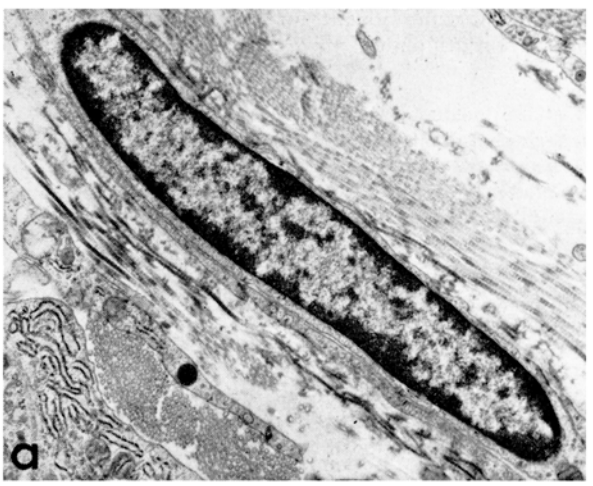
Presence of Modified Fibroblasts in Granulation Tissue and their Possible Role in Wound Contraction

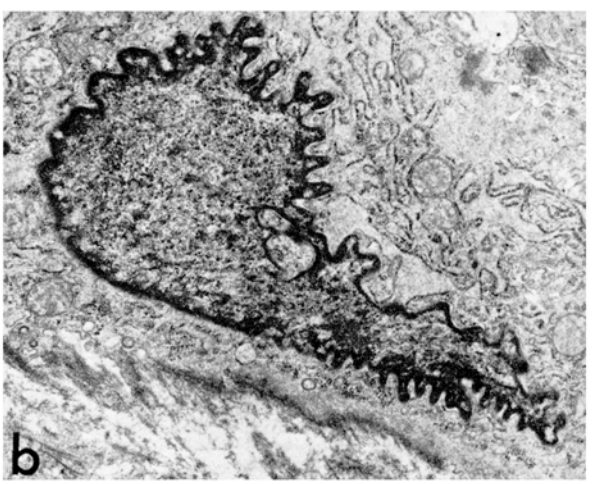
Although the mechanism of wound contraction is not yet clarified, recent histological, histochemical and metabolic studies suggest that the cellular component of granulation tissue (particularly fibroblasts) plays a role in this process[1-3]. It has been shown that cultured fibroblasts may contract in vitro[3,4]; however, at present there is no evidence that they do so during wound healing. We have investigated this possibility, by means of electron microscopy, in 4 experimental models of contracting granulation tissue.

Male Wistar rats weighing 100–180 g were used for the study of: 1. the reparative process after removal from the chest of a square of skin (with the cutaneous muscle) measuring 4 cm^2; 2. the contraction of a tail tendon (2 cm in length) homotransplanted in one of the pelvic fat bodies[5]; 3. the scarring of a small area of the liver capsule after drying for 5 min with a gentle stream of compressed air; 4. SELYE's[6] granuloma pouch produced by the s.c. injection of 20 ml of air and 1 ml of 1% croton oil in corn oil. The controls were fibroblasts from either tail tendon, subcutaneous connective tissue or liver capsule. Granulation tissues were examined at the following time intervals: for wounds 6 and 12 days; for tendon implants and liver surfaces 7, 16 and 21 days; and for the granuloma pouch 2, 6, 10, 16 and 21 days. The tissues were examined grossly and selected areas were fixed by immersion of small cubes for 5 h in 3% glutaraldehyde in cacodylate buffer. These were left in buffer overnight, post-fixed in 2% OsO_4 in collidine buffer, dehydrated, embedded in Epon 815, cut, stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

During the first few days, the fibroblasts multiplied as expected[2], and showed the cytologic structure regarded as 'typical' (Figure 1a) (i.e. numerous cisternae of rough endoplasmic reticulum and many mitochondria). Later, however, sometimes between 8 and 21 days depending on the experimental model (earliest with the skin wound, latest with the granuloma pouch), many fibroblasts exhibited 3 peculiar modifications:

1. *A fibrillar system* developed within the cytoplasm (Figures 1b and c). The term 'system' is purposely employed to emphasize that these were no longer the few fibrils often seen in fibroblasts[2,7], but bundles of packed fibrils resembling those of smooth muscle[8], cultured rat embryonic cells[9], BHK-21 cells[10] or the amoeba[11]. They measured mostly 40–80 Å in diameter (more rarely 120–160 Å) and were usually arranged parallel to the axis of the cell. They showed electron-dense areas (beneath the plasmalemma or scattered within the fibrillar bundles) such as are called 'attachment sites' in smooth muscle[8,12-14] (Figures 1b and c). These features were sometimes so well developed that individual cells could have been labeled 'smooth muscle





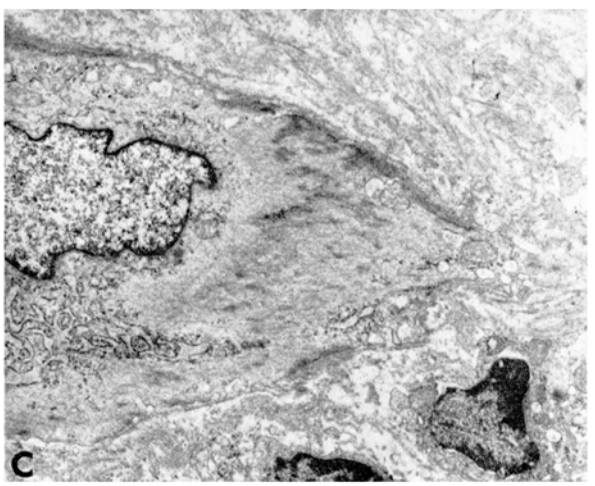


Fig. 1. Fibroblasts from control and granulation tissue. a) Fibroblast from normal subcutaneous tissue: the nucleus is oval and the cell is surrounded by collagen fibres. ×10,500. b) Modified fibroblast from the wall of a 21-day-old granuloma pouch: numerous folds and indentations of the nucleus. In addition to a prominent endoplasmic reticulum, the cytoplasm contains many fibrils located mostly at the periphery of the cell. In the extracellular space are collagen fibres together with a finer fibrillar material without periodicity. ×14,000. c) Another modified fibroblast showing a well developed fibrillar system with dense bodies both within the cytoplasm and at the periphery of the cell, in connection with an extracellular layer of basement membrane-like material parallel to the cellular surface. ×7600.

