Ultrastructural and Cytochemical Studies on the Remodelling of the Tracheal Cartilage

Toshihiko Yajima

Summary. Ultrastructural and cytochemical studies on the remodelling of the rat tracheal cartilage have been carried out. The thickness of the tracheal cartilage was constant, during the observation periods (1 to 54 days after birth). The external perichondrium of the tracheal cartilage consisted of active fibroblasts and intercellular fibrils. The inner part of this perichondrium was a chondrogenic layer, where appositional growth was taking place. On the other hand, the internal perichondrium contained fibroblast-like cells, which were nearly twice as large as the external perichondrial fibroblasts in size and were arranged in three or four layers. The cells had well developed organelles and large vacuoles which contained numerous fragments of fibrils and/or glycosaminoglycan. Many cytoplasmic processes protruded to the cartilage matrix, where the intercellular fibrils were particularly irregular in arrangement. Some vacuoles included collagen fibrils. Based on an intense acid phosphatase activity in these vacuoles and other findings, the fibrils were thought to be phagocytosed collagen of the cartilage matrix. An extensive alkaline phosphatase activity was demonstrated on the plasma membrane of fibroblasts and chondroblasts in the external perichondrium.

The present investigation revealed distinct functional difference between the external and internal perichondrium of the tracheal cartilage. It is resorbed at the internal perichondrium, while it appositionally grows at the external perichondrium. The fibroblast-like cells of the internal perichondrium play an essential role in resorption of the matrix in cartilage remodelling.

It is well known that remodelling processes should occur in every connective tissue during development and growth in order to maintain its shape and structure without any impairment of its function. The remodelling of connective tissue involves new formation in a certain portion and degradation in another of the organic matrices such as collagen and glycosaminoglycan. The role of the fibroblasts and related cells such as chondroblasts, osteoblasts, and odontoblasts in these processes are also widely recognized.

The most suitable morphological example of remodelling is that of bone, that can be achieved by resorption of the osseous substance from one surface and the apposition of it to another surface of the opposite side. Extensive studies have revealed that resorption of a large bulk of calcified matrix in bone is performed within a relatively short period and osteoclasts play a principal role in this process (Cameron, 1961; Yaeager and Kraucunas, 1969; Vaes, 1969; Lucht, 1972a, b; Schofield, Levin; and Doty, 1974; Holtrop, Raise and Simmons, 1974).

Remodelling processes are known to occur also in other connective tissues during their development. It has been reported that the extracellular constituents of connective tissues, particularly collagen may be removed in normal tissues such as the involuting uterus (Luse and Hutton, 1964; Schwarz and Guldner, 1967; Parakkal, 1969a), a catagen hair follicle (Parakkal, 1969b), the endometrial cup (Allen,
HAMilton and Moor, 1973) and the periodontal ligament (Ten Cate, 1972; Listgarten, 1973; Beertsen, Everts and Van Den Hooff, 1974), as well as in pathological conditions such as resorbing carrageenin granuloma (Perez-Tamayo, 1970) and experimental arthritis (Cullen, 1972). All the previous reports, however, have dealt either with transient aspects of the continuously proceeding phenomenon or with unusual and pathological changes, so that it may be difficult to obtain a systematic and standard pattern of the remodelling of connective tissue from those reports. The growing tracheal cartilage seems to be a good model for the study of this field because of the simplicity in its shape and in its cellular and intercellular construction.

By the use of rat tracheal cartilage, an attempt was made in this study to elucidate ultrastructural and cytochemical changes in the cells and matrix elements of cartilage which were involved in its growth and remodelling. Special reference was made to the collagen resorption mechanism in connective tissue remodelling.

**Materials and Methods**

**Animals**

Nine male Wister rats from the same litter (1, 3, 5, 7, 10, 15, 21, 33, and 54 days after the birth) were used for the experiments and maintained on ordinary solid rat pellets and tap water ad lib.

