Int. J. Dev. Biol. 35: 311-320 (1991)

Differentiation of the secondary elastic cartilage in the external ear of the rat

ŽELIMIR BRADAMANTE*[1], LJILJANA KOSTOVIĆ-KNEŽEVIC[1], BOŽICA LEVAK-ŠVAJGER[2] and ANTON ŠVAJGER[1]

[1]*Institute of Histology and Embryology and* [2]*Institute of Biology, Faculty of Medicine, University of Zagreb, Republic of Croatia, Yugoslavia*

ABSTRACT The cartilage in the external ear of the rat belongs to the group of secondary cartilages and it has a unique structural organization. The chondrocytes are transformed into typical adipose cells, the proteoglycan cartilage matrix is reduced to thin capsules around the cells and the rest of the extracelullar matrix is occupied by a network of coarse elastic fibers. It appears late in development (16-day fetus) and needs more than one month for final development. The differentiation proceeds in several steps which partly overlap: the appearance of collagen fibrils, elastin fibers, the proteoglycan matrix, and the adipose transformation of chondrocytes. The phenotype of this cartilage and the course of its differentiation are very stable, even in very atypical experimental environmental conditions. The only exceptions are explants in organ culture *in vitro* and perichondrial regenerates. In these conditions the development of elastic fibers is slow and poor while the production of the proteogycan matrix is abundant. The resulting cartilage then displays structural characteristics of hyaline cartilage rather than those of the initial elastic one.

KEY WORDS: *elastic cartilage, chondrogenesis, elastogenesis, external ear, rat*

Introduction

The elastic cartilage belongs to the group of *secondary* or *accessory* cartilages since it is not a part of the cartilagineous primordium of the body skeleton as is most hyaline cartilage (Schaffer, 1930). It differentiates relatively late in development in restricted parts of the body, among which the auricle (pinna) and the epiglottis are the best known examples. The secondary cartilages are characterized by a great variability in structure and mechanical properties in various mammalian species, even in various races of the same species (Baecker, 1928).

From the point of view of structural organization the elastic cartilage found in the external ear of rats and mice is the most peculiar type because it resembles more the adipose tissue, the primitive chondroid tissue of lower vertebrates (Schaffer, 1930; Merker, 1966), of invertebrates and even plant tissue (Person and Philpott, 1969) than the «true» cartilage. We felt it would be interesting to see whether this structure would undergo variations under the influence of changed environmental conditions. For this reason we undertook the investigation of its normal differentiation *in situ*, the stability of its phenotype under various experimental conditions, including those which cause its perichondrial (appositional) regeneration.

Structural organization

According to Baecker (1928) and Schaffer (1930) the cartilage in the external ear (external auditory meatus and pinna) of the rat can be defined as: a) *cellular* or *parenchymatous*, because of the scarcity of the intercellular matrix; b) *elastic*, due to the presence of elastic fibers in the extracellular spaces, and c) *adipose*, since the chondrocytes are transformed into fat cells («lipochondrocytes») in adult animals (Fig. 1).

The ultrastructure of chondrocytes and the extracellular matrix was previously investigated by Anderson (1964), Geyer and Tews (1971) and Serafini-Fracassini and Smith (1974) in rat and mouse auricular cartilage, and by Holm-Nielsen (1976), Holm-Nielsen and Bytzer (1979) and Akisaka and Yamamoto (1977) in the very similar cartilage in the rat epiglottis. Our own investigation with transmission and scanning electron microscopy showed that the fully differentiated globular chondrocytes in the central zone of the cartilaginous plate strongly resemble white adipose cells (Bradamante and Svajger, 1981; Kostovic-Knezevic *et al.*, 1981).

The single, large cytoplasmic lipid droplet is surrounded by cytofilaments in parallel arrangement (Kostovic-Knezevic and Svajger, 1975) typical for white adipocyte (Luckenbill and Cohen, 1966; Wood, 1967). The thin peripheral rim of cytoplasm with the flattened

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0214-6282/91/$03.00

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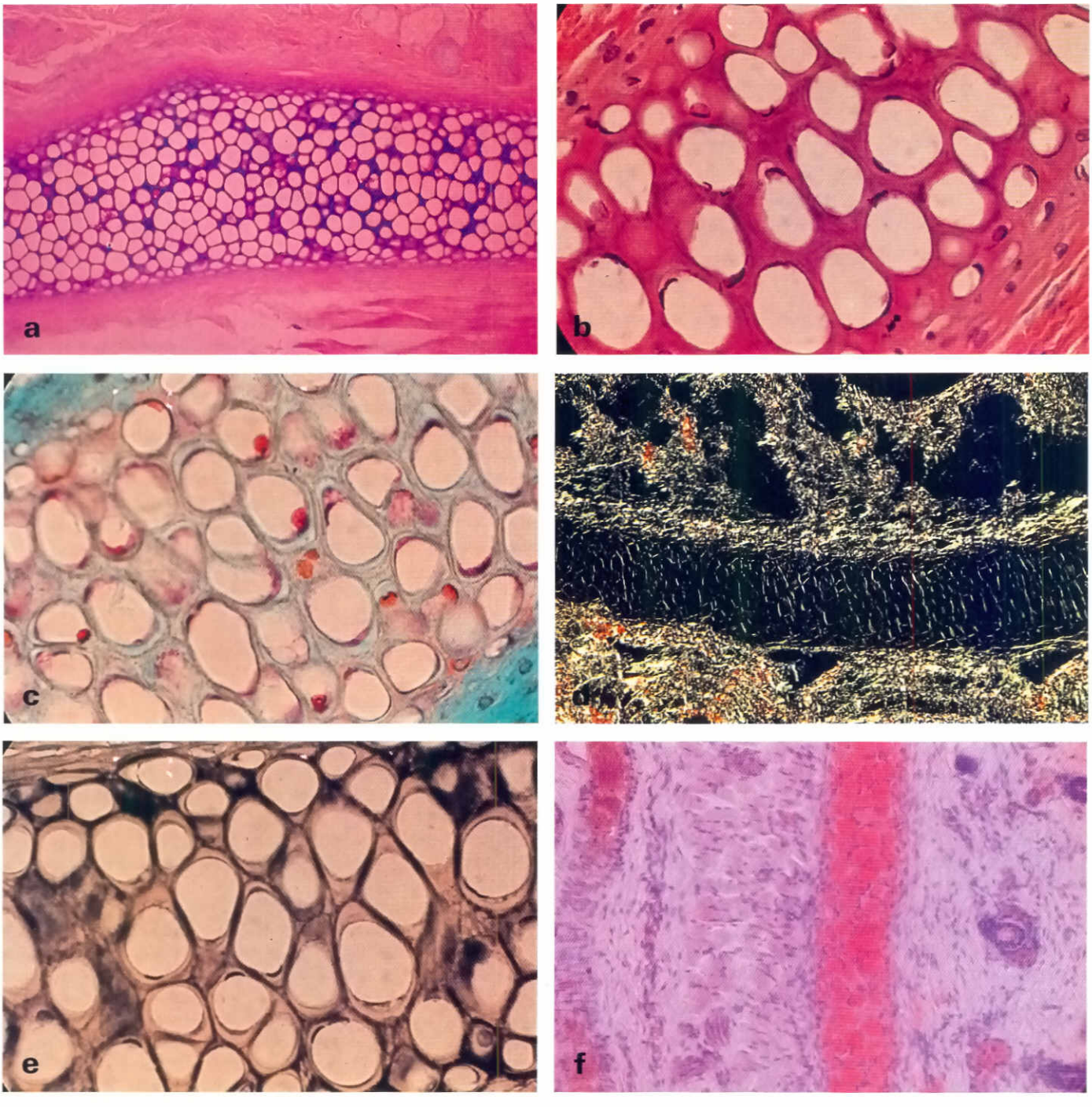

