Fibrillogenesis and Periodicity in the Chick Embryo*

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The histology of light microscopy recognizes three separate and distinct types of extracellular connective tissue fibers; collagen, elastic and reticular. Collagen fibers are the most widely distributed of the three. They consist of wavy, inelastic elements of the order of 1-12 μ thick. They form the substance of tendons, aponeuroses, and ligaments. They also contribute substantially to cartilage and bone. Elastic fibers are small branching cylinders or flat fenestrated plates that contain elastin. They may be stained selectively by special dyes, such as Weigert's elastin stain. Their diameters or thicknesses are usually measurable in micra or fractions thereof. The smallest are near (or beyond) the limit of resolution of the light microscope (0.2 μ). They are somewhat generally distributed but form the bulk of the ligamentum nuchae. This ligament, in the back of the neck, supports the heavy head in cattle, etc. The third fiber type, reticular fibers, are distinguished by their argyrophilia. They become black after treatment by silver-containing stains. Their distribution is general throughout the tissue space. They are highly concentrated in the lymphoid organs where they contribute a pliable endoskeleton.

The electron microscope shows that collagen fibers are made up of subunits, now called unit collagen fibrils. These are cylinders whose diameter varies from about 150 Å to more than 1000 Å. Their most characteristic feature is an axial periodicity (periodic banding of light and dark lines) with a repeating unit of about 640 Å. Unit collagen fibrils are a complex fibrous protein known to be composed of tropocollagen molecules (2,600-3,000 Å long, 14-15 Å thick). Elastic fibers have a homogeneous core of variable density in electron microscopy. Their margins are very dense and are formed by matted fine filaments called microfibrils. Elsewhere microfibrils are found free in the tissue space, with diameters varying from 40 to 160 Å. In this situation they have no counterpart in light microscopy. Although sometimes beady in appearance, microfibrils lack periodicity. They branch occasionally and are hollow when seen in profile. They

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are frequently found in association with boundary (basement) membranes. In fine structure the latter separate epithelium, muscle, nerve and fat from the connective tissues⁴).

The chick embryo at the time of laying possesses an epiblast (anticipating an epithelium) and a hypoblast which consists largely of scattered cells more ventrally located⁵). Extracellular formed elements are already present. These form an intermittent boundary membrane along the basal surface of the epiblast. This structure becomes continuous during the first half-day of incubation. In this time interval a space becomes partitioned off by forward growth of entoderm. This space, which will constitute the part of the adult body known as the tissue space, rests on entoderm below. Above it is limited by the ectodermal boundary membrane along the epiblast. The mesodermal derivatives of the organism, including the cells and extracellular fibers of the connective tissues, will develop within this space. Thus, this area is primarily associated with the connective tissues. Because of its position between and among the various non-connective tissues, it is sometimes known as the interstitium. It was called the milieu interne by Claude Bernard.

Primary fibrils can be demonstrated in the intermittent boundary membrane on the basal surface of the epiblast before incubation has begun (Fig. 1)⁶). These are about 40 to 45 Å in thickness but may be even thinner. On the

Fig. 1. Primary fibrils appear in this face-on view of a boundary membrane. It is bridging a gap between the basal corners of two cells of the epiblast. Chick embryo, 0 hrs. incubation, 130,000 ×.
surface of the boundary membrane toward the interstitium they assume the dimensions and general morphology of microfibrils (Fig. 2). At about the end of the first day of incubation microfibrils free in the tissue space begin to appear. They become increasingly numerous in the first half of the second day. They are generally distributed throughout the tissue space at 36 hours but their strongest concentration is around the notochord (Fig. 3). At this stage close association of microfibrils with the free cells of the tissue space is not conspicuous. Microfibrils are not found in the interstices between cells of the somites. The lumens of gradually forming blood vessels are likewise free of them. Some may intermingle with boundary membranes (Fig. 2). Granular debris is often associated with them. No periodicity is present although a beady appearance is sometimes noticeable. They exist singly rather than in groups and their directional placement seems to be haphazard.

