existence of Dsg3, Dpk and Dsc3 in low Ca++ cells was confirmed by Western blotting. Double-distribution of Dsg3, Dpk and Dsc3 (but not Dsg1/2) in the cytoplasm and/or on the cell high Ca++ without antibodies for 2 h, gold-labels were detected in the newly formed DS as well as Ca++ showed a punctate-linear pattern of PV-IgG, which were colocalized with Dsg3, Dsc3 and low Ca++, and Ca++ concentration in the medium was switched to high. After 2-h incubation in high Ca++, cells were studied by immunofluorescence (IF) and immunoelectron (IEM) microscopy, using anti-Dsg3 affinity purified polyclonal, Dsg1/2, desminocollin 3 (Dnc3) and desminoplakin 1/2 (Dpk) monoclonal antibodies. In low-Ca++ grown cells, IF showed a punctate distribution of Dsg3, Dpk and Dsc3 (but not Dsg1/2) in the cytoplasm and/or on the cell membrane, but not at cell-cell contacts, and IEM revealed Dsg3 on the cell surface “half-DS”. The existence of Dsg3, Dpk and Dsc3 in low Ca++ cells was confirmed by Western blotting. Double-staining IF revealed that high-Ca++ switched cells after pretreated with PV-IgG for 30 min in low Ca++ showed a punctate-linear pattern of PV-IgG, which were colocalized with Dsg3, Dsc3 and Dpk, at cell-cell contacts. When low Ca++ cells were treated with PV-IgG for 5 min and labelled with antihuman IgG-5nm gold for 5 min in a low Ca++ medium, and followed by incubation in high Ca++ without antibodies for 2 h, gold-labels were detected in the newly formed DS as well as on “half-DS”, by IEM. These IF and IEM results demonstrate that PV-IgG causes no steric hindrance in DS formation.

For such a purpose, we performed a semi-quantitative analysis of the Fas-L expression by KC in normal human skin. In situ gold-immunoelectron microscopy on skin ultracryosections was carried out, and thereafter the number of gold particles expressed at the KC surface per cell section (inulaplate) was evaluated. Relatively few, namely, 51.55 ± 28.61 (n = 100), 10-nanometers sized, colloidal gold particles were observed at cell surface of KC resident in the basal layer of epidermis. Extending preliminary results, gold granules were detected even at the plasma membrane of KC resident in the spinous layer, namely, 35.42 ± 15.80 (n = 100) particles per KC section in the lower spinous layer, and 13.99 ± 14.03 (n = 100) particles per KC section in the upper spinous layer. By contrast, the KC labeling of ultracryosections prepared as controls for method specificity was not significantly different in various epidermal layers, namely, 7.83 ± 0.88 (n = 100) per KC section in the basal layer, 8.01 ± 0.88 in the lower- and 0.76 ± 0.87 in the upper-spinous layer. Thus, KC in resting epidermis expresses Fas-L in relatively moderate amounts, and such a low expression is not presumably capable, alone, to fully preserve the homeostasis of certain epithelial tissues, we intended to investigate to what extent the basal layer, 0.81 6 0.3 in control skin. Both the SFS patient and the unaffected sibling showed normal epidermal at 30 K. The suprabasal desmosome size was 350.2 ± 9.3 nm (mean ± SEM) in control skin. Desmosomes in the affected SFS patient’s skin (167.9 ± 7.5 nm) and the unaffected heterozygous sibling with a defect on a single allele. Three skin biopsies from healthy, normal subjects served as controls. The size and number of desmosomes per high power field (HPF) (n=50) was measured at a magnification of ×200 K. The suprabasal desmosome size was 350.2 ± 9.3 nm (mean ± SEM) in control skin. Desmosomes in the affected SFS patient’s skin (167.9 ± 7.5 nm) and the unaffected sibling (283.8 ± 6.6 nm), both showed significant (p < 0.01) reductions in desmosome size, to 47% and 49% of normal epidermis, respectively. The mean number of desmosomes per HPF was 2.3 ± 0.1 in the SFS patient’s skin (39% of the control value). 4.02 ± 0.3 in the unaffected sibling (66%) and 6.02 ± 0.3 in control skin. Both the SFS patient and the unaffected sibling showed similar desmosomal changes compared to control skin (p < 0.01), despite only the homozygous patient exhibiting any clinical symptoms. Desmosome adhesion in the unaffected sibling therefore may be compensated for by sufficient cell-cell binding and desmosomal stability, due to the expression of at least some typeII PPK. These findings attest to the important role of PPK in stabilizing desmosome structures, influencing plaque size and the frequency of such junctions.
Expression of CD1 Family Molecules in Primary and Metastatic Melanoma

Expression of CD1 molecules in metastatic melanoma. ICH using anti-IL10 antibody revealed a strong expression in metastatic but not in primary melanoma. Culturing CD1+ DC in a medium containing IL10 resulted in the down-regulation of CD1 expression in a dose dependent manner. Supernatant from melanoma cells, but not from fibroblasts, down-regulated CD1 molecules. In the mononuclear infiltrate of primary melanoma, an important intra/peritumoral expression of CD1 molecules by dermal DC might play a role in the immune response to primary melanoma.

