GRANULATION TISSUE AS A CONTRACTILE ORGAN

A Study of Structure and Function*

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When an open wound heals, its surface becomes smaller due to an inward movement of the edges; this is the phenomenon of wound contraction.

Following the pioneer work of Carrel (1, 2), it is now widely accepted that the contactile force resides in the granulation tissue that fills the wound (3–5); the nature of this force, however, has not been clearly defined. The traditional view, that shortening of collagen is responsible (6), was challenged by the demonstration that wounds in scorbutic animals can close independently of collagen formation (7). Neither is there evidence that some other extracellular material has a contractile function. Attention has therefore focused on the cells of the granulation tissue, more specifically on the fibroblasts. Under certain conditions these cells are capable of contracting; cultured fibroblasts, extracted with glycerol, contract when treated with adenosine triphosphate (ATP) (8), and neighboring foci of fibroblasts, growing in vitro, pull on one another where they become confluent (9). However, direct proof that fibroblasts contract in vivo has been lacking. Indeed, ultrastructural studies of fibroblasts in healing linear wounds (3) have not revealed features that would be expected of contractile cells.

We have examined the ultrastructure of fibroblasts in open wounds, as well as in several other models of contracting granulation tissue, and found that such cells do in fact develop contractile characteristics. We then demonstrated that isolated strips of granulation tissue respond in vitro to pharmacologic agents in a manner comparable to smooth muscle. This resemblance to smooth muscle was confirmed by chemical and immunologic means. Some of our results have been reported in preliminary communications (10–12).

Materials and Methods

Electron Microscopy.—We examined tissue from four experimental models, using male Wistar rats weighing 100–180 g each divided into groups of 10. (*a*) The reparative process after removal from the chest, or the dorsum, of a square of skin (with the cutaneous muscle) measuring about 4 cm^2 (10): Such wounds begin to shrink in size about a week later and are

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fully closed at about the end of the 2nd wk. (*b*) The shortening of a tail tendon (2 cm in length) homotransplanted into a pelvic fat body (13): This tendon becomes ensheathed by a layer of granulation tissue that progressively contracts between 7 and 21 days, thus causing a decrease in the over-all length of the tendon. (*c*) The injury to the top surface of the liver resulting from drying for 5 min with a gentle stream of air from a compressed air cylinder (14): This produces a thin layer of granulation tissue, the contraction of which causes a striking upward curling of the free edge of the liver, beginning at about 6 days. (*d*) Selye's granuloma pouch (15) produced by the subcutaneous injection of 20 ml of air and 1 ml of 1% croton oil (Magnus, Mabee, and Reynard Div., Paramus, N.J.) in corn oil: The injected material becomes surrounded by a wall of granulation tissue. The resulting egg-shaped pouch slowly shrinks, starting at about 8 days, until it virtually disappears at about 3 months.

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 granuloma pouches 2, 6, 10, 16, and 21 days.

The controls were fibroblasts from normal tail tendon, liver capsule, and subcutaneous connective tissue.

Specimens for light microscopy were fixed in alcohol-formol (80% absolute ethanol and 20% neutral formalin) and stained with hematoxylin-eosin, periodic acid–Schiff, Masson trichrome, cresyl violet, Mallory's phosphotungstic acid, and van Gieson's techniques.

The specimens for electron microscopy were cut into 1-mm cubes and fixed at room temperature for 5 hr in 3% glutaraldehyde in cacodylate buffer at pH 7.2–7.4. They were left in buffer at 4°C overnight, postfixed at 4°C with 2% OsO_4 in collidine buffer at pH 7.3–7.4, dehydrated in alcohol, and embedded in Epon 812 (Ladd Research Industries Inc., Burlington, Vt.). Sections about 1 μ thick were cut with glass knives on a Reichert ultramicrotome (C. Reichert A. G., Vienna, Austria) or LKB Ultrotome (LKB Instruments Inc., Lucerne, Switzerland) and stained with methylene blue and Azur II. Thin sections were cut with diamond knives, mounted on noncoated grids, stained with uranyl acetate and lead citrate, coated with a thin layer of carbon, and examined with a Philips 300 electron microscope (Philips Electronic Instruments, Zurich, Switzerland).

Pharmacology.—We tested strips from the following types of granulation tissue. (*a*) Selye's granuloma pouch (7–50 days old): A spiral cut around the pouch wall (1–2 mm thick) gave a strip of tissue with an average length of 80 mm, a width of 6–8 mm, and a weight of about 900 mg. (*b*) Granulation tissue from the base of healing dorsal skin wounds (7–14 days old): The preparations were shorter and lighter than those from pouches, measuring about 30 mm in length and weighing 200–500 mg. (*c*) Granulation tissue from the capsule that develops around intraperitoneally implanted blood clots (14): 8-ml clots of homologous blood were placed in the peritoneal cavities of rats weighing 200–300 g. By 7 days each clot is surrounded by a thin fibrous capsule, partially adherent to the omentum and to the pelvic fat bodies. We used strips obtained by a spiral cut around the clots (the capsule then readily peeled away from the mass of clot beneath); such strips of tissue had an average length of 30 mm and a weight of about 300 mg.

