microfilament machinery[3,4,14–17]. However, it is difficult to explain its property of disaggregating embryonic cells on the above hypothesis. Experiments by Sanger and Holtzer[8] have shown the action of CB on the cell surface material which holds the cells together, while Schaeffer et al.[11] have proposed an alteration of cell surface charge causing disaggregation of embryonic cells. Our observations of the behaviour of endodermal cells, with and without CH treatment, the rapid recovery of some of the cells which stick to glass surface suggests the possibility that CH like CB acts by bringing about the alteration of cell surface charge as suggested by Schaeffer et al.[11]. However, the possibility of the inhibition of the synthesis of mucopolysaccharides by CH, as reported for CB by Sanger and Holtzer[8], cannot be excluded.

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Contractile filaments in cells of regenerating tendon[1]

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Summary. An extensive cytoplasmic fibrillar system has been observed in fibroblast-like cells of regenerating tendon. It consists of bundles of actin filaments, which often show a cross-striated appearance due to electron dense bodies occurring throughout their length. The functional role of this contractile apparatus seems to be related to the process of movement and orientation of the newly formed cells and to the retraction of the regenerating tendon.

Recent ultrastructural and immunofluorescent studies[3–7] have allowed the distinction between 2 different types of fibroblasts, namely a) the typical fibroblast, containing few randomly dispersed cytoplasmic filaments, probably endowed with contractile activity, and b) the so-called myofibroblast, which is provided with an extensive fibrillar system made up of bundles of parallel actin filaments. This latter type of cell, which can be considered as intermediate between typical fibroblast and smooth muscle cell, has been observed in the granulation tissue of healing wounds[4,5,7], in the chicken aorta[8], in the rat ovary[9], in the palmar nodules of Dupuytren disease[6], and in the tenocytes of newborn rabbit calcaneal tendon[10]. In the current study, evidence is provided for the presence of large amounts of actin arranged in bundles of filaments in the cytoplasm of regenerating cells during the early stages of morphological recovery of tendon.

Material and methods. Adult male New Zealand rabbits were used in the experiments. Under general anaesthesia and aseptic conditions, the right calcaneal tendon was exposed through a short incision of the skin and peritendineous tissues. The tendon was then cut transversely with a scalpel. A gap resulted between the 2 ends of the severed tendon due to retraction of the proximal stump, attracted by the triceps surae muscle; 7 days after operation the newly formed tissue bridging the gap was removed. The controlateral tendon was used as control. For the morphological study, the newly formed tissue and the control tendon were fixed for 10 min with 3% paraformaldehyde – 1% glutaraldehyde in Millonig buffer pH 7.4 by an 'in vivo' dripping method. The tissue was then removed, trimmed into small pieces, placed in the same fixative for 2 h and postfixed in 1% OsO_4. Specimens were dehydrated and embedded in Epon. Light microscopic observations were performed on 1 μm thick sections stained with buffered Toluidine blue (pH 8) or with buffered Blue de Unna (pH 7). For the ultrastructural study, thin sections were stained with lead hydroxide and uranil acetate, and examined in a Siemens Elmiskop 101 electron microscope.

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Some of the regenerating tendons were used only for immunofluorescent study. Tendons were removed, frozen in a dry ice-acetone mixture and cut in 4 μm thick cryostat-sections. Sections were then fixed 10 min in acetone, air-dried and stained by indirect immunofluorescence with a human serum containing high titers of anti-smooth muscle antibodies. The serum specificity for actin was assessed by absorption experiment. Preincubation of the serum with pure actine was able to remove the arterial wall and mesangial staining detectable on mouse kidney sections. The serum was freed by low titers of contaminating antinuclear antibodies by absorption with insoluble nucleoproteins[12], until nuclear fluorescence was no longer detectable on normal mouse kidney and liver cryostat sections. Fluorescein labelled antiserum to human IgG was purchased from Cappel Labor (Downingtown PA. USA).