Control of Basement Membrane Assembly by Nidogen-Laminin Interaction in Organotypic Coculture

Control of basement membrane assembly by nidogen-laminin interaction in organotypic coculture. The absence of laminin (LN) and nidogen (ND) in the basement membrane assembly was observed in organotypic cocultures. The inhibition of ND-LN interaction by Lg-1f either in Epon, following fixation in 2% glutaraldehyde, or in Lowicryl K4M, after fixation in 3% paraformaldehyde. The function of the interface was completely abolished. There was also a dramatic reduction of LN-10 (seen by electron microscopy (EM) within about two weeks. Cross-linking of type IV collagen (CIV) and laminin (LN) by nidogen (ND) is apparently crucial for the regular assembly of epidermal and other BM's. For a direct proof in OTC, we have competitively inhibited ND-LN interaction with a recombinant laminin fragment (Lg-1f) covering the ND binding site. By applying Lg-1f rather on top of the air-exposed epithelium or with medium deposition of ND at the epithelial-matrix interface, the interface was completely abolished. There was also a dramatic reduction of LN-10 (seen by EM) in LN-ND interaction by Lg-1f suppressed BM and also hemidesmosome formation and caused the displacement of keratin filament bundles from the basal cell aspects. Subtle changes in the molecular distribution became manifest by immuno-EM using antibodies against CIV, integrin α6β4 chain, BP230, and pan-keratin which underline the crucial role of proper BM-assembly for the functional integrity of the epidermis.

Alteration of the Lower Part of the Basement Membrane Zone in Epidermolysis Bullosa Acquisita

Alteration of the lower part of the basement membrane zone in epidermolysis bullosa acquisita. Three-dimensional reconstruction of BMZ in laser scanning confocal microscopy. The study included three cases of morphea and three cases of lichen sclerosus (LS) diagnosed according to clinical and histopathological criteria. Biopsies from patients' skin and a control biopsy from normal human skin were labelled with antibodies against β4-integrin (lumina lucida marker), collagen IV and N-terminal end of collagen VII (lumina densa-LD markers) and C-terminal end of collagen VII (sublumina densa-SL marker), studied with the use of laser scanning confocal microscopy. The threedimensional reconstruction of various regions of basement membrane zone (BMZ) showed a decrease in the number and size of the dermal papillae in both LS and morphea as compared to normal skin. In the morphea, there were numerous invaginations of flattened BMZ at the level of lamina lucida and lamina densa, whereas numerous holes were present in LS BMZ. The vascular skin network system, visualized by labelling with anticoagulation IV antibody, revealed increased angiomatosis in morphea, as compared to LS and normal skin. The three-dimensional reconstruction of BMZ and skin vascular networks revealed the different alterations of BMZ and vessels in morphea and LS. Our studies provide further evidence that these two diseases are separate entities – in spite of the not infrequent overlapping of their clinical and histopathological patterns.

Alteration of the lower part of the basement membrane zone in epidermolysis bullosa acquisita. Three-dimensional reconstruction of BMZ in laser scanning confocal microscopy. The study included three cases of morphea and three cases of lichen sclerosus (LS) diagnosed according to clinical and histopathological criteria. Biopsies from patients’ skin and a control biopsy from normal human skin were labelled with antibodies against β4-integrin (lumina lucida marker), collagen IV and N-terminal end of collagen VII (lumina densa-LD markers) and C-terminal end of collagen VII (sublumina densa-SL marker), studied with the use of laser scanning confocal microscopy. The threedimensional reconstruction of various regions of basement membrane zone (BMZ) showed a decrease in the number and size of the dermal papillae in both LS and morphea as compared to normal skin. In the morphea, there were numerous invaginations of flattened BMZ at the level of lamina lucida and lamina densa, whereas numerous holes were present in LS BMZ. The vascular skin network system, visualized by labelling with anticoagulation IV antibody, revealed increased angiomatosis in morphea, as compared to LS and normal skin. The three-dimensional reconstruction of BMZ and skin vascular networks revealed the different alterations of BMZ and vessels in morphea and LS. Our studies provide further evidence that these two diseases are separate entities – in spite of the not infrequent overlapping of their clinical and histopathological patterns.

006

Ultrasound Features of Hereditary “White Nail”.

M. Haftek, B. Bähme, N. Tidman, H. Perrot, D. Keßler, D. Schmitt, F. Wolf* INSERM U144/CNRS, Hôpital Hospital, ²Department of Dermatology, Antiquaille Hospital, Lyon, France and CRF Skin Tumour Laboratory, SBRIL, School of Medicine and Dentistry, London, UK. Hereditary subungal leukonychia is a rare nail disease. We have analyzed microscopically and ultrasonically the white nails of a patient from a family in which the trait is inherited in an autosomal dominant manner as an isolated symptom. No skin lesions or hair abnormalities could be detected. A longitudinal surgical biopsy of the nail from a big toe was split in two parts. One part was fixed in formaldehyde, dehydrated, and processed for histopathology. Another part was further subdivided and embedded either in Epon, following fixation in 2% glutaraldehyde, or in Lowicryl K4M, after fixation in 3% paraformaldehyde. Decalcified nail sections and Lowicryl ultrathin sections were treated with several histochemical methods, in search for abnormalities of keratinisation. The nail matrix presented an abnormal hypergranulomous. The upper part of the nail plate, originating from the proximal nail matrix, had a nonhomogeneous lamellar appearance, with numerous intracellular “lipidic” vacuoles and “empty” spaces separating keratin filaments in bundles. These cells were progressively shed at the nail surface. The cell loss was compensated by hyperproliferation of the distal matrix and of the nail bed keratinocytes, with persistent marked parakeratosis and loose arrangement of keratin bundles. The lower plate made up 80% of the nail thickness and presented the characteristics of a tissue composed of soft keratins. Our morphological findings suggest a mutation in one of the hard keratin genes as a possible cause of the hereditary leukonychia. Genetic linkage studies of the family are in progress.

007

Structure of Skin Appendages During the Prenatal Development in Skin

The evolution of hair, apocrine and eccrine sweat and sebaceous glands during fetal development has been the subject of research for more than 100 years. The aims of our research have been both the estimation of the time of the hair germ’s development and the observation of the initial stage of sweat and sebaceous gland development from epidermis and hair germ, including topographical differences and a detailed analysis of the periods of gland formation.

