A Picropolychrome Staining Technique Applied to Dupuytren's Tissue

A. M. P. FITZGERALD, J. J. R. KIRKPATRICK, I. T. H. FOO and I. L. NAYLOR

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A PICROPOLYCHROME STAINING TECHNIQUE APPLIED TO DUPUYTREN'S TISSUE

A. M. P. FITZGERALD, J. J. R. KIRKPATRICK, I. T. H. FOO and I. L. NAYLOR

From the Plastic Surgery and Burns Research Unit, the Department of Biomedical Sciences and the Department of Pharmacology, University of Bradford, West Yorkshire, UK

Although the histology of Dupuytren's tissue is well-documented, conventional stains do not distinguish between the different types of collagen which biochemistry and immunochemistry suggest are present. Dupuytren's specimens [nodules ($n=26$), cords ($n=15$) and dermofasciectomies ($n=6$)] were stained with haematoxylin and eosin, Van Gieson's, Mallory's, Masson's, and Herovici's picropolychrome stain, and examined for both cellularity and collagen staining characteristics. All stains illustrated the marked cellularity of the nodules, contrasting with a paucity of cells within the cords. The first four stains demonstrated uniformity of the collagen staining within the tissues. Herovici's picropolychrome, however, showed distinct staining patterns for the dermis, nodules and cords, with both purple/red and blue areas. Other studies suggest that those fibres stained purple/red and blue are types I and III collagens respectively. These findings may shed further light on the tissue of origin of Dupuytren's disease.

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Much controversy remains over the tissue of origin of Dupuytren's disease. In 1614, Plater wrongly attributed the condition to shortening of the flexor tendons, an error which was corrected by Cline in 1777 and Cooper in 1822 who were the first to correctly implicate the palmar fascia as the tissue of origin (Verheyden, 1983), although it was not until 1831 that Dupuytren described the condition (Dupuytren, 1832).

Until the early 1970s, the mechanism of contracture was thought to be by shortening of the collagen fibres due to their ability to contract on denaturation in vitro. In 1972, Gabbiani and Majno demonstrated the presence of cells within the nodules of diseased palmar fascia that had morphological characteristics of both fibroblasts and smooth muscle cells, naming them "myofibroblasts". Subsequently, these were shown to have both contractile properties and a means by which to transfer the produced force to the collagen fibres of the cord (Chiu and McFarlane, 1978). Clearly their role in Dupuytren's disease was established but their source of origin continues to be an enigma.

For many years, debate has centred on whether the tissue of origin is within the palmar fascia itself (intrinsic theory) or the dermis (extrinsic theory). As early as 1832, Goyrand suggested that the disease may originate outside the palmar fascia (Elliot, 1989). Supporters of the intrinsic theory have included Skoog (1948) who suggested that micro-ruptures of the palmar fascia led to healing by fibrosis and Millesi (1959) who contested that the nodules initiated within the vertical fibres of the palmar fascia. Biochemical analysis (Menzel et al, 1979; Bazin et al, 1980; Gelberman et al, 1980; Brickley-Parsons et al, 1981) and immunochemical studies (Bailey et al, 1977; Pasquali-Ronchetti et al, 1993) have both demonstrated an increased ratio of type III to type I collagen within the palmar fascia even before clinical symptoms and signs appear. Furthermore, McGrouther (1982) has demonstrated that fibrosis is initially seen at points of friction between the longitudinal and vertical fibres of the palmar fascia.

The main proponent of the extrinsic theory has been Hueston (1984), who proposed that the overlying dermis may have a regulatory role over the palmar fascia. This has been supported by the much lower recurrence rate following dermofasciectomy rather than fasciectomy and the clinical observation that Dupuytren's disease is never found on the dorsal aspect of the palmar fascia.

A traditional method of investigating the tissue of origin has been the examination of stained sections of palmar cords and nodules using classical histological stains. The literature suggests that the four most commonly used stains are haematoxylin and eosin, Van Gieson's, Mallory's and Masson's stains (Larson and Posch, 1962). These stains remain in widespread use today for the staining of connective tissues although unfortunately they produce no selective differentiation of the collagen fibres. In Dupuytren's disease for example, the collagen fibres all appear to be similar.

Clearly, on the basis of such stains, all this is at odds with biochemical and immunochemical analyses performed over the last 15 years. These have established the presence of both types I and III collagen within the diseased palmar fascia in contrast to the negligible amounts of type III collagen within normal control palmar fascia. Furthermore, an increased ratio of type III to type I collagen occurs before the appearance of any clinical signs or symptoms. This has never been demonstrated using simple histological techniques.

