Abstracts from the 13th Annual Meeting of the Society for Cutaneous Ultrastructure Research and the European Society for Comparative Skin Biology, Paris, France, 28–31 May, 1986—Part I

Epidermolysis bullosa acquisita. Electron and immunoelectron microscopic data M. Zultak, E. Quenez, P. Humbert, D. Blanc, S. Coumes-Marquet and R. Laurent, Department Dermatology, CHU Besançon, 25030 Besançon Cx Inserm U 198, 25000 Besançon, France

We report the case of a 62-year-old man whose past medical histories consists of a broken left forearm in 1947, and a chronic psychiatric illness treated with lithium since 1977. First bullae burst out in 1983 and have consistently appeared on his shins, forearms and jugal mucosa since this date. Bullae are flaccid, Nikolski’s sign is positive in their close proximity, and they heal with marked scarring.

Routine histological examination reveals a subepidermal cleavage with an intact roof, and a mononuclear infiltrate with no eosinophils. Out of six biopsies taken from April 1983 to December 1985 for direct immunofluorescence, three were positive and showed IgG and C3 binding, along the BMZ with a linear irregular pattern.

Electron microscopy revealed an alteration of the sublamina densa component with a rupture of collagen fibres and dermal oedema. Focal alterations and limited breakages in the lamina densa are regarded as secondary. Specimens for immunoelectron microscopy are cut in two pieces. One is fixed in 2% formaldehyde cacodylate buffer, and the other one in liquid nitrogen. Sections are incubated with anti-IgG, and revealed with the peroxidase-antiperoxidase method. Examinations reveals a faintly dense, discontinuous and granular deposit under the lamina densa. Indirect immunofluorescence has been consistently negative, as well as the search for an underlying disease. Several therapeutic trials were unsuccessful.

Pemphigoid and Epidermolysis bullosa acquisita: direct immunoelectron microscopic and clinical studies in 32 patients C. Prost, V. Chassade, B. Lapellle and L. Dubertret, Department of Dermatology, Hop H. Mondor, 94010 Creteil Cx, France

By IEM, auto-antibodies (AA) were localized: (a) in the lamina lucida (LL) in 20 patients (group I), (b) on the dermal side of the BMZ in 11 patients (group II). The upper dermis, the anchoring fibril (AF) zone and the lamina densa (LD) were overlayed, according to previous direct IEM findings in 'classic' EBA, in five patients (group Ia). The LL, LD and AF zones were overlayed, but the upper dermis respected in six patients (group Iib). Localizations on LD and AF zones have been reported for EBA antigen (by indirect IEM), and in vivo bound AA in 'inflammatory phase of EBA' (Journal of Investigative Dermatology 1985; 85: 79–84). Localizations on LD and LL have been reported for AA3 antigen (British Journal of Dermatology 1985; 113: 651–659) and in vivo bound AA of CP and LCP (B Albini. Immunodermatology of the skin, New York. Wiley Medical, New York. Archives of Dermato-

Clinical features

| Clinical features         | Group I (n=20) | Group II (n=12) |
|--------------------------|---------------|-----------------|
| Head–Neck +/–            | 9/11          | 6/4             |
| Mucosa +/–               | 1/19          | 6/5 P<0.05      |
| Flexural areas +/–       | 20/0          | 8/3             |
| Extensor areas +/–       | 18/2          | 8/2             |
| Size >/=<1 cm            | 10/8          | 9/1             |
| Bullae tense/flecid      | 15/3          | 6/4             |
| Erythematous PLS +/–     | 19/1          | 9/2             |
| Normal PLS +/–           | 3/17          | 8/2 P<0.05      |
| Nik erythematous PLS +/– | 1/19          | 2/8             |
| Nik normal PLS +/–       | 6/14          | 5/5             |
| Scar-Milia +/–           | 4/16          | 9/2 P<0.001     |

Neuropeptide immunoreactivities in peripheral nerves of the skin—comparative electron microscopic investigations in rat and man U. Schultz-Ehrenburg,* R. W. Veh† and K. H. Andres†, Departments of Dermatology* and Anatomy†, Ruhr-Universität Bochum, FRG

Numerous neuro-regulatory peptides have been described recently in the brain and the gastrointestinal tract. So far, only a few of them have been detected in the skin. With regard to the highly complex sensory and autonomic functions of the skin, others may be important, too.

The present work aimed to obtain a more complete knowledge of the neuropeptideergic innervation of the skin. For this purpose, vibratome sections from human trigeminal ganglion, facial skin and finger tip, and perfusion-fixed rat tongue, whisker follicle and foot pad were immunostained following the ABC-technique prior to embedding in araldite.

At first, the trigeminal ganglion was screened with a battery of 16 commercially available neuropeptide antibodies, because this ganglion contains highly packed neuropeptide-producing cell bodies for all the sensory nerves of the densely innervated facial skin. So far, Met-
enkephalin-like immunoreactivity was found in nerve cell bodies and large myelinated fibres within the trigeminal ganglion. Substance P (SP) and calcitonin gene-related peptide (CGRP)-like immunoreactivities were found in nerve cells and fibres of the ganglion, as well as in unmyelinated fibres of small nerves within the skin and the tongue. The fine and ultrastructural localization of SP-immunoreactivity-containing axons suggested a close correlation to mechano-sensitive structures, questioning the exclusive role of SP in pain reception. The CGRP-like immunoreactivity showed a roughly similar distribution. Additionally, vaso-active intestinal polypeptide (VIP)- and neuropeptide Y (NPY)-like immunoreactivities were found in skin and tongue. In contrast to the above mentioned peptides, VIP and NPY were concentrated in the surroundings of the cutaneous vascular plexuses, around eccrine sweat glands, and close to small capillaries between muscle fibre bundles of the tongue. This distribution, in addition to their absence in the trigeminal ganglion, may suggest a neuroregulatory function of these peptides related to the autonomic nervous system.

