of binding to hydrophobic recognition sites of enzymes and receptors.

Finally, it is remarkable that Car, Ada, Bug, and Neo can very efficiently stabilize enekphalin peptides against degradation by various proteolytic enzymes, if introduced in position 5[8,9].

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Distribution of cytoskeletal elements in cultured skin fibroblasts of patients with Duchenne's Muscular Dystrophy[1]

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Summary. Cultured fibroblasts from patients suffering from Duchenne's Muscular Dystrophy were examined by indirect immunofluorescent techniques using antibodies against actin, myosin, tubulin, and intermediate-sized filaments. The cells display normal patterns of microfilamentous bundles (stress fibres), microtubules, and intermediate-sized filaments suggesting a normal organization of these cytoskeletal structures.

Although the primary defect of Duchenne's Muscular Dystrophy (DMD) is still unknown, evidence has been presented suggesting membrane defects in tissues of neither myogenic nor neurogenic origin[3-5]. In view of the well documented interrelationships of membrane dynamics and cytoskeletal elements[6-11], Shay and Fuseler[12] studied fibroblasts from explants from cardiac and skeletal muscle of dystrophic inbred chicken and found a reduction of the immunofluorescent staining of interphase microtubules compared with those in cells of normal chickens.

We have extended the studies on cytoskeletal elements in DMD and compared the distribution of actin, myosin, tubulin and intermediate-sized filaments in cultured fibroblasts from patients with DMD and from normal persons.

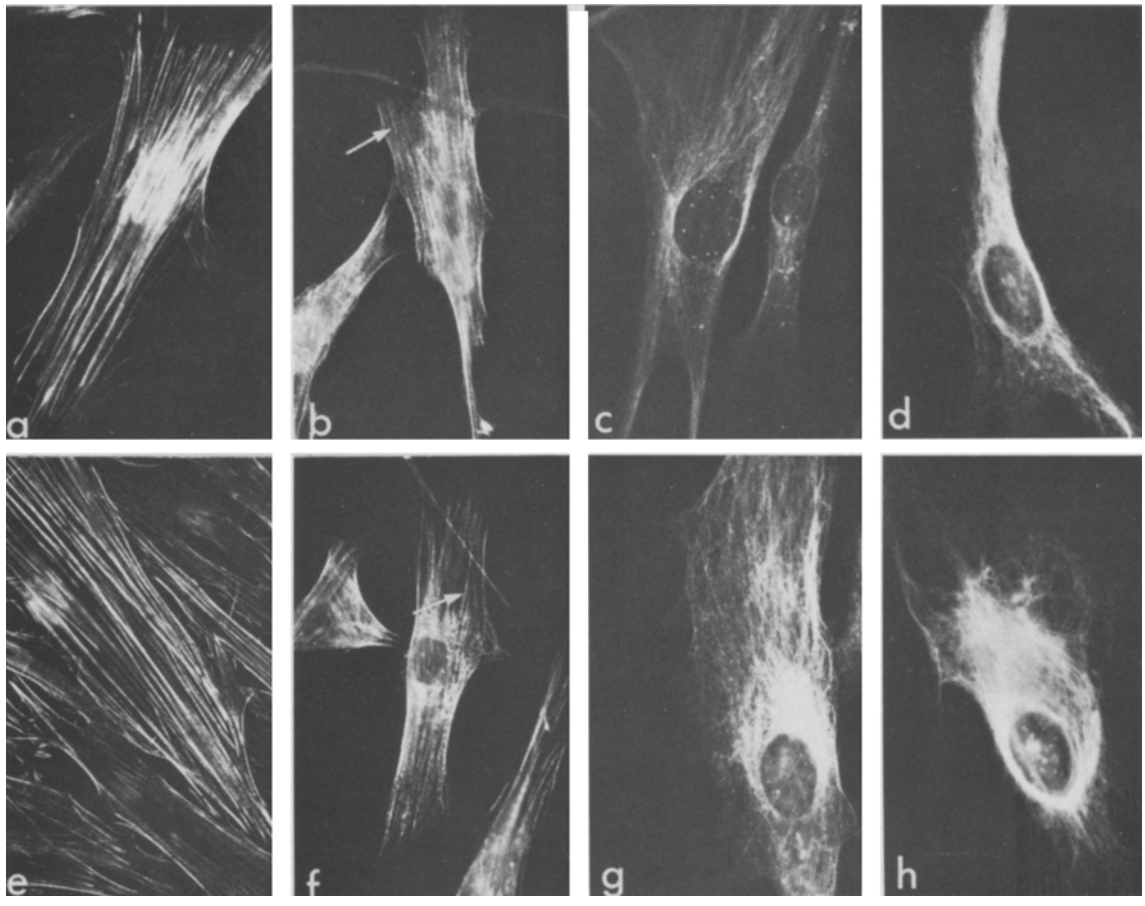
The fibroblasts were obtained from biopsies of 4 patients with clinically confirmed DMD and of 4 normal, sex- and age-matched children (from the Department of Pediatric Surgery of the University of Berne). Cells were grown in 250-ml Falcon flasks in Eagle's minimum essential medium supplemented with 10% fetal calf serum and nonessential amino acids. The cultures were incubated at 37 °C in 95% air and 5% CO_2, and medium was changed twice weekly.

