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An embryological view of cartilage*

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Standard accounts of chondrogenesis state that cartilage develops from mesenchyme and the process is first recognizable histologically in embryos of about 5 weeks (10 mm crown–rump length). In areas where cartilage is developing the mesenchyme condenses, the cells proliferating and becoming rounded. Such cellular condensations, which lead to cartilage formation, are distinguishable from predominantly fibrous condensations, which lead to the subsequent formation of membrane bone (Gardner, 1963).

Although mesenchyme is predominantly of mesodermal origin, it should be realized from the outset that other origins are possible (Stone, 1929; Harrison, 1937; Horstadius, 1950). Thus ectoderm can give rise to this type of tissue, either directly or by way of the neural crest, and it is possible that, to a more limited extent, endoderm does also.

Differentiation of blastema

In primitive mesenchyme the cells are stellate or polymorphous and form a network, the meshes of which are filled with jelly-like, amorphous, intercellular substance.

Mesenchymal cells can differentiate in a number of ways, but when destined to become cartilage they form condensations which result from a number of changes in the morphology of the constituent cells. These cells either become more spindle-shaped or more rounded and the ratio of cytoplasmic to nuclear volume is reduced. There is also a great reduction in the amount of intercellular substance. Thus, in a sense, the mesenchymal cells appear to 'dedifferentiate' in the course of forming such condensations, which may now be referred to as precartilage blastemata.

The blastema cells then start secreting homogeneous hyaline intercellular substance containing fibrils of a similar refractive index to that of the hyaline binding substance, so that they are not apparent in routine histological preparations. The laying down of intercellular substance in developing cartilage is associated with the accumulation of sulphated mucoprotein, and this process has been studied by autoradiography of fetal tissues following the administration of sulphate labelled with radioactive sulphur to the mother (Boström & Odeblad, 1953; Curran & Kennedy, 1955).

So long as the intercellular substance remains reasonably pliable, the cartilage cells can divide and the daughter cells can move away from each other, so that the blastema can expand by interstitial growth.

The cells at the periphery of the blastema condense to form a bilaminar

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perichondrium whose outer layer becomes fibrous while the inner develops as a layer of closely packed, rounded or polymorphous cells – chondroblasts – which are responsible for appositional growth of the cartilage rudiment. However, during growth of a chondrogenic rudiment in the embryo, demarcation of the perichondrium from the already formed cartilage may be quite indefinite, there being often a relatively wide zone of gentle transition.

As cartilage develops and differentiates further, it assumes varying structural features and properties in different parts of the body. The characteristics of the three chief varieties of cartilage – hyaline, fibrous and elastic – are determined in the main by the composition of the fibres laid down in the intercellular substance.

The hyaline variety is found extensively in the embryo and fetus in the form of 'models' for most of the bones, in the larger laryngeal cartilages and in the cartilage of the air passages. Elastic cartilage develops in the pinna, the pharyngo-tympanic tube, the epiglottis and the smaller laryngeal cartilages. Fibrous cartilage develops at the attachment of ligaments and tendons to bone and in some intra-articular discs.

Characteristics of prenatal cartilage

Cartilage in prenatal life is distinguished from the postnatal variety in a number of ways.

The cells of embryonic and fetal cartilage are more closely packed, but are less regular in shape and arrangement, than those of postnatal cartilage. Attempts have been made recently to express in histochemical terms the differences between the cartilage of the tibial growth plate in prenatal life from that found in childhood (Stanescu, Stanescu & Maroteaux, 1973). Another characteristic of cartilage in prenatal life is that it may be well vascularized. A point of considerable interest in this respect is that the angiogenic factor, which Folkman (1974) claims to have isolated from tumours, appears to be inhibited by postnatal cartilage, but not by fetal cartilage.

Role in normal development

The cartilage which appears in the embryo and fetus may:

(*a*) develop into definitive structures, such as the cartilages of the nose, external ear, pharyngo-tympanic tube, larynx, trachea and lungs;

(*b*) form ligaments like the anterior ligament of the malleus, the sphenomandibular ligament and the stylohyoid ligament;

(*c*) provide a temporary, plastic framework for bone development.

(i) *Visceral skeleton*

The role of cartilage in the development of most bones throughout the body needs no emphasizing. Parts of the cartilages of the first two branchial arches form models for middle ear ossicles, while the mesenchyme around the derivatives of the otic vesicle condenses and chondrifies to form the otic capsule which becomes incorporated in the petrous temporal bone (Bast & Anson, 1949; O'Rahilly, 1963; Andersen, Matthiessen & Jørgensen, 1969). The second arch cartilage also forms the basis of the styloid process, and the lesser cornu and upper part of the body of the hyoid bone; while the lower part of the body and greater cornu of the hyoid are ossified from the ventral portions of the third arch cartilage.