**IgA in human sebaceous glands**

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Immunoglobulin A polymers are well known to be an important part of local immunodefence on mucous membranes, and have been demonstrated in various tissues, as for example in the gastrointestinal, urogenital, and bronchial tracts. It has not yet been studied whether these factors are also present in skin. The immunohistochemical methods have shown, that IgA is present in various parts of the skin, in normal human skin and in sebaceous areas. In particular, the highly differentiated cells showed a more intensive staining, with the heaviest deposition in the excretory duct. In electron microscopy, a double-bridge PAP technique. For electron microscopy, a post-embedding technique with colloidal gold was used. On semi-thin sections a marginal staining of sebocytes could be demonstrated. In particular, the highly differentiated cells showed a more intensive staining, with the heaviest deposition in the excretory duct. In electron microscopy, an accumulation of gold granules could be seen in the intercellular spaces of the sebocytes. Intracytoplasmic staining of basal sebocytes was weak to negative. However, more highly differentiated cells showed dense groups of numerous gold granules in the cytoplasm, whereas the fully developed cells revealed an additional diffuse distribution of the IgA determinants in the remaining cytoplasmic protusions between the lipid droplets. Several control experiments showed negative results. These results demonstrate, by two different immunomorphological methods, the occurrence of IgA in the sebaceous glands of human skin. This finding correlates with the protective mechanism of IgA, known from the mucous membranes, and gives evidence for a similar function in sebum.

**Immunoelectron microscopic localization of established and newly discovered epidermal basement membrane components using a post-embedding technique with colloidal gold**

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Knowledge of the distribution of known components within the epidermal basement membrane (EBM) has been based largely on the immunoperoxidase system applied to tissues before fixation. We have used the technique of post-embedding on-section labelling with IgG-colloidal gold to obtain more precise information than is currently available about the localization of two established components of the EBM, and to localize the antigens identified by the polyclonal antibody AA3 (also known as 6/2) and by the monoclonal antibody LH 7:2 (two novel probes of use in the diagnosis of recessive forms of epidermolysis bullosa [EB]). Normal human skin was examined, both unfixed and fixed, in a variety of formaldehyde-based fixatives, or in 0-1% buffered dimethylsuberimidate. Specimens were dehydrated in methanol and embedded in Lowicryl K4M of K11M resins. Ultra-thin sections were incubated with polyclonal antisera to laminin (LN) or type IV collagen (IV C), or with AA3 or LH 7:2 antibodies. Secondary incubations were performed with gold anti-rabbit or goat anti-mouse IgG conjugated with 5 nm colloidal gold particles (Janssen Pharmaceutica).

In all specimens, IV C localized predominantly to the lamina densa (LD) with relatively little labelling of the lamina lucida (LL). LN labelling was also evident mainly within the LD. Both IV C and LN labelling were seen in short rows, suggesting an underlying filamentous network throughout the EBM. LH 7:2 labelled with LD, while AA3 was found in both the LD and LL.

We conclude the EBM does not consist of well-defined compartments containing LN and IV C in the LL and LD, respectively, but that these macromolecules are co-distributed within the LD with filamentous extensions into the LL. This information, together with localization of novel antigens, will help to define further studies on the pathogenesis of EB.

**Herpes gestationis factor reacts with the amniotic epithelial basement membrane**

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Patients with herpes gestationis (HG) have a circulating serum factor called 'herpes gestationis factor', which is a complement binding IgG. This factor is detected by the complement fixing immunofluorescence technique using normal human skin as the substrate. It has been suggested that the 'circulating HG factor' that reacted with skin BMZ may be induced by placental antigens and this auto-antibody may also bind to the fetal tissues and thereby jeopardize the pregnancy. Previous reports indicated that the anti-BMZ antibody found in the HG sera did not bind to homologous and autologous placenta and fetal membranes.

In order to re-examine this question, sera from five clinical and immunopathological proven herpes gestationis patients were studied by complement fixing immunofluorescence and complement fixing immunoelectron microscopy on specimens of skin, amniochorion and placenta. The results demonstrated that the complement fixation auto-antibody (herpes gestationis factor) could bind to the basement membrane zone of skin, amnion and chorion laeve but not that of placental syncytiotrophoblast. C3 deposits were observed as electron-dense products in a band-like pattern along the basement membrane zone of epidermis and amniotic epithelium. Most of them obscured the lamina lucida.

These data suggest that the herpes gestationis factor may be...
induced by the basement membrane antigens of extra-villous cytotrophoblasts.

Immunogold labelling of a desmosome-related antigen for determination of the degree of a keratinocyte differentiation in skin tumours and epidermal cell cultures M. HAFTEK, K. MEISSNER*, J. VIAC AND A. REANO, INSERM U.209, Hop E. Herriot, 69437 Lyon Cx 03, France; *Universitätshautklinik, Hamburg, RFA FRG

The KM48 monoclonal antibody (Ab) was demonstrated to react with a keratinocyte membrane antigen predominantly concentrated at desmosomal regions. Immunoelectronmicroscopy and use of the immunogold technique permitted the quantification of membrane antigenic sites, and a comparison of the expression of the KM48 antigen with ultrastructural evidence of the cell maturation normal epidermal cells (EC) in suspension. It was demonstrated that the number of keratinocyte membrane antigenic sites detected by KM48 mAb and revealed by immunologically linked 15 nm gold particles of GAM IgM conjugate increased along with ultrastructural evidence of the cell maturation.