In 1963, Herovici described a variant of the picropolychrome stain. This contained three stains, one each for the nuclei, cytoplasm and connective tissues. He initially used this for the investigation of nervous tissues but noted that the connective tissues stained both purple/red and blue in a reproducible manner. Surprisingly, this stain was little used in investigations of connective tissues and has never been used to examine

tissue samples from Dupuytren's disease. The relevance of this stain is perhaps shown by the studies of Levame and Meyer (1987) who used immunochemical techniques to confirm that those tissues which stained purple/red were in fact composed of type I collagen and those which stained blue were composed of type III collagen. We therefore set out to compare and contrast Herovici's stain with those already mentioned, in the investigation of Dupuytren's disease.

MATERIALS AND METHODS

Diseased palmar fascia was obtained from 47 consecutive patients who had either undergone fasciectomy, when the specimens were clearly labelled as either nodule ($n=26$) or cord ($n=15$), or dermofasciectomy ($n=6$). There were 39 male and eight female patients with an age range of 47–82 years.

The specimens were fixed in 10% neutral formalin, embedded in lambswax and sections were cut at 7 mm. The sections from each specimen were then stained with haematoxylin and eosin, Van Gieson's, Mallory's, Masson's (standard stains) and Herovici's picropolychrome stain and then studied by light microscopy, using a Leitz Orthoplan microscope. Standard staining procedures were used as described by Gabe (1976) and Herovici (1963). Colour photomicrographs were taken using a tungsten-based Kodak Ektachrome film.

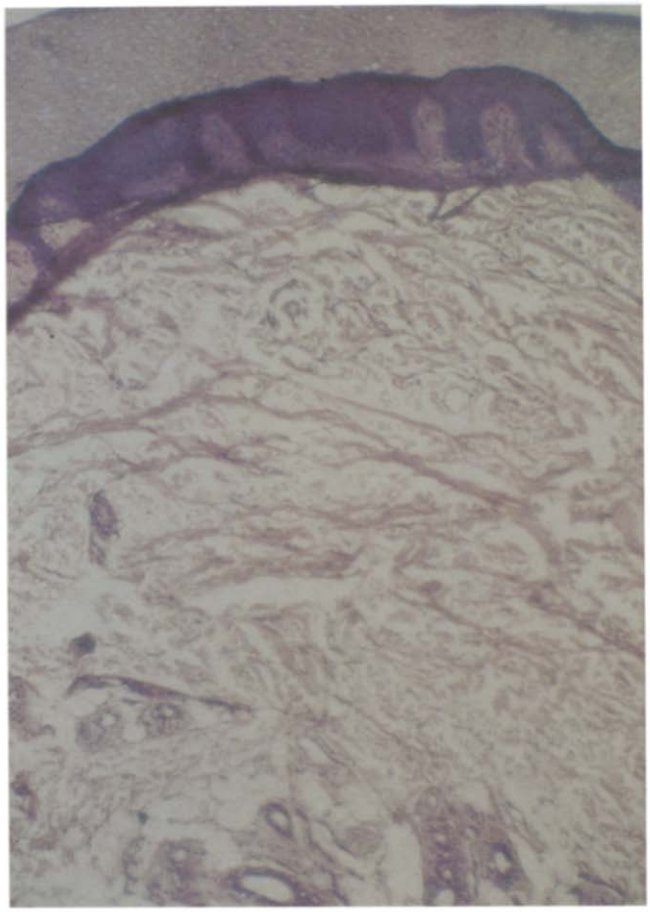

Fig 1 Dermofasciectomy specimen stained with Haematoxylin and Eosin (magnification × 26).

