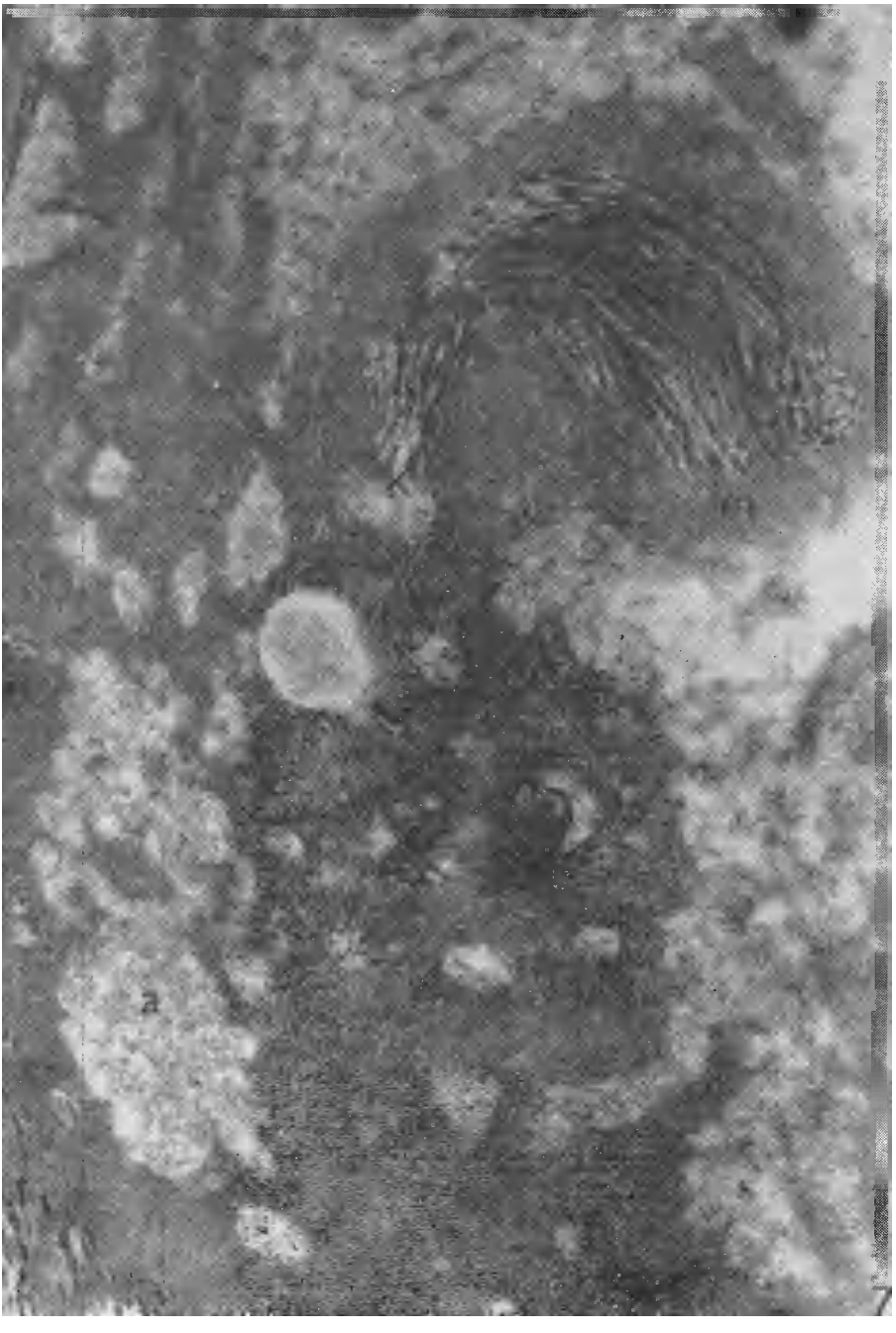


Fig. 7. Part of the irregular surface of the joint cartilage with a bundle of collagen fibres (K) overcovered with a chondral membrane (m), aperiodic fibres (a). × 24 500.

occurrence of these inclusions in the hyaline cartilage is mentioned, whereas these lipid droplets were found to be very frequent in the elastic cartilage (Sanzone and Reith 1976).

Glycogen occurs in larger numbers only in the deepest chondrocytes of this layer (Fig. 6). Accumulation of glycogen granules into small areas occurs and also the overlapping of the individual granules can be observed to that these clusters are strongly osmiophilic.

Cytoplasmic fibrillar structures could be arranged in the same way as in the cytoplasm of chondrocytes of the superficial or middle layer, or they are dispersed diffusely in the cytoplasm. Their dimensions are the same in the cells of all the layers of the cartilage. The present investigations are in accordance with the data of Silberger et al. (1965), Meachim and Roy (1967), Kostovič-Kněževič and Svajger (1975).