**Fixation and preparation**

Each of the nine rats was anaesthetized with sodium pentobarbital (Nembutal), the ventricle of the exposed heart was pierced with the tip of a fine tube and the vascular system was gently perfused for 20 min with a 1% paraformaldehyde-1% glutaraldehyde mixture in 0.1M cacodylate buffer (pH 7.2) after initial perfusion with physiological saline. After fixation was completed, the animal was subsequently perfused for 15 min with a 0.05M cacodylate buffered washing solution containing 4% sucrose (pH 7.2). At the end of the perfusion period, the trachea and femur were carefully dissected out and removed from the animal to be stored in the same cold washing solution.

**Measurements of the trachea**

Length and wet weight of the trachea (from tracheal cartilage I to bifurcation of trachea) and the femur were measured immediately after removal. The diameter was expressed in terms of the mean value of short and long diameters which were measured on the transverse section of the trachea at the middle part.

**Fine structural study**

After the measurement of the trachea, a part of the specimen was cut less than 1mm in thickness transversely and longitudinally using a razor blade prior to post-fixation which will be described below.

**Cytochemical study**

The remaining specimen was used for cytochemical examination. Fifty micron thick slices were prepared with a Smith-Farquar Tissue Sectioner (TC-2). The sections were then incubated in the medium for the following enzymes.
Acid phosphatase (Gomori, 1952): After pre-incubation in 0.1 M acetate buffer (pH 4.5) for 30 min at 37°C, the sections were kept for 15-30 min at the same temperature in a Gomori medium prepared immediately before use: Approximately 10 ml of 0.5 M acetate buffer (pH 5.0) containing 0.01 M sodium β-glycerophosphate (Merck), 3.6 mM Pb(NO₃)₂ and 0.19 M sucrose. Control preparations consisting of analogous media but lacking substrate were examined in all experiments.

Aryl sulfatase (Goldfischer, 1965): 0.02 M p-nitrocatechol sulfate (Merck) and 0.024 M Pb(NO₃)₂ in 0.03 M acetate veronal buffer (pH 5.4). Control preparation consisted of analogous media but lacking substrate.

Alkaline phosphatase (Mayahara et al., 1967): 0.01 M sodium β-glycerophosphate (Merck) and 4 mM Pb(NO₃)₂ in 0.1 M Tris-maleate buffer (pH 9.0). Control preparation consisted of analogous media but lacking substrate.

Preparative procedures

After rinsing in 0.1 M cacodylate buffer (pH 7.2), both sections and blocks were postfixed for 1.5 hrs at 4°C in 1% osmium tetroxide in the same buffer, dehydrated in ethanol and propylene oxide, and embedded in Epon 812 for electron microscopic examination.

Thin sections were cut with glass and diamond knives, after preliminary examination of semifine sections stained with 1% toluidine blue (pH 7.0). They were examined under the Hitachi 11 DS electron microscope either unstained or stained with lead citrate or with uranyl acetate followed by lead citrate.

Results

Dimensional changes

The results of the measurements were given in Tables 1 and 2, and graphically shown in Figures 1 and 2. Linear growth was shown in tracheal diameter and length as well as in femur length. The thickness of the tracheal wall was given by the difference between the outside- and inside-diameters. At the end of the observation period of 54 days, the wall became 1.6 times as thick as the original value. The thickness of the tracheal cartilage, on the other hand, was 0.10 ± 0.03 mm and did not change during that period. The percent-increase per day of both the length and diameter of the trachea was highest on day 1 (Fig. 1). Growth was marked during the first week.

In wet weight, the trachea and femur showed a sigmoidal increase curve similar to that shown in the body weight. The percent-increase in body weight and in wet weight of the femur was highest on day 7, and in wet weight of the trachea on day 10 (Fig. 2).

Gross anatomy

The trachea was supported by C-shaped cartilages that were set one above another, each incompletely encircling the lumen. There were 18 to 20 cartilages in rat trachea.

Light microscopic observation

The open ends of these incomplete cartilaginous rings were bridged by fibrous connective tissue and smooth muscle. The tracheal cartilage was surrounded by
Table 1. Measurements of length and diameter of trachea, and femur length

<table>
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<th>Age (days)</th>
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These values are the average of three or five measurements.

Fig. 1. Graphical representation of measurements of length and diameter of trachea, and femur length (Table 1).
Table 2. Measurements of body weight, and wet weight of trachea and femur

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These values are the average of three or five measurements.

