3 Histopathology

NORMAL PALMAR SKIN AND SUBCUTANEUM

Palmar skin (Fig. 3.1) is similar to the skin of the sole, but differs from skin covering the rest of the body. Skin of the palms and soles is non-hair-bearing and possesses the thickest horny and epidermal cell layers. The average thickness of the epidermis of palmar skin is 1.6 mm, as compared with 0.04 mm on the eyelids. The only adnexal structures present are the eccrine sweat glands and nerve end organs. Eccrine glands have basally located secretory lobules from which a duct emerges to conduct the secretion on to the surface of the epidermis. Special nerve end organs include the Meissner's corpuscles which are located in dermal papillae and the Vater–Pacinian corpuscles which are large nerve end organs located in the subcutis and which mediate a sense of pressure.

As in other parts of the body, the dermis of the palmar skin can be divided into the papillary dermis, which is the thin zone immediately beneath the epidermis and surrounding the epidermal rete ridges and, lying deeper, the thicker reticular dermis. Vital to the dermis are the fibroblasts, which have spindle-shaped cell bodies and nuclei. Three types of fibres can be recognized in the dermis by light microscopy; these fibres are all produced by the fibroblasts. Collagen represents by far the most abundant constituent of the filamentous components of the dermis. The diameter of collagen fibres on light microscopy ranges from 2 to 15 μm. Reticulum fibres are recognizable by impregnating sections of dermis with silver nitrate; they are also known as agyrophilic fibres. Reticulum fibres are first formed during embryonic life and appear
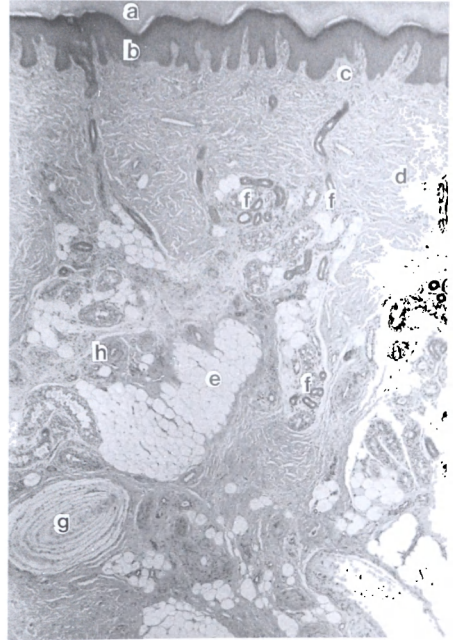

Fig. 3.1 Normal skin and subcutaneum of palm (haematoxylin & eosin; × 10 original). a stratum corneum; b epidermis; c papillary dermis; d reticular dermis; e subcutaneous fat; f eccrine sweat gland and duct; g Pacinian corpuscle; h artery.

around the third month of gestation. As these fibres increase in number and become thicker, they are organized into bundles, lose their agyrophilic properties and become collagen fibres.

25

The elastic fibres, the third type of filament, on the hematoxylin–eosin-stained sections appear as wavy, thin, 1–3 μm eosinophilic refractile fibres. They are especially highlighted by stains like orcein or Verhoeff–VanGieson. Under the electron microscope, elastic fibres lack periodicity, but are seen to consist 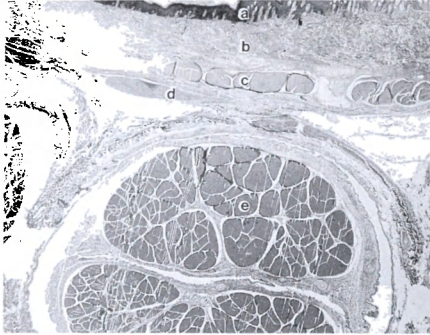of two components. Elastin is the amorphous protein occupying the centre of each fibre whereas the microfibrils — thread-like fibrillary protein — are embedded within the elastin and are numerous at the periphery of individual fibres, giving it a frayed appearance. While collagen is a structural protein and forms a fibrous network of support, elastic fibres provide the normal elasticity and resilient character of the skin. Unique to the palm is the palmar aponeurosis. This three-dimensional ligamentous system of the palm comprises three groups of collagenous fibres (Fig. 3.2), as described in Chapter 12.