Fig. 1. Structure of the adult cartilage. (a) *General appearance, alcian blue-PAS stain, x60.* **(b)** *Hemalaun-eosin stain. Note the absence of basophilia in the extracellular matrix. x180.* **(c)** *Masson stain. Note the bright green capsules around some cells. x140.* **(d)** *Sirius red stain, polarized light. Note fine type II collagen fibrils within the cartilage matrix (green). x80.* **(e)** *Verhoeff's stain for elastic fibers (black). x180.* **(f)** *Sudan III stain for lipids. x100.*

nucleus is characterized mainly by abundant, densely packed filaments. Within the scanty extracellular matrix the thin pericellular capsules contain thin collagen fibrils (about 20μm in diameter) embedded in the ultrastructural equivalent of the proteoglycan cartilage matrix. The intercapsular spaces are occupied by thick, branched elastic fibers and plates (Fig. 2). In peripheral zones of the cartilage plate immature elastic fibers (oxytalan fibers, Fullmer *et al.*, 1974) can also be shown to exist (Bradamante *et al.*, 1975).

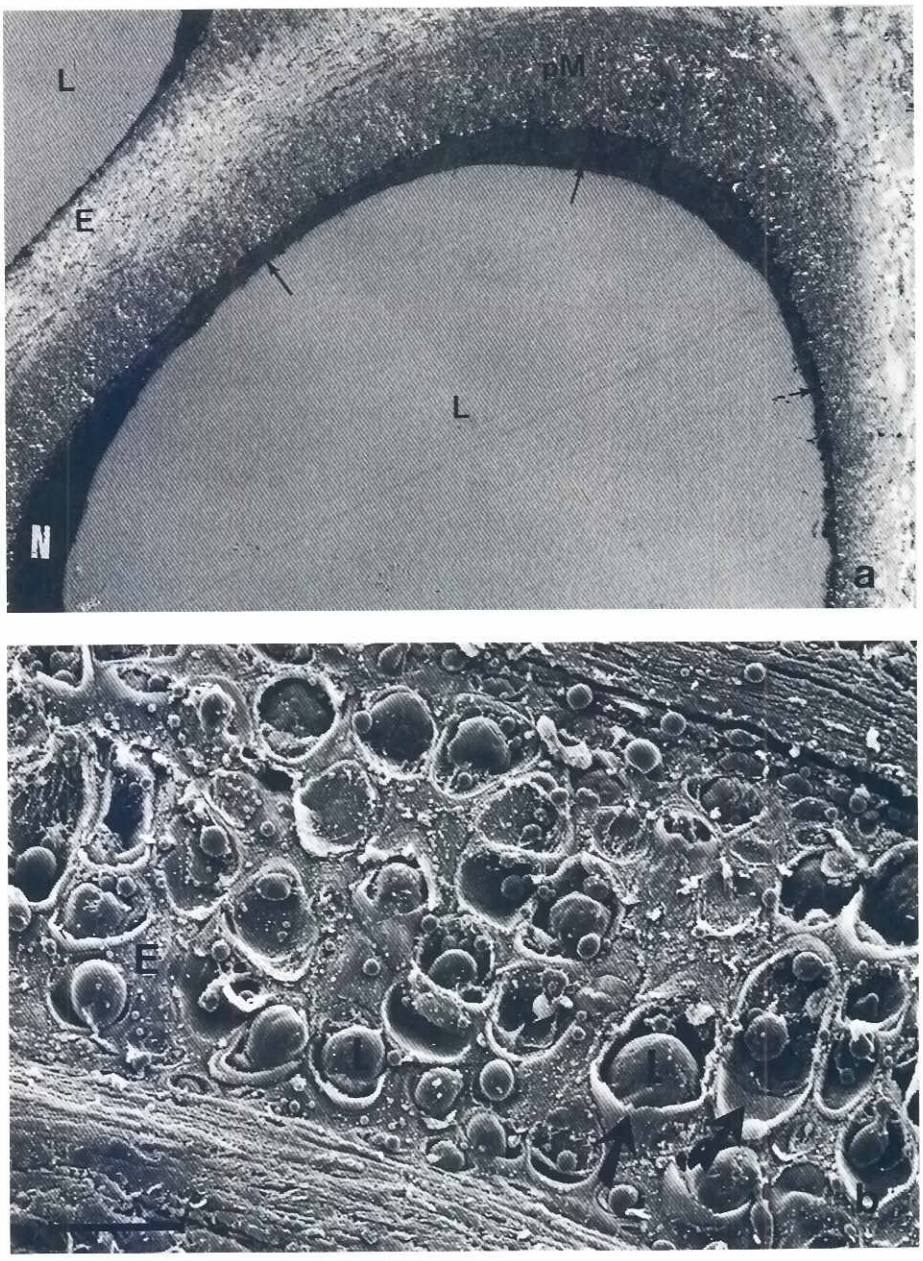

Fig. 2. Ultrastructure of the adult cartilage. **(a)** *The fully differentiated chondrocytes from the central zone of the cartilage plate. The single lipid droplet (L), the flattened nucleus (N), the thin rim of cytoplasm around the lipid droplet (arrows), the pericellular capsule of the extracellular matrix (pM) and the coarse elastic fibers (E). x5800.* **(b)** *The scanning electron micrograph of the cartilage plate. Chondrocytes with lipid droplets (L) reside inside pericellular capsules (arrows). The space between them is occupied by elastic fibers and plates (E). Bar, 40μ m*

Differentiation *in situ*

The differentiation of the hyaline cartilage proceeds in two steps: appearance of collagen fibrils and their impregnation with the proteoglycan cartilage matrix. In the elastic cartilage of humans and some other mammals elastic fibers appear later on, so that the general opinion has been that the elastic cartilage differentiates on the basis of a previously hyaline one (Schaffer, 1930). The auricular cartilage is fully differentiated before birth (Krompecher, 1931; Caponneto, 1932; Goshi, 1966 a,b). In rats, however, the differentiation of the elastic cartilage in the external ear is characterized by: a) more than three sequential steps, and b) long duration (several weeks).

The chondrogenesis in the external ear of the rat and mouse had been studied previously by routine histology (Roland,1936; Danini and Potockaya, 1955; Slais, 1957) and by electron microscopy (Anderson, 1964; Sanzone and Reith, 1977) but the results did not give a complete chronological survey of events. We investigated the differentiation of the rat auricular cartilage from the 15th day of embryonic development up to the age of 5 months by routine histology, histochemistry, on semi-thin sections and by electron microscopy. Special attention was paid to the typical phenotypic traits of the tissue: the components of the extracellular matrix and the adipose transformation of chondrocytes.