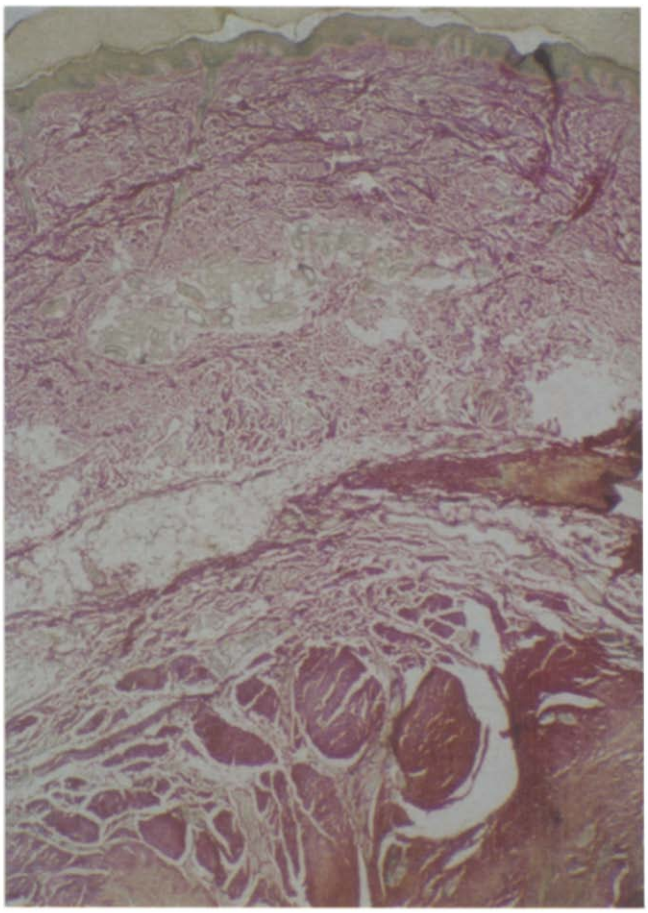

Fig 2 Dermofasciectomy specimen stained with Van Gieson's stain (magnification × 36).

RESULTS

All the stains studied revealed the marked cellularity and heterogeneity of the nodules in contrast to the sparse cellularity and apparent uniformity of the cords. The nodules were found to be densely packed with spindle-shaped cells that had irregular, swollen and hyperchromatic nuclei. The cells themselves formed either a random pattern or recognisable whorls even within different parts of the same nodule. Blood vessels entered the nodules and the peri-vascular areas were particularly densely populated with fibroblasts.

Standard stains revealed the cords to be relatively acellular but consisted mainly of coarse collagen fibres

parallel to the line of the cord. Those cells that were present consisted of mature flattened fibrocytes.

Each standard stain produced its own individual single colour staining pattern for the connective tissues. Haematoxylin and eosin (H and E) stained the connective tissues a uniform pink throughout, with poor definition of the collagen fibres (Fig 1). Van Gieson's, staining the connective tissues red, offered some advantage over H and E in that there was better definition of the collagen fibres allowing the variability in fibre size to be seen. This stain particularly highlighted the sweat glands and ducts (Fig 2). Mallory's stain produced such an intense blue staining pattern that no further information could be gathered over and above those stains already mentioned (Fig 3). Even Masson's trichrome stain once again produced a uniform green colouration of the connective tissues (Fig 4).

In contrast, Herovici's picropolychrome stain indicated a marked heterogeneity in the connective tissues that were coloured both a purple/red and blue (Fig 5).

Unlike the other stains that produced homogeneous colouring of the nodules, Herovici's produced a distinct dual staining pattern, both purple/red and blue (Fig 6).

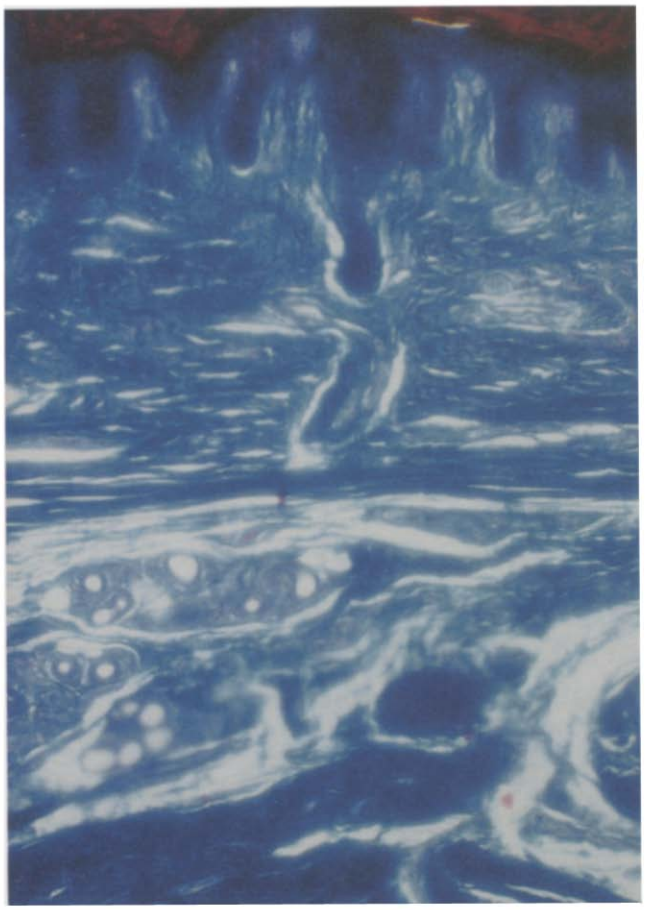

Fig 3 Dermofasciectomy specimen stained with Mallory's stain (magnification × 72).