Fig. 2. Graphical representation of measurements of body weight, and wet weight of trachea and femur (Table 2).
adventitia which contained numerous adipose cells, some blood vessels and nerves. The tracheal tube was lined by a mucose surface epithelium was ciliated pseudo-stratified epithelium with occasional goblet cells, but there were not many ciliated epithelial cells during the neonatal period (Fig. 3).

The cartilages in longitudinal sections of the trachea (viz. cartilages in cross section) were roughly ovoid in shape, and both the internal and external surface of each ring was convex. The distance between the adjacent cartilage rings was considerably less than their own height and was filled with fibrous connective tissue which was continuous to the perichondrium of each ring. The perichondrium was virtually inseparable from the fibrous connective tissue and was thicker on the external than on the internal face of the cartilage ring. Perichondria covering the superior and inferior aspects of the cartilage rings were quite similar in structure to the external perichondrium. The inner part of the external perichondrium was the chondrogenic layer composed of many flattened cells which might convert to the chondroblasts and this perichondrium became thicker and more fibrous with age. The internal perichondrium, however, mainly consisted of ellipsoid cells rich in cytoplasm. The ellipsoid cells appeared sometimes vacuolated. It was noticed that close to those vacuolated cells metachromasia of the cartilage matrix appeared markedly weakened.

The cartilage cells, or chondrocytes of the tracheal cartilage occurred isolated or isogenously grouped in their lacunae. Each lacuna was surrounded by a portion of basophilic ground substance, forming a territorial area or cartilage capsule around one lacuna or one group of lacunae. Chondrocytes came to contain a large amount of lipid droplets with increase in age.

**Electron microscopic observation**

*External perichondrium:* The external perichondrium of the tracheal cartilage consisted of a compact sheet of elongated spindle-shaped fibroblasts and intercellular
fibrils (Fig. 4a). The cells with a large nucleus contained a well-developed Golgi apparatus, rough-surfaced endoplasmic reticulum and a few mitochondria suggesting an active synthesis of collagen and other organic matrix. Clear vacuoles and a number of small vesicles were present near the Golgi region and free ribosomes were scattered throughout the cytoplasm. These fibroblasts were in parallel layers over

Fig. 4. Survey electron micrograph of external perichondrium (a) and internal perichondrium (b) of a 1 day old rat. IP internal perichondrium, EP external perichondrium. LP lamina propria, SE surface epithelium, TC tracheal cartilage. ×2,800
the underlying cartilage and were separated from each other by layers of collagen fibrils which showed a periodic banding (64nm). Collagen fibrils, unlike collagen fibrils of the cartilage matrix, were regularly arranged in bundles running in parallel or crossing at right angles to each other. Sometimes, elastic fibrils were found between such collagen fibrils.

Chondroblasts were fusiform and occurred singly. They contained a large amount of rough-surfaced endoplasmic reticulum with occasionally dilated cisternae, a well developed Golgi apparatus and a few mitochondria. Larger and smaller vesicles with electron dense contents were sometimes seen in different portions of the Golgi zone. The cytoplasm was more electron-dense than that of perichondrial fibroblasts.

Chondrocytes were rounded with a large nucleus and were either single or paired. The Golgi apparatus was much more prominent than in chondroblasts. The three types of vacuoles proposed by Holtrop (1972) in the chondrocytes of mice could be distinguished: 1) Small vacuoles, 50-100nm, were found everywhere in cytoplasm and resembled those found in association with the rough-surfaced endoplasmic reticulum. 2) Vacuoles of intermediate size, 250-400nm, contained a homogenously distributed fine fibrillar material. 3) Large vacuoles, 0.5-1.2 μ, included a fine fibrillar material resembling the contents of the intermediate vacuoles. Moreover, small spherical inclusions were usually present inside the vacuoles, some clearly surrounded by a membrane. Many small vesicles were often seen in the surroundings of a large vacuole. The rough-surfaced endoplasmic reticulum was extended throughout the cytoplasm except for the Golgi area. The cytoplasm occasionally contained lipid droplets and variable amounts of glycogen. Occurrence of numerous bundles of fine intracytoplasmic filaments (2-8nm in diameter) was a distinctive feature of chondrocytes in the later period (33 or 54 days).