As controls, we used strips (50–80 mm long) from various normal tissues: dorsal subcutaneous tissue, tail tendons (seven to nine tied together), tail skin (which does not possess a cutaneous muscle), dorsal skin (including the panniculus carnosus), and cremaster and rectus abdominis muscles.

The preparations were suspended from one end of a frontal lever (Jaquet 1152RM, Jaquet, Basel, Switzerland) and attached to the bottom of a bath containing 20 ml of Tyrode's solution maintained at 37°C and bubbled with 95% O_2 plus 5% CO_2. The lever amplified vertical displacement by a factor of 6; the tungsten recording stylus inscribed on metallized paper advancing on a kymograph (Jaquet EK2025) at a rate of 1 mm/min.

We tested the following drugs: bradykinin triacetate, Sigma Chemical Co., St. Louis, Mo.; histamine dihydrochloride, Hoffmann-La Roche and Co. Ltd., Basel, Switzerland; angio-

tensin (Hypertensin), Ciba Ltd., Basel, Switzerland; vasopressin and methysergide bimaleate, Sandoz Ltd., Basel, Switzerland; epinephrine hydrochloride and papaverine hydrochloride, Vifor SA, Geneva, Switzerland; norepinephrine (Arterenol), Hoechst AG, Frankfurt, Germany; acetylcholine (Dispersa-Baeschlin), E. Baeschlin AG, Winterthur, Switzerland; serotonin creatinine sulfate (5-hydroxytryptamine), L-tryptophan, and L-histidine, E. Merck AG, Darmstadt, Germany; barium chloride, S. Siegfried AG, Zofingen, Switzerland; cyproheptadine hydrochloride (Periactin), Merck Sharpe & Dohme, West Point, Pa.; cytochalasin B, Imperial Chemical Industries, Alderley Park, Cheshire, England.

Chemical Studies.—We extracted actomyosin from 21-day-old granuloma pouch walls, using the method of Murphy and Hasselbach (16). As controls, we used pregnant rat uteri.

The calcium-activated adenosine triphosphatase activities were estimated as follows: Adenosine triphosphate (ATP) (disodium salt, E. Merck AG) was dissolved in 0.2 N NaOH to give a neutral solution. The reaction was carried out at 20°C in a flask containing 0.6 M KCl, 1 mM $CaCl_2$, 0.1 M tris (hydroxymethyl) aminomethane-hydrochloride (pH 7.4), actomyosin extract (3 mg/ml for granuloma pouch and 5 mg/ml for uterus), and 10 mM ATP. At regular intervals after the addition of ATP to the mixture, samples were taken from the flask and put into tubes containing 5% trichloroacetate. The inorganic phosphate content was then estimated spectrophotometrically (17). The activity of adenosine triphosphatase per milligram of protein was calculated on the basis of the amount of phosphate liberated in 30 min.

Immunology.—We used tissue from granuloma pouches (7, 21, 30, and 50 days old) and open skin wounds (7, 9, 13, and 15 days old). Human anti-smooth muscle (HASM)[1] sera were obtained from two patients with chronic active hepatitis. These sera reacted against smooth muscle of rat stomach with dilutions up to $1/320$; tests for anti-mitochondrial and anti-DNA antibodies and for anti-nuclear factor were negative. To demonstrate smooth muscle antigens the double-layer technique was used (18). Cryostat sections of all tissues were treated with HASM serum at a dilution of $1/6$ and stained with sheep anti-human IgG fluorescent serum (Miles-Seravac, Maidenhead, England). Fluorescence was compared with that of sections treated either with normal human serum or serum from a patient with myasthenia gravis (which reacted against striated muscle at dilutions up to $1/640$). Photographs were taken on a Zeiss UV photomicroscope with UGI or II excitor filter and a Zeiss 50/65 barrier filter (Carl Zeiss Inc., Zurich, Switzerland), using Ektachrome HSB (Eastman Kodak Co., Rochester, N.Y.) or Ilford HP4 film (Ilford Ltd., Ilford, England). The same fields were photographed with visible light after staining the sections with hematoxylin and eosin.

RESULTS

Electron Microscopy

Control fibroblasts showed characteristics regarded as "typical" (19) (Fig. 1 *a*): The nuclear contour was relatively smooth (occasionally it had a shallow fold but we never saw deep indentations); the cytoplasm was rich in cisternae of rough endoplasmic reticulum and mitochondria; intracytoplasmic fibrils, though observed sometimes, were always few.

In granulation tissues the number of fibroblasts increased gradually, starting from about the 3rd day. Somewhere between 7 and 21 days (depending on the experimental model) many of these cells progressively developed three striking modifications (Figs. 1 *b* and *c*):

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[1] *Abbreviations used in this paper:* HASM, human anti-smooth muscle; 5-HT, 5-hydroxytryptamine.

A Fibrillar System within the Cytoplasm.—A fibrillar system developed within the cytoplasm, not the few fibrils seen in normal fibroblasts, but bundles of parallel fibrils resembling those of smooth muscle cells (20). Individual fibrils measured 40–80 A in diameter, more rarely 120–160 A, and were usually arranged parallel to the long axis of the cell. Many electron-opaque areas were scattered among the bundles or located beneath the plasmalemma (Figs. 1 *b* and *c*); these were similar to the "dense bodies" or "attachment sites" (20) of smooth muscle. Although these fibrillar structures often occupied a large part of the cell, the remaining cytoplasm contained packed cisternae of rough endoplasmic reticulum typical of normal fibroblasts (Fig. 1 *c*).