Results. In semi-thin sections, the regenerating tissue was made up of rare macrophages and polimorphonuclear cells and by a large number of fibroblast-like cells, the disposition of which varied from a haphazard to parallel arrangement along the tendon major axis (figure 1). On electron microscopic examination, the fibroblast-like cells showed a smooth or slightly wavy nuclear contour, abundant rough endoplasmic reticulum, numerous mitochondria and a well-developed Golgi apparatus. More-

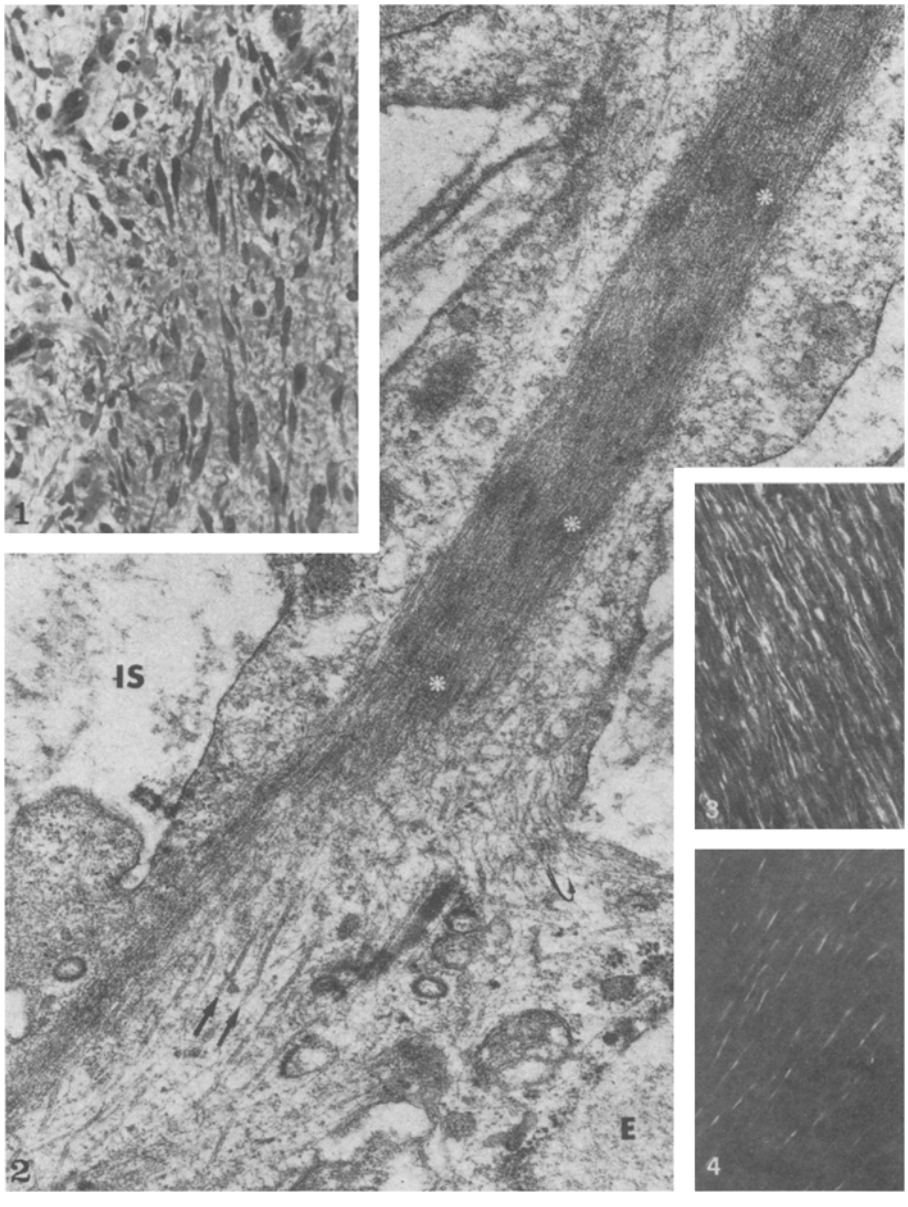

Fig. 1. 7-day-old regenerating tendon tissue. In some areas the fibroblast-like cells are arranged parallel to the major tendon axis, while in others (right side) in which the tissue is less compact, they are oriented haphazardly (left side). ×400. Fig. 2. A large bundle of densely packed thin filaments is visible in the cytoplasm of a myofibroblast. Note the electron dense areas arranged along the bundle (asterisks). Straight arrows point to microtubules, while curved arrows indicate isolated thin filaments. *E*, dilated cisternae of ergastoplasm; *IS*, intercellular space. ×42.000. Figs. 3 and 4. Indirect immunofluorescence of regenerating (figure 3) and control (figure 4) tendons treated with human anti-smooth muscle antibodies. The fluorescence in the cells of the regenerating tissue appears uniformly distributed and more intense than in the control tissue. ×250.