γ) cell surface

As compared with the middle and mainly with the superficial layer the cytoplasm of chondrocytes of the deep layer protrudes in a lower number of projections which are, however, considerably long (Fig. 6). Some of them are 2 μm long and even more, they partition the capsule of the chondrocytes and they reach the intercellular substance. Such an arrangement of the cell surface, size and formation of cytoplasmic processes was also described by Weis et al. (1968), Zimny and Redler (1969), Hirohata and Morimoto (1971).

Desmosomes and cilia were not proved by the author and no data were found in literature about them in connection with chondrocytes of the deep layer of the joint cartilage.

Submicroscopic structure of the cartilage intercellular substance

The intercellular substance of the cartilage consists of the ground fibrillar substance and the ground amorphous substance. In the cartilage these two components are arranged in such a way that we can distinguish (1) the specialized area deposited in the immediate vicinity of chondrocytes and indicated as pericellular matrix (capsule, area, lacuna) and (2) the other intercellular substance deposited outside this area between the chondrocytes, and, or groups of chondrocytes, indicated as intercellular matrix. The differences in the structure of these two areas are in the quantitative representation of the various types of fibres and the amount of the ground amorphous substance.

The ground fibrillar substance is, in its majority, formed by typical collagen fibrils (Figs. 1, 3, 6). In the electron microscope these fibrils show "transversal striping" with regular changes of light and dark parts 64 nm apart (Fig. 7). This phenomenon is indicated as periodicity (Boni and Monteleone 1957). In the joint cartilage the most frequently are represented collagen fibrils of a thickness of 30—80 nm. Besides these typical fibrils also much wider fibrils appear, of a much as 150 nm and more, with very striking transversal striping. Thick fibres of this type also occur near the dying out chondrocytes. The present investigations of these fibres as far as their occurrence and size are concerned fully correspond with the findings of Weiss et al. (1968), Meachim (1969), Ghadially and Roy (1969) and others.

The other component of the ground fibrillar substance are fine fibrils of a thickness of 4—10 nm of various length, the largest amounts of which

are found in the region of the chondrocyte capsule (pericellular matrix) where they form the essential part of the intercellular substance (Fig. 1—4). These filaments lack the periodicity and only on the basis of observations using an electron microscope it cannot be decided if they are collagen fibres in this case as well. In the pericellular matrix they are the only fibrillar component as this area lacks collagen fibres which appear only very rarely under normal conditions. Filaments 4—10 nm wide can occur in small amounts between the collagen fibres in the superficial zone of the intercellular substance of the cartilage (Fig. 7) and they are an exclusively fibrillar component, the so called lamina splendens. These fine filaments were observed and described by Weiss et al. (1968), Meachim(1967, 1969), Ghadially and Roy (1969), Meachim and Stockwell (1973), Stockwell and Meachim (1973) and many others not only in the intercellular substance of the human joint cartilage but also in other mammals.

The ground amorphous substance forms the filling between the fibrillar components and connects the fibres together. Chemically, it is formed by protein--polysaccharide complexes, the so called acid mucopolysaccharides, or, more exactly, glycose-aminoglycans. Their typical representatives are especially chondroitinsulphate and keratinsulphate. These protein-polysaccharides, known as proteoglycans, have a molecular weight of about 4×10^6 and are aggregated into large complexes. The ground amorphous substance was studied in detail both biochemically and histochemically. Thus, many data were acquired about the composition and distribution of the individual components in the cartilage under normal conditions even with various disorders of chondrocyte metabolism. A survey on this problem was given by Muir (1973). In an electron microscope it can be illustrated both as a fine network in the region of the pericellular matrix and between the typical collagen fibres in the intercellular matrix (Figs. 6, 7). The network is formed of fine fibres mutually connected in acute angles. Periodicity can be indicated in some of the filaments; however, it was not proved that they are collagen fibres or protein-polysaccharide complexes, or aggregates of both (Smith et al. 1967). It is still questionable whether these fibrillar formations are really a component of the ground amorphous substance or a special constituent of the fibrillar component because in their shape and size they remind structures observed in the electron microscope after precipitation of protein-polysaccharides (Rosenberg et al. 1979).