110 skin biopsies were taken from foetuses aged 8–36 gestation weeks, as well as 4 from newborns. The samples were obtained from hands, head, perianal and pubic regions. The samples were stained using HE, Mallory, Masson, PAS and Bielschowsky-Lent cell method. The earliest visible germ of hair were observed in lps and forearms in the 8th–9th week of fetal life. The first sebaceous glands appear in the 3rd–4th week of the third month. There are 3 stages of that process: The first concerns palms and soles, the second the axillar region and the third the whole surface of the skin. The first 80% of the nail's thickness and presented the characteristics of a tissue composed of soft keratins. The cell loss was compensated by hyperproliferation of the distal matrix and of the nail bed keratinocytes, with persistent marked parakeratosis and loose arrangement of keratin bundles. The lower plate made up 80% of the nail thickness and presented the characteristics of a tissue composed of soft keratins. Our morphological findings suggest a mutation in one of the hard keratin genes as a possible cause of the hereditary leukonychia. Genetic linkage studies of the family are in progress.

009

Laser Scanning Confocal Microscopic Study in Lichen Sclerosus and Morphea

A. Koziolka, K. Wojcik, S. Jablońska, and C. Kowalewski Department of Dermatology, Warsaw University of Medicine, Poland

The study included three cases of morphea and three cases of lichen sclerosus (LS) diagnosed according to clinical and histopathological criteria. Biopsies from patients’ skin and a control biopsy from normal human skin were labelled with antibodies against β4-integrin (lumina lucida marker), collagen IV and N-terminal end of collagen VII (lumina densa-LD markers) and C-terminal end of collagen VII (sublumina densa-SL marker), studied with the use of laser scanning confocal microscopy. The threedimensional reconstruction of various regions of basement membrane zone (BMZ) showed a decrease in the number and size of the dermal papillae in both LS and morphea as compared to normal skin. In the morphea, there were numerous invaginations of flattened BMZ at the level of lamina lucida and lamina densa, whereas numerous holes were present in LS BMZ. The vascular skin network system, visualized by labelling with anticoagulation IV antibody, revealed increased angiomatosis in morphea, as compared to LS and normal skin. The threedimensional reconstruction of BMZ and skin vascular networks revealed the different alterations of BMZ and vessels in morphea and LS. Our studies provide further evidence that these two diseases are separate entities – in spite of the not infrequent overlapping of their clinical and histopathological patterns.
011

Giant Cell Tumor of Tendon Sheath – Localized Type (Pigmented Villonodular Tenosynovitis), Case report

J. Stork, D. Kodetova,* and F. Vosnak
Department of Dermatovenereology and *Department of Pathology, Charles University, Prague, Czech Rep.

The authors describe the case of a 57-year-old woman presenting with an enlargement of her right 4th toe without any bone involvement on X-ray examination. Punch biopsy findings included nodules separated by collagenous septae formed by medium-sized polygonal mononuclear and foam cells, positive for anti-PCG positive (macrophage marker) with an admixture of B and T lymphocytes. The diagnosis of a giant cell tumor of a tendon sheath was suspected. Because of the tumor extension the amputation of the whole digit had to be performed. Histological examination revealed additional features consisting of scattered multinucleated giant cells, xanthoma cells, clefslike spaces and hemosiderin pigment which confirmed the diagnosis. Bone invasion was not present. Two years after the surgical intervention there were no signs of tumor recurrence.

012

Neutrophilic Eccrine Hidradenitis Secondary to Serratia Marcescens Infection Demonstrated by Electronmicroscopy

J. Katnatis,* P. Cumbemate, J. Faisant, and M. Dupin
*Department of Dermatology, Ed. Hôpital Hospital and †Military Hospital “Drozignes”, †Laboratory of Pathology, Lyon, France

Neutrophilic eccrine hidradenitis (NEH) is a rare dermatosis of unknown origin, developing usually after the administration of chemotherapeutic treatments for haematopoetic malignancies. Only a few cases have been reported to date. Very rarely, NEH may have an infectious origin. We report the case of a 31-year-old man operated for ependymoma who presented a typical eruption of NEH, although he had not received chemotherapy. The eruption was comprised of successive crops of small, erythematous papules that began on the legs and progressively spread to the thighs and the abdomen. Histological examination of a skin biopsy performed under strictly aseptic conditions showed focal necrosis of eccrine secretory coils extending to the excretory ducts and to adjacent blood vessels; these were surrounded by a dense infiltrate of pyocyanic neutrophils.

Electronmicroscopic examination of a skin biopsy showed a dense, mainly neutrophilic inflammatory infiltrate surrounding eccrine sweat glands. Several neutrophils contained cytoplasmic inclusions with a granular content of variable electron-density (phagosomes), and some of them contained membrane-bound round or oval-shaped bacteria. Culture of a skin biopsy revealed Serratia Marcescens (SM) in the absence of other bacteria. The dermatosis improved after antibiotic therapy (ceftriaxone plus tetracyclin) but recurred twice and cultures isolated again showed SM. Up to now, only three cases of NEH of infectious origin have been reported. (One of them was due to SM). Our case demonstrates the usefulness of EM, as it showed for the first time the presence within the lesions of the responsible microorganism and suggested antibiotic therapy as an appropriate treatment.

013

Olmsted Syndrome, Light and Electronmicroscopic Study of a New Case

J. Katnatis, V. Callot, and M. Larrégue
Departments of Dermatology, *Ed. Hôpital Hospital, Lyon, and †Athlète Hospital, Itéines, France

Olmsted syndrome (OS) is a rare keratodermization disorder (18 patients published so far), associated with mutilating congenital palmo-plantar keratoderma (PPK) with perionychal and intertrigenous erythematous keratoderatic lesions. We report herein a new case of OS, that was studied by light and electronmicroscopy (EM). The patient was a 7-year-old boy born to consanguineous parents of Algerian origin, presenting with the major features of OS, i.e. a thick, mutilating PPK with ainhum-like constrictions and flexion contractures of the fingers, nail dystrophy, plantar, scrotal, axillary, inguinal, and perioral erythematous lesions. He also suffered from severe growth retardation, muscular atrophy and joint laxity of the lower limbs.