Cell-to-cell suspensions of trypsinized spinal-cell carcinoma tumours, and of marginal epidermis from the same patients, were studied with the same immunoelectronmicroscopy method. Tumour-cell morphology varied largely from neoplastic cells of spinal layer type to deeply de-differentiated cells that only seldom bore signs of a keratinocyte identity. Quantification of immunogold-labelled antigenic sites revealed significant reduction in KM48 antigen expression by tumour cells when compared with the keratinocytes from marginal epidermis. The degree of reduction depended on the degree of keratinocyte de-differentiation. Similar parallelism could be observed in normal EC cultures. Cultured EC demonstrated ultrastructural features of relative keratinocyte de-differentiation and were not labelled with the KM48 mAb. After increase of cell culture stratification by delipidation of the culture medium, more differentiated cells bearing KM48 antigen could be found. Immunogold labelling of the desmosome-related KM48 antigen can be used for determination of the degree of epidermal de-differentiation in ultrastructural studies.

Effects of gamma-interferon on HLA-DR and HLA-DQ antigen expression by human Langerhans cells. D. SCHMITT, M. GOMES*, C. DEZUTTER-DAMBUYANT, M. HAFTEK AND J. THIVOLET INSERM U.209, Service de Dermatologie, Hop. E. Herriot, Lyon, France; *Service de Dermatologie, Faculté de Médecine, Lisbonne, Portugal

Indirect immunogold labelling was used to identify cell membrane HLA-class II antigens on human Langerhans cells (LC) before and after incubation with gamma-interferon. Isolated epidermal cells were obtained by skin trypsinization and enriched in Langerhans cells by Ficoll–Hypaque sedimentation and the panning technique. Dispersed human Langerhans cells were maintained in culture medium at 37°C during 24 h in the presence or in the absence of gamma-interferon.

After 24 h of culture, Langerhans cells were fixed and the HLA-DR antigen class II density of the cell membrane to 15-7 gg//im. In the presence of gamma-interferon, the density of HLA-DR antigenic sites was significantly increased (mean 26-4 gg//im). In contrast, the modification of the HLA-DQ antigenic site density before (1-4 gg//im) and after 24 h of culture (13-3 gg//im) was not significantly modified in the presence of gamma-interferon (17-7 gg//im). In controls, the T6 antigen expression was not induced by exposure to gamma-interferon.

These results suggest that gamma-interferon may induce a modulation of the expression of HLA-class II antigens expressed by human Langerhans cells, and especially the density of HLA-DR antigenic sites. These results suggest also that gamma-interferon may play a role in regulating skin-associated immune responses through enhanced expression of HLA-DR antigens.

Further immunoelectronmicroscopy investigations on colloidal gold immunocytocchemistry applied to cutaneous sections for a suitable detection of surface antigens of resident and infiltrating cells in normal and pathologic skin C. FERRARI, G. C. MANARA, G. MANFREDI AND G. DE PANFILIS Departments of Dermatology and History, Parma University, Parma, Italy

Immunocolloidal gold (IcG) methods show several important advantages in comparison to other immunoelectronmicroscopy (IEM) techniques, such as immunoperoxidase (IP) and immunoferritin. The present investigation was performed to achieve an as suitable as possible IcG labelling of plasma membrane-associated antigens of resident and infiltrating cells on skin sections.

A novel pre-embedding in situ double step IcG technique was developed, slightly modified in comparison to the procedure we previously described in preliminary investigations. Skin biopsies were fixed in 4% paraformaldehyde, washed in dimethylsulfoxide and cut by a cryostat. Ten to twenty micrometres-thick sections were fixed in 4% paraformaldehyde, incubated with a panel of monoclonal antibodies, and, after exhaustive washings, incubated with a gold-labelled anti-mouse serum. After further washings, sections were post-fixed and treated for the standard electron microscopy procedures.

A suitable specific and sensitive IcG labelling of the cell surface of resident and infiltrating skin cells was achieved, together with a good ultrastructural preservation of subcellular and tissue details. Due to the more gentle fixation, the present pre-embedding procedure presumably allows to better preserve the antigenicity of plasma membranes in comparison to post-embedding procedures. The advantages of the present IcG method in comparison to the IP technique deal with higher specificity, higher sensitivity, and the possibility of quantitative evaluation of the labelling.

Expression of amnion antigens on normal human skin: an immunoelectron microscopy study using a panel of monoclonal antibodies J. P. ORTONNE*, B. L. HET, P. VERRANDO*, F. BERNERD, G. PAUTRAT, J. P. LACOUR* AND A. PISANI* *CIRAD, Sophia Antipolis, 06565 Valbonne Cx, France
We have previously demonstrated, using polyclonal antibodies, that epidermis and amnion epithelium share several antigens. In the same line of research, a panel of monoclonal antibodies was prepared using amnion cells as antigenic sources. Their reactivity on normal human skin was studied by indirect immunofluorescence and by immunoelectron microscopy (IEM).

The monoclonal GB showed strong binding at the dermo-epidermal junction. IEM revealed electron-dense products in the lamina lucida, sometimes apparently related to hemidesmosomes.

The monoclonal GB stained the intercellular spaces from the basal layer to the stratum granulosum. IEM showed that the antigen recognized by GB was exclusively located on the keratinocyte membrane including the desmosomal.

Two other monoclonal antibodies stained, respectively, the cytoplasm of basal (GB) and suprabasal epidermal keratinocytes. By IEM, the immunoreactivity of these two monoclonal antibodies was diffuse, although in some cells it appeared to be associated with intermediate filaments. The biochemical characterization of the antigens recognized by these monoclonal antibodies is in progress, and further studies are required to establish their usefulness for the study of normal and abnormal skin.