Arrangement of the ground fibrillar component in the intercellular substance of the cartilage

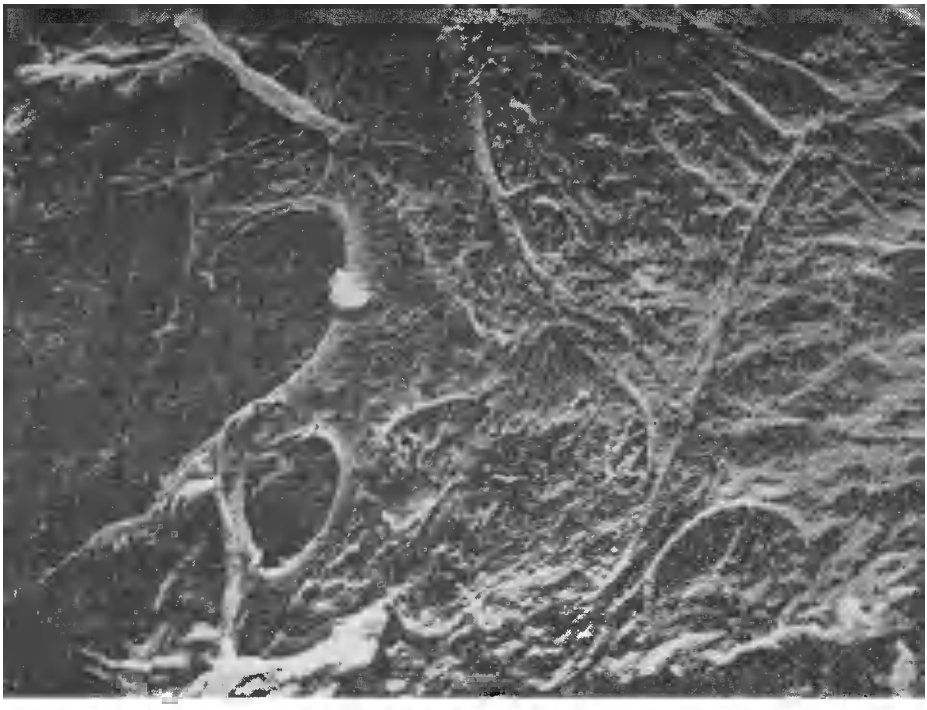
The superficial layer of an adult human cartilage is covered with a variously thick, 1—3 μm, layer consisting of two basic components. One of the components are fine fibrils 4—10 nm wide which are embedded into the ground amorphous substance which, in its majority, covers it. The reticular structure is distinctly evident under great enlargement and especially well in those areas where the amorphous substance was removed is its irregular arrangement evident (Fig. 7). The superficial marginal layer was described by MacConaill (1951) in a light microscope and was called lamina splendens. Wolf (1969, 1975) indicated it as a chondral membrane. On the basis of his investigations MacConaill (1951) considered it to be a thin layer of hyaluronic acid; however, in later studies on an electron miscroscopic level (see above) it was found to be composed of fine

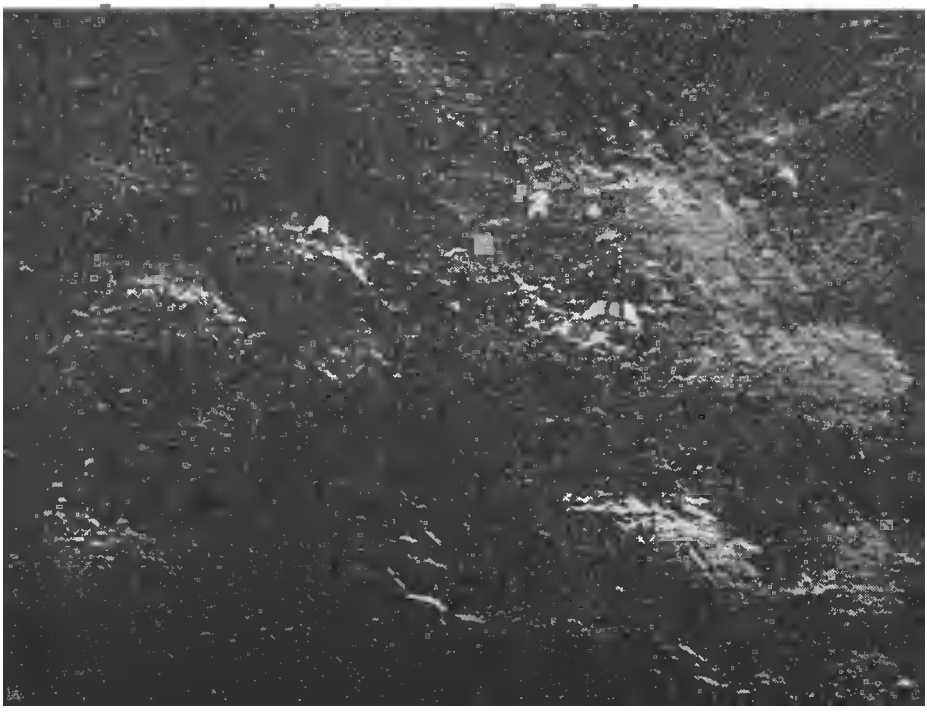
fibrils and ground amorphous substance. The thickness of this layer was stated to range from 0.5 to 3 μm in adult intact cartilage. In the present material the thickness of this layer ranged between 0.5 and 2 μm. According to the author's opinion the relatively large differences in the thickness are mainly due to the place of sampling, age of the individual and type of joint. In this respect it is in accordance with the data of Weiss et al. (1968), Meachim and Roy (1969), Meachim and Stockwell (1973). The greatest effect on the thickness of the marginal layer is that of the age of the individual. Cameron and Robinson (1958) e.g. state that in newborn infants this layer is as much as 12 μm thick and with increasing age it decreases. The surface of the normal joint cartilage is not quite smooth. In places higher or lower prominences appear the basis of which are collagen fibres reaching from the deeper layers (Fig. 7). However, in a normal cartilage they are covered with a continuous superficial layer. This observation is fully in accordance with literature data (Meachim 1969; Weiss and Mirrow 1972; Bozděch et al. 1974) and corresponds with observations using a scanning electron microscope.

