OBSERVATIONS ON FIBRILLOGENESIS IN THE CONNECTIVE TISSUE OF THE CHICK EMBRYO WITH THE AID OF SILVER IMPREGNATION*

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Plates 20 and 21

Silver impregnation of collagenous microfibrils has been applied in electron microscopy in recent years by von Herrath and Dettmer (3), Dettmer, Neckel, and Ruska (1), Dettmer (2), Schwarz (5), Tomlin (6), and Irving and Tomlin (4). The main purpose of these authors was to determine whether the silver-impregnation picture of the so called reticulin fibers differed from that of the collagenous fibers, and how it might change with the increasing width of the microfibrils. For this purpose the impregnation of microfibrils which were isolated from the tissues was a satisfactory technique, and it provided important information about the mechanism of silver impregnation. However, it seemed to be desirable to obtain in electron microscopy the same advantages of silver staining as in the light microscope, that is, to combine the impregnation with ultrathin sectioning.

Methods and Materials

Connective tissue was taken from the legs of 5- to 20-day chick embryos at the region where the flexor tendons of the foot were developing. In this way, one obtains both loose connective tissue as seen in Fig. 2 and embryonic tendon as seen in Fig. 1. Small pieces of tissue were fixed in neutral formalin or in buffered osmic acid (pH 8). After washing, the tissues were transferred in bulk to the different solutions prescribed in Gömöri's modification of the Bielschowsky technique. After dehydration, they were embedded in methacrylate. Sectioning was done with the Porter-Blum microtome. The sections were studied with an RCA model EMU electron microscope without the removal of the plastic. The electron micrographs presented in this paper will show that in this way very satisfactory impregnation can be produced. However, the result was sometimes spoiled by the precipitation of rather coarse silver particles upon the fibrils, or by a precipitation of fine particles all over the tissue. Occasionally complete failure of the impregnation occurred. Up to now failure has happened most frequently after osmic fixation. Further experimentation with the method is needed in order to decide upon the optimal time for the different steps of the procedure, and to determine optimal conditions of temperature, pH, and other factors.

The Gömöri technique was also applied to frozen sections, about 30 μ thick, of the same formalin-fixed material, which were then embedded in methacrylate. The impregnation of the microfibrils in ultrathin sections from this material was found to be very satisfactory, but the

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tissue as a whole was too much damaged by freezing and thawing to be useful for electron microscopy. The results were similarly unsatisfactory when silver-impregnated paraffin sections were re-embedded in methacrylate and sectioned for electron microscopy.

OBSERVATIONS

Fig. 1 shows part of a longitudinal section of a tendon from a 12-day old chick embryo. Bundles of microfibrils are situated alongside the fibroblasts corresponding to the argyrophil fibers as seen in light microscopy. All microfibrils in the bundles are marked by a serial alignment of silver particles. These particles, which have diameters ranging from about 100 to about 200 A, are rounded, but of irregular shape; apparently they are not so regularly aligned along the axis of the fibrils as to reflect a periodic structure which may be present in these early fibrils. Single, very fine fibrils are frequently observed at the edges of the bundles and are often found very close to the nuclear membrane. In well impregnated specimens as seen in Fig. 1, no silver granules are to be found outside the fibrillar bundles.

Fig. 2 represents fibroblasts of the loose embryonic connective tissue. Except for a narrow continuous layer surrounding the nucleus, the cytoplasm of these fibroblasts is seen as sections of interconnected strands which often are continuous from one cell to another. Some of the microfibrils are observed in their longitudinal course, others which cross the plane of sectioning are cut short, and some bundles are cut transversely (arrows). It is of special interest that, as a rule, the fibrils follow the cytoplasmic strands; the bundles are often seen to divide with the branches of the cytoplasmic strands or to match their curvatures.