Two days after the incomplete fusion of the 1st and 2nd branchial arch (15-day embryos) the chondrogenic blastema appears underneath the epidermis and until the 1st week of postnatal life it does not change significantly in its general appearance. The differentia-

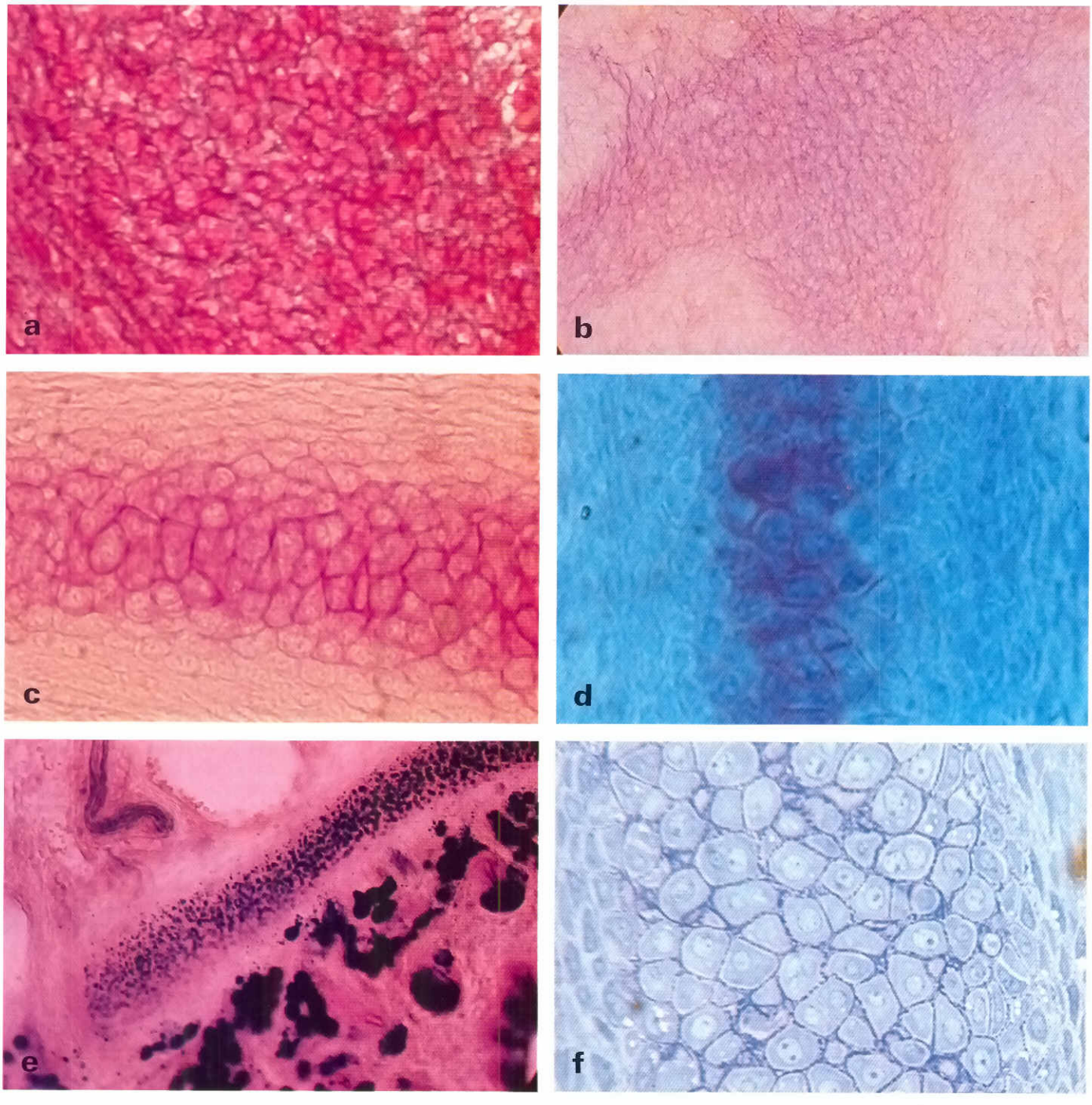

Fig. 3. Differentiation of the cartilage. (a) *Collagen (reticulin) fibers. 17-day fetus. Gomori's silver impregnation. x200.* **(b)** *Oxytalan fibers. 20-day fetus. Aldehyde fuchsin after oxydation. x450.* **(c)** *Elastic fibers. 10-day rat. Aldehyde fuchsin. x320.* **(d)** *Pericellular proteoglycan matrix. Il-day rat. Toluidine blue metachromasia. x480.* **(e)** *Intracellular lipid droplets. 22-day rat. Sudan black. x60.* **(f)** *Semithin section through the cartilage of a 9-day rat. Note cytoplasmatic vacuoles in some cells (lipid droplets) and scarce extracellular matrix. Toluidine blue. x350. Reproduced from Kostovic-Knezevic* et al., *1981 with permission of Springer-Verlag, Berlin.*

tion of the cartilage begins around the external meatus and progresses upwards following the growth of the auricle. As revealed by histochemistry it proceeds in five steps (Fig.3), each of which is characterized by the appearance of a new component: (1) *Collagen (reticulin) fibers* (a delicate network in 16-day fetuses); (2) *Oxytalan (Pre-elastic) fibers* (first appearance of single fibers in 16-day

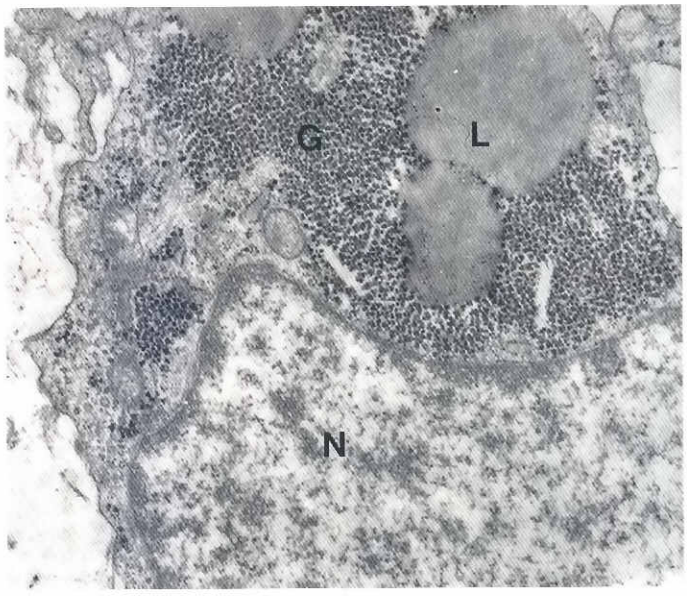

Fig. 4. Chondroblast of a 19-day fetus. (a) *Nucleus (N), lipid droplet (L) and accumulations of glycogen particles (G). x21000. Reproduced in a modified form from Kostovic-Knezevic* et al., *19886 with permission of Spnger-Verlag, Berlin.*