Extracellular matrix vesicles were identified throughout the tracheal cartilage matrix as round or oval profiles, ranging in diameter from 20nm to 1μ and bound by a trilaminar membrane.

Internal perichondrium: In the internal perichondrium ellipsoid fibroblast-like cells, which were nearly twice as large as the external perichondrial fibroblasts, were arranged in three or four layers (Fig. 4b). These cells possessed well developed Golgi apparatus, rough-surfaced endoplasmic reticulum, mitochondria and large membrane-bound vacuoles which might generally characterize a fibroblast or chondroblast. Many cytoplasmic processes of the cell protruded into the cartilage matrix, where intercellular fibrils were particularly irregular in arrangement. The most striking feature of these fibroblast-like cells was the occurrence of numerous intracellular vacuoles which were seen on the side of the cells facing the cartilage. The vacuoles contained numerous fragments of fibrils and/or a substance which closely resembled the cartilage matrix (Fig. 5). The large vacuole containing cells were constantly observed at the internal perichondrium during the neonatal period. At the end of the first week after birth, they were decreased in number, but did not disappear. A few of the vacuole-containing cells were seen even in 33 or 54 day old rats. Occasionally, the cells were literally filled with vacuoles (Fig. 5b).

The vacuoles were classified into three types according to their structure: The first type of vacuoles had a round or oval shape ranging from 0.5 to 1.5μ in diameter (Fig. 6a.). Their content consisted of fine granular particles of moderate density,
delicate fibrils and small vesicles. Occasionally, a dense material with a fibrillar pattern could be seen in the vacuoles. This first type of vacuoles apparently resembled the multivesicular bodies found in the Golgi area of chondrocytes and chondroblasts. The second type of vacuoles included one or two cross-striated (64 nm) fibrils.

Fig. 5. a and b. Two typical fibroblast-like cells at the internal perichondrium with numerous cytoplasmic processes. Large membrane-bound vacuoles (arrows) which contain several inclusions such as fibrils, fine granules and filaments appear within their cytoplasm. a. ×8,000, b. ×16,000
and fine filaments associated with dense granules (Fig. 6b). These vacuoles can be considered the intermediate or transitional form between the first and the third type to be described below. They ranged from 0.5 to 0.75 μ in diameter. The third type vacuoles contained thick fibrous structures which had 64 nm periodicity and resembled collagen (Fig. 6c, d). The vacuoles had an elongated shape measuring about 0.3 μ in diameter and 2 μ in length. These fibrous structures in the vacuoles seemed to be aggregated laterally to form thicker structures, unlike extracellular collagen fibrils of the perichondrium and cartilage matrix. Whereas isolated fibrils generally measured from 6 to 20 nm, such fibrillar aggregates had a thickness of up to 120 nm. Electron dense amorphous materials occasionally accompanied the fibrilar aggregates. Most of the aggregates lay parallel to the long axis of the vacuole.

Fig. 6. Three types of vacuoles within fibroblast-like cells. a. The first type vacuole with fine granular particles of moderate density, delicate fibrils and small vesicles. ×36,000  b. The second type vacuole with one or two thin cross-striated fibrils, fine filaments and several dense granules. ×50,000  c. and d. The third type vacuole containing collagen like fibrils with clear striation. c. ×72,000, d. ×50,000
Fragments of cell organelles such as those seen in lysosomes could not be recognized in the three types of vacuoles. Small vesicles were present around the vacuoles in the cytoplasm. Some of the vesicles appeared to be fusing with the membranes of the vacuoles. On the opposite side of the cell, i.e., on the luminal side, dense bodies were found which looked like residual bodies. A few cells showed signs of degeneration. Fragments of degenerated cells were seen in close relation to the perichondrium. In general, the fine structure of the chondrocytes on this side of the tracheal cartilage was similar to that of the cells in the external side.

