absorption of dissolved oxygen was by far the dominant source of oxygen for the fish, with photosynthesis being a small contributor. The fish were able to maintain a high rate of metabolism and hence a high demand for oxygen, even though the concentration of oxygen in the water was low. This ability to maintain high rates of metabolism despite low oxygen concentrations is referred to as hypoxia tolerance. Some species of fish are able to tolerate hypoxia for extended periods, while others show signs of distress and mortality even at relatively low oxygen concentrations. The mechanisms underlying hypoxia tolerance are not well understood, but may involve increased oxygen transport efficiency, increased oxygen extraction efficiency, and increased capacity for anaerobic metabolism. The study of hypoxia tolerance in fish has important implications for the conservation of many fish species that live in habitats with low oxygen levels, such as rivers and estuaries.
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Chart 1. Contraction of doxorubicin-induced ulcers in rat skin with time. Bars, S.E.

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tures underwent significant change. Rough endoplasmic reticulum was dilated or in small loops. Multiple vacuoles with double membranes were also seen. Through 8, 12, and 14 days, many of these vacuoles persisted (Figs. 1 and 2). Many of the cells were disrupted with lysis of the cell membrane. Considerable swelling of mitochondria was noted in some cells.

Rough endoplasmic reticulum persisted in unusual patterns with aggregation and rosettes through 3 weeks. Intracellular mitochondria were frequently disrupted, and many blood vessels were filled with cellular debris.

Not until 4 weeks after doxorubicin injection were cells seen suggestive of early myofibroblasts. At this time, small bundles of 60- to 80-Å microfilaments occurred with electron-dense bodies plus occasional desmosomes (Fig. 3). Of the fibroblasts present at 4 weeks, 10 to 15% had small bundles of microfilaments. At 6 weeks, cells were again seen containing microfilament bundles suggestive of myofibroblasts and also containing many microtubules. Weekly biopsies through 11 weeks continued to demonstrate cells with characteristics of myofibroblasts but with quite small bundles of microfilaments and vaguely defined electron-dense bodies. Microtubules were prominent in the cells during this period (Fig. 4). Mitochondrial degeneration and dilated rough endoplasmic reticulum persisted in some cells. By 12, 14, and 20 weeks, cells characteristic of myofibroblasts were no longer seen, and by 14 and 20 weeks, the rough endoplasmic reticulum had returned to a normal appearance. Dense collagen was noted in all specimens after the second week.

Thus, degenerative changes including bizarre rough endoplasmic reticulum, double-membrane vacuoles, and swollen mitochondria were seen from the first through 12 weeks, with the tissues appearing more normal at 14 and 20 weeks. Myofibroblasts containing microfilament bundles with electron-dense bodies, desmosomes, and microtubules were seen only from 4 through 11 weeks.

DISCUSSION

The prolonged morbidity of clinical doxorubicin ulcers suggests persistent damage to the tissue. In previous experimental studies, we have shown that surgically induced lesions of the same size heal at a much faster rate than do doxorubicin-induced ulcers (7, 9). Two possible mechanisms have been suggested as the cause of this prolonged morbidity (9). Doxorubicin binds directly to the bases of DNA (12) and hence interferes with nuclear function. Thus, it would interfere with the cell replication necessary for healing of tissues.

An additional possible mechanism might be direct interference with wound contraction mechanisms. Contractile fibroblasts (myofibroblasts) have been demonstrated by Gabbiani et al. (3) to be the probable cause of wound contraction (4, 15). These cells share electron microscopic characteristics of both fibroblasts and smooth muscle cells. In addition, pharmacological stimulation and relaxation with agents known to cause effects in smooth muscle produce similar effects in granulation tissue. Finally, immunofluorescent studies have demonstrated similarity of myofibroblasts to smooth muscle cells (3, 4).

Myofibroblasts contain microfilament bundles with electron-dense bodies similar to those of smooth muscle cells. In addition, they have intercellular desmosomes and gap junctions which connect the individual cells and allow them to exert pull on each other. We have also demonstrated prominent microtubules in actively contracting myofibroblasts and have theorized that these intracellular structures are necessary for effective cellular contraction (10).

Jaenke (5) demonstrated via electron microscopy that in heart muscle damaged by doxorubicin, disruption of the intracellular contractile microfilaments occurred in addition to nuclear damage. Our study was conducted to evaluate whether any of the myofibroblast structures related to effective contraction were specifically affected by doxorubicin injury.

In fact, doxorubicin injury leads to delayed development of myofibroblasts. In surgical wounds in rats, myofibroblasts began to appear within 2 days (7), whereas in this study, they were not clearly seen until 28 days. The myofibroblasts themselves did not appear to be unusual, although the microfilament...
bundles were somewhat smaller than in normally contracting wounds. Extracellular basal lamina and convoluted nuclei, originally described as features of myofibroblasts (3), were rarely seen, as is typical of myofibroblasts in rats versus those in humans or pigs (7). No specific alteration in intracellular myofibroblast appearance could be demonstrated in the doxorubicin-damaged tissues.

Electron microscopy can never be truly quantitative because of sampling error problems, and thus, it is possible that intracellular effects might not have been seen on the samples studied. However, multiple samples were taken and studied in exactly the same fashion by the same electron microscopist, experienced from previous studies (7, 10) of myofibroblasts in identifying them and their characteristics. Thus, this study represents a reasonable approximation of the myofibroblast population. In contrast to the lack of changes in the myofibroblasts, multiple intracellular degenerative changes occurred which persisted through 14 weeks after doxorubicin injury. The persistent swollen mitochondria, rough endoplasmic reticulum appearing dilated or in small circles, and vacuoles with double membranes all suggest chronic intracellular damage.

Probably, these changes represent disruption of cellular processes due to persistent damage of the nucleus. In cardiac muscle, the muscle bundles are preexisting, whereas in doxorubicin-damaged skin, contractile cells must be developed by the local tissues to produce contraction. A reduced ability of the damaged tissues to differentiate and produce contractile cells probably explains the reduced contraction of doxorubicin-induced skin ulcers.

REFERENCES

Fig. 1. Fibroblast in 8-day-old doxorubicin ulcer. Rough endoplasmic reticulum is dilated and in smaller loops than in normal active fibroblast. Multiple vacuoles with double membranes (arrows) are present. × 20,000.

Fig. 2. Persistent abnormality (small loops) of rough endoplasmic reticulum in fibroblast in 2 week doxorubicin skin ulcer. Mitochondria are swollen. × 25,000.
Fig. 3. Myofibroblast features in cells at 4 weeks after doxorubicin skin injury. Rough endoplasmic reticulum more normal (cf. Figs. 1 and 2). A, small bundle of microfilaments with electron-dense bodies (arrows); B, intercellular connection (desmosome). × 40,000.

Fig. 4. Myofibroblast at 9 weeks. Microfilament bundles with electron-dense bodies (*) plus prominent long microtubules (arrow). × 40,550.
Ultrastructure of Doxorubicin (Adriamycin)-induced Skin Ulcers in Rats

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