The relationship between the fibrils and the cytoplasm can be demonstrated most clearly when, as in Fig. 3, the fibers are cut nearly, and in some instances precisely, at right angles to the axis. One finds the cross-sections of the microfibrils embedded in a material which is continuous with the cytoplasmic strands and of the same character with respect to density.

DISCUSSION

The electron microscopy of ultrathin sections of silver-impregnated embryonic connective tissue demonstrates that the silver staining technique is both selective and complete: (*a*) no tissue structure other than the argyrophil fiber collected silver particles; (*b*) the blackening of the fiber results from the complete silvering of all the microfibrils composing it. In cross-sections of fibers (Fig. 4) one finds the individual microfibrils marked by a group of silver particles which can often be seen to surround an unstained area, sometimes in the form of a ring. We agree therefore with the authors mentioned previously that the silver particles aggregate at the surface of the microfibrils in the material in which they are embedded.

With respect to the relationship of fibers and cells, longitudinal sections such as the one shown in Fig. 1 do not afford a basis for a decision as to whether the fibers are situated inside or outside the border of the cells. The cross-sections in Figs. 3 and 4, however, suggest that the fibers are, at that particular stage of development, enclosed in the ramified body of the fibroblast. The material surrounding the microfibrils is not noticeably different from that in the vicinity of the nucleus; however, the matrix of the fibrils and the rest of the cytoplasm are unlikely to be identical in their physico-chemical qualities. The fact that the silver particles precipitate within the fibers but nowhere else in the cytoplasm seems to indicate a differentiation of the cytoplasm at the sites of fibrillogenesis.

The observations reported in this paper are very similar to those made by Wassermann (7) on the early fibrillogenesis in the regenerating rat tendon. A systematic study of the formation of fibrils in the connective tissue of the chick with aid of the silver-impregnation technique is needed for further discussion of fibrillogenesis as far as structural changes are concerned.

SUMMARY

A technique is described which combines silver impregnation and ultrathin sectioning for the electron microscopic demonstration of fibrils in the connective tissue of the chick embryo. The electron micrographs presented in this paper provide evidence for the specificity and completeness of the silver-impregnation technique. It has been shown that, in this particular tissue after fixation in neutral formalin and at the stage of development represented by our material, the argyrophil fibers are embedded in a material which is continuous with the body of the fibroblasts.

Addendum.—While this paper was in press a study on "The morphogenesis of avian tendon" by Sylvia Fitton-Jackson appeared in *Proc. Roy. Soc. London, Series B*, 1956, **144,** 556–572. The author using the same material as we, could show "that some small groups of fibrils are included in an area that is indisputably cytoplasmic." Fig. 8 of Jackson's paper to which this statement refers corresponds to our Figs. 3 and 4. Since Jackson's tissue was fixed in buffered osmic acid, her findings support strongly our statement based on formalin-fixed material, that the fibrils are embedded in a material that is continuous with the cytoplasm of the fibroblasts.

