AN UNUSUAL ARRANGEMENT OF RIBOSOMES IN MESENCHYMAL CELLS

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It has been established for some time that cytoplasmic ribosomes may be arranged in characteristic linear or spiral configurations (1, 2). Recently it has been shown that a multiple ribosomal structure is the active unit in protein synthesis (3–6) and hypothesized that individual ribosomes of a unit are held together by a strand of messenger RNA since the collection of ribosomes dissociates rapidly when exposed to low concentrations of ribonuclease (7–9). In studies of the rabbit reticulocyte, which synthesizes a single protein, hemoglobin, Warner *et al.* (3) and Slayter *et al.* (9) have described, in fractionated material, extended arrays of ribosomes connected by a strand approximately 10 A in width. The most frequent structure they encountered was a pentaribosomal unit, although some larger units contained six or even seven ribosomes. These structures, termed polyribosomes or polysomes, were in linear arrays when seen in positive contrast whereas in negative contrast clusters predominated. They considered that the linear array is the actual configuration *in vivo* and that the clusters seen in negative contrast were the result of drying. The thin thread, 10 A wide, which connects ribosomes, was thought to be a single strand of RNA. This work recently has been reviewed by Hultin (10).

In our current studies of the rat umbilical cord, we have observed, at certain stages of gestation, an unusual arrangement of ribosomes in relation to the endoplasmic reticulum of mesenchymal cells. The array of ribosomes, in sectioned material, appears to bear a resemblance to the polyribosomes described, in fractionated material of the rabbit reticulocytes, by Slayter *et al.*

MATERIALS AND METHODS

Umbilical cords for study in this series were obtained from fetuses removed under anesthesia from pregnant rats at 17, 19, and 21 days' gestation. For each gestation stage, the fetuses of two pregnant females were examined. Small lengths of umbilical cord were placed immediately upon removal into 1 per cent cold (about 4°C) buffered osmium tetroxide. Tissues were dehydrated rapidly in the cold and embedded in Maraglas. Thin sections were cut with glass knives on a Porter-Blum microtome, mounted on uncoated grids, and stained with lead hydroxide. The sections were examined with an RCA EMU 3F operated at 50 kv.