**Cytochemical observation**

*Acid phosphatase*: A strong activity of acid phosphatase was demonstrated in the fibril containing vacuoles of the fibroblast-like cells in the internal perichondrium (Fig. 7). Some vacuoles of the first type, however, lacked the reaction product. Vacuoles of the second and the third type rarely lacked the evidence of the enzyme activity. The activity was also found on the residual bodies. There was no activity of this enzyme extracellularly in the control sections incubated in a medium without substrate. On the other hand, the lysosomal bodies of fibroblasts and chondroblasts in the external perichondrium also showed a moderate activity of acid phosphatase. In the chondrocytes, the reaction product was deposited over Golgi cisternae, Golgi-associated vesicles and conventional lysosomes. However, no deposition occurred in the vacuoles of intermediate and large size and not all Golgi-associated vesicles contained the reaction product.

*Aryl sulfatase*: No deposition of reaction product, indicating aryl sulfatase activity (pH 5.4), was demonstrated in the cytoplasm of fibroblasts and chondroblasts in

![Fig. 7](image-url). Reaction products of acid phosphatase activity are mainly localized within the second and the third type vacuoles. \( \times 35,000 \)
the external perichondrium. In the fibroblast-like cells of the internal perichondrium, activity of this enzyme was present in the vacuoles and lysosomal bodies. Not all vacuoles contained the reaction product. In the chondrocytes the products were deposited in lysosomal bodies and some vacuoles. Control sections were free of the enzyme activity.

Alkaline phosphatase: An extensive alkaline phosphatase activity was observed on the surface and in the vicinity of the plasma membrane covering the external side of the fibroblasts and chondroblasts which were engaged in an active synthesis of collagen fibrils and matrix in the external perichondrium (Fig. 8a). Reaction pro-

Fig. 8. Alkaline phosphatase activity in the external perichondrium (a) and internal perichondrium (b). Reaction products are mainly found on the plasma membrane of fibroblasts in the external perichondrium, on the other hand, rarely seen in the internal perichondrium. ×6,000
duct could be demonstrated neither on the interior side of the cells nor in the intracellular vacuoles or lysosomal bodies. In the internal perichondrium, a very weak activity of alkaline phosphatase was shown on the surface of the plasma membrane covering the internal side of the fibroblast-like cells, while elongated fibroblasts demonstrated a strong activity of the enzyme (Fig. 8b). There was no evidence of the enzyme activity in the vacuoles of the fibroblast-like cells.

Discussion

Histological and histochemical studies of the tracheal cartilage have been carried out by Pelc and Glucksman (1955), Ö (1959) and Kaelis (1969) on the light microscopic level, and by Porter and Bonneville (1973), Seegmiller, Ferguson and Sheldon (1972) and Rhodin (1974) on the electron microscopic level. But to date, reports on the growth and remodelling of the tracheal cartilage are few. Savastin-Asling and Asling (1973) recently reported the structure of cells at the resorption front of the tracheal cartilage in the rat.

Dimensional changes

Although linear growth was shown in the tracheal length and diameter, their percent-increase per day was high during the first week. This may be explained in that the lumen of the trachea is especially expanded by active respiratory movements immediately after birth (Ham and Baldwin, 1941; Ham, 1975) and this likely accelerates the growth and remodelling of the tracheal cartilage in this period.

The thickness of the tracheal cartilage was constant (0.10 ± 0.03 mm) during the period of the present observation. The possible mechanisms for this are: 1) that the resorption of the tracheal cartilage at the internal perichondrium and the appositional growth at the external perichondrium proceed at the same rate, and 2) that C-shaped tracheal cartilage grows towards the ends of the C. That the first mechanism is actually occurring and plays the essential role in the maintainance of the shape and thickness of the cartilage could be confirmed by the present study. The ultrastructural evidence for the appositional growth at the external perichondrium has been demonstrated, while the resorption activity in the fibroblast-like cells in the internal perichondrium has been supported by the strong activity of acid phosphatase in their collagen containing vacuoles. The second mechanism, on the other hand, could not be ruled out as morphological signs of growth were found at both ends of C-shaped cartilage in this study. There, the fibroblasts and chondroblasts appeared identical in morphology and activity with those in the external perichondrium.