Scanning electron microscopically controlled epidermal sheet separation for psoriasis for cell cultivation H. J. Schulze and G. Mahrle Department of Dermatology, University of Cologne, FRG

A clean-cut dermo-epidermal separation is a requirement for further culturing of epidermal cells. In this study, we will present a gentle and distinct method to separate the dermo-epidermal junction (DEJ) directly above the lamina densa (LD), which shows good results in acanthotic skin for isolating all basal cells from the germinative compartment without any contamination by dermal fibroblasts. Methods for DEJ separation, such as sodium bromide, hyaluronidase, dithiothreitol, phosphate-buffered saline and dispase, are discussed and comparatively monitored with the scanning electron microscope (SEM).

Probes of fresh human skin, measuring 0.6 cm², were taken from volunteers with plaque psoriasis. Optimal results for separation were obtained by incubation with either 2 u.c. sodium bromide at 37°C for 30 min, or dispase (1-5 U/ml) at 4°C for 10 h. Each method separated the DEJ completely and regularly above the LD of psoriatic skin, preserving characteristic epidermal "Wurzelfüschen" and dermal micropapillae in SEM. Concerning cell viability, dispase preparation was superior to sodium bromide and less cytotoxic. Viable Ki-67-positive, keratin-positive, vimentin-negative keratinocytes were isolated and cultivated on collagen-coated dishes, in combination with 0.3% trypsin for 2 min.

Morphological differentiation of psoriatic hair-follicle keratinocytes in culture M. C. Lenoir*, C. M. A. A. Gossé, B. Shroot* and J. J. M. VerMorken*†. Centre International de Recherches Dermatologiques (CIRD), 06565 Valbonne Cx, France; †Research Unit for Cellular Differentiation and Transformation, University of Nijmegen, Netherlands

Psoriatic human hair-follicle keratinocytes were cultured on bovine eye lens capsules in Epicult dishes. They were then examined after either 3 weeks or 6 weeks in culture, using light and electron microscopy. In comparison to control cultures derived from non-psoriatics, there were significant differences in 3-week-old cultures: (1) stratification in general was more extensive; (2) suprabasal cells were flat instead of round; (3) there were almost no depositions of basal lamina or cellular debris on the growth substrate; (4) numerous membrane coating granules and a few keratohyaline granules were present; (5) the differentiation pattern resulted in an earlier appearance of corneocyte-like cells, and clusters of these cells appeared to have been shed into the culture medium. When older cultures of psoriatic and control hair-follicle keratinocytes (6 weeks) were compared, several differences observed in the 3-week-old psoriatic and control cultures were found to have disappeared. However, some morphological differences persist that could be readily observed in light microscopic preparations, and that might allow discrimination between normal and psoriatic cultures: (1) the lower cell layers contained predominantly flattened cells in psoriatic cultures, instead of round as in control cultures; (2) the differentiation pattern was irregular in psoriatic cultures, instead of regular as in control cultures; (3) the differentiated zone was very compact and relatively marked in comparison to the lower cell layers in psoriatic cultures. In our cell culture system, hair-follicle keratinocytes, derived from involved and uninvolved psoriatic skin, differentiated in culture and showed, in the absence of dermal influences, an abnormal behaviour when compared with control hair-follicle keratinocytes. Moreover, some characteristics that are also encountered in psoriatic lesions were found; these allow discrimination between psoriatic and control cultures.

Experimental behavior of a new human prepared collagen V. Mitz*, I. Golstein*, J. Malambo*, F. Vilde*, F. N. Mari† and J. Gay*, †Hôpital Boucicaut, 75015 Paris; †Institut Pasteur, 75724 Paris Cx, France

It has been possible to reconstruct a matrix of human collagen from human skin and placenta. This matrix is available for experimental research. The electron microscopy studies have shown the high quality of fibres and their density.

This collagen has been introduced, after sterilization, in the subcutaneous layer of the skin of the dorsum of rats, and also applied on healing wounds. There have not been, so far, acute rejection phenomena. Application for use in humans thus seems possible, for various indications.

Retroviruses in Kaposi sarcoma cells in AIDS K. Konrad, P. Schenk and K. Rappensberger Department of Dermatology I and Department of Otorhinolaryngology II, University of Vienna, Vienna, Austria

Human T-cell lymphotropic virus type III (HTLV-III) has been identified as the causative agent for AIDS. The virus has been shown to occur in cultured human lymphocytes, in blood, in various body fluids and, recently, also in Kaposi sarcoma (KS) cells in a lymphonode.

We report on the appearance of budding retrovirus particles in KS cells of oral KS lesions from a patient with AIDS. Several biopsies were taken from the tumorous KS lesions on the hard palate. The material was processed for routine transmission electronmicroscopy.

Very occasionally, retrovirus particles were seen budding from the plasma membrane, or had separated from the cell surface of KS cells.
The viruses had a diameter of 100–120 nm and exhibited dense cylindrical-shaped cores. The ultrastructure of these virus particles is identical to the morphology of the recently described HTLV-III.

To our knowledge, this is the first report on the presence of retroviruses in KS cells of the oral mucosa. This observation will revive the discussion of the possible importance of the virus for the development of KS. One should bear in mind that these retroviruses are able to replicate in KS cells, but without causative relation to the tumour. Thus, the characterization of the virus-producing cells, as well as the quantitative aspects of virus production, are certainly of great interest.

'Oral hairy leukoplakia'—ultrastructural and molecular—biological analysis of two LAV/HTLV III positive cases G. Gross*, H. Zentgraf†, H. Wiegand†, H. Ikenberg‡, C. Fluckiger§ and U. D. Frese† *Department of Dermatology, University of Freiburg, FRG; †German Cancer Research Center, Heidelberg, FRG; ‡Institute of Virology, University of Freiburg, FRG; §Dept of Dermatology, University of Basel, CH

In 1984, Greenspan and co-workers (Lancet 1984; 3: 831–834) described a type of leukoplakia of the lateral parts of the tongue associated with 'full-blown' AIDS.