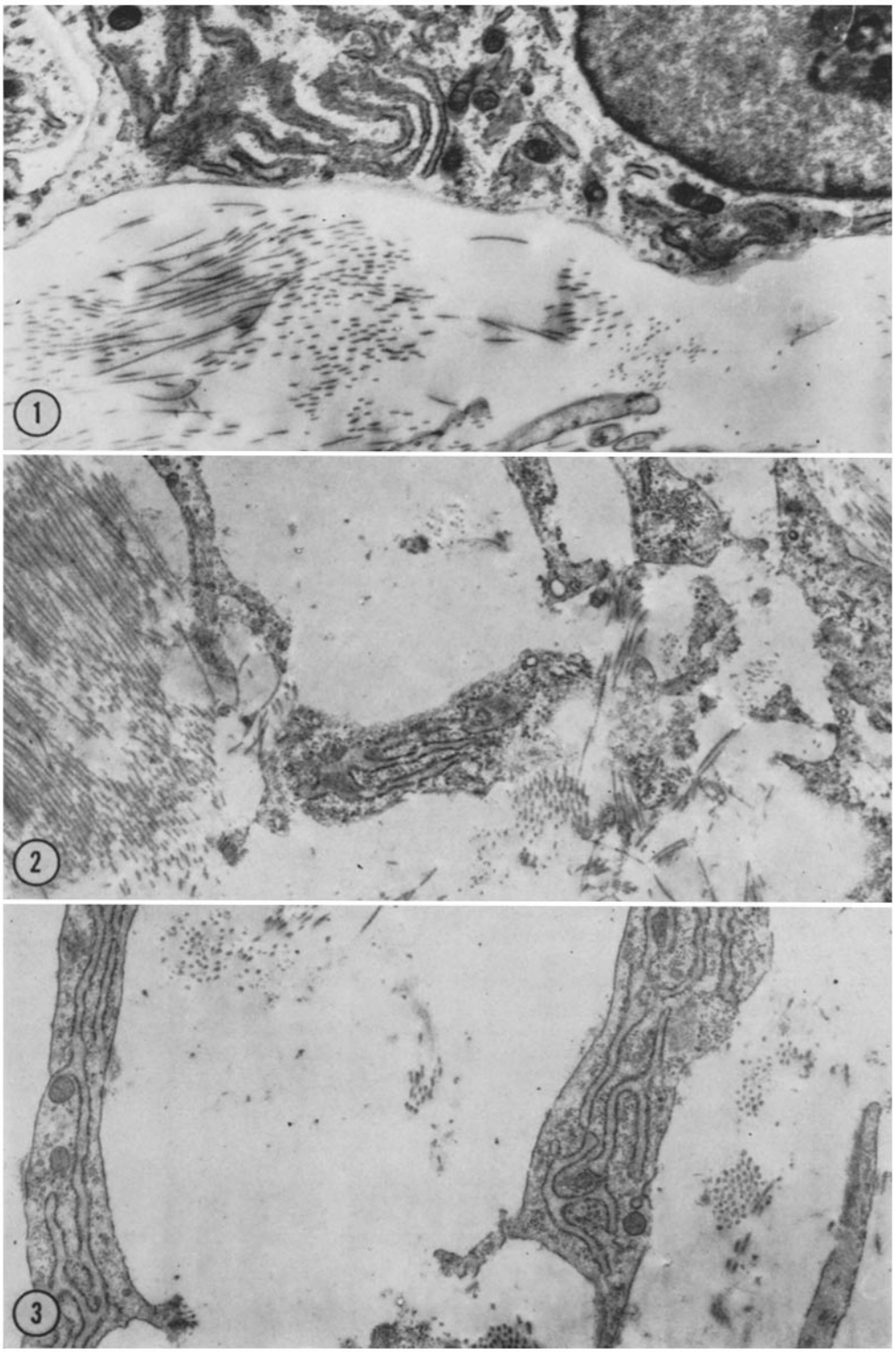
OBSERVATIONS

After 17 days' gestation in the rat, the mesenchyme of Wharton's jelly is very cellular and fine fibrils of collagen are scattered loosely within the matrix (Fig. 1). Mesenchymal cells are characterized by a marked development of the endoplasmic reticulum. Profiles of the latter are closely packed within the cytoplasm and in general are very irregular in outline. They contain a moderately electron-opaque, homogeneous material within their interior. Ribosomes attached to the membranes may occur singly but frequently they are aligned in clusters or in double rows, many of which exhibit curved or spiral forms (Fig. 4). With the resolution available, it was not possible to visualize any connecting thread between individual ribosomes. After 19 days' gestation, the mesenchyme does not appear as cellular as after 17 days' gestation and component cells are separated by a greater amount of matrix containing

FIGURE 1 17 days' gestation. Shown is a portion of a mesenchymal cell, containing well developed endoplasmic reticulum with associated polyribosomes, and the matrix, in which are scattered fibrils of collagen. × 11,000.

FIGURE 2 19 days' gestation. Processes of mesenchymal cells lie in a matrix which contains numerous fibrils of collagen. × 11,000.

FIGURE 3 21 days' gestation. Portions of two mesenchymal cells are shown. Collagen fibrils within the matrix are arranged mainly in discrete bundles. × 11,000.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numerous fine fibrils of collagen (Fig. 2). Mesenchymal cells again exhibit a considerable development of the endoplasmic reticulum, which still is characterized internally by a homogeneous electron-opaque material. Many ribosomes occur in aggregates (Fig. 5) but there is the impression that they are not seen so frequently as in the earlier stage. By 21 days' gestation (just prior to term), component cells of the mesenchyme are attenuated and widely separated, and collagen fibrils within the matrix are found, in the main, in discrete bundles (Fig. 3). The endoplasmic reticulum is still a prominent feature of mesenchymal cells but the majority of attached ribosomes occurs singly or in single linear arrays (Fig. 6), and only a few exhibit the complex arrangement noted in the earlier stages.

DISCUSSION

It is considered that the arrangement of ribosomes noted in this study is unlikely to represent an artefact since it was observed to vary considerably in extent in the three stages studied. Materials for all stages were subjected to a similar method of preparation. This arrangement of ribosomes has also been observed by us in material embedded in methacrylate and in Araldite.

The arrangement of ribosomes appears to bear a resemblance to that noted in fractionated material of rabbit reticulocytes by Slayter *et al.* (9). In their material, however, they described single chains whereas in the present study the ribosomes appeared commonly in more complex arrays.

Previous work has established a multiple ribosomal structure as the active unit in protein synthesis (3–6). Marks *et al.* (11) have noted that 80 to 90 per cent of ribosomes in a population of relatively immature rabbit reticulocytes appear as polyribosomes in electron micrographs of sectioned cells. They found that the maturation of reticulocytes to erythrocytes is associated not only with a decrease in the number of polyribosomes but also with a decrease in their functional capacity, as evidenced by their ability to incorporate amino acids into polypeptides. They considered it likely that these alterations in polyribosomes account for the decrease in protein synthesis which occurs as reticulocytes mature. The significance of the association of messenger RNA with ribosomes has been investigated by Henshaw *et al.* (12). They separated free from bound particles in rat liver cytoplasm by glucose density gradient centrifugation and found that both *in vivo* and in a cell-free system the membrane-bound particles actively incorporate amino acids into protein in the absence of exogenous stimulation, whereas the free ribosomes are almost completely inert. On the other hand, *in vitro* the free ribosomes can be stimulated to incorporate amino acids by the addition of synthetic messenger RNA. These findings would seem to indicate that the membrane-bound ribosomes are associated with messenger RNA, whereas the unbound particles are devoid of messenger RNA and are receptive to added messenger in an *in vitro* system.