A skin biopsy from the palm showed hyperkeratosis, hypergranulosis and psoriasiform hyperplasia of the stratum malpighii. The muticindex and nucleolar organizer regions (AgNORs) of the epidermis were increased as compared with a control skin specimen.

Immunohistochemically, there was normal expression of high MK keratins, subnormal expression of filament, and highly increased expression of involucrin and calprotectin. S100 + epidermal Langerhans cells were sparse, and one of them was seen by EM to be in mitosis. By EM, connexinocytes contained dense matrix, lipid droplets and occasional remnants of cytoplasmic organelles. Keratohyalin granules were coarse, polygonal or star-shaped and occasionally absent. Keratinocytes seemed slightly diminished in number. Epidermal keratinocytes had large nuclei with visible nucleolus. Several keratinocytes contained centrioles, even at the level of the stratum malpighii. Mitochondria were abundant and occasionally contained myelinoid inclusions. The mode of inheritance (autosomal or X-linked recessive?) and the mechanisms responsible for skin lesions are not known. Our results suggest that the cutaneous lesions develop as a result of epidermal hyperproliferation.

014

Congenital Gengival Granular Cell Tumor (CGGCT)

D. Innocenzi,* R. Boldrini, and C. Bosman*†
*Department of Pathology, Pediatric Hospital “Bambino Gesù”†, Rome, and *Department of Dermatology and Pathology University “La Sapienza”, Rome, Italy

The term “congenital epulis” should be correctly referred to as “CGGCT”, a disease which is frequently confused with the granular cell tumor or Abrucksoff tumor, this neoplasm is actually considered of Schwannian origin and can easily be identified by immunohistochemistry, due to the positive staining of its cytoplasm with S100 protein, CEA, CD57 and collagen IV. The CGGCT, evenly built up from eosinophilic granular cells, represents a different nosographic entity, with its immunohistochemical pattern negative for S100 protein and positive for vimentine, in relation to its histocytic origin. At the ultrastructural level, in both tumors the granular aspect of the cytoplasm is due to a large number of autophagosomes, each containing myelin-like electron dense bodies. Some cells in CGGCT are evocative of histiocytic type and thus deprived of basal lamina; on the other hand, this laminar is consistently well evident, and sometimes multilayered, in cells of Schwannian origin. This case report refers to a 3-month-old baby showing a congenital gengival tumor. The diagnosis was achieved by histologic, immunohistochemical and ultrastructural examination.

015

Multiple “Spitz” Naevi in a Boy with Oculocutaneous Albinism

D. Innocenzi,* R. Boldrini, M. C. El Hachem, and C. Bosman*†
*Department of Pathology and Dermatology, Pediatric Hospital Bambino Gesù, and †La Sapienza University, Rome, Italy

A 7-year-old boy affected by oculocutaneous albinism (OCA) recently and progressively developed multiple (up to 300) pink salmon papular lesions, widely disseminated over the trunk and the limbs, each of about 5 mm in diameter. One of these lesions was biopsied and evaluated with light microscopy, immunohistochemistry and electron microscopy. The lesion proved to be a nonpigmented Spitz naevus, whose melanocytes showed as strongly positive for S100 protein and weak for HMB 45 cytoplasmatic reaction. In the cytoplasm of melanocytes, ultrastructural investigation showed aberrant melanosomes in the form of spherical organelles containing granular-electro dense material or concentric electron-dense lamellae. To our knowledge, this report represents the first case of multiple Spitz naevi associated with oculocutaneous albinism.

016

Ultrasructural Demonstration of Skin, Muscle and Other Organ Involvement Caused by Borrelia Burgdorferi Persistence

D. Halinska and P. Bartl
Department of Electromicroscopy, Centre of Epidemiology and Microbiology, National Institute of Public Health, Prague, Czech Rep.

Because the localization of the causative spirochete (B. burgdorferi) in infected tissues is unknown, we used electron microscopy to find spirochetes in the skin (erythema migrans and acrodermatitis chronica atrophicans lesions), muscles, hearts, synovial membranes, placenta (and in the brain autopsy of humans with active or advanced Lyme disease (LD).

Spirochetes were cultured only from skin and placenta samples in a special BSK medium. Ultrastructural findings were analyzed antigenetically with monoclonal antibodies and by molecular biology techniques. Ultrastructurally, the spirochetes were often situated between collagen fibers and along fibroblasts, some of which were deeply invaginated by these Borrelial organisms. In the active phase of LD, they were found in or around blood vessels in dermis, heart and other muscle tissue. Spirochetes were generally not observed in or near areas of inflammatory infiltrates. They are able to adhere and invade human peripheral monocytes, leucocytes and B-lymphocytes. Features which may be related to the persistence of Borrelia were the occurrence of unknown phagocytosis, coiling and tube included the segmental uptake of spirochetes with leaky lysosomes and invagination of a large cell’s membrane area. The studies extend our understanding of the behavior of the spirochete in vivo by identifying common locations of Borrelia and by finding the disparity between infection and inflammation.
017
Persisting Erythematous Symmetric Orbital Swelling: Ultrastructural Features Disclosing Breast Cancer Metastasis
I. Hausner, T. Zimmermann, U. Jappe, C. Cornelius, and W. Hartschuh
Department of Dermatology and *Department of Gynecology, University of, Heidelberg, Germany
A 56-year-old female patient presented with protracted erythematous swelling of upper and lower lids persisting for half a year. The histopathological diagnosis had been xanthelasma. Because of progreession and additional skin tumours arising in perinasal, inimal, temporal and mandibular regions as cutaneous nodules, new deep biopsies were taken from lesions of the lower lid and from the neck. Histological and immunohistochemical investigations of the lid biopsy revealed an unusual, rather mononuclear infiltrate consisting of histiocyte-like-cells, similar to those found in xanthelasma but also of signt-nest cells, resulting in the suspicion of a metastasis of a signt-nest cell carcinoma. By electron microscopy, numerous individual or grouped tumour cells were found within the reticular and deep dermal regions of the neck. They showed characteristic intracytoplasmatic lamina as well as a secretory vesicle pattern typical for invasive lobular carcinoma. Indeed, the cells showed a strong expression of Gross Cystic Disease Fluid Protein-15 (GCDFP), an apocrine differentiation marker. The folloewin examination established an invasive lobular carcinoma. Immunohistochemistry revealed that the drug intake triggered the lichenoid and autoimmune process. In this rare variant, skin metatases are the primary symptoms of an invasive lobular breast carcinoma. This case presents an examle of tumour diagnostics in which electron microscopy presents a valuable tool, in combination with other techniques, to improve diagnostic accuracy.