Fig 4 Dermofasciectomy specimen stained with Masson's stain (magnification × 27).

The type III collagen as suggested by its blue colouration was more apparent lying towards the palmar surface. The nodules were found to always lie upon the superficial aspect of the palmar fascia.

The cords were found to be predominantly purple/red staining fibres which are identified as type I collagen fibres lying parallel to the direction of the cord, blue type III collagen fibres being found between the type I collagen fascicles. It was interesting to note that the blue fibres had a distinctly finer structure being more fibrillar in nature than their purple/red counterparts.

Another incidental finding was that the dermis was stained both purple/red and blue, the proportion of blue staining fibres being greater in the papillary than the reticular dermis.

The dermofasciectomy specimens readily revealed the presence of Pacinian corpuscles lying immediately above the palmar fascia having an onion-like appearance the layers of which were all stained blue apart from its centre which stained purple/red (Fig 7a). This contrasts with all the other stains that indicated a uniform appearance (Fig 7b).

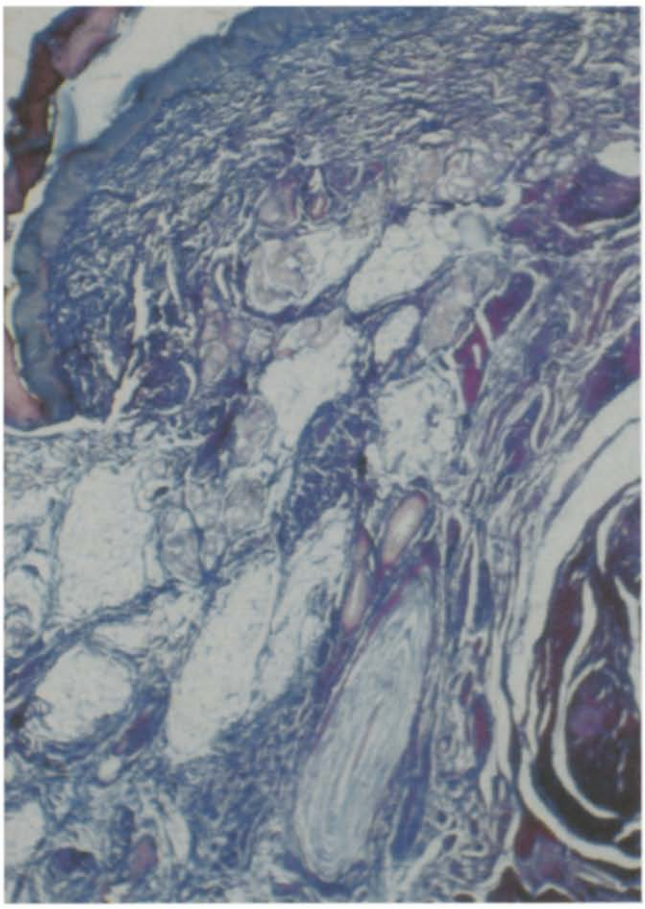

Fig 5 Dermofasciectomy specimen stained with Herovici's picropolychrome stain (magnification × 68).