embryos, abundant network in 18-day embryos); (3) *Elastin fibers* (clearly visible not earlier than 1 day after birth, with a well developed network existing at the age of 12 days and elastogenesis completed in the entire external ear at the age of 12 days); (4) *Cartilage round substance* (hardly detectable metachromatic deposits between postnatal days 4 and 6, only single cells completely surrounded by a capsule at day 9, the chondrification of the entire cartilaginous plate being complete at the age of about 3 weeks). The deposition of the ground substance does not progress more than the thickness of the capsule. (5) *Lipid droplets* (first sudanophilic droplets at postnatal day 10; at the age of 20 days each chondrocyte contains 1 or 2 layer droplets; the final adipose transformation is achieved in 1.5-month-old animals in the basal part and one month later in the entire cartilage plate).To sum up, on the basis of light microscopic observations it seems that the main biosynthetic and secretory events in the chondroblasts start during the last week of intrauterine life and the first two weeks of postnatal life (Svajger 1970; Bradamante and Svajger, 1977).

Our study of the ultrastructure of chondrogenesis in the rat external ear (Kostovic-Knezevic *et al.*, 1986) showed that chondroblasts display all the main characteristics of glycoprotein-secreting cells. The prominent features were: a) large deposits of glycogen (often surrounding lipid droplets) in 19-day fetuses which decreased remarkably during the 1st postnatal week, and b) numerous closely-packed cytofilaments situated especially in peripheral parts of the cytoplasm and close to deposits of glycogen and lipid droplets (Fig.4) in newborn rats and older animals.

The cytoplasmic lipid droplets were first observed in 19-day fetuses and they increased in number and size during the first postnatal week, at the end of which they were first detectable by histochemical methods. According to the opinion of Sanzone and Reith (1977) this «early lipid» could be regarded as the storage fat which is metabolized. Later on, during the final adipose transformation of chondrocytes, they are replaced by «adult fat» as a permanent phenotypic trait of this type of cartilage which is regulated genetically rather than metabolically. In this connection it is interesting to note an early experiment of Saccerdotti (1900), who found no quantitative changes of the intercellular fat in rabbit auricular cartilage even after starvation causing a 50% reduction in body weight (even if this might also be due to the absence of a capillary network within the cartilage).

The proteoglycan cartilage matrix (matrix granules+matrix filaments= «stellate reticulum» of Serafini-Fracassini and Smith, 1974) was observed in our material 4-6 days before it could be detected by histochemical methods. A loose reticulum was present already in 19-day fetuses and it was more prominent in newborn rats.

Concerning the fibrillar components of the matrix the observations did not essentially differ from those made by light microscopy. Randomly oriented, 25-30-nm thick collagen fibrils were present in 17-day fetuses, and in the first postnatal week they displayed an increased thickness and regularity of the axial periodicity.

The elastogenesis proceeds in the conventional way as described by Fahrenbach *et al.* (1974, Fig.5). In the central zone of the cartilage plate the intermediary stages, as defined in terms of histochemistry and ultrastructure (Fullmer *et al.*, 1974; Cotta-Pereira *et al.*, 1977) appeared in the regular sequence: (1) *Oxytalan fibers* (bundles of 10-13 nm thick fibrils; 17-day fetus); (2) *Elaunin fibers* (oxytalan fibers poorly impregnated with amorphous elastin, newborn rats); (3) *Elastin fibers* (apparently devoid of microfibrils, 5- to 9-day-old rats). The process of elastogenesis progresses towards the periphery of the cartilage plate. Therefore even in adult animals all the constituents of the «elastic system fibers» (Cotta-Pereira *et al.*, 1977) are simultaneously present in the cartilage: elastic fibers in the central zone, elaunin fibers in the boundary zone, and the oxytalan fibers in the peripheral zone. A similar feature was demonstrated in the tracheal and bronchial mucosa (Böck and Stockinger, 1984).

Stability of the phenotype

As described above, the elastic cartilage in the external ear of the rat is a special type of cartilage which differentiates by a sequence of different biosynthetic events. Due to the long duration of differentiation, the flat shape, the scarcity of the basophilic ground substance and the presence of thick eosinophilic elastic fibers, it can be distinguished from the hyaline cartilage even at earlier stages of differentiation, before the onset of adipose transformation of its chondrocytes. In order to check the stability of the phenotype of this cartilage we placed it in various atypical environments. The direct question was: will the new environment change the normal course of differentiation of the elastic cartilage or eventually switch it more or less towards the phenotype of the hyaline cartilage? The experiments we carried out with this aim were the following :

(1) Transplantation of the chondrogenic blastema of 14- and 15-day rat embryos (with or without the overlying epidermis) into the anterior chamber of the eye of adult rats for 30 days (Svajger and Levak-Svajger, 1971).

(2) Transplantation of the chondrogenic blastema of 15- and 16-day embryos, the precartilage of the 3-day-old rats and the cartilage of 15 day- and 2 month-old rats under the kidney capsule of adult rats for 30-100 days (Svajger and Levak-Svajger 1975a).

