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17241. Observations on the Formation of Connective Tissue Fibers.*

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Some information on the origin and development of collagenous fibers has been obtained from the study of cultured tissues[1-4] and wound healing.[5] In such material fine fibrils can be observed to develop between the cells. These subsequently increase in number and finally coalesce to form larger fibers. It is generally thought that the fibrils are organized from an intercellular protein that is produced by or at least influenced by the surrounding cells, but the precise mechanism is not understood.

One approach to the examination of this problem is by way of direct observations on the early submicroscopic sequences involved in fiber formation. To that end we have utilized the combined methods of electron microscopy and tissue culture.

The material studied has included fibrous arrays formed *in vitro* in association with explants of chick embryo skin and foregut, rabbit thymus and rat pericardium.‡ In preparation for microscopy the cultures containing these arrays were washed in a balanced salt solution, fixed briefly over vapors of OsO_4 and thereafter mounted on electron microscope screens by the same technics that are used for cells.[6]

Only areas of the culture in which the plasma clot had been completely lysed were suitable for microscopy. In such areas the presence or absence of connective tissue fibers could be determined with the light microscope. Generally the cell population was sparse. Some units appeared as macrophages and fibrocytes and were spread out quite thinly on the coverglass whereas others of unknown nature remained rounded up and were dispersed over the surface of the fiber mat.

As was more of less expected, it was found that these fibrous mats contain a great many more fibers than are apparent with the light microscope. In fact, the predominant type, hereinafter referred to as unit fibers, has a diameter usually less than 500 Å (Fig. 1 to 5). They are long slender strands which, over the

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1 Maximow, A., *Centr. allg. Path.*, 1929, **43**, 145.

2 McKinney, R. L., *Arch. exp. Zellforsch.*, 1929, **9**, 14.

3 Momigliano-Levi, G., *Z. Zellforsch.*, 1932, **16**, 389.

4 Bloom, W., and Santstrom, R. H., *Anat. Rec.*, 1935, **64**, 1.

5 Stearns, M. L., *Am. J. Anat.*, 1940, **67**, 55.

‡ All micrographs used for illustrations are of fibers that had developed from explants of chick embryo skin after 9 days in culture.

6 Porter, K. R., Claude, A., and Fullam, E., *J. Exp. Med.*, 1945, **81**, 233.

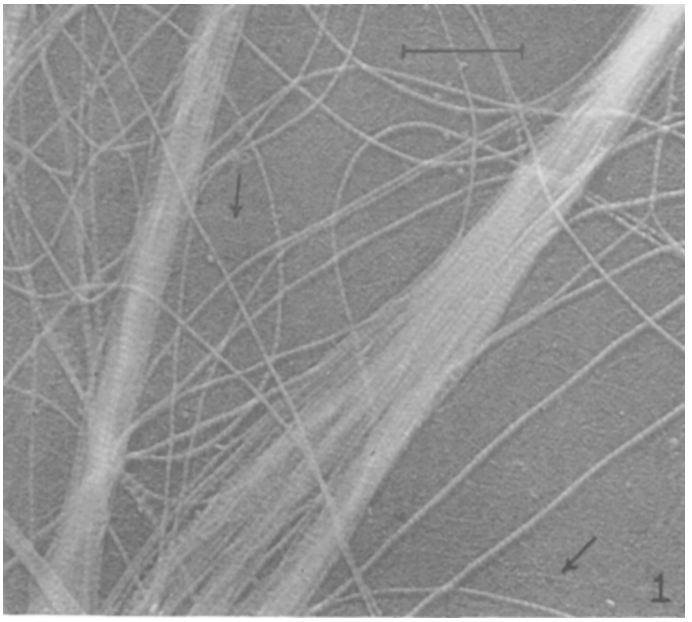

FIG. 1.
Micrograph showing numerous unit fibers and two large collagen-like strands made up of unit fibers. Beaded protofibrils are present in the background and indicated by arrows. Specimen lightly shadowed with gold, 12° angle. Mag. 15,734.