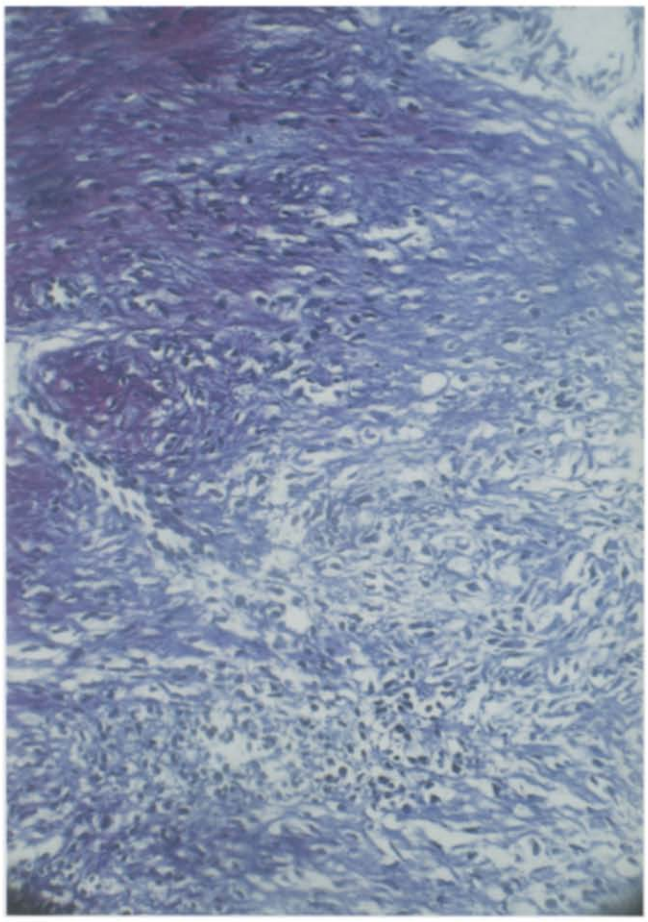

Fig 6 Dupuytren's nodule stained with Herovici's picropolychrome stain (magnification × 90).

DISCUSSION

As early as 1897, Anderson used histological techniques to determine the tissue of origin of Dupuytren's disease and believed that an "inflammatory process" was initiated in the subcutaneous adipose layer and subsequently spread to involve the palmar fascia (Larson and Posch, 1962). Meyerding et al (1941) described the presence of pathological changes within the skin of Dupuytren's specimens and therefore proposed that the disease started in the connective tissues above the palmar fascia as a fibrous response to an inflammatory reaction which then spread to involve the palmar fascia, ultimately producing the contracture. There is, therefore, a precedent for the use of histology in the scientific investigation of Dupuytren's disease, and with the use of Herovici's stain that role may be extended.

The five stains used were all able to demonstrate the highly cellular nodules with their varied hyperchromatic nuclei. However, standard stains could only display uniformity of the collagen fibres. Herovici's stain was able to distinguish between two types of collagen fibre, the coarse purple/red type I collagen fibres and the finer blue type III fibres in both a reliable and reproducible manner. Why should this be so important? Brickley-Parsons et al (1981) have demonstrated an increased ratio of type III to type I collagen in the apparently normal fascia of an affected hand, even before clinical signs have appeared.

We feel that this simple histological technique has advantages over both biochemical and immunochemical methods. Biochemical analysis requires homogenization of tissues under investigation and cannot therefore provide information with respect to the spatial distribution of collagen types, only that they are present and in what proportion. Immunochemical analyses often require frozen sections, are time-consuming and can be expensive.

A striking use of this stain was found in its ability to differentiate between collagen types within Pacinian corpuscles unlike any of the other stains. This is interesting, as previous histological studies of Dupuytren's disease (Broadbent, 1955; Nezelof and Tubiana, 1958) have shown enlarged Pacinian corpuscles although no obvious structural abnormality has previously been