Recently, Ross and Benditt (13) have described a configuration of the ribosomes of the endoplasmic reticulum of fibroblasts in wounds from guinea pigs which appears to be similar to that noted in the present study. They observed that the arrangement is lost in scorbutic cells but reappears within 8 hours after the administration of ascorbic acid. Coincident with the appearance of ribosomal aggregate formations, mature collagen fibers appear within the matrix. Thus in fibroblasts there appears to be a close correlation between the degree of aggregation of ribosomes and the ability to produce mature collagen. In this study of mesenchymal cells of the umbilical cord, ribosomal arrays have been noted commonly after 17 and 19 days' gestation, periods when there is an obvious increase in amount of fibrillar material within the matrix. By 21 days' gestation, immediately prior to term and when there appears to be little addition to the fiber content of the matrix, few arrays are observed. Thus it would appear that the degree of order among the ribosomes can be correlated with the state of gestation and, perhaps too, with the rate of collagen synthesis.

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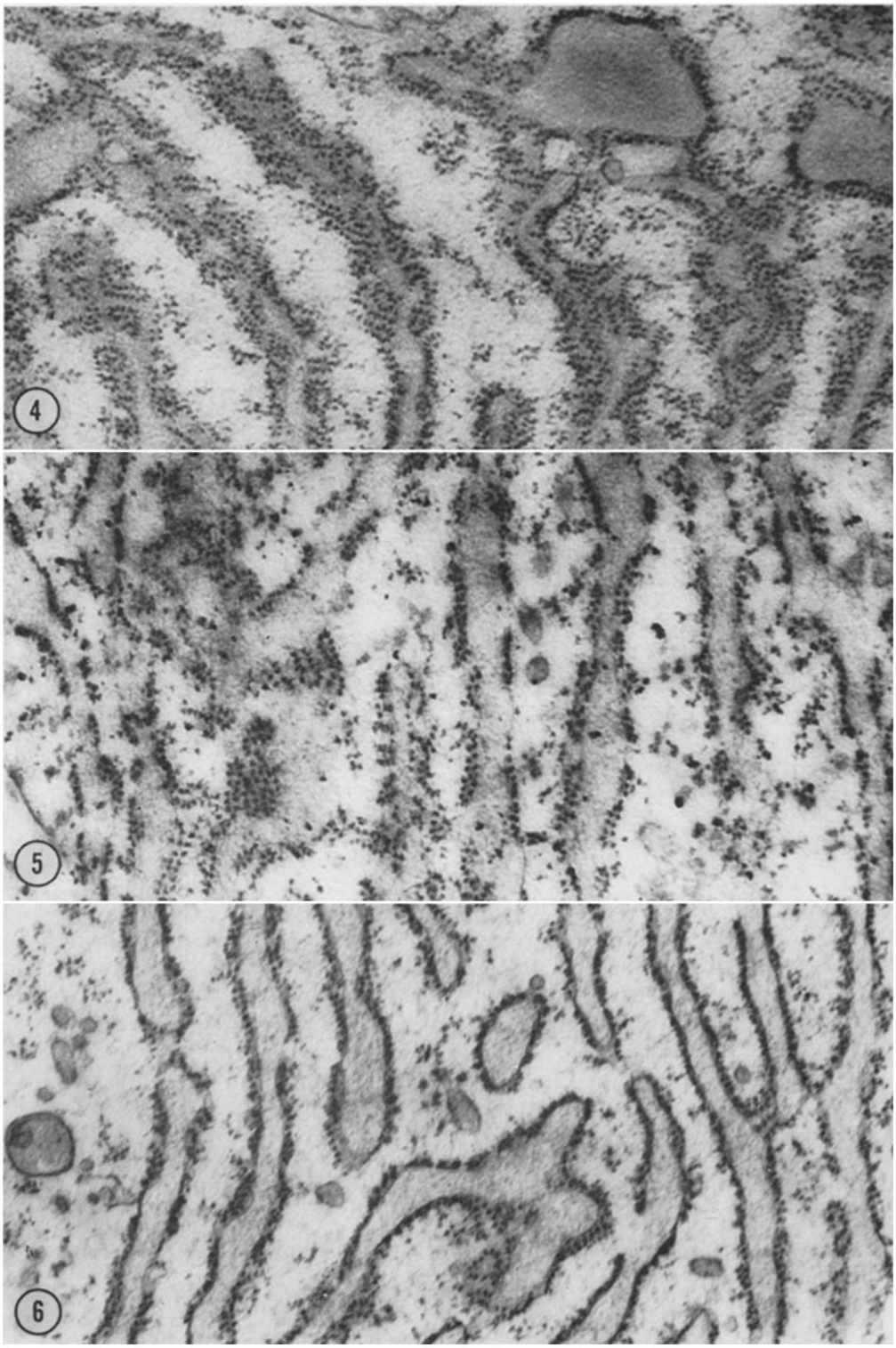
FIGURES 4 to 6 Portions of mesenchymal cells after 17, 19, and 21 days' gestation, respectively, illustrating the degree of order of ribosomes in relation to the membranes of the endoplasmic reticulum. Note the homogeneous material within profiles of the latter. × 42,000.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Addendum

Since this manuscript was submitted for publication, the work of Ross and Benditt (13) has been reported in detail. Ross, R., and Benditt, E. P., Wound healing and collagen formation. IV. Distortion of ribosomal patterns of fibroblasts in scurvy, *J. Cell Biol.*, 1964, **22**, 365.

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