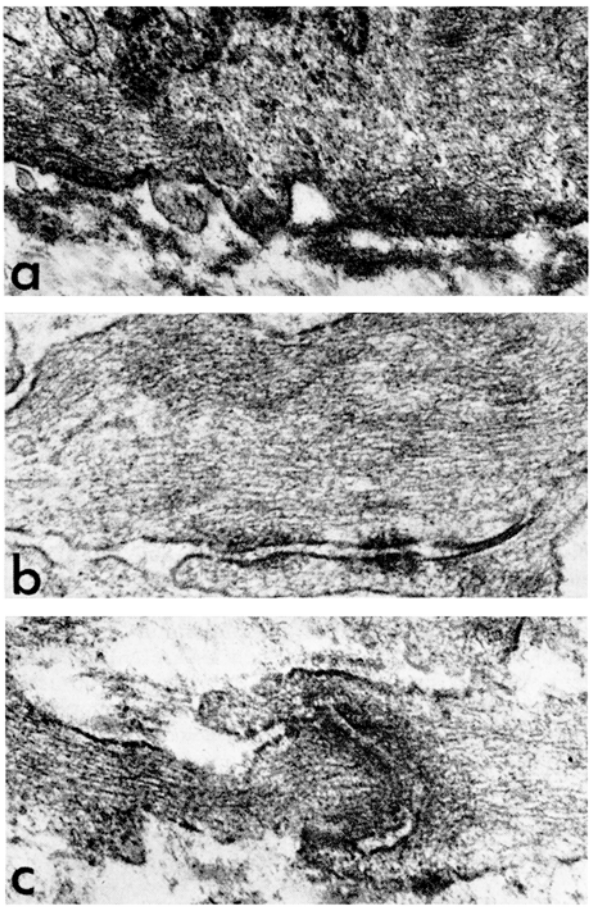


Fig. 2. Surface differentiations of modified fibroblasts (from the liver capsule 21 days after drying). a) Cytoplasmic dense bodies in relation with a layer of basement membrane-like material. ×40,000. b) and c) Maculae adhaerentes between 2 fibroblasts; note the intracellular fibrils. ×60,000.

cells' if another part of their cytoplasm had not displayed a structure otherwise 'typical' of a fibroblast.

2. *Nuclear deformations.* Whereas the nuclei of control fibroblasts (as well as those of other cells in the granulation tissues, e.g. histiocytes, mast cells or fibroblasts poor in fibrils) were mostly smooth or showed only a few wavy indentations, many of the modified fibroblasts contained very irregular nuclei, with deep folds or many shallow nicks recalling the shape of an accordion (Figure 1b). This was particularly evident in the granuloma pouch and on the liver surface. Such changes have been correlated with cellular contraction in at least 3 systems: smooth muscle[15], myocardial fibres[16] and endothelial cells treated with histamine-type mediators[17].

3. *Surface differentiations.* Many peripheral 'attachment sites' were related to an extracellular layer of basement membrane-like material parallel to the cell wall (Figures 1c and 2a). In several cells, such layers covered a large part of the surface (Figure 1c). This complex, which is present on the surface of smooth muscle cells[8,12], is also reminiscent of the hemidesmosomes described in pericytes or endothelium[13,18]. Moreover, localized intercellular connections were found, in the nature of maculae adhaerentes (Figures 2b and c). These have been described between foetal[19], neonatal[14] or cultured[20] fibroblasts but have not previously been observed between such cells in adult animals even during wound healing[19].

The presence of nuclear deformations, associated with a well-developed cytoplasmic fibrillar system, suggests that these modified fibroblasts have undergone contraction. The surface differentiations give a mean whereby they could transmit the resulting mechanical pull to the surrounding tissue elements. This mechanism could play an important role in the contraction of wounds and in other forms of connective tissue shrinkage.

It is likely that, in the experimental models used here, these modified fibroblasts have developed from the more conventional tissue fibroblasts. In normal animals, intermediate forms between fibroblasts and smooth muscle cells are present[7] and smooth muscle cells may behave like fibroblasts in that they produce collagen and elastin[14]; moreover, smooth muscle cells resembling fibroblasts have been described in the uterus of rats treated with oestrogens[14] and in pathologic or experimental arteriosclerotic lesions[21–23]. It seems obvious, then, that fibroblasts and smooth muscle cells are much more closely related than classical histology would have allowed one to suppose, and that either cell may be capable of modulating towards an intermediate type.

In conclusion, our findings support the view[1] that fibroblasts play a role in granulation tissue contraction; furthermore they indicate that this contraction is due, at least in part, to the modification of fibroblasts into cells which are capable of an active spasm[24].

Résumé. Au cours de la contraction du tissu de granulation, de nombreux fibroblastes acquièrent des caractéristiques ultrastructurelles qui les rendent semblables à des cellules musculaires lisses. Il est probable que ces éléments modifiés jouent un rôle dans le processus de contraction des plaies.

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