Under the chondral membrane in the superficial layer of the cartilage collagen fibres are deposited of a thickness of 30—80 nm, length of several μm. They are closely clustered, in some cases they form bundles orientated mostly parallel with the surface of the cartilage. Bundles of collagen fibres oriented vertically to the surface of the cartilage occur sporadically. The fibres are connected into bundles by a small amount of ground amorphous substance. Between the collagen fibres of the superficial layer chondrocytes are deposited, whereas in the chondral membrane no cells occur under normal conditions. The present findings correspond with literature data of Weiss et al. (1968), Meachim and Roy (1969), Ghadially and Roy (1969), Meachim and Stockwell (1973), Stockwell and Meachim (1973) and others.

In the middle layer of the cartilage a change occurs in the arrangement of the ground fibrillar substance even in proportion between this component and the basic amorphous substance. Collagen fibres of the same appearance and size as in the superficial layer form bundles of two arrangements and courses. In places where they press onto the pericellular matrix they run parallel to the surface of the chondrocyte and they envelope the whole area. Outside this area the bundes of collagen fibres are more or less disarranged. A similar arrangement of the ground fibrillar substance in this cartilage layer was described by Weiss et al. (1968), Meachim and Roy (1969), Ghadially and Roy (1969), Meachim and Stockwell (1973) and Stockwell and Meachim (1973). McCall (1969a, b) using a scanning electron microscope proved that these bundles form a disarranged network provided they are not subjected to pressure. If the cartilage is pressed the fibres and bundles are oriented vertically to the direction of the pressure.

The intercellular substance of the deep layer of the joint cartilage is characterized by a disarranged course of collagen fibres which form bundles here to a much lower extent than in the preceding layer (Fig. 6). Some of the bundles are oriented normally to the surface of the cartilage and are deposited between the column-like arranged chondrocytes. The ground amorphous substances was found in a larger amount than in the superficial layer so that the intercellular substance in the depth of the cartilage is of an expressively net-like appearance. In the majority of cases the pericellular matrix occupies only a narrow area by the cellular membrane of the chondrocytes. This area frequently completely disappears unilaterally and the collagen fibres from the surrounding intercellular matrix penetrate





to the cellular membrane of the chondrocytes (Fig. 6). These observations correspond with previous findings of Little et al. (1958), Weiss et al. (1968), Meachim and Roy (1969), Meachim and Stockwell (1973), Stockwell and Meachim (1973). McCall (1969a, b) observed in a scanning electron microscope that while in the middle layer the spatial arrangement of collagen fibres changes under the effect of pressure, the arrangement of fibres of the deep layer remains, in essence, unchanged.