(ii) *Skull*

The cartilaginous basal region of the developing skull is mostly replaced by bone, but cartilage persists for some years at the spheno-occipital joint. Although most of the cartilaginous elements of the neurocranium develops from mesenchymal condensations that arise *in situ* in the outer layer of the ectomeninx, there is evidence that the trabeculae cranii, rostral to the cranial tip of the notochord, may have come from branchial arch mesoderm and so originally from neural crest (De Beer, 1937; Horstadius, 1950).

(iii) *Appendicular skeleton*

When it comes to considering the role of cartilage in the development of the post-cranial skeleton, it should be noted that the pre-cartilage blastema for the appendicular skeleton is established first in the region of the girdles, and then the process of blastema formation proceeds proximo-distally. Differentiation in the upper limb is earlier than in the lower limb and, in general, larger elements chondrify and ossify before smaller ones. However, the process of endochondral ossification is too well known to merit much comment here. Suffice it to say that cartilaginous models of the bones are laid down and, in the long bones at any rate, ossification has started by the eighth week, when the fetus measures about 30 mm from its crown to its rump. The process of growth of cartilage and its replacement by bone proceeds until, at birth, a long bone appears as a bony shaft with cartilaginous extremities. These extremities (with two exceptions) become ossified after birth, starting with the appearance within them of secondary epiphyseal centres of ossification. The cartilage between the epiphyseal ossification and the bony shaft is the epiphyseal plate, and the cartilage at the extreme end of a bone is the articular cartilage.

(iv) *Post-cranial axial skeleton*

The rather special developmental features of the post-cranial axial skeleton are worthy of some note. The sclerotomic condensations of pre-cartilage around the notochord show evidence of their segmental origin and each condensation has a cranial less condensed portion and a caudal more condensed part. The condensed part becomes level with the centre of each paired myotome and mostly differentiates into intervertebral disc material. The most caudal part of the preceding and the cranial part of the succeeding sclerotome fuse to form the pre-cartilaginous vertebral body (Prader, 1947*a*, *b*; Tonbury, 1958).

More dorsally and laterally the less condensed portions of the sclerotomes form the pre-cartilage of the neural arches and transverse processes, whereas the dense parts form the intervertebral ligaments.

Nature of the notochord

The development of the axial skeleton raises the question of the possible relation of the notochord to cartilage. In the lowest chordates the sole structure which deserves to be called a skeleton is the notochord and its sheath (Balfour, 1881), and these structures constitute, in all vertebrates for a considerable period of their early embryonic life, the sole representative of the axial skeleton.

The notochord is at first a cellular rod, and the superficial cells form a delicate sheath which soon thickens to become a well-developed structure. The cells of the notochord vacuolate extensively and so come to constitute a meshwork of protoplasm surrounding vacuoles. In those species in which the notochord persists into adult life, the meshwork becomes highly complicated and is filled with gelatinous material, not all of which is intracellular. In such species the notochordal sheath becomes very thick: indeed, in elasmobranchs, at one stage, the sheath forms a definite unsegmented cartilaginous tube. The notochordal sheath is possibly the basis of the centra of the future vertebrae.

Secondary cartilage

A feature of note in the embryology of cartilage is the occurrence of 'secondary cartilage'. This is seen in membrane bones, especially the mandible and clavicle. In the former the ramus 'membrane' is partly transformed into cartilage before ossification takes place (De Beer, 1937). A similar transformation occurs in the clavicle, chondrogenesis occurring at either end of the bone (Koch, 1960; Andersen, 1963). The full significance of this secondary cartilage is not understood, but the fact that it occurs emphasizes the similarity of the developmental mechanisms in 'membrane' and 'cartilage' bones.

Cartilage and the development of joints

The role of cartilage in the development of joints (Haines, 1947; Barnett, Davies & MacConaill, 1961) may be summarized as follows:

The contiguous ends of bone primordia are connected at first by less differentiated mesenchyme. If this mesenchymal tissue becomes chondrified, as for example between the first rib and the sternum, a synchondrosis is established. In the case of the diarthroses between 'cartilage' bones and between the cartilaginous portions of 'membrane' bones, there is no indication of the site of the future synovial joint until after the differentiation of the cartilaginous models of the constituent bones.