Collagen containing vacuoles

The occurrence of collagen containing vacuoles in the fibroblast-like cells of the internal perichondrium may be interpreted in different ways. The first possibility is that the banded fibrils in question might represent an artifact resulting from precipitation of collagen fibrils in the cell vacuoles during the specimen preparation (Trelstand, 1971; Imura, Tanaka and Takase, 1975). The second possibility is intracellular synthesis of collagen; the fibrils in the vacuoles might represent either the intracellularly synthesized collagen or the precursors of collagen (Avery and Han, 1961; Meek, 1965; Gothlin and Ericsson, 1970; Carlson, 1973; Inage, 1975). An
alternative possibility for this is a pathological or abnormal process in the course of collagen formation (Welsh, 1966; Welsh and Meyer, 1967; Imura, Tanaka and Takase, 1975). The third possibility is phagocytosis of collagen fibrils (Luse and Hutton, 1964; Schwarz and Guldner, 1967; Parakkal, 1969a, b; Perez-Tamayo, 1970; Ten Cate, 1972; Listgarten, 1973; Cullen, 1973; Göthlin and Ericsson, 1973; Allen, Hamilton and Moor, 1973; Beertsen, Everts and Van den Hooff, 1974).

Support for the first and the second possibilities can be found in the following reports. Sheldon and Kimball (1962) demonstrated that following papain administration, chondrocytes in rabbit ear cartilage showed several Golgi vacuoles loaded with collagen-like fibrils. They proposed that the fibrils with a long spacing striation (200nm periodicity) possibly appeared due to a fixation artifact resulting in the precipitation of monomeric collagen. Welsh and Meyer (1967) suggested a pathologic process of the cell as the thick bundles of collagen fibrils in the cell vacuoles appeared abnormal and unlike any extracellular fibrils. Göthlin and Ericsson (1970), observing the intracellular collagen containing vacuoles in the fibroblasts, chondroblasts and osteoblasts of the fractured callus, suggested that the vacuoles were transporting intracellularly synthesized collagen, though they more recently supported the collagen phagocytosis hypothesis (vide infra). Imura, Takano and Takase (1975) found segment long spacing (SLS) fibrils (250nm periodicity) in cell vacuoles of a secondary chondrosarcoma, and assumed that the tropocollagen molecules were aggregated into SLS fibrils under a rather unusual physical chemical condition in the cell.

The third hypothesis of collagen phagocytosis seems to account much better for the ultrastructural and cytochemical findings of the present study. The collagen containing vacuoles occurred in the very cells of the internal perichondrium which were not involved in the new formation of cartilage but were believed to be destroying and absorbing it. Thus, it seems most unlikely that they represent the figure of collagen synthesis. In their recent study using thorium dioxide as a tracer, Göthlin and Ericsson (1973) demonstrated that this marker was taken into the collagen containing vacuoles, thus showing unequivocally that collagen was phagocytosed.

The demonstration of acid phosphatase activity within the collagen containing vacuoles in the fibroblast-like cells strongly supports that the cells were resorbing the cartilage and the vacuoles were digesting the phagocytosed collagen. A similar localization of this enzyme in the vacuoles including collagen fibrils or intercellular matrices was observed in macrophages of involuting uterus (Parakkal, 1969a) and lung (Cohn and Weiner, 1963), and fibroblasts of developing periodontal ligament (Deporter and Ten Cate, 1973). Acid phosphatase is a useful marker for lysosomal enzyme activity in cytochemical investigations and the relationship of this hydrolytic enzyme and connective tissue absorption is well established (Woessner, 1968; Reynolds, 1969; Perez-Tamayo, 1970).

In the same manner as acid phosphatase, aryl sulfatase activity in the collagen containing vacuoles and lysosomal bodies within fibroblast-like cells, and in lysosomal bodies within chondrocytes is supposed to be associated with degradation of proteoglycan (Goldfisher, 1965).

Deporter and Ten Cate (1973), Ten Cate and Syrbu (1974) reported on the alkaline phosphatase activity in the collagen containing vacuoles of ligament fibro-
blasts, and suggested that alkaline phosphatase was involved in phagocytosis and degradation of collagen. In the present study, however, alkaline phosphatase activity was not confirmed to occur within the collagen containing vacuoles: it was mainly found in the cells of the external perichondrium. This enzyme is supposed to be concerned in the formation of connective tissue matrix (McKelvie and Mann, 1948; Siffert, 1951; Kroon, 1952) and in the differentiation of chondroblasts (Teaford and White, 1964).