Nuclear Deformations.—The nuclei consistently showed multiple indentations or deep folds (Figs. 1 *b* and *c*), an appearance quite unlike that of normal fibroblasts (or other cells in the same granulation tissues, such as macrophages or mast cells).

Surface Differentiations.—There were numerous intercellular connections between these modified fibroblasts (Figs. 2 *a* and *b*); their structure identified them as maculae adhaerentes or desmosomes, often with a clear-cut intermediate line. In addition, part of the cell surface was often covered by a well-defined layer of material having the structural features of basal lamina, and generally separated from the cell membrane by a translucent layer. Where it was covered by basal lamina, the cell often showed dense zones in the fibrillar bundles immediately beneath the surface membrane (Fig. 2 *c*); the resulting complex was reminiscent of the hemidesmosomes that bind endothelial cells, pericytes, or smooth muscle cells to their basal lamina (21, 22).

Pharmacology

Granuloma Pouch.—With strips from pouches 8–50 days old, 5-hydroxytryptamine (5-HT) induced a brisk contraction. A typical response is shown in Fig. 3. When a strip was tested with successive, increasing doses of 5-HT (with no wash after each dose), a dose-response curve resulted (Fig. 4). A good effect was obtained with a concentration of 1×10^{-5} g/ml (final concentration in the bath), and so most of the tests were run with that dose (22 tests); the contraction began to be recorded almost immediately upon contact with the drug and reached a maximum in 5–10 min (at 5 min the strip had shortened by 4.16%, with a standard error of ± 0.29, at 10 min by $4.35 \pm 0.30\%$). The strip tended then to remain contracted (for as long as 2 hr); any relaxation that occurred was very slow, and the tracing never returned to the base line. When the same strip was tested with identical doses several times in succession, separated by washes, contractions became progressively smaller.

Other smooth muscle stimulants that induced contraction in this system (in decreasing order of effectiveness) were angiotensin 1×10^{-5} g/ml (five tests) (Fig. 5), vasopressin 0.25 IU/ml (six tests), norepinephrine 1×10^{-5} g/ml (three tests), bradykinin 1×10^{-5} g/ml (three tests), and epinephrine 1×10^{-5}