018
Photosensitivity to Amiodarone – Clinical and Ultrastructural Changes
K. Euler, D. Sulbotta, *J. Gregor, and M. Nozikova
Department of Dermatology, *Department of Histology and Embryology, ¹Department of Internal Medicine H. Faculty of Medicine in Hradec Kralove, Charles University Prague, Czech Republic
Amiodarone is an antiarrhythmic drug with many side-effects including the induction of photosensitivity and the development of blue-greyish pigmentation in the sun-exposed skin areas. We examined 64 patients treated by Amiodarone (mean dose 200 mg/day) and 32 controls without any photosensitivity. We established the skin phototype, the minimal erythema dose – MED (using polychromatic light of xenon lamp), early onset of erythema and its lasting, pigmentary response after phototests. Obtained data were evaluated by two-sample t-test and by the X2-test of independance in contingency table. Skin biopsy specimens were obtained from the hyperpigmented facial skin area of a 59-year-old women treated with amiodarone (400 mg/day) for 3 years. The specimens were processed for light and electron microscopy. For paraffin sections, routine staining methods as well as techniques detecting various pigments were used. Ultrastructural sections for transmission electron microscopy were stained with uranyl acetate and lead citrate. Moreover, semithin epoxy resin sections were prepared for light microscopical examination. The incidence of clinical photosensitivity (erythema, burning, papules) as well as hyperpigmentation related to amiodarone on sunlight-exposed skin areas were present in 9.4% (6 patients). The average MED, early onset and delayed duration of erythema and pigmenratory response after polychromatic light irradiation were not statistically different (P below 0.05) between amiodarone treated patients and controls. Light microscopy revealed mononuclear infiltrates situated in the upper and middle dermus, predominantly in a perivascular location. In these infiltrates, numerous cells showed macrophage morpholgy together with prominent cytoplasmic accumulations of granules. The granules had staining properties similar to lipofuscin. Electron microscopy, the granules were membrane-limited and dense, showing a nonhomogeneous content. The appropriate patient’s education and sun protection is necessary, especially if the amiodarone dosage is more than 200 mg/day.

019
Subcutaneous Benign Triton Tumor
R. Boldrin, D. Innocenzi, *R. Limus, and C. Bonnano
*Department of Pathology, Pediatric Hospital Bambino Gesu, and **Department of Dermatology and Pathology University “La Sapienza”, Rome, Italy
Giant cell fibroma, pleomorphic hamartoma, neurovascular hamartoma or choriocarcinoma are all synonyms of a rare entity also called the benign triton tumor. Most of the case reports in literature consist of histological investigations of scrofuloderma, and negative for NF, SYF and smooth muscle specific actins.

020
Acquired Progressive Kinking of the Hair: Diagnosis by Scanning Electron Microscopy
F. Brenz, J. Breit-Maly, D. Moer, H. Herzmans, and A. Tauer
*Department of Dermatology, *Division of Special and Environmental Dermatology, Department of Dermatology, University of Vienna Medical School, ¹University Clinic of Oral and Maxillofacial Surgery, University of Vienna Medical School, Vienna, Austria
Acquired progressive kinking of the hair (APKH) is a rare hair disorder of unresolved pathogenesis characterized by kinking, curling, darkening and thinning of the hair in a circumscribed scalp area. Male patients are predominantly affected with a predilection site at the frontal and parietal region. APKH usually occurs in adolescence and early adulthood. In male patients it often evenes into androgenetic alopecia, whereas in females it tends to spontaneous resolution. Hair changes similar to APKH may develop after retno treatment and hair transplantation. We describe a 35-year-old female patient with APKH of 8 years’ duration in the occipital, retroauricular and parietal region. She also had schizophrenia. By light microscopy the hair appeared twisted. Scanning electron microscopy disclosed canalicular grooves along the shaft of abdominal hair (pili canaliculi). Focal, a desquamation of cuticular scales was observed. Some spontaneous improvement was observed at a follow-up examination one year later.

021
Furosemide-Induced Lichen Planus Pemphigoides. Ultrastructural Study
C. Bédane, S. Boulinguez, F. Labrousse, M. Rumeau-Trividic, and J. M. Bonnetblanc
Institute of Pathological Anatomy and *Department of Pathology, Pediatric Hospital Bambino Gesù, and C. Bosman*
Department of Dermatology and Pathology, Hobart Boulevard Genexe, and **Department of Dermatology and Pathology University “La Sapienza”, Rome, Italy
Furosemide is a thiazide diuretic and one of the most commonly prescribed diuretic drugs. The ultrastructural examination of samples retrieved from paraffin inclusions revealed some spindle cells, which contained in their cytoplasm parallel bundles of contractile filaments merging in the upper and middle dermis, predominantly in a perivascular location. In these infiltrates, treatment related to amiodarone on sunlight-exposed skin areas were present in 9.4% (6 patients). The average MED, early onset and delayed duration of erythema and pigmenratory response after polychromatic light irradiation were not statistically different (P below 0.05) between amiodarone treated patients and controls. Light microscopy revealed mononuclear infiltrates situated in the upper and middle dermus, predominantly in a perivascular location. In these infiltrates, numerous cells showed macrophage morpholgy together with prominent cytoplasmic accumulations of granules. The granules had staining properties similar to lipofuscin. Electron microscopy, the granules were membrane-limited and dense, showing a nonhomogeneous content. The appropriate patient’s education and sun protection is necessary, especially if the amiodarone dosage is more than 200 mg/day.