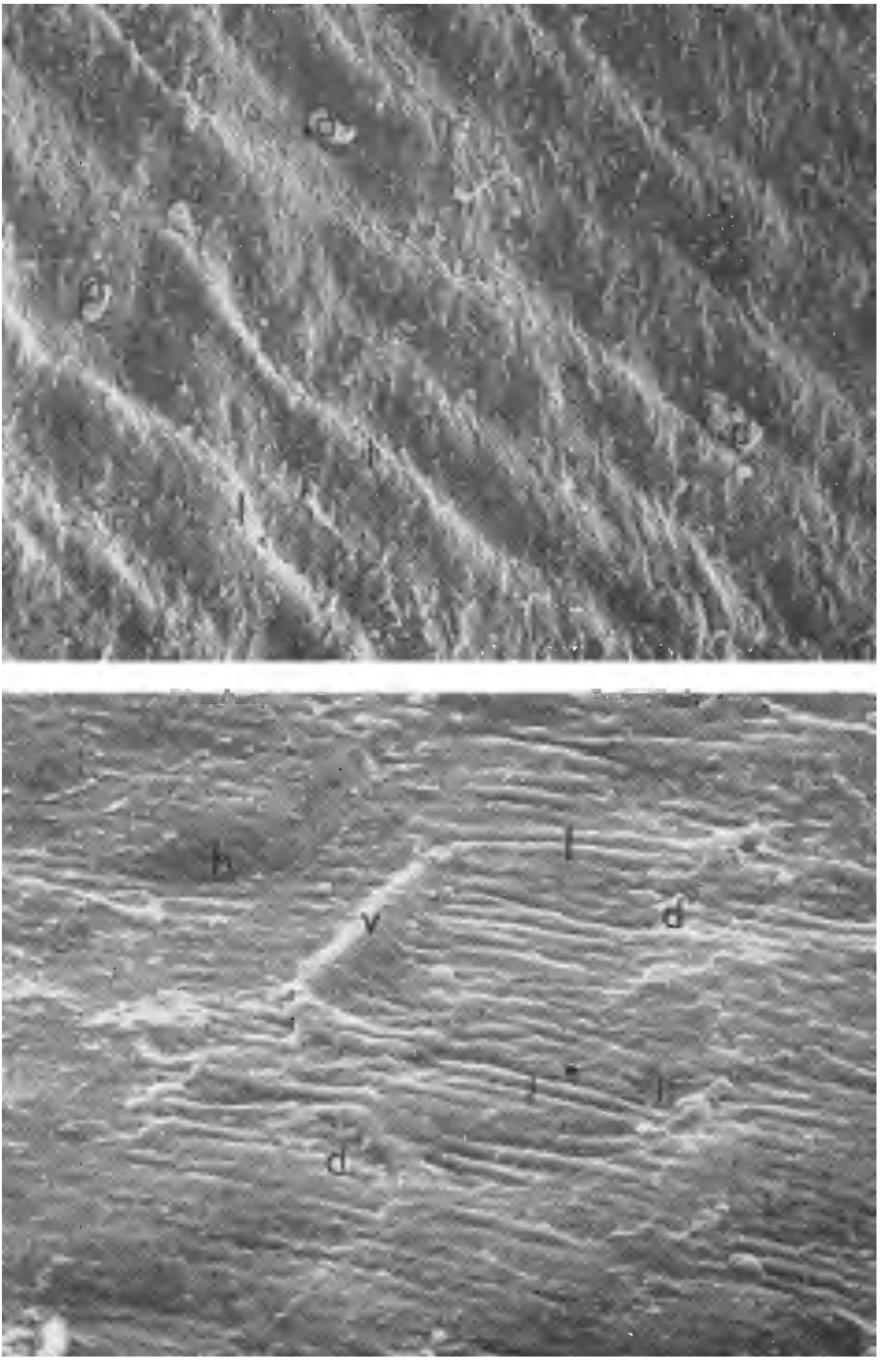
The surface of joint cartilage in a scanning electron microscope

The first information concerning the surface of the joint cartilage of the rabbit and dog was given by Elliott (1936) who described chondrocytes deposited near the surface of the cartilage in a light microscope and observed traces of their degeneration which was manifested by the appearance of empty capsules. He called this process the cartilage erosion. In much greater detail was cartilage dealt with by Cameron and Robinson (1958) who studied the joint cartilage of a newborn infant in electron microscope and studied also the formation of its surface. They found that even at this age there are irregularities on the surface of the cartilage and that chondrocytes form protuberances. Similar data about the surface of the joint cartilage of mice of various age were given by Silberger at al. (1961). On the other hand, Davies et al. (1962), Barnett et al. (1963) found the rabbit cartilage to be very smooth, with irregularities not exceeding 0.2—0.3 μm and with sporadic ridges 0.8 μm high. In all the cases the superficially deposited chondrocytes were covered by a layer of intercellular substance and no depressions deep enough to contain cells were found. Meachim et al. (1965) dealt with changes of the ultrastructure of human joint cartilage in the initial stage of arthrosis. In the transmission electron microscope they observed for the first time small depressions which, according to their opinion, were formed by rupture or erosion of superficially deposited capsules of chondrocytes. Weiss et al. (1968) dealt with the appearance of the surface of a normal adult joint cartilage and described irregularities on its surface of a similar appearance as described by Davies et al. (1962) and Barnett et al. (1963) in rabbit cartilage.