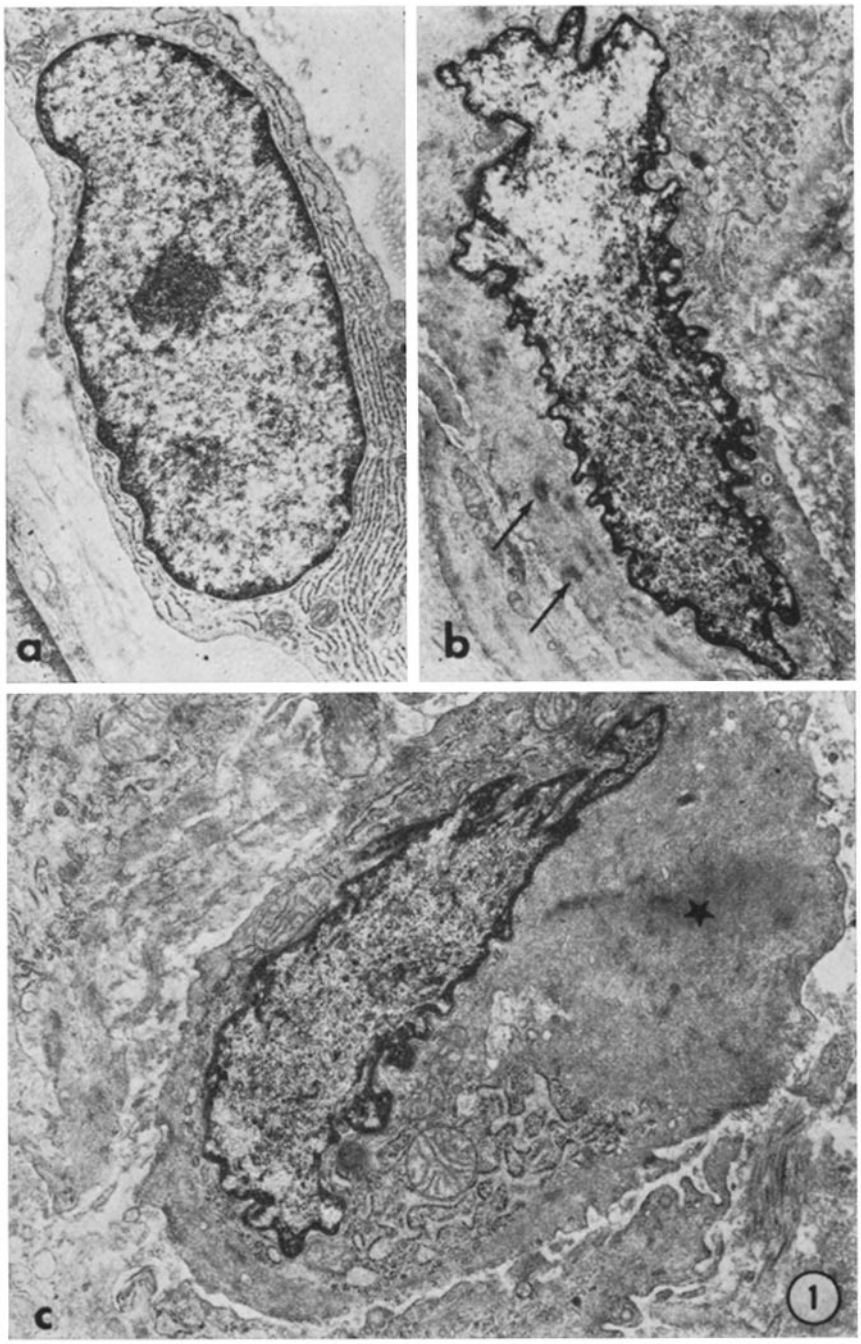

FIG. 1. Electron micrographs comparing the characteristics of fibroblasts and myo-fibroblasts. (*a*) Normal fibroblast from rat subcutaneous tissue. Note mitochondria, small peripheral vesicles, and regular arrangement of the endoplasmic reticulum. × 10,000. (*b*) Myo-fibroblast in a 21 day old granuloma pouch. A large part of the cytoplasm contains bundles of densely packed fibrils, with "attachment sites" (*arrows*) typical of smooth muscle. Note the numerous nuclear indentations. × 12,300. (*c*) Another myo-fibroblast (21 day granuloma pouch). Much of the cytoplasm is filled with fibrils, which give a smooth muscle-like appearance (the *star* marks an electron-opaque body); there is still abundant endoplasmic reticulum, recalling that of a fibroblast. The nucleus shows several folds. Extracellular tissue consists of collagen fibers, microfibrils, and dense homogeneous material. × 15,000.

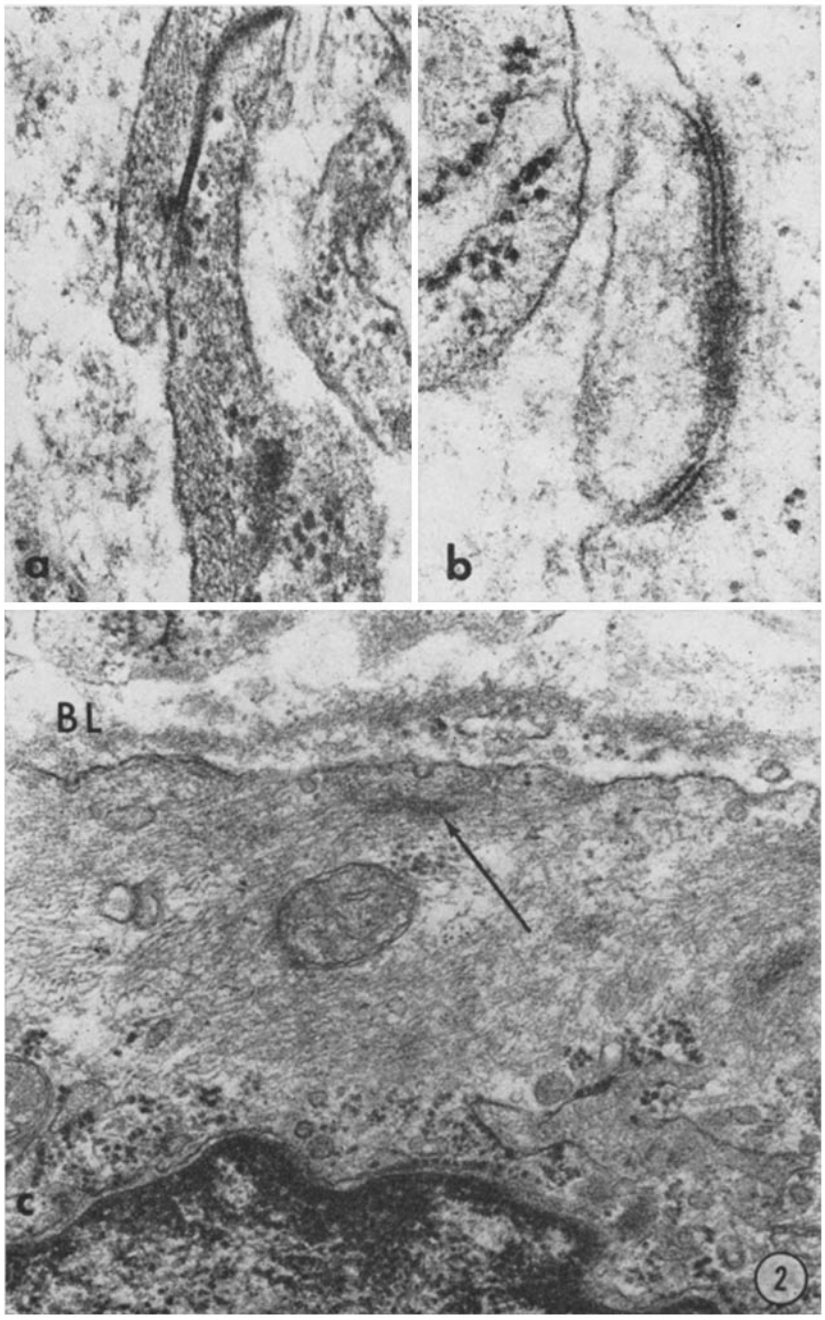

FIG. 2. Cell-to-cell and cell-to-stroma connections of myo-fibroblasts. (*a*) A typical desmosome with an intermediate line. × 63,000. (*b*) Macula adhaerens consisting of peripheral cytoplasmic condensations immediately beneath the cell membrane at the zone of contact between two cells. × 100,000. (*c*) Basal lamina (*BL*) parallel to the cell membrane. Note the bundles of intracytoplasmic fibrils with a dense body immediately beneath the cell membrane (*arrow*). × 32,000.