Once the cartilaginous models are laid down the mesenchyme between adjacent ends become arranged to form an 'interzone'. In the centre of these interzones the cells become flattened, while at the periphery they are continuous with the perichondrium of the cartilaginous models. As the cartilaginous models grow, the central part of an interzone becomes compressed, while one or more cavities appear in the circumferential part. The compressed central cells soon disappear, so that the cartilaginous ends of the bones come into contact and there is a distinct joint cavity. The immediate lining layer of the joint cavity is the synovial membrane. The mesenchymal tissue around the developing joint forms the capsule and this is continuous with the perichondrium of the constituent cartilage models. The cells lining the articular surfaces and capsule form a flattened epithelial layer – the synovial mesothelium; later, probably as a result of joint movement, these mesothelial cells disappear from the articular surfaces exposing the cartilage.

Some diathroses develop intra-articular fibrocartilages from portions of the capsular sleeve and adjacent perichondrium. As the synovial cavity forms, it extends over the surfaces of such accessory cartilages.

Developmental pathology

The borderland between the embryology and the pathology of cartilage highlights the diversity of the developmental role of the tissue.

Heterotopic cartilage may occur in the form of nodules in the walls of branchial cysts or fistulae, or as islands of cartilage in the neck or around the tonsil, and they may be considered to be of branchial arch origin (see Willis, 1962). 'Cervical auricles', small tags of skin containing cartilage, found on the side of the neck are also encountered and may be considered to represent displaced auditory hillocks.

Developmental abnormalities of cartilage may have a genetic basis, as in diaphyseal aclasis and multiple exostoses, a substantial proportion of the cases being attributable to the transmission of a dominant gene. By contrast, dyschondroplasia (multiple enchondroses; Ollier's disease) is said not to be determined genetically (see Willis, 1962, for discussion). Achondroplasia can be transmitted as an autosomal dominant, but most cases appear to be due to a fresh mutation.

Intrinsic and extrinsic factors in chondrogenesis

The evaluation of the relative roles of intrinsic and extrinsic factors in chondrogenesis is greatly aided by grafting experiments (Willis, 1936; Fell & Grüneberg, 1939) and by the use of organ culture techniques (Fell & Robison, 1929; Fell, 1939; Jacobson & Fell, 1941; Chen, 1952).

The cells which form the cartilaginous models of bones appear to be determined very early on in development. This has led to the concept of the limb bud as a self-differentiating mosaic of mesenchyme, some parts of which are destined to become chondrified. This mosaic pattern is thought to be imposed on the limb bud by organizing influences which are under genetic control, and is not the result of local mechanical factors. Dissociation of the cells of the chondrogenic blastema of early chick limb buds by tryptic digestion of the intercellular matrix (Moscona & Moscona, 1952) is followed by reorganization and differentiation into typical skeletal elements when the blastema cells are allowed to reaggregate in culture. Another example of early determination and independence from extrinsic mechanical factors is seen in organ culture of mouse body wall, in which it has been shown that the sternal bars develop independently of the ribs and that during culture these bars can move towards and fuse with each other (Chen, 1952).

The development of form in precartilage blastemata, foreshadowing the characteristic shapes of the cartilaginous models of the definitive bones, is thus considered to be determined very early in development and to be the result of intrinsic and not of extrinsic influences acting on the developing skeleton. Extrinsic influences are considered to be more concerned with the later-appearing details of skeletal shape. In early stages of development extrinsic factors appear to be important only in providing suitable conditions in which the intrinsic factors can act adequately to produce skeletal form. In the later stages of development the importance of extrinsic factors increases, and there is evidence that skeletal muscle contraction and movement are essential for primary joint cavity formation, and for the establishment of the normal shape of the articular surfaces (Sissons, 1956; Drachman & Sokoloff, 1966).

Criteria of differentiation

Studies of regeneration blastema cells suggest that cartilage, once differentiated, is far more stable in its differentiation than some other tissues, and chondrocytes are not thought to 'dedifferentiate' into pluripotent cells (for discussion see Burgess, 1974).