McCall (1968a, b) gave the first data about the surface of the joint cartilage of a man in a scanning electron microscope. He studied the necrotic material from the femur head of a two-year-old child and of adults. He distinguishes two types of surfaces of joint cartilage. In young individuals groups of cells are evidently covered by a fine fibrillar network, whereas in adults the surface is formed by parallelly running ridges. Similar findings on adult joint cartilage were also described by Walker et al. (1969). In the same year Gardner and Woodward using light microscopy, transmission and scanning electron microscopy demonstrated depressions on the surface of the joint cartilage of a guinea-pig of 400 μm in diameter with smaller wells, 20—40 μm, at their bottom. Later stu-

Fig. 8. Surface of the joint cartilage in a scanning electron microscope.
Partly removed chondral membrane (m) under which is evident the irregular surface of the joint cartilage (s). × 5700.

Fig. 9. Surface of the joint cartilage of a child with rounded processes (v) and depressions (h). On the surface are fine ridge-like lines (l). Cellular detritus (d). Scanning electron microscopy. × 10 250.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dies published by Cotta and Puhl (1970), Johari (1970), Clarke (1971a, b, 1973, 1974), Fujita et al. (1971); Gardner and McGillivray (1971), Zimny and Redler (1972), Puhl and Iyer (1973), Puhl (1974), Zimny and Redler (1974) provided much new knowledge about the structure of the surface of a normal joint cartilage, possibly about the changes of the shape of the surface under an experimental intervention (Ghadially et al. 1974a), or they dealt with studies of changes occurring on the surface of the joint cartilage with pathological processes in joints as compared with the surface of normal tissue (e.g. Inoue et al. 1969; Bozděch et al. 1972; Horký et al. 1974a, b; Bozděch et al. 1974; Redler 1974) and others.

The normal joint cartilage is covered by a continuous layer of fine fibrillar structure on the surface which is well evident in an undamaged state. This layer indicated in transmission electron microscopy as lamina splendens or chondral membrane is relatively fragile after fixation and easily damageable so that its artificial severing could easily occur (Fig. 8). Under this superficial layer, when enlargened, evident is the net-like arrangement of collagen fibrils of the superficial layer of the intercellular substance of the cartilage (Fig. 8.). In young individuals the chondral membrane overcovers the typically formed surface where evident are oval protuberances of 10—30 μm in diameter (Fig. 9.) Both on the protuberances and in the depressions between them there are fibrillar structures and cellular detritus originating probably from the synovial liquid. The superficial membrane has a fine fibrillar structure and on it are evident not high ridges separated by shallow cuts parallelly orientated. (Fig. 9). Also Clark (1974), Puhl (1974), Zimny and Redler (1974) and others describe the surface formed in such a way. According to the opinion of these authors the processes are dependent on the presence of chondrocytes in the vicinity of the young cartilage. Puhl (1974) indicated such an arrangement of the surface of the joint cartilage as a tertiary structure of the surface.

The joint cartilage of a young adult individual has a different appearance. According to the place of sampling (due to a different load of the joint cartilage) the surface of the cartilage is either only slightly undulated (Fig. 10), when the individual ridges run nearly parallelly, or deeply furrowed. In the first case the ridges are low, they reach about 3—5 μm and the whole extent of the surface is covered with a continuous chondral membrane of a fine fibrillar structure. On its surface are indicated fibrillar structures and in the depressions between the ridges are deposited both cells and detritus from the synovial liquid (Fig. 10). On the basis of studies of cartilage in transmission electron microscope we can judge that the base of these ridges are collagen fibres reaching into the superficial layer of the cartilage, possibly immediately under its chondral membrane. Of the same opinion are also Walker et al. (1969), Inoue et al. (1969), Cotta and Puhl (1970), Clarke (1971a, b), Gardner and McGillivray (1971), Puhl (1974), Redler (1974) and others. In contradistinction Clarke (1974), according to results of studies of the surface of the joint cartilage near the fracture and farther away,

Fig. 10. Parallelly running ridge-like lines (l) on the surface of the cartilage of an adult person. Cellular detritus (d). Scanning electron microscopy. × 5450.

Fig. 11. Surface of cartilage of an adult individual from the loaded joint region. Besides the parallelly running ridge-like lines (l) are evident transverse mounds (v) and depression s (h). Cellular detritus (d). Scanning electron microscopy. × 2370.