Fig. 3.2 a Transverse section of normal palmar skin through distal palmar crease (haematoxlyin & eosin; × 10 original). a Epidermis; b dermis; c longitudinal fibres; d deep transverse fibres; e flexor tendon.
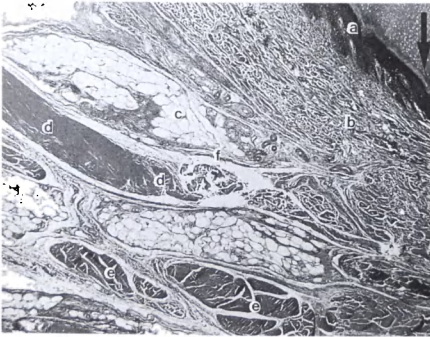

Fig. 3.2 b Longitudinal section of normal palmar skin just proximal to the proximal digital crease. Left: direction of proximal palm (haematoxlyin & eosin; × 25 original). Arrow = proximal digital crease. a Epidermis; b dermis; c subcutaneous fat; d longitudinal fibres merging with dermal collagen; e deep transverse fibres; f delicate vertical fibres.

HISTOPATHOLOGY

Even with the light and electron microscope techniques of the present day, there can be little improvement on Meyerding et al's (1941) paper: 'The Etiology and Pathology of Dupuytren's Contracture'. They wrote: 'other investigators have focused their attention on the palmar fascia to the exclusions of the surrounding tissue' and 'that Dupuytren's contracture is not merely a disease of the palmar fascia, but involves all structures from the skin down to the tendon sheaths'. As shown in Figure 3.3, we now know that this is certainly the case, and that the fibrosis, especially in more advanced cases, extends to the overlying dermis and surrounding subcutaneous tissue.

Meyerding et al described changes in the palmar fascia as well as in the adjacent tissues. In the former, they observed that: 'the characteristic change is the proliferation of fibroblasts in the nodules of the contracture'. More importantly, they noted the variable cellularity in lesions of Dupuytren's disease (DD) (Fig. 3.4). Meyerding was the first investigator to attempt a functional interpretation of the histopathological changes by assuming that the cellularity of the nodules was indicative of the activity of the disease. He suggested a gradation of one to four to reflect the activity of the proliferative process. With regard to the surrounding connective tissue changes, he noted that in advanced cases of DD 'evidence of subcutaneous adipose tissue is not revealed and sweat glands are rare or completely absent' (Meyerding et al 1941). This was the result of an increase in
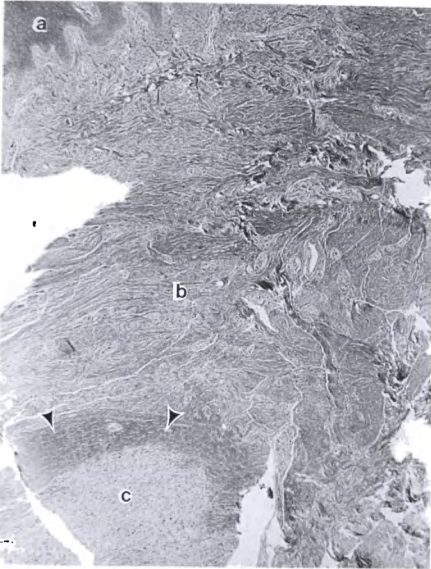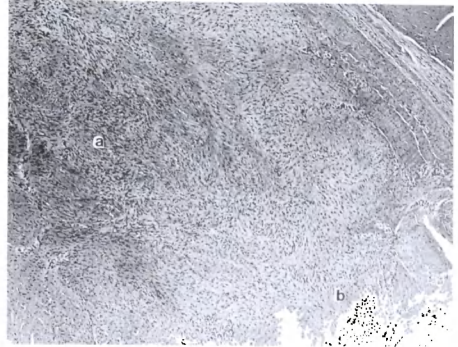
Fig. 3.3 Advanced lesion of DD. Note the nodular configuration and extension into dermis (haematoxylin & eosin; × 10 original). a epidermis; b dermis; c pale staining, cellular fibrous tissue of DD. Arrows = thick collagen fibres at periphery of lesion.