g/ml (three tests). The response was always quick, but the slope of the curve was somewhat different with different drugs. Agents that had no effect were: histamine 1×10^{-5} and 1×10^{-4} g/ml (five tests each); acetylcholine 1×10^{-6}–1×10^{-3} g/ml (five tests at each multiple of 10); tryptophane and histidine

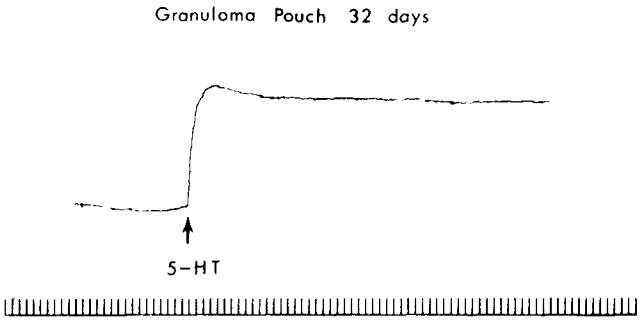

FIG. 3. Contraction of strip of tissue from a 32 day granuloma pouch in response to 5-hydroxytryptamine (5-HT) (1×10^{-5} g/ml). One division of the scale (1 mm) represents 1 min.

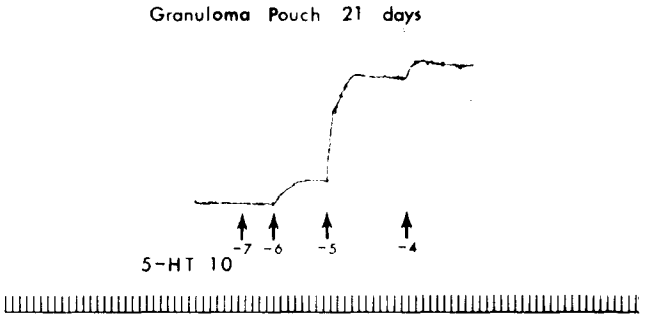

FIG. 4. Responses of strip from a 21 day granuloma pouch with successive, increasing doses of 5-HT (1×10^{-7}–1×10^{-4} g/ml).

1×10^{-5} g/ml (one test each); barium chloride 1×10^{-4} and 1×10^{-3} g/ml (six tests each).

The smooth muscle relaxant, papaverine, 1×10^{-4} g/ml alone on fresh strips of granuloma pouch (10 tests) induced a slow and progressive relaxation; such strips showed an elongation of $0.69 \pm 0.06\%$ at 5 min, $1.52 \pm 0.17\%$ at 10 min, and $2.39 \pm 0.29\%$ at 20 min. When applied after any contracting agent, papaverine caused a relaxation that began within 1–2 min and often the tracing dropped below the original base line (eight tests) (Fig. 5). All the contracting agents were virtually ineffective when applied after papaverine.

The reactivity of the tissue depended on the age of the pouch. At 7 days 5-HT

had no clear-cut effect. The first definite response was registered at 8 days, and the maximal response by 15–20 days; thereafter the reactivity stayed at this plateau for 4–5 wk. Contractions, although somewhat smaller, were obtained with strips from 50-day pouches.

Granulation Tissue from Open Wounds.—In our early experiments, strips from 10- and 11-day-old wounds gave only small contractions with vasopressin and

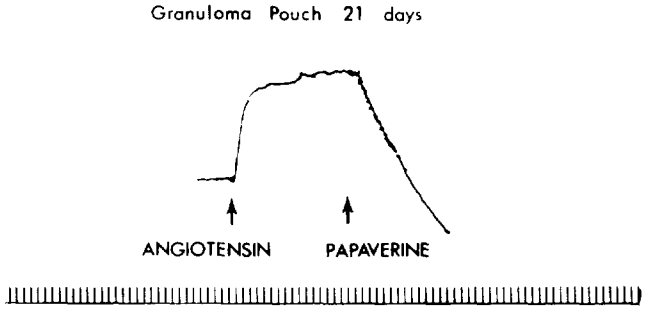

FIG. 5. Strip from a 21 day granuloma pouch. Contraction in response to angiotensin (1×10^{-5} g/ml), followed by relaxation due to papaverine (1×10^{-4} g/ml).