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EXPLANATION OF PLATES

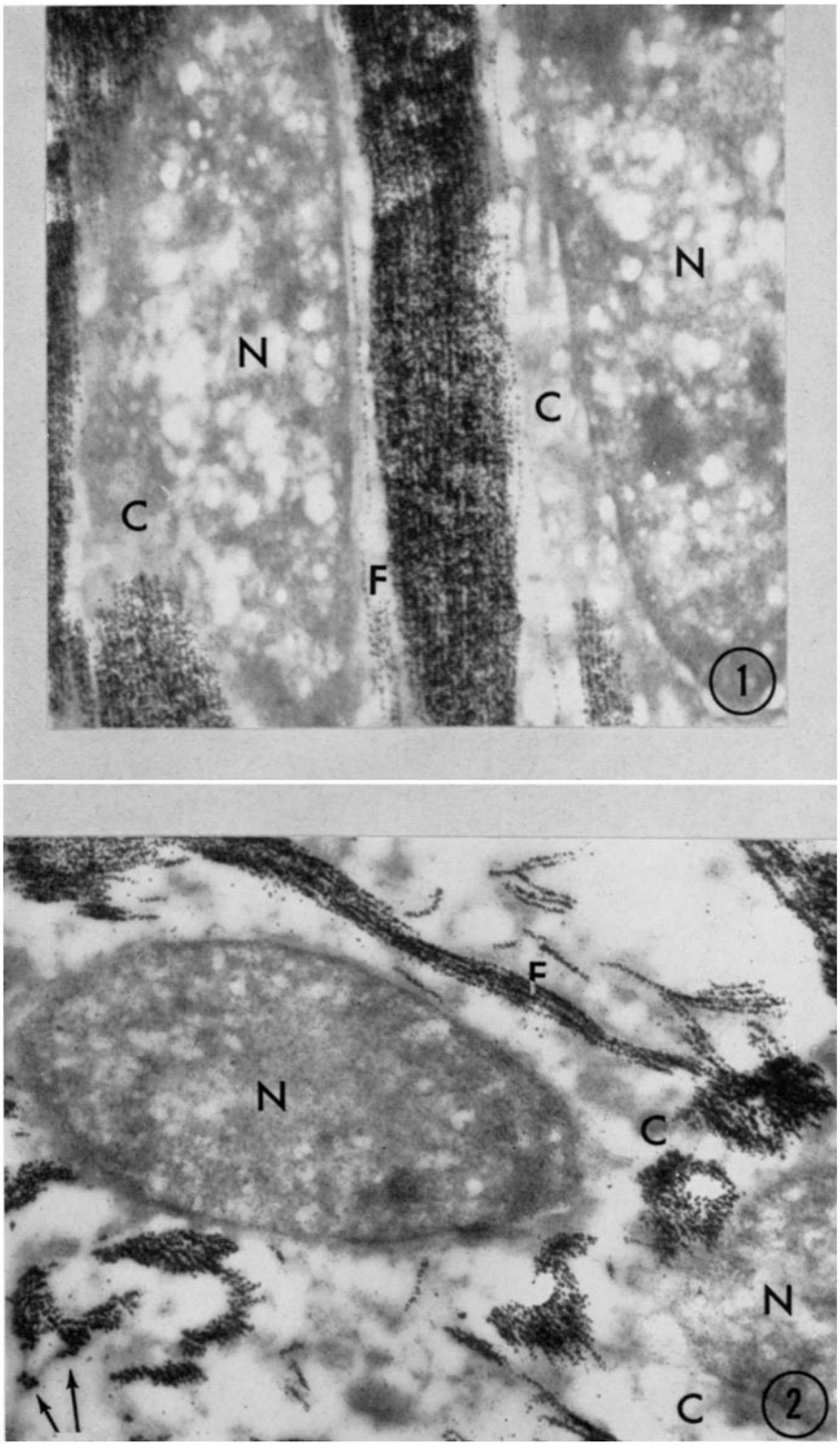
N, nuclei.
C, cytoplasm.
F, fibrils.

All the figures represent prints of electron micrographs of sections after formalin fixation and silver impregnation.

PLATE 20

FIG. 1. A longitudinal section of tendon from a 10-day chick embryo. × 21,000.

FIG. 2. Fibroblast from loose connective tissue of 10-day chick embryo. Notice the relationship of fibers and cytoplasm (arrows). × 21,000.