(3) Introducing the chondrogenic blastema of 16-day fetuses either

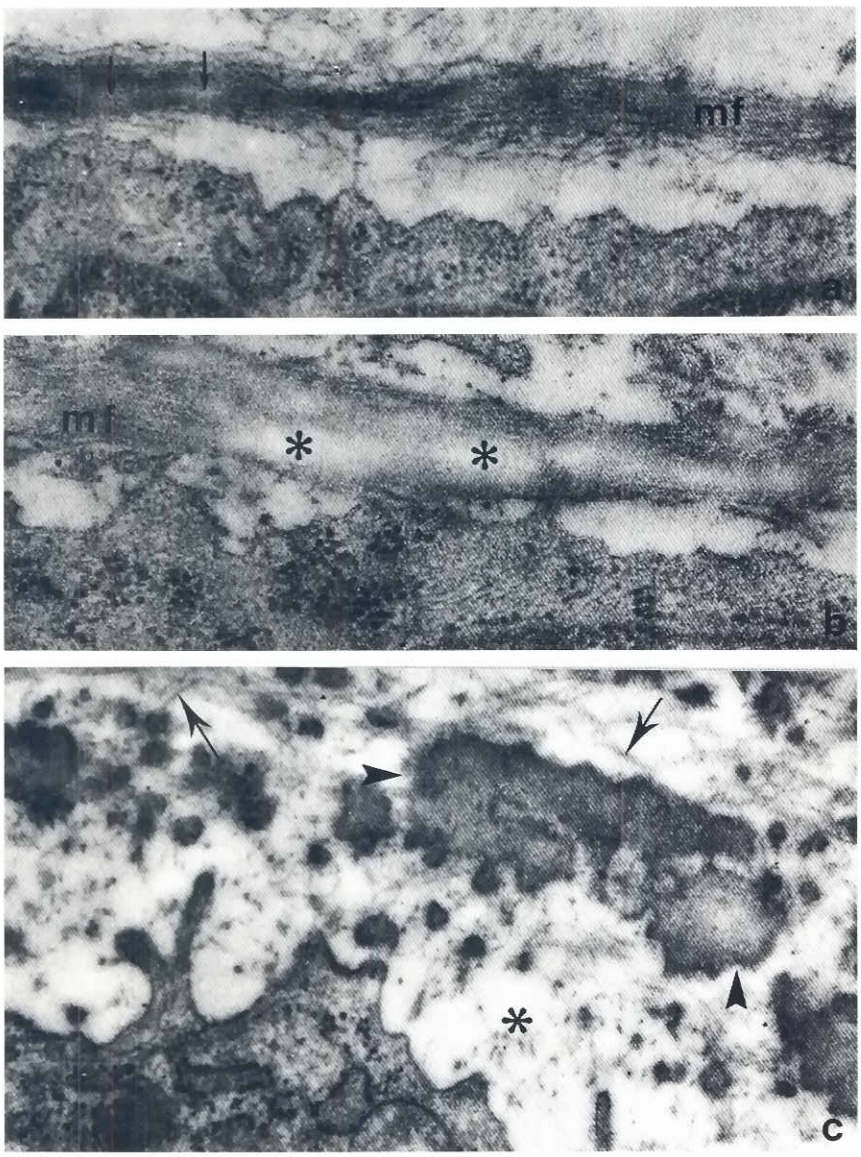

Fig. 5. Course of elastogenesis in the cartilage matrix. (a) *Assembly of 10-13 nm microfibrils (mf, oxytalan fibers) with the initial small deposits of elastin (arrows). Newborn rat. x60000.* **(b)** *Deposits of elastin (asterisk) within the bundle of microfibrils (mf, elaunin fiber). Newborn rat. x48000* **(c)** *Ripe elastic fibers (arrowheads) in the pericellular space of a 9-day rat. Microfibrils (arrows) and matrix granules (asterisk). x30000. Reproduced in a modified form from Kostovic-Knezevic* et al., *19886 with permission of Spnger-Verlag, Berlin.*

into the amniotic cavity of 9-day rat egg cylinders (head fold stage) or between the ectoderm and mesoderm of egg cylinders at the same stage, and the subsequent transplantation of these combined grafts under the kidney capsule of adult rats for 15 days (Svajger and Levak-Svajger, 1975b).

(4) Transplantation of the 1st branchial arch of 10-day embryos under the kidney capsule for 18 days (Svajger and Levak-Svajger, 1976) or of the mesenchyme of the 1st and 2nd branchial arch (as one piece) of 10- to 13-day embryos into the anterior chamber of the eye for 30 days (Svajger and Levak-Svajger, 1971).

(5) Explantation of the chondrogenic blastema of 14-, 15- and 19-day embryos in organ culture on the fowl plasma clot for 5-21 days (Svajger, 1971).

In all these experiments the chondrogenic blastema pursued its phenotypic traits and reached the degree of differentiation analogous to that during development *in situ* for the equivalent period of time (Fig. 6). The only partial exception was differentiation *in vitro* (experiment 5 listed above) in which the slow differentiation and the flat shape were retained, but the differentiation of elastic fibers was delayed and poor while the deposition of the ground substance was more abundant. As a result an intermediate type between the hyaline and the elastic cartilage developed (Fig.7). In grafts such as branchial arches, in which from the same mass of mesenchyme in addition to the elastic cartilage the hyaline cartilage also developed, the latter displayed its type-specific traits: the more advanced stage of differentiation, the abundance of the ground substance and the lack of elastic fibers. The existence of such a specificity is reinforced by the experience of Weiss and Moscona (1958) that a type-specific reaggregation and morphogenesis of cartilage occurs from a mixed suspension of dissociated limb bud and scleral mesenchyme.

Perichondrial (appositional) regeneration

In order to analyze the capacity of the rat auricular cartilage for regeneration, the cartilage with the adjacent perichondrium of 7-