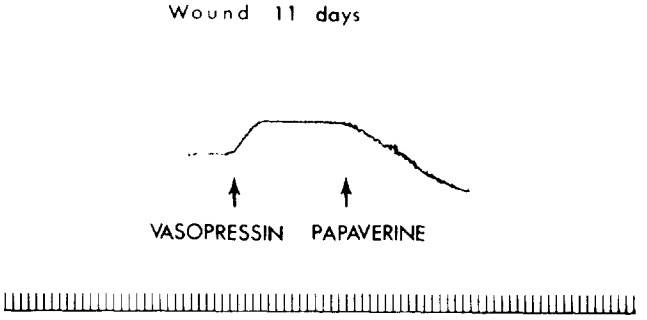

FIG. 6. Tissue from an 11 day skin wound. Contraction in response to vasopressin (1×10^{-5} g/ml), followed by relaxation due to papaverine (1×10^{-4} g/ml).

angiotensin. However, when such strips were gently stretched in the bath for 2 hr (increasing their length by about 50%) and then allowed to stabilize at the new base line before testing, more vigorous contractions occurred. Most effective were vasopressin (Fig. 6) (six tests), bradykinin (three tests), and angiotensin (five tests). Epinephrine (two tests) had a slight effect, whereas histamine (two tests) had none, and surprisingly neither had 5-HT (six tests), the most powerful agent on strips of granuloma pouch.

Papaverine alone (10 tests) caused the wound strips to relax in a manner practically identical to strips from granuloma pouches. When applied after any contracting agent, papaverine also caused a relaxation (eight tests) (Fig. 6).

Blood Clot Capsules.—These capsules provided a source of granulation tissue other than subcutaneous tissue. We tested the effects of 5-HT (seven tests), vasopressin (six tests), and papaverine (seven tests); the responses were similar to those obtained with strips of granuloma pouch.

Control Tissues.—No contraction or relaxation was registered with strips of dorsal subcutaneous tissue (i.e. the tissue from which the granuloma pouch develops) (four strips tested), tail tendons (four tests), tail skin (one test), dorsal skin (two tests), and cremaster (two tests) and rectus abdominis (one test) muscles. The agents tried in each case were 5-HT, bradykinin, angiotensin, and histamine (each at a concentration of 1×10^{-5} g/ml), vasopressin 0.25 IU/ml, and papaverine 1×10^{-4} g/ml.

Effects of Anoxia, Inhibitors, and Poisons.—For these experiments we used strips of granuloma pouch.

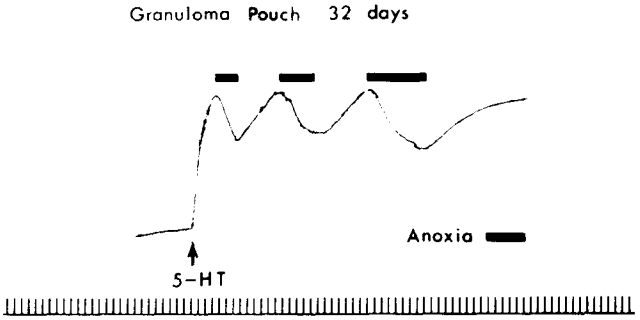

FIG. 7. Influence of intermittent anoxia on a strip from a 32 day granuloma pouch after contraction with 5-HT (1×10^{-5} g/ml).

The effect of anoxia was tested in two ways: (*a*) by contracting a strip with 5-HT, then stopping the oxygen flow for periods of 3–6 min (five tests). At each period of anoxia, the strip relaxed partially but without reverting to the base line; when oxygenation was resumed, the strip returned to the original contracted state. The result was a wavy curve (Fig. 7). (*b*) By taking a fresh strip, submitting it first to anoxia, and then to a stimulating dose of 5-HT. Periods of anoxia up to 240 min decreased the response but did not abolish it (three tests). Controls were oxygenated strips maintained in the bath for similar periods. To test an extreme condition, three strips were left overnight at room temperature in a bath that was not bubbled with oxygen; the next morning they still gave a slight response to 5-HT.

We used the following inhibitors: (*a*) Methysergide bimaleate, a potent anti-5-HT agent (23). Strips pretreated with this drug at a concentration of 1×10^{-5} g/ml (eight tests) failed to respond to 5-HT; the effect could not be reversed by washing, but the same strips still responded normally to vasopressin and angiotensin. Doses of 1×10^{-6} g/ml of methysergide relaxed strips already contracted

with 5-HT (three tests). (*b*) Cyproheptadine, a drug with both anti-histaminic and anti-5-HT activities (24) had similar effects to methysergide at a concentration of 5×10^{-5} g/ml (11 tests). (*c*) Cytochalasin B, which inhibits contraction due to microfilaments (25), caused by itself a slight relaxation at a concentration of 1×10^{-6} g/ml (three tests). It also inhibited the contraction induced by 5-HT, and this inhibition could be partly reversed by washing.

As a metabolic poison, we used potassium cyanide (KCN). At a concentration of 1×10^{-5} g/ml (four tests), this immediately relaxed stripes of granuloma pouch previously contracted with 5-HT; however, after these strips were washed, 5-HT was still able to induce a small but definite contraction. In a dose of 1×10^{-2} g/ml (two tests), KCN on its own caused an immediate and deep relaxation; thereafter, even after washing, 5-HT and vasopressin had no effect. Similar results were obtained when these experiments were repeated with strips of rat duodenum.

Chemical Studies

The yield of actomyosin obtained by extraction from granuloma pouch walls (4 mg of actomyosin/g wet weight of pouch tissue) was comparable with that obtained with identically prepared extracts of pregnant rat uteri (3.5 mg/g wet weight). The calcium-activated adenosine triphosphatase activities of these extracts were similar, each splitting approximately 10 nmoles of adenosine triphosphate/mg of protein per min.