In order to characterize the viruses present within the tissue of oral hairy leukoplakia (OHL), biopsies and epithelial swabs from two LAV/HTLV III-positive individuals were analysed, respectively, by means of light-microscopy, immunocytochemistry, electron-microscopy and DNA–DNA hybridization on filters.

In case I, virions of 45–55 nm in diameter were present at a low concentration, whereas herpesviruses could not be found. In case II, both herpesviruses were disclosed intranuclearly, and coronaviruses were disclosed within the intercellular spaces. A third virus type, structurally similar to HTLV II and HTLV III, was additionally seen in almost destroyed epithelial cells of the tongue. By means of molecular hybridization on epithelial swabs from the OHL of both patients, EBV- and HPV-DNA sequences were demonstrable. These findings are in contrast to that of Greenspan and co-workers (New England Journal of Medicine 1985; 313, 25: 1564–1571), which attributed the lesions exclusively to a reactivated Epstein-Barr-Virus or a Papillomavirus infection. Special attention should be focused on the detection of LAV/HTLV III within epithelial of OHL of the tongue.

Further investigations are planned to elucidate whether OHL indeed represents an AIDS-specific leukoplakia, or whether it is also seen with the same frequency in individuals suffering from acquired immunodeficiency different from AIDS, such as, for instance, renal allograft recipients.

Congenital self-healing histiocytosis E. Thomine, M. C. Boulle, P. Lauret and J. Hemet, Laboratoire d'Anatomo-Pathologie, Clinique Dermatologique, CHU Rouen, France

Two cases of congenital, self-healing histiocytosis are reported. The most recent case was studied with electron microscopy and immunostaining by OKT 6 and S 100 antibodies. The other, seen before the advent of immunostaining, was peculiar by its association with a giant cell placentitis.

Most of the tumour cells were OKT 6 and S 100 positive. In contrast to the findings of immunostaining, the ultrastructure study showed only few cells containing Langerhans cell granules, frequently attached to the cell membrane. These Langerhans cells were not scattered over all the Grids but were gathered into small groups. The other tumour cells contained intra-cyttoplasmic histiocytic organelles: smooth and rough vesicles, mitochondria, REE, Golgi and lysosomal inclusions. Frequent, laminated, myelin-like bodies were found but never in association with Langerhans cell granules in the same cell.

This ultrastructural heterogeneity contrasts with the immunostaining homogeneity. Only few recent cases have been studied by both techniques. But, according to Hashimoto (Journal of the American Academy of Dermatology 1984; 11: 447–453) and Caputo (Archives of Dermatology 1982; 118: 267–272), we consider that self-healing congenital histiocytosis is among the X type of histiocytic proliferations.

Non-neoplastic circulating Sezary-like cells in a cutaneous T-cell lymphoma: ultrastructural, immunological and T-cell receptor gene rearrangement studies A. Bendelac, N. T. J. O'Connor, M. T. Daniel, C. Boitard, C. Michel, L. Lerche, P. Lesabre and J. F. Bach Departments of Immunology, Hospital Necker, Paris and Haematology, John Radcliffe Hospital, Oxford, UK, and Hopital Saint Louis, Paris, France

The Sezary cell is a structurally distinctive cell which represents various conditions of T-helper phenotype lymphocytes, ranging from clonal malignant proliferations including cutaneous T-cell lymphomas, to reactive non-neoplastic expansions encountered in some benign disorders. Histological examinations in one case of cutaneous (nodular) lymphoma had showed massive lymphoid proliferations in the dermis with a mature T-helper cell phenotype. No significant nuclear abnormalities nor epidermotropism of lymphoid cells were detected. There was no evidence of lymph node nor visceral involvement. Immunotyping of peripheral blood lymphocytes (5·10^4/l) revealed a highly increased T4/T8 ratio (19/1), and an abnormally faint expression of the T3 antigen on two thirds of T3+ lymphocytes. Electron microscopy with morphometric evaluation of circulating lymphocytes showed a majority of moderately to highly convoluted Sezary-like cells. However, the T-cell receptor (TCR) gene study did not show any DNA rearrangement in Ficoll-separated blood lymphocytes, even though a clonal T-cell proliferation was simultaneously evidenced in cutaneous tumours. Since dilution experiments have shown that a monoclonal population of T-cells can be detected when their DNA represents only 5% of that present in a sample, these data provide good evidence for the polyclonality of the circulating Sezary-like cells. Our study clearly demonstrates that reactive, non-neoplastic Sezary-like cells may be present even in T-cell lymphomas, and stresses the usefulness of TCR gene rearrangement studies for diagnostic and prognostic procedures in T-cell lymphoproliferations.

Ultrastructural study on Paget's disease S. Calvieri, M. Zampetti and D. Ribuffo, Clinica Dermatologica, Universita' degli Studi di Roma 'La Sapienza', Rome, Italy

Recent investigations confirmed the presence of an epidermal stem cell in the human epidermis. Other studies, moreover, have shown that epidermal stem cells as target cells in the process of experimental differentiation and neoplastic growth.
Ultrastructural research on mammary and extramammary Paget's disease was carried out to identify a possible cellular sequence of neoplastic differentiation. Indeed, with ultrastructural studies of the lesions, we were able to find stem cells, typical Paget's cells, and other elements morphologically resembling either epidermal stem cells or Paget's cells.

These results allowed us to suggest an intraepidermal histogenesis of the Paget's cells.