022
Feline Orthopoxvirus-Infection Transmitted from Cat to Man: A Case Report
Institute of Pathological Anatomy and *Department of Dermatology, Central Hospital Salzburg, T Clinic Virology, Institute of Virology, University of Veterinary Sciences, Vienna, Austria
We report on a 56-year-old female patient who presented at the Department of Dermatology with an inflamed, exacerated lesion on the left side of her neck measuring about 1.5 × 4 cm. There was deep subcutaneous infiltration without signs of abscess formation. She had already been treated by a general practitioner with systemic antibiotics for about three days without success. Further exploration revealed an initial contact (scratch) with a cat living in the patient’s house. Diagnostic investigations included a skin biopsy. A scrap biopsy, scrotum biopsy and off. Satellite lesions developed despite local treatment and parenteral clindamycin. Immunofluorescence testing for herpes simplex virus type 1 and 2 proved negative, although histologic examination was found to be consistent with herpes-induced ulceration. Initially, negative staining of scrap tissue and material from a fresh pustule was negative for bacterial and viral structures. Subsequent transmission electron microscopical (TEM) examination of the same material – fixed, pelleted, and embedded in resin – clearly showed multiple typical poxvirus particles, predominantly in remnants of scaled-off layers of degenerated keratinocytes, as well as virus-particles in intermingled phagocytes, leading to the diagnosis “orthopoxvirus infection”. These results of the TEM examination, which were verified by PCR and sequencing (performed at the Institute of Virology, University of Veterinary Sciences, Vienna) again demonstrate the value of using electron microscopical techniques in the diagnosis of infectious skin diseases.
023
Aptosis Resistance in Human Melanoma Cells
M. Raisova,* A. Hossain, C. Barzelberg,* T. Wieder,† P. Daniel,† J. T. Eberle,* C. E. Orfanos,* and C. C. Gelenz*  
*Department of Dermatology, University Medical Center Benjamin Franklin, the Free University of Berlin and †Department of Histology, Onco- and Tumor Immunology, University Medical Center Charité, Humboldt University Berlin, Germany  
The CD95 (Fas) system is known as an important pathway for the induction of apoptosis in cells and tissues. Human melanoma cells have recently been shown to be heterogeneously sensitive to CD95-mediated apoptosis. Defective cytochrome c release and the resulting loss of caspase-3 activation was recognized to be essential for the susceptibility of human melanoma cells to CD95/Fas-induced apoptosis. Cytochrome c release from mitochondria is regulated by the relative amounts of apoptosis-promoting and -inhibiting Bcl-2 proteins in the outer membrane of these organelles. The assignment of Bax/Bcl-2 expressions by quantitative Western blotting in 11 melanoma cell populations revealed a relation to the susceptibility to CD95-mediated apoptosis. We could show that a relative low Bax/Bcl-2 was characteristic for resistant cells and a relative high Bax/Bcl-2 ratio for sensitive cells. Furthermore, Bcl-2 overexpression abrogated apoptosis triggered by CD95L on the critical role of the Bax/Bcl-2 ratio in melanoma cells. We suggest that apoptosis resistance in human melanoma cells bone turn out to be either gene therapy altering the Bax/Bcl-2 ratio or by the drugs directly targeting mitochondria as, e.g., betulinic acid.

024
Effects of a Ceramide-Containing Emollient in an Experimentally Induced Skin Barrier Dysfunction
M. Kucharekova, J. Schalkwijk, and P. G. M. van der Valk  
Department of Dermatology, University Medical Center Nijmegen, the Netherlands  
Recently a new generation of emollients, containing lipids chemically related to the physiological content of the intercellular domain of the horny layer, has been introduced. In the present study we compared the efficacy of containing emollient (CCE) with the conventional emollient (Vaselinum album/cremor lanette ana) by repeated exposure of SDS-sensitized volunteers to sodium dodecyl sulfate (0.2%) for 4 h a day for 5 consecutive days. The investigation sites were treated once a day with the above-mentioned agents. Daily, irritant reaction was assessed by erythema scoring and measurement of transepidermal water loss (TEWL). After 5 days, punch biopsies were taken from all sites. Immunohistological assessment was carried out with respect to epidermal proliferation, keratinization and epidermal differentiation.  
Tape stripping resulted in an erythematosus reaction and an increase of TEWL associated with up-regulation of proliferative cells and expression of cytokeratin 16. Both the CCE and the positive control significantly decreased the erythematosus response (p < 0.01 and p < 0.03 resp.). TEWL was also influenced significantly by treatment with CCE (p < 0.01), but not significantly by treatment with the positive control (p > 0.05). Concerning immunohistological markers, CCE significantly suppressed the amount of proliferating cells (Ki-67) compared to the control site. Repetitive exposure of SDS induced a variable degree of erythema. The erythematosus response enhanced in intensity up to day 4 in four participants. The gradual increase of TEWL values associated with up-regulation of proliferative cells was observed in all participants. Treatment with both CCE and the positive control did not significantly prevent erythema. Barrier dysfunction, however, was prevented significantly (p < 0.05 resp.) by the positive control, but not by CCE (p > 0.05). The immunohistological analysis in this model showed a significant suppression of proliferating cells by the positive control. Both investigated emollients enhanced the recovery of the skin barrier from mechanical and chemical damage. However, the unique properties of CCE are best expressed following mechanical damage, suggested that its shielding from chemical damage is not optimal. Further research on various skin conditions is required to find out to what extent the use of CCE has a clinical advantage.