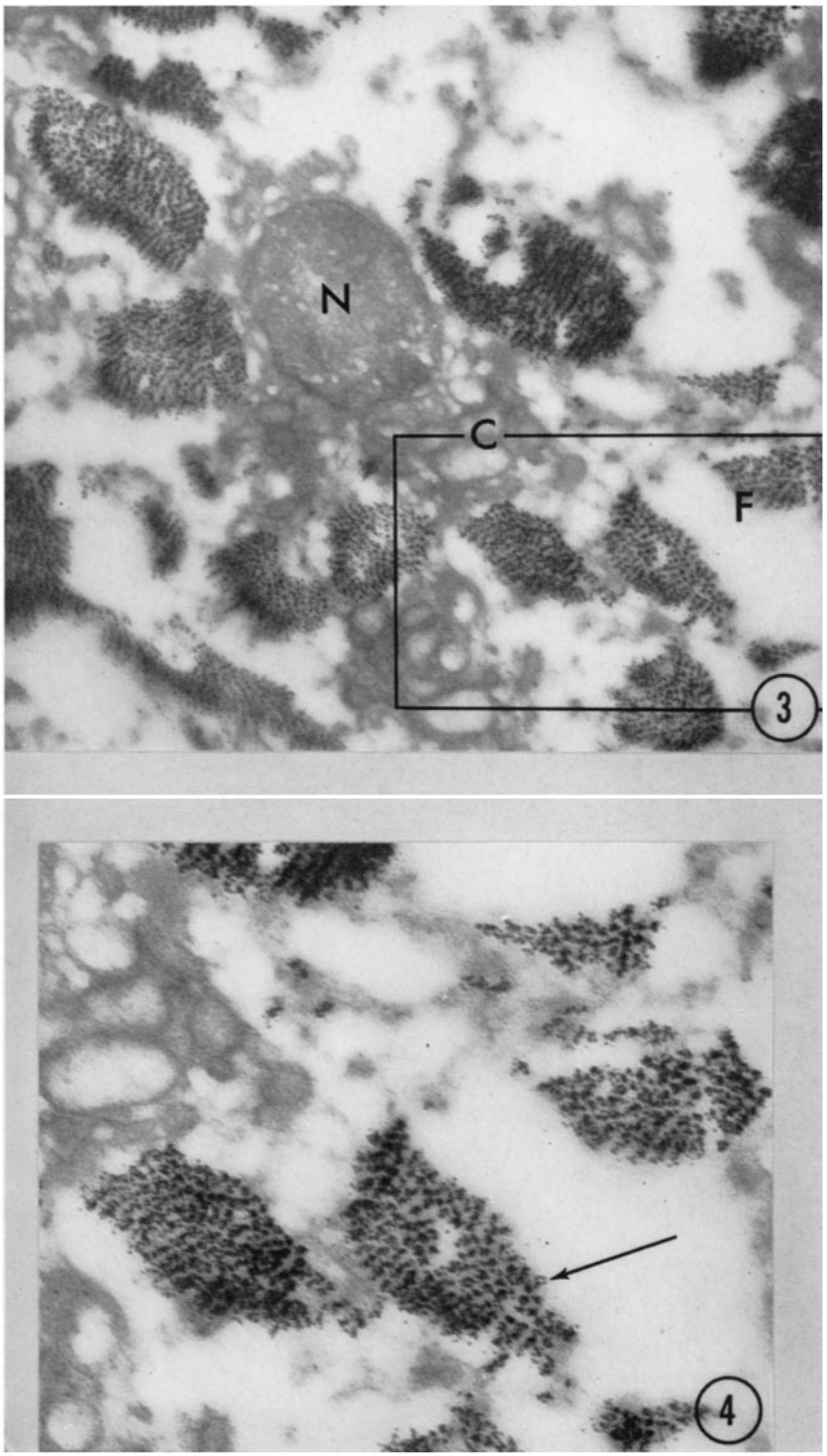


(Wassermann and Kubota: Electron microscopy of silver-impregnated fibrils)

PLATE 21

FIG. 3. Fibroblast from loose connective tissue of 10-day chick embryo. The fibers are seen in cross-section. Note that they are embedded in a material which is continuous with the cytoplasm. × 32,000.

FIG. 4. Higher magnification of area marked in Fig. 3. Notice that in cross-sections of the fibers the microfibrils are represented by groups of silver particles often surrounding an unstained area or forming rings (arrow). × 54,000.



(Wassermann and Kubota: Electron microscopy of silver-impregnated fibrils)