Cutaneous histiocytoid (epithelioid) hemangioma: clinicopathological, ultrastructural and immunohistochemical study of a case G. Pettinato, L. Insabato, A. De Ghira, A. Manco and V. Ruocco,* Institute of Pathology, Second Faculty of Medicine, and *Institute of Dermatology, First Faculty of Medicine, University of Naples, Italy

The histiocytoid hemangioma (HH) is an unusual but distinctive vascular tumour of the subunits or dermis, occasionally involving deep soft tissue or arising from vessels (Human Pathology 1979; 10: 707–707). This lesion is reported also as epithelioid hemangioma (Soft Tissue Tumors, C. V. Mosby, St Louis, 1983: 391–397), angiomythoid hyperplasia with eosinophilia (Journal of the American Academy of Dermatology 1985; 12: 781–796), a typical pyogenic granuloma (Human Pathology 1977; 8: 653–653) and, in the Japanese literature, as Kimura's disease (Japanese Journal of Dermatology 1966; 76: 61). We report a case of HH in an 8-year-old girl who presented an ill-defined blue-reddish nodularity in the anterior skin of the leg. The tumour was in the dermis and showed a vague lobular arrangement consisting of numerous, small capillary-sized vessels lined by distinct epithelial-appearing endothelial cells. These cells protended into the lumen with a characteristic hob-nail appearance. A number of epithelioid endothelial cells showed cytoplasmic vacuolization, mimicking primitive vascular lumen formation and intranuclear cytoplasmic inclusions. A mixture of inflammatory cells, particularly eosinophils, surrounded the vessels. The ultrastructural study showed many features of endothelium, including pinocytotic vesicles, thick basal lamina and Weibel–Palade bodies. Using an immunoperoxidase method, factor VIII-related antigen was localized in epithelioid cells, confirming the endothelial nature of the tumour.

Ultrastructural aspects of comparative skin carcinogenesis D. Komitowski, Institute of Experimental Pathology, German Cancer Research Centre, D-6900 Heidelberg, FRG

In order to better understand species-related differences in morphology and biology of experimental skin tumours, we compared, by means of electron microscopy, tumours induced by the same methods in different species and strains, and correlated their ultrastructural features with those of the normal skin. We treated mice (NuNu, BALB/c, NMRI), mastomys and rats with the carcinogen DMBA and the tumour promoter TPA according to the schedule of two-stage carcinogenesis. Skin lesions after the treatment and samples from unchanged control skin were investigated, both by light and by electron microscopy.

The results suggest that, when explaining species-related morphology of the tumours, important ultrastructural features of epidermis are: (a) diversity of cell types; (b) arrangement of the cell layers; (c) pattern of intercellular junctions; (d) cytoplasmic organization of tonofilaments, and (e) keratin formation. As was best evident comparing skin of mice and mastomys, even minor deviations in the forms of keratinization, types of epidermal cells and structure of desmosomes can be indicative for apparent differences in the tumour morphology. Papillomas are typical of mice and keratoacanthomas of mastomys.

Our findings demonstrate that ultrastructural organization of epidermis is important in interpreting morphological events of skin neoplasia.

The living skin equivalent: a new tool for studying dermal and epidermal differentiation in vitro L. Dubertret, B. Coulomb, C. Prost, P. Salig, E. Bell, C. Lefebreton and M. Heslan, Department of Dermatology, Hop H. Mondor, 94010 Creteil, France

The improvement of human fibroblast and keratinocyte differentiation in vitro, according to morphological, enzymological and biochemical markers, is an important aim for improving biological, pathophysiological and pharmacological studies on human skin.

A human living skin equivalent can be reconstructed in vitro: the constitution of the dermal equivalent requires two major components: fibroblasts that are multiplied in monolayer, and collagen matrix. Fibroblasts are mixed with the collagen solution and culture medium and a gel forms very rapidly. The fibroblasts extend cytoplasmic processes and collect collagen fibrils: the fibril condensation and ordering by fibroblasts give a tissue-like consistency to the gel. In this dynamic tissue model, cell permeability to reagent, and enzymatic differentiation of fibroblasts, are similar to those of the fibroblasts in vivo and quite different from those of the fibroblasts cultivated in monolayer, usually used for pharmacological or toxicological studies. The epidermis can be reconstructed on the dermis by inserting small biopsies that serve as a source of keratinocytes into a dermal equivalent made up immediately before. The growth of this new epidermis is evaluated by planimetric measurement, measure of DNA content and thymidine incorporation. The differentiation of this new epidermis is evaluated by morphological studies. Light and electron microscopy studies of the newly developed epidermis showed, at 10 days, a well organized basal layer made up of cuboidal cells; the desmosomes were numerous. Higher in the epidermis, the keratinocytes were flat with no visible nuclei, forming a thick and compact layer that desquamated. A thin granular layer with typical keratohyalin granules was observed as well as many membrane coating granules, generally absent in vitro. In the stratum corneum, cells were characterized by a thick cornified envelope. The keratin electrophoresis confirm the quality of the epidermal differentiation. Soon after grafting for giant naevi or burns fibroblasts, the skin equivalent produces many elastic fibres and the epidermal ridges reappear, giving excellent mechanical properties to the graft.

Thus, a new culture technique is available for studying modulation of human skin differentiation.