However, the consideration of the process of differentiation of a mesenchymal cell into a chondrocyte leads one inevitably to the question, 'When and what is cartilage?' To base the answers on morphological criteria alone would now seem inadequate. It has been postulated convincingly (Levitt & Dorfman, 1974) that improved knowledge of the biochemistry of cartilage has permitted the formulation of a definition based on chemical criteria. Thus, it seems reasonable to define a chondrocyte as a cell that synthesizes large amounts of chondroitin sulphate proteoglycan (the structure of whose protein core is not yet known) and produces collagen of a unique structure and composition – $[\alpha 1(\mathrm{II})]_3$.* It was the discovery of $\alpha 1(\mathrm{II})$ chains in chick cartilage (Miller & Matukas, 1969) which furnished a biochemical marker for the differentiation of cartilage cells.

The combination of biochemical analysis with the study of chondrogenesis and behaviour of chondrocytes in culture systems (see Levitt & Dorfman, 1974) continues to illuminate the distinction between those factors which promote the process of differentiation of precursor cells to differentiated chondrocytes, and those factors that facilitate the expression of a differentiated characteristic.

Chondrogenesis in culture is dependent on suitable culture conditions, and it is of considerable interest (particularly in view of what was said above about the notochord) that a number of studies suggest that matrix materials secreted by the spinal cord and the notochord may be a requisite for the differentiation of cartilage from somite mesenchyme.

Differentiation of cartilage from mesenchyme elsewhere makes it difficult to postulate a specific cartilage-inducer role for spinal cord and notochord, but the matrix materials which these organs produce may act as promoters or stabilizers of chondrogenesis. It should also be realized that limb-bud tissue possesses several enzymes necessary for the synthesis of chondroitin sulphate, and this type of tissue actually does synthesize small amounts of sulphated glycosaminoglycan prior to the morphological appearance of cartilage.

It has been established that exposure of cultured chondrocytes to 5-bromo-2′-desoxyuridine–(BUdR)–reduces their ability to produce cartilage matrix (see Levitt & Dorfman, 1974). On the evidence available it seems probable that BUdR affects the cells' ability to synthesize the core protein of proteoglycan. In the undifferentiated mesenchymal cell it inhibits the acquisition of an ability to synthesize the protein,

* The fundamental structural unit of collagen – tropocollagen – consists of three polypeptide chains which are helically coiled and associated in a triple helix. Cross-linking occurs between chains and between molecules. The amino acid sequence of specific chains is becoming known in detail and it has been found that a number of different individual chains exist in different species and tissues. Also, carbohydrate side chains are present in varying amounts in collagen chains; glycosylation varies in different collagens. Individual chains are referred to as α, two chains cross-linked as β, and three chains cross-linked as γ. Chains are designated as $\alpha 1$ and $\alpha 2$. Because a variety of different $\alpha 1$ chains are now known, these are designated as $\alpha 1(\mathrm{I})$, $\alpha 1(\mathrm{II})$, $\alpha 1(\mathrm{III})$. Thus the tropocollagen molecule of calf skin is designated as $[\alpha 1(\mathrm{I})]_2\alpha 2$, whereas that of chick cartilage is $[\alpha 1(\mathrm{II})]_3$.

whereas in the differentiated chondrocyte the inhibition is transient and incomplete. BUdR treatment of undifferentiated mesenchyme cells also prevents them subsequently expressing the cartilage phenotype as regards collagen synthesis.

Clearly there exist, in somites as well as in limb buds, mesenchymal cells that differ from those destined to become chondrocytes. It has been established, using *in vitro* methods, that such non-chondrogenic mesenchymal cells from limb buds show poorly developed endoplasmic reticulum and Golgi apparatus, and produce scanty extracellular material; the collagen synthesized by such cells is of the $(\alpha 1)_2\alpha 2$ type; the level of chondroitin sulphate synthesis is low, and the addition of xylose to the culture results in negligible stimulation of chondroitin sulphate synthesis. The progeny of BUdR-treated presumptive chondrogenic cells (which do not differentiate to cartilage because of the BUdR treatment) produce the same type of collagen as do the non-chondrogenic mesenchymal cells. They also produce a low level of chondroitin sulphate proteoglycan, *but* they do contain a well-developed endoplasmic reticulum and are markedly stimulated by xylose. Thus perhaps they have undergone some degree of differentiation, despite the inhibiting effect of the uridine analogue.

It would seem that, in the case of developing cartilage, we now have two identifying biochemical markers – chondroitin sulphate proteoglycan and a tropocollagen molecule designated as $[\alpha 1(II)]_3$ – and these chemical parameters appear to correlate well with the morphological and staining characteristics utilized to identify cartilage. The sequence of events that results in the transition of a mesenchymal cell to a chondrocyte is still not understood, but it may be hoped that studies such as those mentioned will begin to elucidate this complex problem at the molecular level.

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