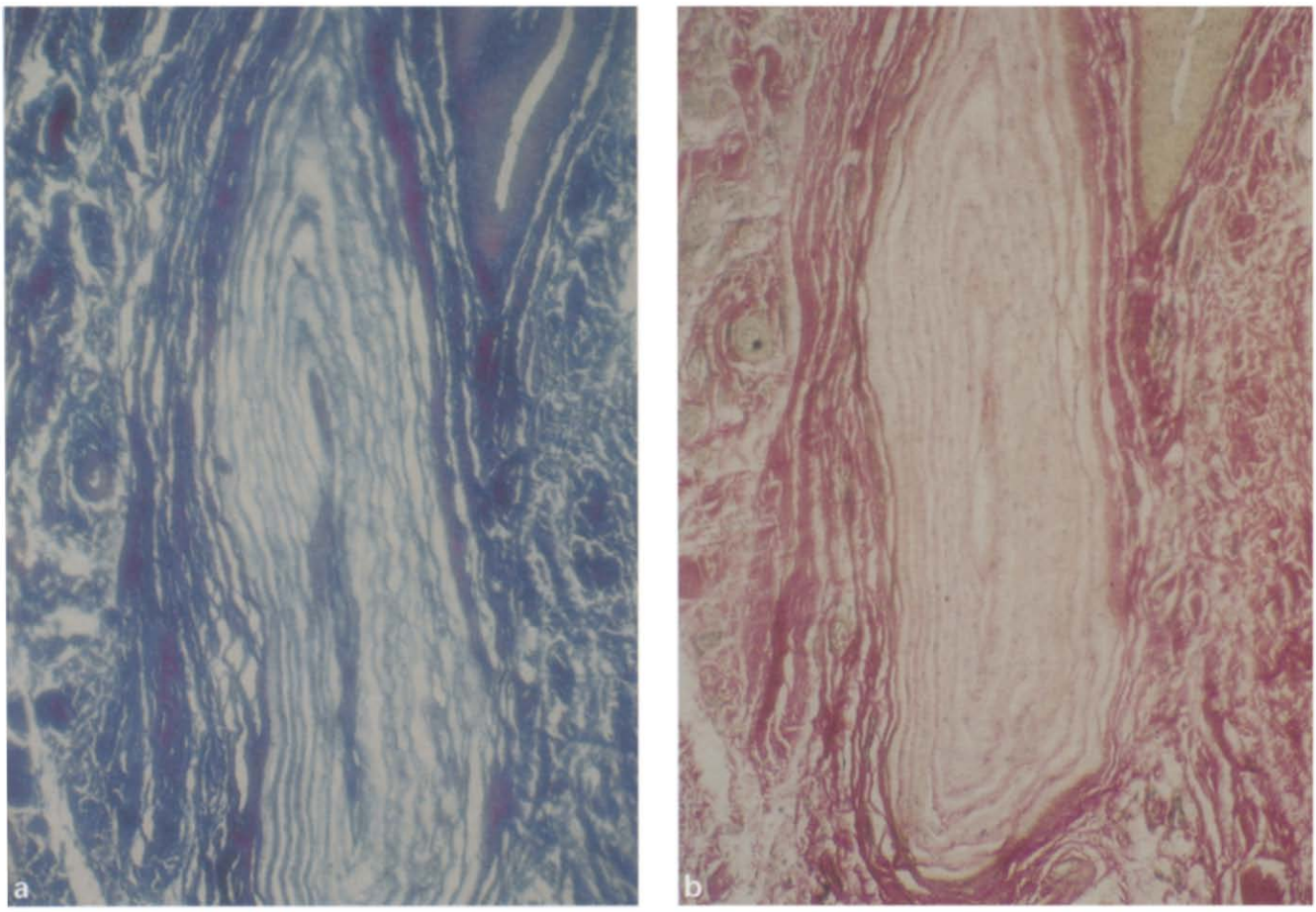

Fig 7 (a) Pacinian corpuscle stained with Herovici's picropolychrome stain (magnification × 20) and (b) Pacinian corpuscle stained with Van Gieson's stain (magnification × 20).

illustrated within the corpuscle itself although further investigation with Herovici's stain may suggest otherwise. It is interesting to speculate that sensory perception may be facilitated by the greater deformability of type III collagen rather than the rigidity of some of the other collagen types.

Luck (1959) categorized the process of Dupuytren's disease into three successive stages, namely proliferative, involutional and residual. Subsequently Meister et al (1979), using immunochemical techniques, proved that as the process passed through each stage the proportion of type III collagen decreased. Herovici's stain now provides an opportunity to view this histologically.

We have demonstrated histologically for the first time, the presence of type III collagen with the palmar fascia. Since nodules have staining characteristics similar to those of the dermis rather than the cord, the origin of the cells within the nodule may be from the dermis rather than the pre-existing fascia.

Herovici's stain in this study has shown that in Dupuytren's disease the proportion of type III collagen diminishes from the papillary dermis through the reticular dermis and subcutaneous tissues down to the nodules and finally the cords where there is little type III collagen. Could it be that fibroblasts producing type III collagen are migrating down from the dermis to the palmar fascia? It may even be possible that these fibroblasts could be the agent through which the dermis regulates the palmar fascia as Hueston (1984) surmised. May in fact these fibroblasts actually be myofibroblasts? McCann et al (1993) showed that myofibroblasts lie within the dermis of dermofasciectomy specimens, explaining the low recurrence rate associated with this operation (Brotherston et al, 1994). It is well-known that if the diseased fascia is simply removed, recurrence is a problem following fasciectomy. This problem could be due to fascia at the edge of the area removed but since it is the same finger which is involved in the recurrence could not a simpler explanation be the migration of cells from the dermis to the site and their subsequent proliferation?

Studies to characterize the role of collagen types I and III and myofibroblasts are currently in progress and may answer these questions.

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Mr A. M. P. Fitzgerald, Plastic Surgery and Burns Research Unit, Department of Biomedical Sciences, University of Bradford, Bradford, West Yorkshire BD7 1DP, UK