Fig. 3.4 Varying cell density within an active lesion of DD (haematoxylin & eosin; × 40 original). a cellular and dark staining area; b fibrous and less cellular area.

Fig. 3.5 Active lesion of DD resulting in loss of normal subcutis and adnexal structures (haematoxylin & eosin; × 40 original). a Encroachment of subcutaneous fat by DD. Arrows = perivascular lymphocytic infiltrate.
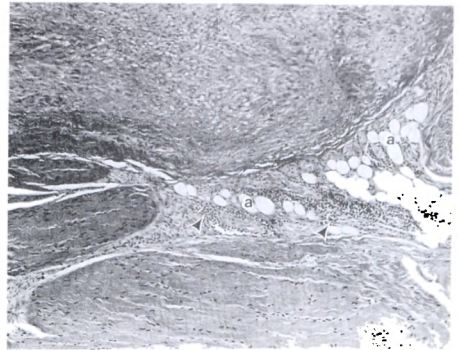
the size and number of connective tissue bands which normally separated the lobules of fat. There was also an increase in the number of capillaries in the interstitial connective tissue and the capillaries were infiltrated by lymphocytes (Fig. 3.5).

Such detailed and accurate accounts of the histopathological changes noting the involvement of not only the fascia, but the adjacent subcutaneous tissue and overlying skin, surpassed all previous descriptions.

Luck (1959) further classified the disease into three stages: proliferative, involutional and residual. He subdivided diseased tissue into essential fibrous nodules, reactive tissue and residual tissue. He defined the essential fibrous nodule as the initial lesion in the proliferative stage; he stated that histologically the nodule was a focus of proliferating fibroblasts that resembled a fibroma. He wrote: 'in this focal fibroplasia, the fibroblasts

do not align themselves with lines of stress and have, in fact, no purposeful arrangement' (Fig. 3.6). The involutional stage was characterized by fibroblasts aligning themselves with major lines of stress that pass through the nodules (Fig. 3.7). Luck considered that the fibrous cords represented reactive functional hypertrophy in response to the repeated tension stresses on the hand of fascia from which the nodule took its origin, hence the term reactive tissue. Finally,
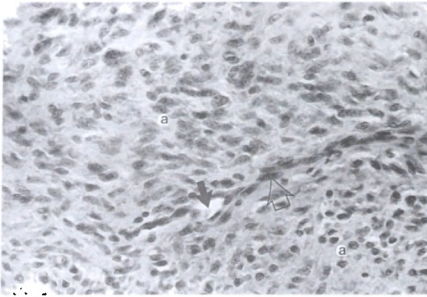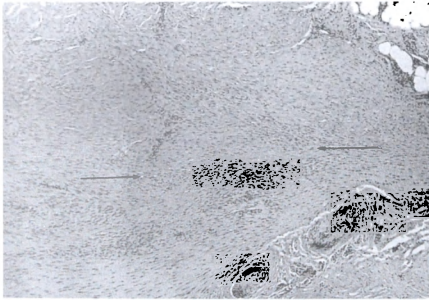
Fig. 3.6 Essential fibrous nodule as defined by Luck (1959) (haematoxylin & eosin; × 100 original). a Haphazardly arranged fibroblasts. Solid arrow = capillary lumen; hollow arrow = endothelial cells.

Fig. 3.8 Dense fibrous cord of residual stage (haematoxylin & eosin; × 25 original). Arrows = spindle-shaped nuclei of fibrocytes in between dense collagenous fibres.