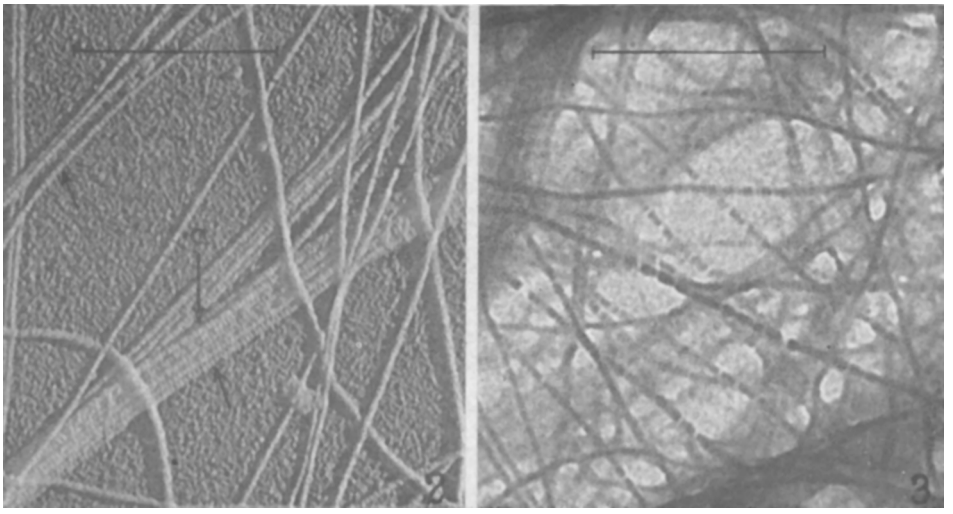

FIG. 2. Micrographs of gold-shadowed preparation showing banded structure of unit fibers (arrows). The periodicity is approximately 270 Å. At (a) there is some evidence of the development of the larger 640 Å period of collagen. Mag. 26,900.

FIG. 3. Micrograph of unit fibers in which the bands or striae are grouped in three's to form the repeating axial pattern of collagen. The intra-period bands are observed to be unequal in size and density. Mag. 30,500.

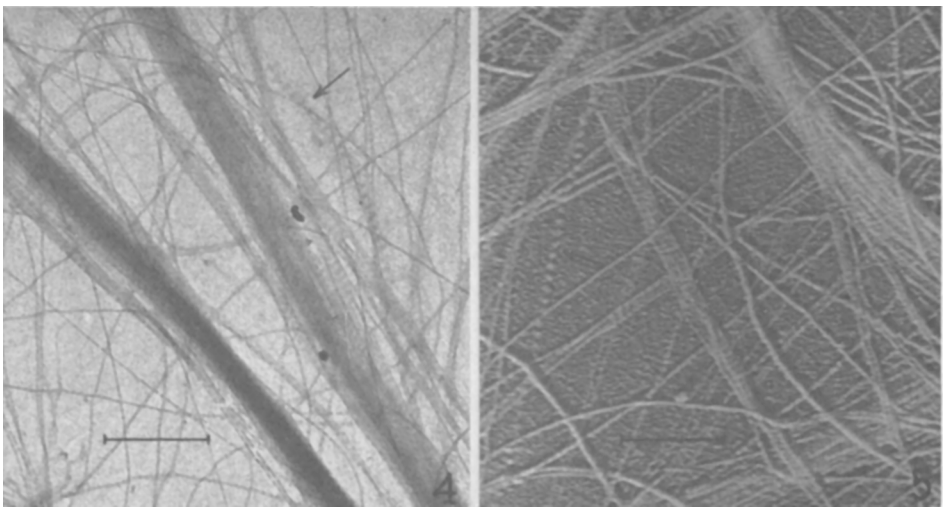

FIG. 4. Micrograph showing large-period component (arrow) of preparations organized as a fiber with densities spaced uniformly at approximately 100 Å. Mag. 13,700.
FIG. 5. Micrograph of similar material (arrow) shadowed with gold, 12° angle. Mag. 14,000.

entire width of a single field of a micrograph (12 μ), may not vary appreciably in width. At their ends they taper off gradually into slender threads 50 Å or less in diameter. The unit fibers are all striated or banded, but there is some variation in the organization of the striae or bands. In many fibers, and more especially in those of small diameter, the striae are of uniform size and evenly spaced at approximately 270 Å (Fig. 2). In other unit fibers, which are the larger and presumably more completely formed, the striae are compressed into groups of 3 which have an over-all length of 650 to 800 Å (Fig. 3). When thus grouped, the bands show some differences, 2 appearing larger and denser than the third. The pattern of these bands is repeated in each group or major period along the fiber axis so that not only each period but the whole fiber is polarized (Fig. 2 and 3).