The fibroblasts, used at the same number of generations, were grown on glass cover-slips and examined as nonconfluent cultures. For the visualization of actin and myosin, cells were fixed with ethanol (30 sec, room temperature) and then briefly rinsed in PBS. For microtubular structures and intermediate-sized filaments, fixation was in methanol (5 min, -20 °C) followed by a short immersion in acetone (−20 °C). Then the preparations were air dried.

We used: 1. human anti-actin antibodies[13,14] purified by affinity chromatography, at a concentration of 80 µg/ml; 2. rabbit antiserum containing antibodies against human uterine smooth muscle myosin; the titer of the serum, measured in rat intestinal smooth muscle, was 1/1280[15]; 3. rabbit antiserum containing antibodies against pig brain tubulin[11]; 4. rabbit serum containing auto-antibodies against intermediate-sized filaments of fibroblasts[16]. All 3 sera were used at dilutions 1:10 in phosphate buffered saline (PBS; KH_2PO_4 14 mM, Na_2HPO_4 53 mM and NaCl 154 mM, pH 7.2).

Incubation with anti-actin and antimyosin antisera was at room temperature for 15 min; incubation with antitubulin and anti-intermediate-sized-filaments sera was at 37 °C for 45 or 60 min. After washing, the cells were further incubated for actin, with fluorescein conjugated Ig fraction of a goat serum against human IgG (Miles Seravac, Lausanne, Switzerland, dilution 1:5); for myosin, tubulin and intermediate-sized filaments, with fluorescein conjugated Ig fraction of a goat serum against rabbit IgG (Behring-Werke, Marburg Lahn, West Germany, dilution 1:20). Incubation was 15 min for actin and myosin (room temperature) and 30 or 45 min for tubulin and intermediate-sized filaments (37 °C). Cells were observed with a Zeiss UV microscope equipped with epiillumination and specific filters for fluorescein. Photographs were taken with a plan Apochromate 40× objective, using Ilford HP5 black and white film.

Upon microscopic examination, we did not detect any differences in the staining pattern of cytoskeletal proteins between fibroblasts from patients with DMD and normal subjects (figure). Decoration with antibodies to actin and myosin revealed thick stress fibres extending over long distances of the cell body (figure, a, b, e, f). As depicted in the figure (b and f), stress fibres appeared stippled after staining with antimyosin antibodies, which is characteristic



Immunofluorescence micrographs of human fibroblasts from normal subjects (a–d) and from patients with muscular dystrophy (e–h) after decoration with antibodies to actin (a and e), myosin (b and f), tubulin (c and g), and with a rabbit serum against intermediate-sized filaments of the fibroblastic type (d and h). Note the cable-like stress fibres consisting of microfilaments visualized with anti-actin and antimyosin antibodies (a, b, e, f). Arrows point to stippled cables after decoration with antimyosin antibodies (b and f). A dense microtubular meshwork is visible in both normal and diseased fibroblasts (c and g). Intermediate-sized filaments display a normal arrangement in both types of fibroblasts (d and h). × 750.

for the distribution of myosin[17,18]. After staining with antitubulin antibodies, a dense network of cytoplasmic microtubules was visible in the perinuclear region radially extending towards the cellular margin (figure c and g). Intermediate-sized filaments formed thick aggregates in the perinuclear region and (similarly to microtubules) radiated into the cellular periphery (figure, d and h).

Using the indirect immunofluorescent method, we have shown that the distribution of cytoskeletal filamentous structures, namely of microfilaments, microtubules and intermediate-sized filaments, is normal in cultured fibroblasts from patients with DMD. In particular, we observed positive staining of cytoplasmic microtubules suggesting a normal organization of the microtubular network of cultured interphase cells. Similar results concerning the distribution of microtubules have been reported recently for fibroblasts of dystrophic chickens and humans[19]. We could not confirm the lack of stainability of microtubules as was found by Shay and Fuseler[12] in fibroblasts of cardiac and skeletal muscle of dystrophic chickens.

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