Fig. 3.7 Reactive tissue during the involutional stage of DD. The nodular configuration of the proliferative lesion is lost. (Haematoxylin & eosin; × 25 original). Arrows = alignment of fibroblasts along lines of stress with loss of the nodular configuration of initial lesion.
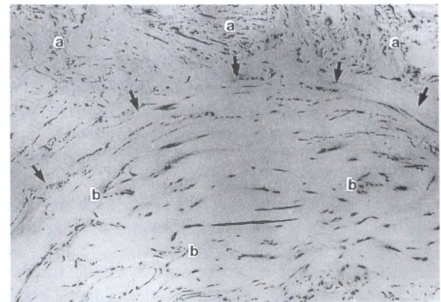

Fig. 3.9 A Dupuytrens nodule during the proliferative stage of the disease. The section was reacted with antibodies against factor VIII in order to demonstrate the endothelial cells of the capillaries (factor VIII antibodies peroxidase stain; × 10 original). a Normal vessels in surrounding subcutaneous tissue; b arborescent network of vessels in DD. Arrows = nodular boundary of Dupuytren's nodule.

with the complete involution of the nodule came the residual stage which he described as follows: 'the nodule disappears, leaving only a focus of dense adhesions and the reactive proximal fibrous cord which is almost acellular and tendon-like' (Fig. 3.8).

MacCallum & Hueston (1962) continued the investigation with their publication entitled 'The Pathology of Dupuytren's Contracture'. In their microscopic observations they recognized two rather than three phases of activity, and they correctly pointed out that the two phases may freely intermingle or be separated in a lesion. Their attention focused on the increased vascularity in the surrounding tissue and within the hyperplastic foci where they stated that: 'the new fibroblasts are arranged around sheets of branching blood vessels' (Fig. 3.9). They were first to suggest that: 'the sequence [of production of the Dupuytren's lesion] can be followed from vascular invasion and perivascular cellular proliferation to a maturing
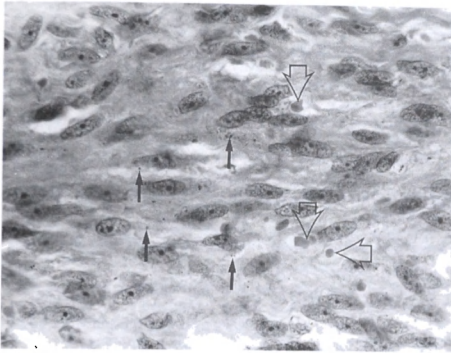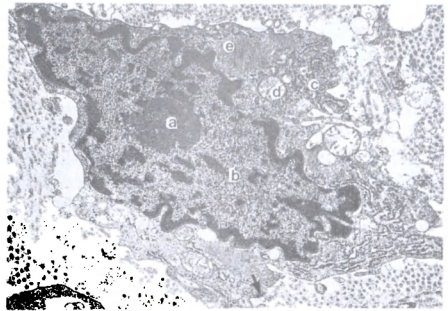
Fig. 3.10 Proliferative stage of Dupuytren's nodule. The haemosiderin pigment derived from breakdown of extravasated red cells is yellow-brown in colour (haematoxylin & eosin; × 1000). Solid arrows = yellow-brown haemosiderin pigment; hollow arrows = extravasated red blood cells.
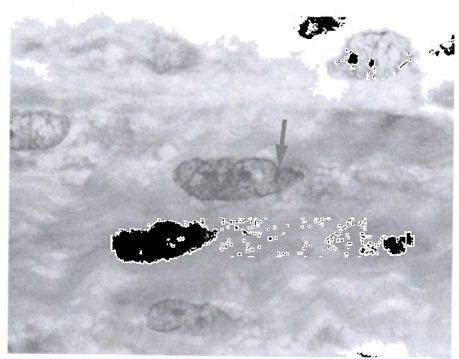
Fig. 3.11 Myofibroblasts in a Dupuytren's nodule (haematoxlyin & eosin × 1250 original). Arrow = myofibroblast with cross-banded nucleus.

Fig. 3.12 Electron micrography of a myofibroblast from a Dupuytren's nodule (× 14 000 original). a nucleolus; b nucleus with irregular nuclear membrane; c cytoplasm; d mitochondria; e intracellular fibrils with electron densities; f collagen. Arrow = hemidesmosome.

nodule and finally to a relatively acellular dense atrophic tendinous band'. They also noted microhaemorrhages and deposits of haemosiderin pigment, in about one in ten specimens of DD usually in the hyperplastic foci (Fig. 3.10). This finding was interpreted as evidence of past interstitial haemorrhage from recently formed ingrowing capillaries.