Cultured human epidermal sheets (CES) used as skin allografts: a sequential electronmicroscopic study J. Kanitakis, G. Mauduit, M. Faure, D. Schmitt and J. Thivolet, INSERM U.209, Hop E. Herriot, 69437 Lyon Cx3, France

Human keratinocytes were grown on 3T3 feeder-cell layers into multilayered epithelia. These were used as epidermal allografts to
Ultrastructure and growth of human hair follicle keratinocytes in vitro E. Imcke, M. Detmar, A. Mayer-da-Silva, H. Tiell and C. E. Orfanos, Department of Dermatology, University Medical Centre Steglitz, The Free University of Berlin, FRG

The aim of this study was to characterize, by electron microscopy and autoradiography, human hair follicle keratinocytes (ks) cultured in vitro. Plucked anagenic human scalp hair follicles were plated on collagen-coated coverslips and were incubated in McCoy's 5A medium, supplemented with 10% human serum, 100 IU/ml penicillin, 100 µg/ml streptomycin, 0.4 µg/ml hydrocortisone, 12 ng/ml EGF, 10^-5 M cholera toxin, and 4 µg/ml human insulin. After 3 weeks, the cultures were 3–4 mm in diameter, and were studied by light- and electron-microscopy. After 8, 10, 12, 14, 21 and 28 days, the cultures were incubated with 3H-thymidine (1 µCi/ml, for 16 h) for autoradiography. Beginning at Day 5, the cultures displayed an exponential growth pattern, with doubling of the surface area every 3 days. Light microscopy showed a monolayer of polygonal peripheral cells, stratified in the central areas. The origin of these cells was most likely the outer hair root sheath. Autoradiography revealed a high proliferative activity in the peripheral areas (labelling index 40%), compared to only moderate proliferation in the central areas of the cultures. By electron microscopy, ks with abundant cytoplasm were found, interconnected by desmosomes. Tonofilaments were always present with different patterns of organization, not related to the position of the cell. Keratinocytes with large, well-differentiated nuclei and normal mitochondria, RER and Golgi apparatus, showed small amounts of tonofilaments aggregated in small, not compact bundles. Other ks showed degenerated or absent nuclei, scarce organelles and increased amount of tonofilaments and thickness of the cell membranes. Keratinosome-like bodies were present; non-trichohyaline granules were also found.

Attachment and association of keratinocytes in vitro: electron microscopic and timelapse kinematographic studies B. Thiele, B. Bonnekoh and G. Maibele, Department of Dermatology, University of Cologne, FRG

Isolated keratinocytes were seeded on collagen (type I)-coated plates and studied by time-lapse cinematography in a special climate box. For electron microscopy, the cells were fixed and embedded within the plates. The results justified to differ between a phase of cell rearrangement until colonies were formed about 48 h after seeding and a succeeding phase of consolidation.

During the phase of rearrangement, settled isolated, flat keratinocytes were moving with a speed of 10–25 µm/h. They showed polarity and formed macropodia, by means of which they contacted other cells, helped to settle spherical cells, and favoured colony formation. At the ultrastructural level, only keratinocytes displaying a microvillus surface pattern were able to attach to the collagen and associate with other cells. A net of striated and non-striated fine filaments seemed to be involved in the attachment of keratinocytes on collagen.

During the phase of consolidation, cells were arranged in a similar way to fetal epidermis covered by a microvillus periderm-like upper layer. Desmosomes and a basal dense lamina occurred, but no real half desmosomes were observed. A distinct difference of in vitro-grown epidermis compared to fetal epidermis was the lack of palisading of basal cells.

Azelia acid inhibits proliferation and modulates differentiation of keratinocytes in vivo and in vitro A. Mayer-da-Silva, R. Muller, H. Gollnick and C. E. Orfanos, Department of Dermatology, University Medical Center, Steglitz, the Free University of Berlin, FRG

The effects and mechanism of action of azelaic acid (AA) were studied on human and on neonatal mouse keratinocytes in vivo and in vitro, respectively.

In vivo. Based on a double-blind clinical trial, three skin biopsies were obtained from each of 27 volunteers: one before topical application, and two after 3 months' application of a base-cream alone (placebo site).

By electron microscopy, no significant differences were detected between the specimens taken before AA application and those after placebo. In contrast, keratinocytes with disordered cytoplasmic organization, frequent perinuclear oedema, cytoplasmic vacuoles and swollen mitochondria were observed at the verum site. Moreover, a decreased amount of keratohyaline granules was seen. The remaining granules were smaller and their electron-density was irregular and reduced. Also, the tonofilament bundles of the malpighian layers seemed reduced. Melanocytes and Langerhans cells showed a more fibrillar cytoplasm and swollen mitochondria with destroyed cristae.

In vitro. In cultured mouse keratinocytes, the 3H-thymidine incorporation to DNA was reduced in the presence of AA; a 10 mM culture medium indicating an antiproliferative effect of AA. Concent-
trations higher than 50 mM were cytotoxic. In addition, by serial extraction, a pronounced reduction of keratohyaline macroaggregate proteins and non-cross-linked keratins was detected, whereas the other fractions were less reduced. By SDS-PAGE, no qualitative differences in the keratins fraction were found, but a 36 kD protein of the cytosolic fraction was dramatically decreased.

Our results indicate that AA clearly inhibits keratinocyte proliferation and modulates the early phases of their differentiation.

Collodion baby: TEM and freeze fracture study A. TAIRE, J. E. SURLEY-BAZELLE, M. DEMARQUEZ and J. MALEVILLE, Department of Pediatric Dermatology and Neonatology, Hopital des Enfants, 33077 Bordeaux Cx, and Department of Electron Microscopy, University of Bordeaux I, Talence, France

The collodion baby syndrome is a transitory condition of variable outcome in which a collodion-like membrane covers the entire body surface. We have investigated the pathophysiology of this rare skin disorder using TEM and freeze fracture (FF).

The patient, a boy, was the first child of first-degree cousins. Membranes shedding was achieved within 3 weeks, and the skin exhibited only minimal scaling at 2 months of age.

Abdominal skin biopsies were obtained at 8 days of age and processed routinely for light and electron microscopy. For freeze fracture, following glutaraldehyde fixation, specimens were impregnated with glycerol, mounted, and then fractured at —115°C using a Balzers freeze-etch apparatus. Observation of replicas and standard TEM was carried out on a Jeol 100S electron microscope at 80 kV.