The larger strands of the preparations—those that can be seen with the light microscope—are compound in structure (Fig. 1 to 5). They are composed of varying numbers of unit fibers, and hence, vary in diameter. The size most frequently encountered in these preparations is 0.5 μ or less. Usually these strands, when not stretched, show the characteristic periodicity of collagen[7,8] (640 Å) with striations extending across a part or all of the bundle. The typical collagen-like striation of these compound strands shows 2 prominent intra-period striae and one less prominent, in each repeating pattern. The more dense striae correspond to the double elevation apparent in the image of the metal-shadowed, dried fiber.

In part of the preparations where areas of the supporting plastic membrane are exposed, a fine fibrous component is frequently encountered. This consists of very slender filaments or protofibrils, 50 to 100 Å in diameter, which are similar to the ultimate tapered ends of the unit fibers. They are usually beaded. The distance between the bead varies considerably, but not infrequently it is about 270 Å, giving them a periodicity similar to that in small unit fibers. Presumably these protofibrils represent the primary association of the collagen macromolecules.

Neither these fine filaments nor the larger unit fibers have been observed in the material examined to arise as formed structures from cells.

There is a fourth component of these preparations that appears as aggregations of even-

[7] Schmitt, F. O., Hall, C. E., and Jakus, M., *J. Cell. and Comp. Physiol.*, 1942, **20**, 11.

[8] Gross, J., and Schmitt, F. O., *J. Exp. Med.*, 1948, **88**, 555.

ly-spaced parallel bands or densities. The organization of these is usually in the form of a fiber (Fig. 4 and 5), but occasional patches of irregular outline have been observed. The periodicity displayed measures as a rule between 1000 to 1100 Å. The densities in shadowed preparations appear as prominent elevations and there may be extremely little if any material connecting them. There is no evidence of intra-period banding. In most of the collagen preparations examined, this component has been found scattered rather sparsely among the fibers, but it has also been observed without associated fibers. We are therefore unable to decide whether it is related in any way to collagen formation, but are inclined to the view that it represents a component of the connective tissue ground substance.

The available evidence indicates that the major fibrous components of these preparations, exclusive of the last, are collagen. The periodicity of the large compound fibers is like that of collagen, the fibers resist tryptic digestion, and staining technics have identified collagen fibers in cultures paralleling these used for electron microscopy.

The sequence of events leading to mature fiber formation, while not directly observable, can be reasonably reconstructed from the character and associations of the various components of any fibrous array. Thus the relation of the unit fibers to the larger strands is clear; they are obviously the component units and appear to have come together into these parallel arrays much the same as unit fibers of fibrin aggregate to produce the larger strands of the formed clot.[9] But the origin of the unit fibers is less evident. They are apparently not spun off the cells. Instead, it is probable that they are formed, as in fibrin, through a lateral association of several protofibrils and a progressive deposition of molecular collagen on their surfaces.[10] Since the more slender unit fibers show a fine even periodicity, it appears that the larger collagen pattern of the mature fiber is a secondary development. These and other phenomena of connective tissue fiber formation will be considered more completely in a later report.

[9] Hawn, C. V. Z., and Porter, K. R., *J. Exp. Med.*, 1947, **86**, 285.

[10] Porter, K. R., and Hawn, C. V. Z., *J. Exp. Med.*, 1949, **90**, 225.

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17242. The Cellular Transfer of Cutaneous Hypersensitivity to Tuberculin in Man.

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It has been demonstrated by Chase[1] that transfer of specific cutaneous hypersensitivity of the "delayed type" to tuberculin can be accomplished in unsensitized guinea pigs by the injection of leucocytes isolated from peritoneal exudates produced in sensitized guinea pigs.

It is the purpose of this report to describe the transfer of cutaneous hypersensitivity to tuberculin in man by the intradermal injection of viable leucocytes isolated from the peripheral blood of non-tuberculous humans.

Materials and Methods. Utilizing the method of Minor and Burnett[2] it has been possible to isolate and concentrate viable leucocytes from human blood by the addition of bovine fibrinogen, Fraction I (Armour), which accelerates the rate of erythrocyte sedimentation leaving a plasma suspension of leucocytes in the supernatant portion.

[1] Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 134.

[2] Minor, A. H., and Burnett, L., *J. Hematology*, 1948, **7**, 799.