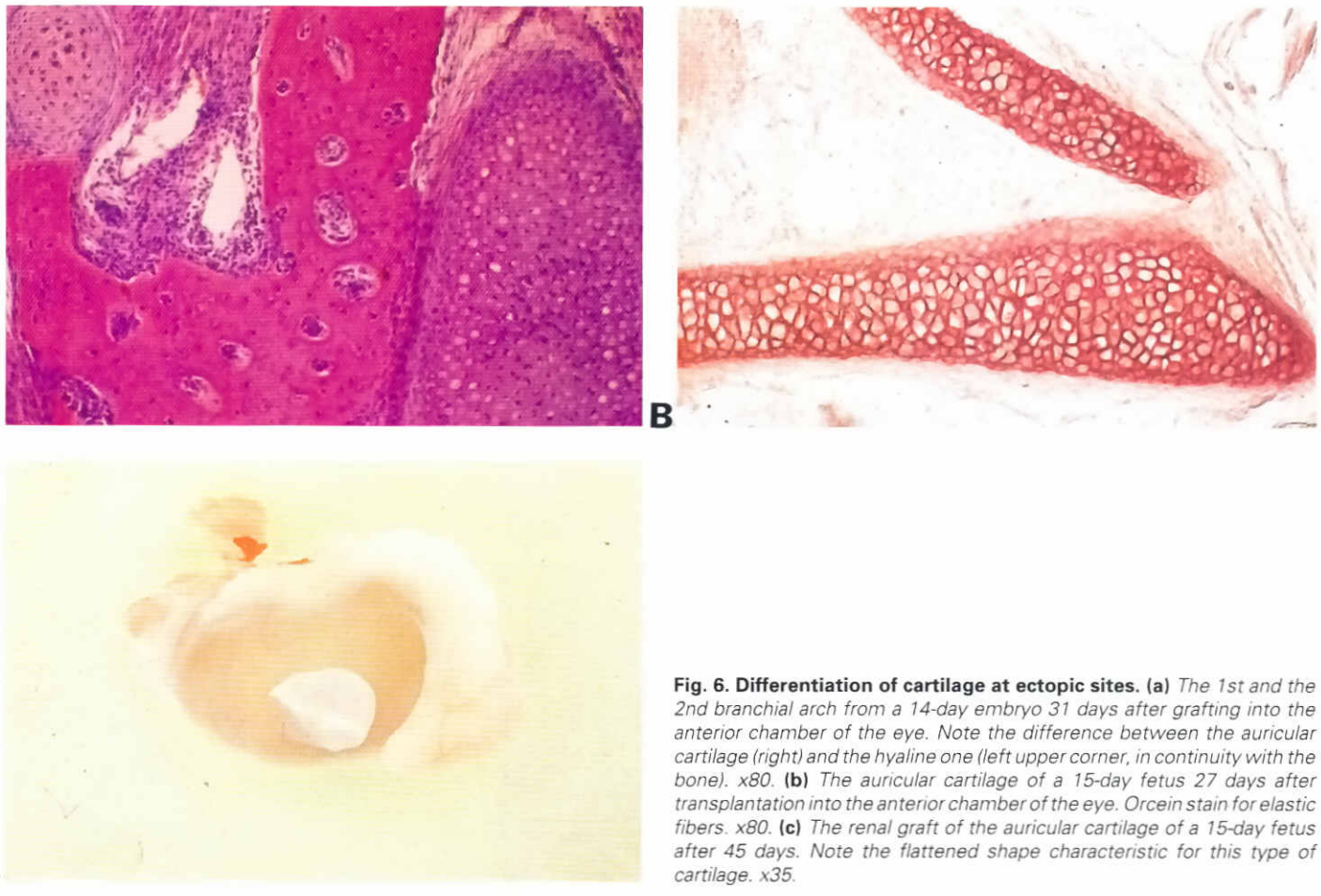

Fig. 6. Differentiation of cartilage at ectopic sites. (a) *The 1st and the 2nd branchial arch from a 14-day embryo 31 days after grafting into the anterior chamber of the eye. Note the difference between the auricular cartilage (right) and the hyaline one (left upper corner, in continuity with the bone). x80.* **(b)** *The auricular cartilage of a 15-day fetus 27 days after transplantation into the anterior chamber of the eye. Orcein stain for elastic fibers. x80.* **(c)** *The renal graft of the auricular cartilage of a 15-day fetus after 45 days. Note the flattened shape characteristic for this type of cartilage. x35.*

15- and 30-day and 3-month-old rats was treated with elastase or with testicular hyaluronidase, which caused an almost complete depletion of the main components of the extracellular matrix: the elastic fibers and cartilage ground substance, respectively. After enzymatic digestion cartilages were grafted under the kidney capsule for 7-22 days. An interstitial regeneration occurred only in some grafts pre-treated with elastase. In other grafts, pre-treated with either of the enzymes, only a perichondrial regeneration occurred in the form of large cartilage plates or, more frequently, in the form of nodules of various size. In both types of regenerates the histological structure was different from that of the original cartilage. It was characterized by the absence or low degree of adipose transformation of chondrocytes, the abundance of cartilage ground substance and the presence of only scarce and very thin elastic fibers (Fig. 8). A thickening of ground substance capsules was sometimes observed around single chondrocytes or small groups of them within the original elastic cartilage (Svajger *et al.*, 1979). This type of perichondrial regeneration is not a specific reaction, because it was also observed after mechanical injury (stroke with the hammer) in some renal isografts of untreated mature auricular cartilage after 65 days and in various experimental conditions (Svajger *et al.*, 1979). It was also demonstrated that in humans during aging an appositional growth of the elastic cartilage of the epiglottis occurs and that the newly formed subperichondrial zone displays the same characteristics as the regenerates in the above-described experiments (Juric-Lekic *et al.*, 1982).

Concluding remarks

The elastic cartilage in the external ear of the rat is a peculiar type of cartilage which in several respects recalls the primitive cartilage or the chondroid tissue of invertebrates or lower vertebrates. The main traits of its phenotype (adipose, transformation of chondrocytes, scarcity of the ground substance, abundance of coarse elastic fibers), as well as its late appearance and the long duration of differentiation, which involves several distinctive steps, are in contrast to those of the hyaline cartilage. Its phenotype and the course of differentiation are very stable even in substantially altered environmental conditions such as the position underneath the early embryonic neuroectoderm which *in situ* induces the differentiation of the hyaline cartilage in sclerotomes.

The only exception seems to be the differentiation in conditions which are not convenient for elastogenesis, such as organ culture *in vitro* when the phenotype partially switches towards that of hyaline cartilage. The same happens during the perichondrial regeneration of the elastic cartilage. This might be due to the high rate of proteoglycan synthesis and the characteristic low rate of elastin synthesis. One could therefore tentatively speculate that during differentiation of the rat auricular cartilage *in situ* the early onset of elastic fiber formation in some way inhibits the byosynthesis of the proteoglycan cartilage matrix («translational competition», Whittaker, 1968) thus leading to its spatial restriction to thin pericellular rims when the network of elastic fibers is already