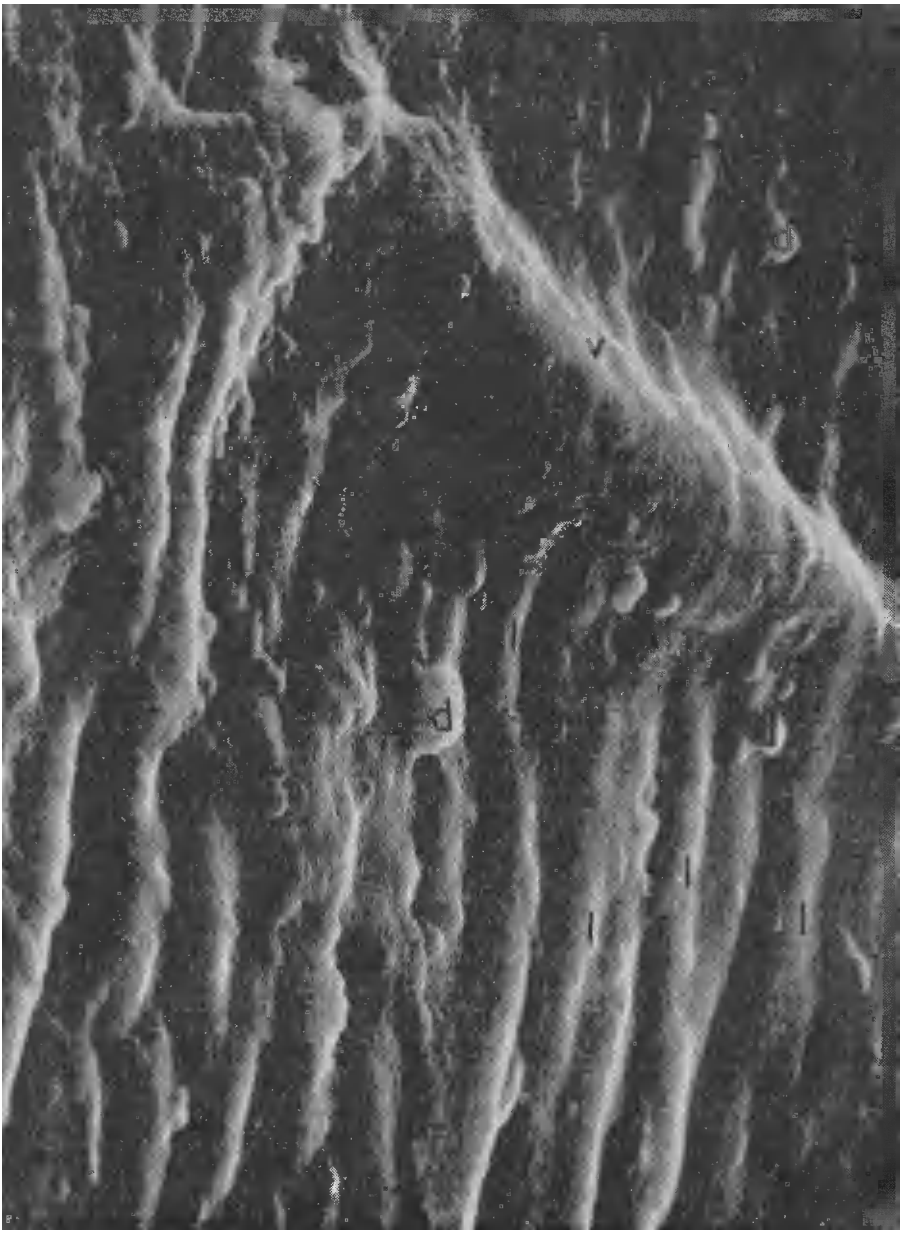

Fig. 12. Detailed illustration of part of the surface of a joint cartilage.
Distinctly evident are ridges (l) and the transverse mound (v) in which the parallelly running lines end. Between the processes the cellular detritus (d). Scanning electron microscopy. × 9500.

judges that they are artificial structures evoked mechanically because he did not observe any furrowing of the surface of the cartilage farther from the fracture. However, his opinion is individual and the author of the present study tends to the results of Redler (1974) and Zimny and Redler (1974) who proved, using different techniques and also by immediate fixation and processing without desiccation, that these structures exist in vivo. These findings are supported also by studies of the relation between the joint cartilage and synovial liquid where the formation was found of certain trapped-pools on the surface of the cartilage during its interaction with the synovial liquid under pressure (Walker et al. 1969). According to Meachim and Stockwell (1973) and Stockwell and Meachim (1974) this arrangement of the joint surface is important for maintaining the normal function of the joint as it is important for lubrication.