Unit collagen fibrils begin to appear in the tissue space during the latter half of the second day. The earliest and smallest of these are about the size of large microfibrils. However, each fibril possesses constant diameter along its length with dark margins and a lucid core. A satisfactory criterion for distinguishing between microfibrils and unit collagen fibrils is axial periodicity in the latter. This develops slowly. At 48 hours incubation it is barely demonstrable in most of the fibrils present and is only slightly better developed at 72 hours. The regular

Fig. 2  This oblique view of a boundary membrane shows fine primary fibrils in its depths with coarser microfibrils near its surface. Chick embryo, 30 hrs. incubation, 97,000 ×.
Fig. 3  These unit collagen fibrils are still undergoing growth. Chick embryo, 7 days incubation, 55,000 x.

Fig. 4  A heavy concentration of extracellular fibrils is found at the edge of the notochord. The morphology of this area suggests very rapid growth. Chick embryo, 54 hrs. incubation, 34,000 x.
Fig. 5 A free cell of the mesenchyme, still largely undifferentiated, gives rise to microfibrils. Chick embryo, 72 hrs. incubation, 58,000 x.

Fig. 6 A cell that appears to be well on its way to becoming a fibroblast gives rise to fibrils that show primitive periodicity at high magnifications. These fibrils will develop structure similar to those of Fig. 3. Chick embryo, 72 hrs. incubation, 23,000 x.
periodicity associated with adult fibrils becomes evident at about 90 hours. However macroperiods and microperiods seem to undergo developmental changes for at least one week (Fig. 3).

Specific cells are physically associated with fibrillogenesis at various stages of embryonic development. The cells of the epiblast are in close proximity to the primary fibrils from the start of incubation. When microfibrils begin to appear free in the tissue space (second day) their highest concentrations are near the margin of the central nervous system and around the notochord (Fig. 4). It is not until the third day and thenceforward that extracellular fibrils are conspicuously associated with cells that are free in the tissue space (Figs. 5, 6).

Fibrillar aggregates in the early embryo provide subunits for the fiber types of light microscopy. Collagen fibers are simple aggregates of unit collagen fibrils. Reticular fibers, however, do not emerge as a separate structural entity at the electron microscopic level. The surface argyrophilia that defines reticular fibrils in light microscopy may enclose any of the extracellular formed elements of the tissue space. There is marked topographical variability. In the alveolar wall of the lung, for example, reticular fibers consist of small bundles of unit collagen fibrils. In the lymph nodes sleeves of tissue space form reticular fibers. These are lined by intermittent boundary membranes. On the inside scattered unit collagen and even small elastic fibers extend through them. Elastic fibers in light microscopy correspond to the homogeneous matrix seen in the electron microscope. The microfibrils on the periphery of elastic fibers in electron microscopy are individually too small to be visualized by the light microscope. Primary fibrils and unit collagen fibrils are also too small to be visualized by light microscopy wherever they stand alone.

The evidence provided by the chick embryo encourages a unitary concept of extracellular fibrillar components. A single developmental line of fibrils accounts for all known fibrillar aggregates in both light and electron microscopy. The main stem of embryonic development is derived from primary fibrils in boundary membranes. Primary fibrils give rise to microfibrils in the tissue space. The latter can transform to unit collagen fibrils. This line of development that unfolds during the first week of incubation in the chick is essentially a recapitulation of evolitional events. It may reasonably serve as a prototype for the interpretation of all extracellular fibrous materials, wherever encountered.

REFERENCES

DISCUSSION

Dr. Mitio Niizima

I would like to know your opinion about the origin of the boundary membrane you showed in your slides.

Dr. Frank N. Low

A dual origin is suggested by the first few days of development in the chick. The earliest boundary membranes develop on the basal surface of the epiblast (future ectoderm) and seem to come from it. During the second day entodermal and some mesodermal boundary membranes develop. They are always closely applied to a parent cell without proximity of differentiating fibroblasts. These events suggest origin from the cell with which the membranes are most closely associated. However, masses of what appear to be formless cytoplasm without enclosing plasmalemma (interstitial bodies) are present in the tissue space. These appear to contribute to the amorphous component of boundary membranes. They are probably derived from free mesenchymal cells. A similar dual origin for the coarser “basement lamella” of collagen has been indicated by Hay and Revel in an autoradiographic study of regenerating newt limb (Devel. Biol., 7(1963), 152).