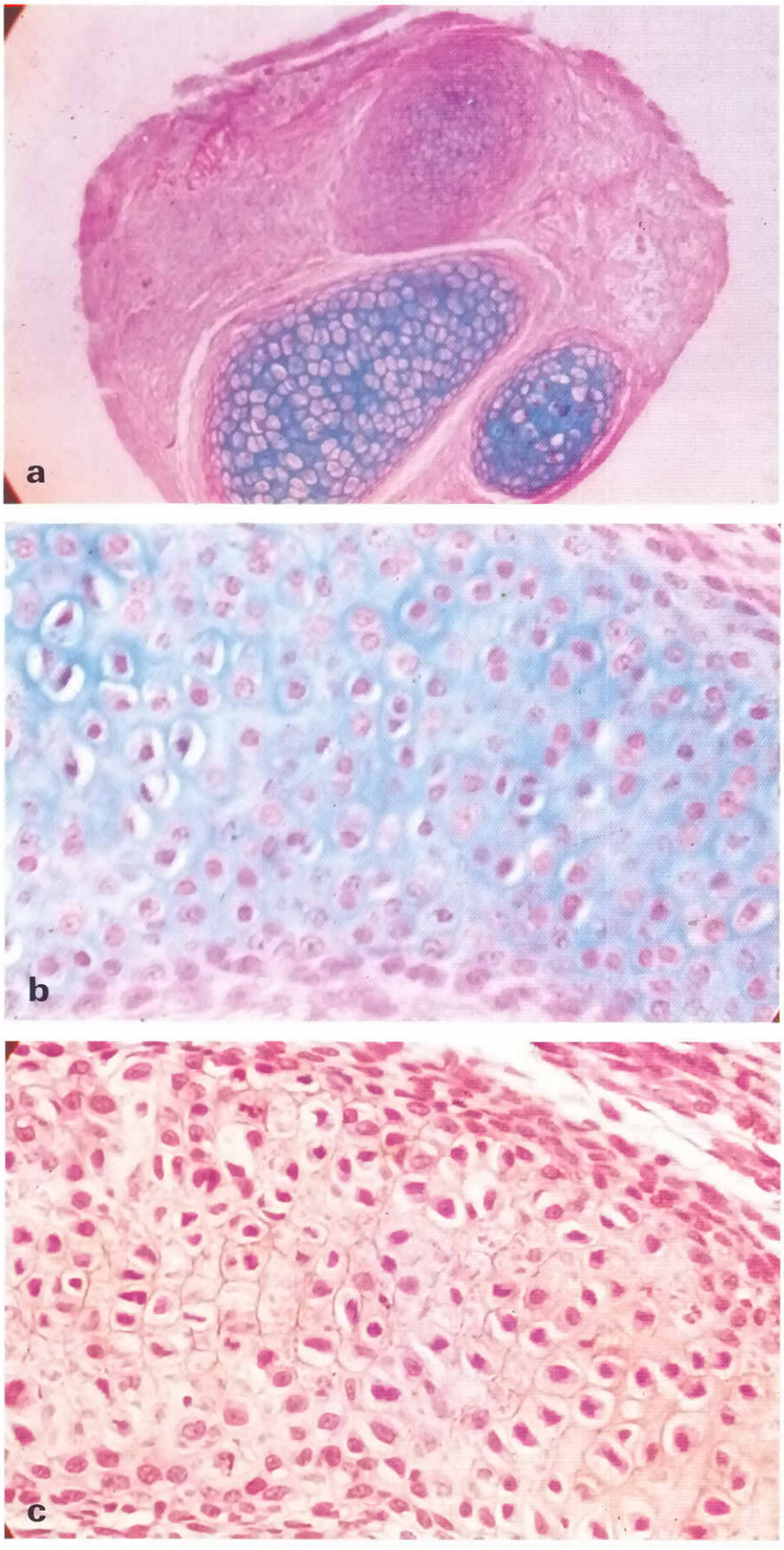

Fig. 7. Differentiation of cartilage in culture. (a) *Explant on the fowl plasma clot of the prechondral mesenchyme (14-day fetus) of the auricle together with the surrounding mesenchyme of the head after 13 days* in vitro. *Note the clear difference between the nodule of auricular cartilage and two nodules of hyaline cartilage. x100.* **(b, c)** *The auricular precartilage of a 19-day fetus after 16 days of cultivation* in vitro. *The alcian blue staining* **(b)** *shows a hyaline phenotype while the orcein staining* **(c)** *shows the absence of elastic fibers. x200.*

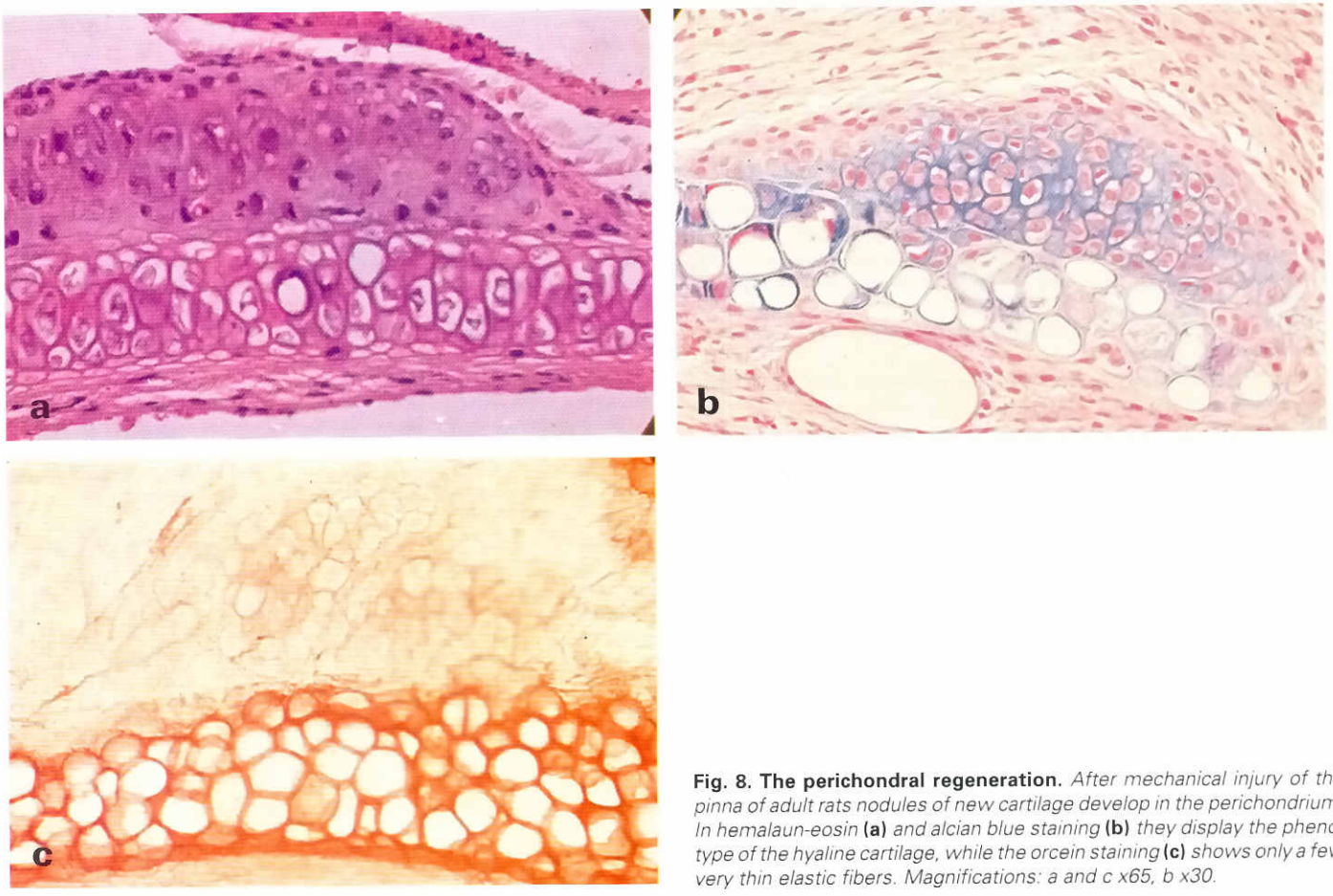

Fig. 8. The perichondral regeneration. *After mechanical injury of the pinna of adult rats nodules of new cartilage develop in the perichondrium. In hemalaun-eosin* **(a)** *and alcian blue staining* **(b)** *they display the phenotype of the hyaline cartilage, while the orcein staining* **(c)** *shows only a few very thin elastic fibers. Magnifications: a and c x65, b x30.*

formed. However, beyond this speculation the mechanisms regulating the phenotype of the elastic cartilage in the external ear of the rat remain unknown.

Acknowledgments

The original work was supported by a grant from the Scientific Fund of the Republic of Croatia. Part of this research was supported by the National Sciences and Engineering Research Council of Canada (Grant No. A5056 to Dr. B.K. Hall), Biology Department), by the Science Research Fund of Dalhousie University, Halifax, Canada. The first author was supported by a Nova Scotia-Yugoslavia Exchange Fellowship. He would like to thank Dr J.G.Mobbs, of the Anatomy Department of Dalhousie University, Halifax, N.S., Canada, for his expert technical advice.

References

AKISAKA, D. and YAMAMOTO, H. (1977). Freeze fracture image of elastic cartilage in rat epiglottis. *J. Electron Microsc. 28:* 12-19.

ANDERSON, D.R. (1964). The ultrastructure of elastic and hyaline cartilage of the rat. *Am. J. Anat. 144:* 403-433.

BAECKER, R. (1928). Zur Histologie des Ohrknorpels der Sauger. Z. *Mikrosk. Anat. Forsch. 15:* 274-367.

BÖCK, P. and STOCKINGER, L. (1984). Light and electron microscopic identification of elastic, elaunin and oxytalan fibers in human tracheal and bronchial mucosa. *Anat. Embryol. 170:* 145-153.

BRADAMANTE, Z. and SVAJGER, A. (1977). Pre-elastic (oxytalan) fibers in developing elastic cartilage of the external ear of the rat. *J. Anat. 123:* 735-743.