Gabbiani et al (1971) used electron microscopy technique to study granulation tissue and discovered a special type of fibroblast that was capable of 'modulating' into cells very similar to smooth muscle cells. In 1972, Gabbiani & Majno reported on six cases of DD in which they specifically looked for these specialized or adapted fibroblasts, the so-called 'myofibroblasts' which they believed were the driving force behind the contraction of the palmar fascia and with it the overlying skin and digits. In this study, new features were reviewed both at the light and electron microscopic levels. With the light microscopy, they reported on the presence of intercellular fibrils which were visible as 'red streaks or filaments' with Masson's trichrome stain in cells of Dupuytren's nodules; these cells had 'crossbanded' nuclei (Fig. 3.11). Ultrastructurally, they reported that these cells had three distinctive features (Fig. 3.12). First, a complex system of

intracellular fibrils with electron densities similar to the dense bodies in smooth muscle cells was present. Second, the nuclei contained frequent indentations and folds which the authors thought corresponded to the nuclear cross-banding seen under light microscopy. Third, they described the surface differentiations of these cells which consisted of basement membrane and hemidesmosomal structures. Indeed, ample ultrastructural morphological evidence was presented to support their hypothesis that these myofibro-

blasts possessed the essential intracellular bundles of fibrils, and cell surface adaptations for transmission of forces. The nuclear foldings were indirect evidence that these cells had been shortened during contraction. Their observations were later confirmed by Hueston et al (1976) and Chiu & McFarlane (1978) who reported that myofibroblasts were seen in the nodules,

Another innovative technique using fluorescent dye tagged on to antibodies was used by Hueston et al (1976) who demonstrated 'smooth muscle antigens' in 10 cases, thus confirming that cells akin to smooth muscle cells were present in lesions of DD.

Articles by Iwasaki et al (1984), Ushijma et al (1984) and Nezelof (1985) have further reviewed the histopathology of DD and a summary of pertinent changes at various stages of the disease is given in Table 3.1.

CONCLUSIONS

Histopathology is the basis of our knowledge. To date, however, it is probable that all significant histological features of DD have been recognized; it is unlikely that histological appearance alone could lead to any further breakthrough in our understanding of the disease.

The use of monoclonal antibody against desmin has demonstrated a subpopulation of desmin-positive cells in the proliferating lesion of DD which is undetectable with the haematoxylin & eosin stain (Shum & McFarlane 1988). Similar studies by Schürch et al, reported in the next chapter, using innovative techniques and multidisiplinary research in areas of molecular biology, bio-

Table 3.1 Summary of histopathological changes*

Features of early lesions
1. Focal increase in vascularity with ingrowth of capillaries and proliferation of endothelial cells.
2. Proliferation of perivascular cells, many of which are desmin-positive and probably akin to perivascular smooth muscle cells.
3. Formation of angiocentric, nodular lesion by centrifugal spread of proliferating cells.
4. Extravasation of red cells focally from the newly formed capillaries.
5. Production of reticulum but not elastic fibres.

Features of intermediate lesions
1. Proliferating cells are undergoing morphosis from cells with round nuclei and scanty cytoplasm to spindle-shaped cells with indented, elongated nuclei and a moderate amount of cytoplasm. These are the morphological features of myofibroblasts.
2. Lesion becomes less cellular with increasing stromal collagen.
3. Alignment of cells along lines of stress.
4. Fibrous infiltration of adjacent skin and subcutaneous tissue.
5. Focal deposition of a haemosiderin pigment as a result of degeneration of extravasated red cells.
6. Lymphocytic infiltration.

Features of late lesions
1. Relatively acellular cords of densely packed and aligned collagen fibres.
2. Cells appear as typical fibrocytes with hyperchromatic, wavy and spindle-shaped nuclei between dense collagen bundles.
3. Few cells are positive for desmin-type intermediate filaments.

*As different stages of microscopic changes occur even within a small nodule, careful examination of all tissue removed is necessary. The activity or chronological age of a lesion is best indicated by the area with the highest cellularity. All of these features are seen in haematoxylin & eosin sections under light microscopy.

chemistry and cytogenetics, should provide more meaningful interpretation and new insight into the familiar morphological tissue changes in DD.