In the present observation, metachromasia of the cartilage matrix was markedly weaker in the vicinity of the vacuolated fibroblast-like cells than elsewhere and this change is also considered to be a proof of the digestion and vanishing of acidic glycosaminoglycan. Growth of collagen fibrils into the large, thick bundles within the vacuoles as demonstrated in the present study (Fig. 6c, d) may be ascribed to the relative deficiency of acidic glycosaminoglycan in (phagocytotic) vacuoles (Seegmiller, Fraser and Sheldon, 1971).

Fig. 9. Schematic representation of the possible processes of the tracheal cartilage resorption. See text in detail. C collagen fibrils, DC digestive vacuole, ER endoplasmic reticulum, E endocytosis, G Golgi apparatus, MVB multivesicular body, N nucleus, PG proteoglycan, RB residual body, SG secretory granule, TI the first type vacuole, TII the second type vacuole, TIII the third type vacuole, Golgi vesicle containing lysosomal enzymes (primary lysosomes), active lysosomal enzymes.
In the experimental studies of embryonic chick limb-bone rudiments in organ culture (Fell and Dingle, 1969; Glauert, Fell and Dingle, 1969; Appleton, Pelk and Dingle, 1969; Dingle, Fell and Glauert, 1969), it was shown that addition of sucrose to the culture medium caused intense cytoplasmic vacuolation in the perichondrial, osteogenic and cartilage cells, and some vacuoles came to contain banded fibrils identical in appearance with those in the surrounding matrix. These experiments indicated that the fibrils were endocytosed.

From the above discussion it seems safe now to conclude that the fibroblast-like cells of the internal perichondrium, which are supposed to be differentiated from fibroblasts (Maximow, 1927; Ross, 1968) or chondroclasts (Knese, 1970), take up fragments of collagen fibrils and digest them. Ten Cate and Syrbe (1974) also indicate that the fibroblast was the cell responsible for both the synthesis and degradation of collagen in the periodontal ligament, a connective tissue with a high rate of remodelling.

**Possible processes of tracheal cartilage resorption**

Dingle (1969) and Glauert, Fell and Dingle (1969) recently suggested “two stage digestion” of extracellular macromolecules in living connective tissues. Figure 9 shows schematic representation of the possible processes of tracheal cartilage resorption based on the results of this study and on the paper of Dingle (1969). Certain forms of primary lysosomes (such as the enzyme containing Golgi vesicles) fuse with the plasma membrane and open on to the extracellular surface. In the extracellular matrix, liberated enzymes cause partial degradation of collagen and other components. Then, the incompletely degraded products (and the liberated enzymes) are absorbed by endocytosis into digestive vacuoles of the resorbing cells. There is also the biochemical evidence that fibroblasts discharge hydrolases into the extracellular space and take them up again (Hickman and Neufeld, 1972). Within the vacuoles enzymes further added from primary lysosomes complete the digestive process. The second and the third type of vacuoles in this scheme are the digestive ones, but the role of the first type is not clear.

The present ultrastructural and cytochemical studies on the remodelling of the tracheal cartilage suggested that there is distinct functional difference in the external and internal perichondrium. The fibroblast-like cells of the internal perichondrium play the essential role of cartilage resorption in the processes of remodelling. The resorption mechanism with regard to this remodelling system seems to be quite similar to that of fibrous connective tissues.

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気管軟骨の改築に関する微細構造学的、細胞化学的研究

矢崎 俊 彦

ラットの気管軟骨の改築過程を微細構造的、細胞化学的に研究した。気管軟骨の厚さは観察した生後1日から54日間において一定していた。気管軟骨の外がわの軟骨膜は活発な線維芽細胞と細胞間膠原線維よりなり、それには典型的な付加成長がみられた。一方、内がわの軟骨膜においては、外がわの軟骨膜の線維芽細胞の2倍近い
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