Immunology

To establish the specificity (for smooth muscle) of our samples of human anti–smooth muscle (HASM) sera, we prepared sections of rat stomach, cecum, kidney, ureter, bladder, prostate, testis, tendon, skeletal muscle, and skin. In all these tissues the distribution of fluorescence coincided with the distribution of smooth muscle as seen in hematoxylin-eosin sections. In the kidney we found slight staining of glomeruli as has already been described (26). Skeletal muscle was also labeled, but this result is sometimes obtained with normal human serum (27). Fibroblasts from normal tissues were never labeled.

Using the same sera, cytoplasmic labeling was first recognizable in a few cells of granuloma pouches on the 7th day. It reached a maximum between 20 and 30 days (Fig. 8) when fluorescent cells were widely distributed through the whole wall, with the exception of the innermost layer that contained mainly polymorphs and macrophages. Later, as the older granulation tissue became replaced by dense collagen, the outermost layer of the wall lost its fluorescence; by 50 days about half of the wall thickness was labeled.

In the open wounds labeled cells were detectable by 9 days, were more prominent at 11 days, and were still present although less numerous at 13 and 15 days.

No labeling of smooth muscle or of granulation tissue fibroblasts was obtained with either normal serum or serum from a patient with myasthenia gravis.

It should be noted that it was not possible to identify the responsible antigen in this system; it was probably not actomyosin itself because (*a*) pretreatment of HASM serum with purified rat uterus actomyosin (16) did not abolish fluorescence, although pretreatment (18) with uterus homogenate did; (*b*) in agar gel immunodiffusion (28) no precipitation lines formed when HASM serum was allowed to diffuse against actomyosin; and (*c*) HASM serum failed to specifically label the deposit left by a dried, acetone-fixed drop of a suspension of actomyosin precipitate. However, for our present purposes, this does not alter the conclusion that a common antigen is present in smooth muscle and in granulation tissue fibroblasts.

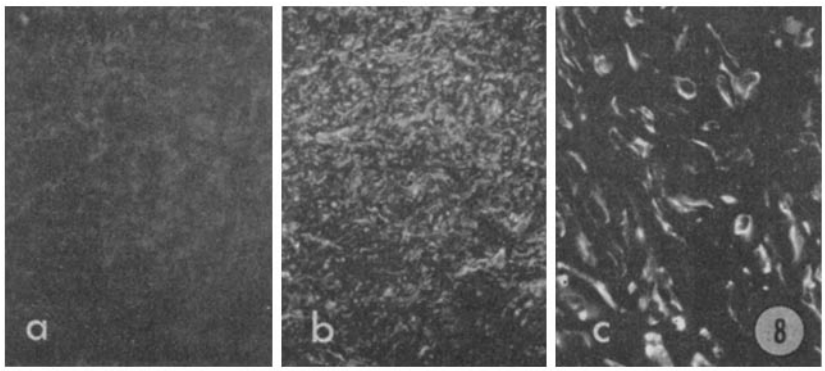

FIG. 8. Immunofluorescent staining of myo-fibroblasts (21 day granuloma pouches). (*a*) Control preparation treated with normal human serum, followed by fluorescent anti-human IgG; no labeling. × 100. (*b*) Serial section of the same tissue treated with human anti–smooth muscle serum, followed by fluorescent anti-human IgG; intense labeling. × 100. (*c*) Higher magnification to show the cellular staining. × 420.

DISCUSSION

Our results show that fibroblasts in granulation tissue progressively assume ultrastructural, chemical, immunologic, and functional characteristics that could well make them responsible for the contraction of such tissue in vivo:

(*a*) The cytoplasm contains a potentially contractile apparatus. This consists of thick bundles of fibrils resembling those in smooth muscle. That this similarity is not simply morphologic is demonstrated by the high content of chemically extractable actomyosin in granulation tissue, and by the selective localization of anti–smooth muscle serum to the cytoplasm of these modified fibroblasts.

(*b*) The nuclear shape provides indirect evidence of contraction. Nuclear indentations and folds have been correlated with cellular contraction in several systems: smooth muscle (29), myocardium (30), venular endothelium (31) and, more recently, the fibroblasts in Dupuytren's contracture (32).

(*c*) The cell surfaces have devices for transmitting contraction to other cells

as well as to the stroma. These take the form of desmosomes and attachments to basal laminae. They are, of course, essential if cellular contraction is to explain an over-all shrinkage of granulation tissue.

(*d*) When tested pharmacologically, strips of granulation tissue behave similarly to smooth muscle. The experiments with metabolic inhibitors (anoxia, KCN) or with specific antagonists (such as methysergide or cyproheptadine) indicate that the contraction of the strips is a cell-mediated phenomenon.

We therefore suggest that, at least in part, the characteristic contraction of granulation tissue depends, ultimately, upon the contraction of these modified fibroblasts. We have proposed the name "myo-fibroblasts" for such intermediate-type cells (11).