BRADAMANTE, Z. and SVAJGER, A. (1981). Scanning electron microscopy of elastic cartilage in the rat external ear. *Folia Anat. Yugoslav. 11:* 25-34.

BRADAMANTE, Z., KOSTOVIC-KNEZEVIC, Lj. and SVAJGER, A. (1975). Light and electron microscopic observations on the presence of pre-elastic (oxytalan) fibers around the mature cartilage in the external ear of the rat. *Experientia 31:* 979-980.

CAPONNETTO, A. (1932). Sullo sviluppo delle fibre elastiche nelle cartilagine dell'orecchio esterno dell'uomo. *Arch. Ital. Anat. Embriol. 30:* 94-101.

COTTA-PEREIRA, G., RODRIGO, F.G. and DAVID-PEREIRA, J.F. (1977). The elastic system fibers. In *Elastin and Elastic Tissue* (Eds. L.B. Sandberg, W.R. Gray and W.R. Franzblau). Plenum Press, New York, pp. 19-31.

DANINI, E.S. and POTOCKAYA, L.E. (1955). On the structure and development of the «cellular cartilage». *Arkh. Anat. Gistol. Embriol. 32:* 35-40.

FAHRENBACH, W.H., SANDBERG, L.B. and CLEARY, E.G. (1974). Ultrastructure study on early elastogenesis. *Anat. Rec. 155:* 563-576.

FULLMER, H.M., SHEEZ, J.H. and NARKATES, A.J. (1974). Oxytalan connective tissue fibers: a review. *J. Oral Pathol. 3:* 291-316.

GEYER, G. and TEWS, K. (1971). Electron microscopic study of the fibrillar components in the extracellular space of elastic cartilage. *Anat. Anz. 128:* 491-496.

GOSHI, N. (1966a). Electron microscopy of chondrogenesis in the auricular cartilage of the human fetus. I. Observations on later embryonal stages. *Kumamoto Med. J. 19:* 132-143.

GOSHI, N. (1966b). Electron microscopy of chondrogenesis in the aurlcular cartilage of the human fetus. II. Observations on early embryonal stages. *Kumamoto Med. J. 19:* 144-159.

HOLM-NIELSEN, E. (1976). The elastic cartilage in the normal rat epiglottis. I. Fine structure. *Cell Tissue Res. 173:* 179-191.

HOLM-NIELSEN, E. and BYTZER, P. (1979). High resolution scanning electron microscopy of elastic cartilage. *J. Anat. 129:* 823-831.

JURIC-LEKIC, G., BRADAMANTE, Z. and SVAJGER, A. (1982). Phenotype modification in zones of appositional growth of human elastic cartilage. *Folia Anat. Yugoslav. 12:* 93-100.

KOSTOVIC-KNEZEVIC, Lj., BRADAMANTE, Z. and SVAJGER, A. (1981). Ultrastructure of elastic cartilage in the rat external ear. *Cell Tissue Res. 218:* 149-160.

KOSTOVIC-KNEZEVIC, Lj., BRADAMANTE, Z. and SVAJGER, A. (1986). On the ultrastructure of the developing elastic cartilage in the rat external ear. *Anat. Embryol. 173:* 385-391.

KOSTOVIC-KNEZEVIC, Lj. and SVAJGER, A. (1975). Cytoplasmic filaments associated with lipid droplets in chondrocytes of the rat auricular cartilage. *Experientia 31:* 581-582.

KROMPECHER, S. (1931). Die Entwicklung des elastischen Knorpels. *Anat. Anz. Erg.-Heft 71:* 42-49.

LUCKENBILL, L.M. and COHEN, A.S. (1966). The association of lipid droplets with cytoplasmic filaments in avian subsynovial adipose cells. *J. Cell Biol. 31:* 195-199.

MERKER, H.-J. (1966). Das elektronenmikroskopische Bild des grundsubstanzarmen Knorpels in den Schwanzflossen von *Petromyzon fluviatilis*. *Z. Zellforsch. 70:* 554-568.

PERSON, P. and PHILPOTT, D.E. (1969). The nature and significance of invertebrate cartilages. *Biol. Rev. 44: 1-16.*

ROLAND, E.J. (1936). Morphogenesi dell'orecchio esterno. *Ric. Morf. 15:* 81-142.

SACCERDOTTI, C. (1900). Über das Knorpelfett. *Virchows Arch. (A) 159: 152-173.*

SANZONE, C.H.F. and REITH, E.J. (1977). The development of the elastic cartilage of the mouse pinna. *Am. J. Anat. 146:* 31-72.

SCHAFFER, J. (1930). Das Stützgewebe. In *Handbuch der mikroskopischen Anatomie des Menschen.* Bd.II/2 (Ed. W.v. Mollendorff). Springer Verlag, Berlin, pp 1-374.

SERAFINI-FRACASSINI, A. and SMITH, J.W. (1974). *The Structure and Function of Cartilage.* Churchill Livingstone, Edinburgh-London, pp.206-219.

SLAIS, J. (1957). Development and structure of the cartilage in the rat auricle. *Vest. Csl. Spol. Zool. 21:* 25-43.

SVAJGER, A. (1970). Chondrogenesis in the external ear of the rat. *Z. Anat. Entwickl.-gesch.131:* 236-242.

SVAJGER, A. (1971). Differentiation of rat auricular cartilage in organ culture. *Period. Biol. 73:* 39-43.

SVAJGER, A., KOSTOVIC-KNEZEVIC, L.J., BRADAMANTE, Z. and LEVAK-SVAJGER, B. (1979). Regeneration phenomena in rat auricular cartilage transplanted under kidney capsule after digestion of matrix with elastase and hyaluronidase. *Proc. Jugosl. Acad. Sci. Arts 383(18):* 363-375.

SVAJGER, A. and LEVAK-SVAJGER, B. (1971). Differentiation of rat auricular cartilage in the anterior chamber of the eye. *Period. Biol. 73.* 33-37.

SVAJGER, A. and LEVAK-SVAJGER, B. (1975a). Behaviour of the fetal, young and adult rat auricular cartilage in renal homograft. *Proc. Jugosl. Acad. Sci. Arts 371(17):* 113-119.

SVAJGER, A. and LEVAK-SVAJGER, B. (1975b). The failure of the rat embryonic ectoderm to induce phenotypic changes of the auricular cartilage. *Proc. Jugosl. Acad. Sci. Arts 371(17):* 107-112.

SVAJGER, A. and LEVAK-SVAJGER, B. (1976). Differentiation of the first branchial arch mesenchyme of the rat embryo in renal homografts and in organ culture. *Bull. Sci. Yugosl. (A) 21:* 139-140.

WEISS, P. and MOSCONA, A. (1958). Type-specific morphogenesis of cartilage developed from dissociated limb and scleral mesenchyme *in vitro*. *J. Embryol. Exp. Morphol. 6: 238-246.*

WHITTAKER, J.R. (1968). Translational competition as a possible basis of modulation in retinal pigment cell cultures. *J. Exp. Zool. 161:* 157-171.

WOOD, E.M. (1967). An ordered complex of filaments surrounding the lipid droplets in developing adipose cells. *Anat. Rec. 157:* 437-448.