025
UV-Light of 313 nm Induced 12(S)-Hydroxyeicosatetraenoic Acid Receptor Down-Regulation in Porcine Keratinocytes
P. Arenberger, I. Obstova, B. Havlikova, E. Matoukova,* and P. Bartk†  
Department of Histoveteranology, Charles University School of Medicine, Prague, Czech Republic; *Czech Academy of Sciences, Prague, Czech Republic; †State Institute of Public Health, Prague, Czech Republic  
UV-light induces different changes in skin which may be shown by electron microscopic examination. DNA damage or apoptosis are well-known as disturbances caused by UV-radiation. On the cell surface, receptors could be also affected during treatment with artificial UV sources or during skin irradiation for cosmetic reasons. Because 12-hydroxyeicosatetraenoic acid (12-HETE) is considered to be the main epidermal eicosanoid, and is assumed to have both pathophysiological effects on inflammatory skin diseases such as psoriasis and atopic eczema as well as a physiological role in cutaneous biology, we decided to show the UV-light effect on 12-HETE cell surface receptors. Therefore, the present work studied the effects of single and repeated irradiations with selected UV-B light of 313 nm from a Waldmann F 85/100 W [TL-01] built on the 12(S)-HETE-receptor in porcine epidermal cells.  
UV-light in vitro (0.5 J/m2) and in vivo (50–150 J/m2) induced a down-regulation of 12(S)-HETE receptors in a dose-dependent manner. The above described effect occurred after a latency period of 6 h and reached its maximum at 7.5 h. In vitro, a single UV irradiation (150 J/m2) or repeated irradiation (50 J/m2) developed a 55% receptor down-regulation (Bmax); however, the receptor affinity remained unchanged. The down-regulation of 12-HETE receptors on keratinocytes developed after the UV-B irradiation may contribute to the explanation of its effects in phototherapy or photaging.

026
Congenital Cutaneous Smooth Muscle Hamartoma
W. Hartschul and I. Hauser  
Dermatological Department, University Clinic Heidelberg, Germany  
A four-month old infant presented with a nonpainful, unspectacular lesions on the trunk. The slowly growing patches consisted of perifolicular papules without prominent hair and were not hyperpigmented. After excision, the lesions revealed the typical histological aspect of smooth muscle fibers interdigitating with collagen bundles and elastic fibers within the reticular dermis as well as ultrastructural smooth muscle differentiation with funifom, characteristically lobulated cells, surrounded by a continuous, often multifocal external lamina, dense bodies and blunt end nuclei with deep indentations; immunohistochemistry further confirmed the findings by positive reactions with actin and desmin antibodies. Therefore, the lesions turned out to be hamartomatous proliferations of smooth muscle cells. The features disclosing the diagnosis of this rare condition are described and discussed with respect to other cutaneous myogenic conditions.

027
3-Dimensional Reconstruction of the Free and Attached Gingiva of SSc-Patients
M. Bacharach-Buhler, T. Porwol,* H. Acker,* P. Altmeurer, and J. Jackowiski  
Dermatological Department of the Ruhr-University, Essen; *Max-Planck-Institute for Molecular Physiology, Dortmund, Germany, †Department of Oral Surgery, Faculty of Dental Medicine, University of Witten, Germany  
Documented oral manifestations of systemic sclerosis (SSc) include impaired mouth opening with periodontitis after routine tooth extraction and gingival curettage. The specimens were embedded in Technovit 7100 and studied by 3D-reconstructions concerning the height and width of the oral epithelium and the connective tissue papillae which are separated from each other by tonofilaments attached to the numerous ribosomes and vacuoles. Mastocytes were found among reduced interincisal distance, teleangiectasis, xerostomia, increased frequency of diseased, missing teeth, periodontal disease, increased periodontal ligament width, and osseous resorption in SSc-patients. Long cytoplasmatic processes of basal cells were penetrating the basement membrane was intact.  
The Ultrastructural Study of Squamous Cell Carcinoma and Keratoacanthoma
I. Zalewiska-Kuliecka, D. Mikulka, and A. Nowak  
Department of Dermatology, Pomeranian Academy of Medicine, Szczecin, Poland  
The squamous tumors (206 squamous cell carcinomas (95%) and 45 keratoacanthomas [45 keratoacanthomas]) underwent histopathological examination in the light microscope (staining: HE, PAS). Additionally, specimens from 25 patients with squamous cell carcinoma and 12 patients with keratoacanthoma underwent examination in the transmission electron microscope [EM – 1200 (JE)]. The electron microscopy of squamous cell carcinoma revealed reduced number of spaces between the cells of spinous layer, occasionally the desmosomes were found within the cytoplasm of these cells. Numerous cytoplasmatic processes replaced desmosomes and hemidesmosomes on the surface of the cells in the spinous and basal layers. Lack of continuity was found in the basement membrane. Long cytoplasmatic processes of basal cells were penetrating through the basement membrane. Syenoplastic and myoepithelial cells were found among the keratoacanthocytes. The mastocytes were already degranulated or were loosing the granules. They were attached to the surrounding cells with numerous long processes. The amorphous tonofilament condensation or big amorphous structures – presumably the first phase of development of keratin pearls – were found within the cytoplasm of these cells.
Focal parakeratosis was found within the cornous layer in the electron microscopy study of keratoacanthoma. The squamous layer was hyperplastic and the intercellular spaces were dilated. The desmosomal junctions between cells were more frequently intact than in the squamous cell carcinoma. The morphological analogies of desmosomes were found within the cytoplasm of the spinous cells. Numerous cellular microvilli were localised on the surface of the squamous cells. Homogenous keratotic foci were found among the cells and within the cytoplasm. The cells with high degree of keratin were filled up with homogenous corneous mass made of aggregated tonofilaments attached to the numerous ribosomes and vacuoles. Mastocytes were found among skin cells and homogenous corneous masses; some of them were loosing the granules. The basement membrane was intact.