It remains to be seen whether the morphologic and functional features of myo-fibroblasts are compatible with their proposed histogenetic origin from fibroblasts. At present most authors agree that fibroblasts of granulation tissues are formed locally from preexisting cells of the same type, or possibly from more primitive mesenchymal cells (3). Although the majority of the cells in our experimental models of granulation tissue are myo-fibroblasts, as judged by electron microscopy and fluorescence, it may be argued that these cells are derived from smooth muscle, e.g., of local blood vessels. We believe that this is unlikely because it would imply that the commonest connective tissue cell, the fibroblast, takes little part in the formation of granulation tissue. The relationship between fibroblasts and myo-fibroblasts receives further support from the fact that fibroblasts cultivated in vitro normally develop extensive cytoplasmic fibrillar systems (33) and intercellular connections (34). In preliminary studies of such fibroblasts obtained from normal rat dermis, we have observed that the addition of 5-HT (1×10^{-5} g/ml) to the culture medium causes cellular contraction within 15–20 min, whereas tryptophane (1×10^{-5} g/ml) has no effect under the same conditions (11).

The pharmacologic experiments reported here do not, by themselves, exclude the possibility that structures other than fibroblasts (e.g. blood vessels) are responsible for the contraction of granulation tissue strips. To clarify this point, we have compared the number and distribution of small vessels in strips of skin and subcutaneous tissue (which do not respond to the active agents) with those in strips of granuloma pouch; no significant difference was found. Moreover, we have produced granuloma pouches by injecting rats subcutaneously with 20 ml of air followed by 5 ml of a 3% solution of carrageenan in water (instead of the croton oil in corn oil). After 2 wk, these carrageenan pouches have a wall consisting mostly of macrophages, blood vessels, and few fibroblasts; they remain soft, flabby and uncontracted in vivo and do not contract in vitro in response to pharmacological agents that are active on the usual croton oil pouch. The number, distribution, and age of the small vessels in the two types of pouches are virtually identical, thus supporting the view that granulation tissue blood vessels are not responsible for our results.

The pharmacologic tests show some differences between the reactivity of granulation tissue strips and that of classical smooth muscle preparations. For example, granulation tissue strips failed to react to barium chloride and acetylcholine, agents which normally cause contraction of smooth muscle. Furthermore, the pattern of response is somewhat different, in that the peak of contraction is reached more slowly and maintained longer by the granulation tissue strips; in some instances (e.g. after stimulation by 5-HT) the contraction remained stable at the peak for more than 2 hr. This "spastic" behavior suggests the presence of a contractile system similar to the "catch muscles" of invertebrates (35), and falls well into place with the biological process of wound contraction which is relatively slow but continuous.

There also appear to be differences in the reactivity of granulation tissues from different sources. Thus, granuloma pouch strips are sensitive to 5-HT whereas wound strips do not respond to this agent, under the same conditions of testing. This we cannot explain.

Previous studies using linear wounds have failed to show the presence of myofibroblasts in granulation tissue (3). Since the healing of a linear wound takes place with little or no contraction, the discrepancy is understandable.

Some normal "fibrous" tissues, such as the splenic capsule (36) and the tunica albuginea of the testis (37), contract in vivo and in vitro. This property has, however, been correlated with the presence of smooth muscle cells (36, 37).

It is of interest that cells ultrastructurally similar to the myo-fibroblasts described here have been recently observed in certain normal tissues such as chicken aorta (38) and rat ovary (39), although their significance is not yet understood. In addition, smooth muscle cells with fibroblastic features have been described in the uterus of rats treated with estrogens (19) and in human or experimental arteriosclerotic lesions (40, 41). Furthermore, there is evidence that smooth muscle can produce collagen and elastin (19). These data indicate that smooth muscle can assume morphologic and functional characteristics of fibroblasts; our experiments suggest that the reverse process is also possible, i.e., that fibroblasts can become modified into smooth muscle-like cells with a contractile capacity. This modification occurs not only in contracting granulation tissue but also in the nodules found in the palmar aponeurosis of patients with Dupuytren's contracture (32), one of the so-called fibromatoses (42). The nodular cells of Dupuytren's contracture resemble smooth muscle ultrastructurally (32), and in recent experiments we have found them to label with human anti-smooth muscle serum.

In conclusion, it appears more and more obvious 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. Further work is needed to clarify the nature and mechanism of myo-fibroblast formation. However, the results reported here already support the conclusion that, under certain conditions, fibroblasts can

differentiate into a cell type structurally and functionally similar to smooth muscle, and that this cell plays an important role in the contraction of connective tissue, a process that is beneficial in wound closure but potentially harmful in other situations.

SUMMARY

Contracting granulation tissues contain fibroblasts that develop characteristics typical of smooth muscle: (*a*) They contain an extensive cytoplasmic fibrillar system. (*b*) They show immunofluorescent labeling of their cytoplasm with human anti-smooth muscle serum. (*c*) The nuclei show complicated folds and indentations, indicative of cellular contraction. (*d*) There are cell-to-cell and cell-to-stroma attachments. (*e*) It is possible to extract similar quantities of actomyosin (having the same adenosine triphosphatase activity) from granulation tissue and from pregnant rat uterus. (*f*) Strips of granulation tissue, when tested pharmacologically in vitro, behave similarly to smooth muscle.

All these data support the view that, under certain conditions, fibroblasts can differentiate into a cell type structurally and functionally similar to smooth muscle and that this cell, the "myo-fibroblast," plays an important role in connective tissue contraction.

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