over, in the cortical cytoplasm, thin filaments usually arranged in large bundles. running parallel to the long axis of the cell, were often detectable (figure 2). The filaments measured 50–70 Å in diameter and often the bundles showed a cross-striated appearance due to the presence of electron-dense bodies throughout their length. Numerous microtubules were also visible.

In indirect immunofluorescence, the cells of the regenerating tissue displayed an intense reaction to human anti-smooth muscle antibodies (figure 3). Staining appeared more intense than in the controlateral tendon (figure 4). The fluorescent cells were uniformly distributed both in the peripheral part and in the centre of the regenerating tissue. No fluorescence was detectable when normal human sera were employed in the same test.

Discussion. In a previous study[10] tenocytes were shown to have morphological and immunochemical characteristics of contractile cells. These findings suggested that tenocytes may be considered myofibroblasts and tendon a contractile organ. Our observations demonstrate that contractile structures are also present in the cells of the granulation tissue during the early stages of tendon regeneration in an amount by far larger than in normal tendon.

In adult tenocytes, the contractile apparatus has been considered to be involved in the modulation of the contractile and retractile activity of the muscle-tendon functional unit[10]. Since during the early stages of the regeneration this modulation is temporarly interrupted, contractile structures could then play a determinant role in a) the process of adhesion, movement and orientation of the newly differentiated cells, and b) in the retraction of the regenerated tissue. These new functions could require a contractile apparatus more developed than that necessary for the normal modulatory activity. A system of cytoplasmic contractile filaments similar to those described in this study has been observed in amoeba[13] and in cultured chick embryo fibroblasts[14], where they seem to be critical for cell-adhesion and mobility. It is likely that such contractile structures play a similar role in the myofibroblasts of regenerating tendon. In fact, between the 2 ends of a cut tendon, a large gap is formed which is initially filled with fibrin clot and blood cells. Successively, cells of the peritendineous sheaths proliferate and progressively invade the blood clot[15,16]. The main role of the contractile apparatus could, therefore, be related to the ameboid movements which allow the cells of the peritendineous sheats to invade and substitute the initial blood clot. At this stage, a retraction of the newly formed tendon probably occurs. The contractile apparatus might at this time play an important role, as has been shown to be the case in the wound contraction described by Gabbiani et al.[4,5,7]. Only at later stages when the cells of the regenerated tissue acquire the morphological and functional characteristics of adult tenocytes[17], the fibrillar system would assume the function played in normal tendon.

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Erythropoiesis and plasma tocopherol levels in irradiated mice

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Summary. Plasma protein and tocopherol concentrations, haematocrit and 59-iron incorporation into erythrocytes have been measured in vitamin E-deficient and supplemented mice before and after exposure to 500 R of 260 kVp X-ray. Supplemented animals had greater haematocrit, plasma tocopherol and protein levels initially. After irradiation plasma tocopherol concentration decreased drastically in the vitamin E-supplemented mice.

The haematopoietic system is particularly sensitive to ionizing radiation[1]. In view of the role of alpha tocopherol in protecting cell membranes from peroxidation[2], protecting red blood cells from hemolysis[3,4] and its role in haematopoiesis[5], the tocopherol status of an organism may be expected to have a great influence on the radiation-response of that organism.

Several studies have been carried out to test the modifying influence of vitamin E on radiation damage[6–9] and the results have been conflicting. This may be due to the fact that tissue tocopherol levels are not readily increased by injection of some forms of tocopherol[10] and furthermore normal lab diets are sufficiently high in tocopherols that further supplementation with vitamin E has little effect

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