ABSTRACTS
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
168
029
Autologous Minigrafting for Difficult Areas of Leukoderma
L. Nieuweboer-Krobotova and W. Westerhof
Netherlands Institute for Pigmentary Disorders, Amsterdam, and Department of Dermatology, Academic Medical Centre, University of Amsterdam, the Netherlands
Minigrafting is regarded as one of the treatments for leukoderma. Melanocyte transplantation for difficult localizations is not a common treatment and it has specific criteria.
We evaluated the efficacy of minigrafting in depigmentation of the eyelids, retroauricular areas, lips, nipples, fingers and male genitals.
Two test minigrafts of 2 mm were implanted in achromic lesions of patients with vitiligo focalis, segmentalis vulgaris and leukoderma after laser treatment. Patients were selected for pigment cell transplantation when the spread of pigment was observed within two months. Repigmentation was judged by the same dermatologist every month.
Thirty-three patients (22 with vitiligo and 1 with leukoderma) after laser treatment were grafted with the minigraft test.
The results of the treatment were scored visually and with the use of photography. They showed 75–99% repigmentation in 16 patients (69%), 51–74% in 2 patients (9%), 25–50% in 3 patients (13%) and 0–24% in 2 patients (9%). The time of follow-up varied from 1 to 12 months after grafting. Except for two patients with vitiligo showing a positive Koebner phenomenon and developed depigmentation of the minigrafts, we did not observe undesirable effects such as scarring and infection at the donor site, or cobblestone-like texture and incomplete or spotty repigmentation at the acceptor site. The group of patients with stable leukoderma showed the best results in areas of nipples, face and male genitals.
Autologous minigrafting is a successful treatment for difficult areas in stable leukoderma.

031
Porphyria Cutanea Tarda – Case Report
H. Michalikova, M. Jirova, and L. Pock*
Department of Dermatology, 3rd Faculty of Medicine, Charles University, and *Dermatohistopathological Laboratory, Akcurska 484, Prague 8, Czech Republic
Porphyria cutanea tarda (PCT) is a chronic, disfiguring porphyria, caused by a deficiency of uroporphyrinogen III decarboxylase in the liver cell. Skin and liver findings are typical. The hepatotoxic factors are the starting factors of the acquired sporadic form. The liver cell damage appears to be the major factor in the pathogenesis of disease. Activity of uroporphyrinogen III decarboxylase is reduced about 50%. High uroporphyrin excretion in the urine is typical. Alcohol is an important factor, participating both in manifestations of sporadic PCT and in the development of its exacerbations.
We report a 46-year-old man with skin fragility, trauma-induced blisters and erosions. The patient developed the lesions in Spring 2000, and they were localized in the sun-exposed areas/backs of the hands and fingers, face and neck. He usually drinks about 5 beers a day, sometimes a lot of vodka. During the first hospitalisation at another Department of Dermatology in Prague, the increase of porphyrins excretion in the urine was not detected. The first biopsy of lesional skin showed subepidermal vesicles and there was a suspicion of epidermolysis bullosa acquisita. (Many cases of PCT have been misdiagnosed as epidermolysis bullosa acquisita). The diagnosis of PCT was suggested following newly developed clinical symptoms and after taking biopsies for the second histological examination with direct immuno-fluorescence.
In our hospital, laboratory parameters showed highly elevated porphyrins in urine – uroporphyrin III/UP, lower increase of coproporphyrin III/KP, and no porphyrin precursors. The following laboratory values were pathological: elevated transaminases, serum iron and ferritin levels. After phlebotomy, the patient was systematically treated with hydroxychloroquine in a dose of 200 mg twice a week. Topical treatment included antiseptic bath treatment and ointment. This therapy was successful; the healing of skin lesions was followed by slight atrophy, scarring, hyperpigmentation and depigmentation. The rate of UP and KP in the urine had decreased to the normal value.

032
Evaluation of the Atopic Skin Residence by Staphylococcus Aureus Using an Impression Method, Effects of UVB 311 nm Phototherapy
M. Selerova, V. Tomova,* and M. Nozickova
Department of Dermatology, Hospital Norù Jičín, *Antibiotic centre, Regional public health Department, Ostrava, and TDepartment of Dermatovenereology, Charles University School of Medicine, Hradec Králové
Porphyria cutanea tarda (PCT) is a chronic disorder in porphyrin metabolism, due to a deficiency of uroporphyrinogen III decarboxylase in the liver cell. Skin and liver findings are typical. Alcohol is an important factor, participating both in manifestations of sporadic PCT and in the development of its exacerbations.
We examined and tested 22 patients, ranging in age from 25 to 55 years. All of them were the patients with PCT, which was confirmed by laboratory values: elevated transaminases, serum iron and ferritin levels. The cases of two of our patients are described. In both of them, patch tests with the coloured material of their clothes led to a reaction of the contact allergic dermatitis type, while patch tests with TROLAB, the European Standard series of common contact allergens (produced by Hermal) yielded negative results.
The discussion is focused on the problems connected with determining the specific dyes used to colour the textile clothing materials.

033
Autoimmune Reactions in Patients with Plaque Psoriasis
T. V. Ihara
tschuk
Sevskoe District Central Clinic, Kiev, Ukraine
The purpose of our work was to study cellular reactions to neurospecific proteins by sensitizing neurotrophils in patients suffering from psoriasis.
We examined and tested 22 patients, ranging in age from 25 to 55 years. All of them were diagnosed with Type II psoriasis, and their test indices were studied in the Neuroimmunology laboratory of the Neurosurgical Hospital.
All of our investigations showed that cell mediated sensitization was increased by more than twofold. However, the level of autoantibodies for tissue antigens was within the normal range.
In our opinion, determining the parameters of autoimmune reactions in every specific case may assist in evaluating the patient’s immune response capability, and hence in determining the choice of appropriate, effective immunocorrective therapy.