In the region where the cartilage is more loaded further changes in the arrangement of its surface occur. Under a clear enlargement it is distinctly evident that the surface is further articulated (Fig. 11). The ridge-like protuberances increase so that they measure as much as 8 μm and are arranged parallel to the direction of the joint movement. From place to place high mound-like formations occur vertical to these lines. Their height is twice as high and the lower ridges end in them (Fig. 11). On the surface of the cartilage we can observe numerous shallow depressions on average about 20—30 μm, 1—5 μm deep. The ridge-like processes usually do not reach into these depressions (Fig. 11). Such depressions on the surface of the joint cartilage were described by Gardner and Woodward (1969) in the joint cartilage of a guinea-pig; however, in their case these depressions occurred on the bottom of the larger depressions of an average of 200—400 μm. Such formations were not observed in the author's material and were not described in literature in human cartilage. The depressions observed in the present study were regularly present on the surface of a normal joint cartilage of an adult human (Clarke 1971a, b, 1974) and Puhl (1971) indicates them as tertiary structure of type III. According to Clarke (1971a, b, 1974) these depressions are caused by a collapse of the area of chondrocytes deposited deeper under the superficial membrane, whereas the processes are due to the presence of the acellular matrix. The size found by the author in essence supports this opinion, similarly as the observations of Clarke (1971b) who artificially removed by a tangential cut the superficial layer of the cartilage and proved in a scanning electron microscope that the diameter of the capsule of the chondrocytes corresponds with the superficial lacunae. All the above mentioned authors are in accordance when explaining the origin of ridge-like lines (Fig. 12). They presume that collagen fibres are their base, and/or their bundles overcovered with a chondral membrane. On the basis of the author's own studies on the arrangement of the fibrillar ground substance in the transmission electron microscope we can agree with this opinion. As yet we cannot explain the origin and purpose of the high vertical ridges (Fig. 12) interrupting the low lines. Provided they are not artefacts (Clarke 1971a, b) we can presume that their role is to withhold the synovial liquid on the surface of the joint cartilage when the cartilage is not under load and thus to form its reserve for the first phase of movement of the joint.

The appearance of the surface of the joint cartilage of an old person is different; the ground amorphous substance decreases due to which the fibrils of the superficial layer of the cartilage become partly uncovered. The collagen fibres, and/or their bundles swell and linearly arranged knot-like formations are formed in the direction of the joint movement. The majority of authors consider changes of this

type to be a sign of a pathological process, of the defibration of the cartilage and signs of arthrosis (Inoue et al. 1969, 1974; Gardner and McGillivray 1971; Bozděch et al. 1972; Clarke 1973, 1974; Puhl and Iyer 1973; Bozděch et al. 1974; Puhl 1974; Redler 1974).

Submikroskopická struktura kloubní chrupavky člověka

Transmisním a rastrovacím elektronovým mikroskopem byla studována kloubní chrupavka 43 jedinců stáří 5—75 let. V práci je podán pokud možno ucelený přehled o ultrastruktuře chondrocytů povrchové, střední a hluboké vrstvy kloubní chrupavky, uspořádání mezibuněčné hmoty a jsou uvedeny základní údaje o utváření povrchu chrupavky v závislosti na stáří objektu. Zvláštní pozornost je věnována mezibuněčné hmotě chrupavky, která z hlediska elektronové mikroskopie byla dosud málo studována. Komponenty mezibuněčné hnoty jsou uspořádány tak, že můžeme rozlišovat jednak specializovaný okrsek v blízkosti chondrocytů označovaný jako pericelulární matrix, jednak ostatní mezibuněčnou hmotu, tzv. intercelulární matrix. Povrch chrupavky je kryt specializovanou vrstvou označovanou jako lamina splendens. Výsledky vlastního studia konfrontujeme s literárními údaji nejen pokud se týkají kloubní chrupavky lidské, ale všímáme si údajů o těchto strukturách i u nižších savců, neboť řada poznatků platí obecně pro tento typ tkáně. Práce shrnuje dosud známá fakta o kloubní chrupavce a vytváří nutný základ pro posuzování stupně změn, ke kterým dochází při některých jejích poškozeních.

Субмикроскопическая структура суставного хряща человека

Трансмиссионным и растровым электронным микроскопом изучался суставный хрящ 43 человек в возрасте 5—75 лет. В работе приводится цельный обзор ультраструктуры хондроцитов поверхностного, среднего и глубокого слоев суставного хряща, распорядок междклеточной массы, сообщаются основные данные об образовании поверхности хряща в зависимости от возраста объекта. Особое внимание уделяется межклеточной массе хряща, которая с точки зрения электронной микроскопии до настоящего времени изучалась недостаточно. Компоннеты межклеточной массы расположены так, что можно различить не только специализированный участок вблизи хондроцитов, обозначаемый перицеллюлярным матриксом, но и остальную межклеточную массу, т. н. интерцеллюлярный матрикс. Поверхность хряща покрыта специалным слоем, называемым lamina splendens. Результаты собственной работы сопоставляются с литературными данными, касающимися не только хряща человека, но и низких млекопитающих, т. к. ряд данных действителен в общем плане для упомянутого типа ткани. Работа подводит итоги известным до сих пор фактам о суставном хряще и образует необходимую основу для оценки степени изменений, имеющих место вследствие его повреждения.

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