By light microscopy, the stratum corneum (SC) appeared compact and thickened, containing some ghost-like horny cells. By TEM, corneocytes were normally arranged and ensheathed in electron-lucent material, which appeared stratified in some intercellular spaces. Stratum granulosum (SG) was one-cell thick and keratohyalin granules were found normal. In the upper stratum spinosum, keratinosomes were in great abundance, as well as mitochondria of bizarre shape. Images of lamellar body-contents expelled into the intercellular spaces at the SG-SC junction were consistently observed. In FF replicas, fracture planes in the SC remained mostly within the plasma membrane planes, indicating more resistant structures in the intercellular spaces, due to abundant extracellular lipids.

Our findings suggest that the collodion baby syndrome represents a transient disorder of production of intercellular mortar which accumulates in huge quantities and impairs normal desquamation. We are currently studying a pharmacological model of intercellular mortar and its pathophysiological implications for that syndrome.

Epidermolytic hereditary palmoplantar keratoderma: ultrastructural and autoradiographic study of three cases L. ATTALAH, R. LAURENT, M. ZULTAK, S. COUMES-MARQUET and P. AGACHE, Department of Dermatology, CCHU, 25030 Besancon Cx, France; INSERM U.198, Rte de Dole, 25000 Besancon, France

Epidermolytic hereditary palmoplantar keratoderma (EHPPK) exhibits autosomal dominant inheritance and characteristic histological and ultrastructural patterns, resembling bullous ichthyosiform erythroderma, in which kinetic studies indicate an increased epidermal mitotic index. We report here the results of ultrastructural and autoradiographic investigations in three patients coming from three different kindreds with EHPPK. Fresh palmar and plantar skin biopsy samples are taken from patients and controls, incubated with (H)-thymidine (specific activity 49 Ci/mmol) and processed for autoradiography. Sections are stained with Toluidine Blue.

Labelled basal and suprabasal cells are counted and the labelling index is expressed as a percentage of total basal cells. The rest of the biopsy is processed for routine histological and ultrastructural examination. Labelling index is significantly increased in patients with EHPPK. Ultrastructural changes are similar in all three patients, and most marked in the upper layers of the living epidermis: oedema and vacuolization of cytoplasm, scarcity and clumping of tonofilaments, globular and heterogenous keratohyaline granules with filamentous inclusions.

One patient was treated with etretinate and the other one with 13-cis-retinoic acid without any improvement.

Voerner's and Voerner-like palmoplantar keratosis G. MAHRLE and B. KUCHMEISTER, Departments of Dermatology, Kohn and Hamburg, West Germany

Two patients with diffuse palmoplantar keratosis (PPK), who shared a similar clinical picture (no progression, no transgression, no associated symptoms), the genetic transmission (autosomal-dominant), and histopathology (granular degeneration), will be presented. Both seemed to fulfil the diagnostic criteria of PPK Voerner until ultrastructural studies demonstrated that the two cases were not identical.

Besides cytolytic vacuoles, huge clumpy keratohyalin granules and plenty of densely aggregated tonofilament bundles in both cases, tonotubules (external diameter 43 nm) were observed only in one. Cells containing tonotubules did not display tonofilaments and vice versa. Bundling, association with keratohyalin and desmosomes, and staining with antikeratin showed that tonotubules represent keratin.

We would like to separate the PPK with tonotubules, as Voerner-like PPK, from true Voerner's PPK with tonofilaments. This seems to be the second case of Voerner-like PPK ever reported, and is similar to that of Anton-Lamprecht and Werner, presented in the last SCUR Meeting in Florence.

A histopathological and ultrastructural study of hyperkeratosis lenticularis perstans M. J. TIDMAN, M. L. PRICE and D. M. MACDONALD, Laboratory of Applied Dermatopathology, Guy's Hospital, London, UK

The clinical and histopathological features of hyperkeratosis lenticularis perstans, or Flegel's disease (Der Hautarzt 1958; 9: 362), were studied in 12 subjects, all female. In each case, the development of typical closely spaced keratotic papules, approximately 1–5 mm in diameter, arising on the lower legs, dorsa of feet, upper arms and pinnae was delayed until middle adulthood.

Histopathologically, there are discrete foci of compact eosinophilic hyperkeratosis with some parakeratosis. The underlying epidermis is thinned and spongotic, the oedema being most apparent in the basal layers. There is a focal lymphohistiocytic infiltrate within the papillary dermis beneath each epidermal lesion. No evidence of perforating lesions was found, contradicting the concept (Acta Dermatologica 1970; 50: 385; Journal of the Royal Society of Medicine 1984; 77: 16)
that Flegel's disease lies on a continuous spectrum with Kyrie's disease (Archives of Dermatology and Syphilology 1916; 123: 466).

In view of the contradictory reports concerning the presence or absence of membrane coating granules (MCG) in Flegel's disease, a non-quantitative assessment of these structures was made by transmission electron microscopy of lesional skin from four subjects. MCG of normal size and architecture were present in all lesions at the level of the upper stratum spinosum. Their characteristic lamellar inclusions were frequently observed within the intercellular spaces.

These findings strongly refute the hypothesis that Flegel's disease is associated with a major structural defect of MCG.

Developmental ultrastructural features of appendageless skin in hydrocortisone-treated and in scaleless mutant chick embryos A. MAUGER, M. DEMARCHEZ, N. BENMOUSSA and P. SENGEL, UA CNRS 682, Laboratoire de Zoologie et Biologie animale, Université scientifique, technologique et médicale de Grenoble, France

The development of cutaneous appendages (feathers and scales) in the chick embryo results from precisely timed dermal-epidermal interactions, during which characteristic ultrastructural features are expressed in particular regions of appendage anlagen. Some of them are common to feathers and scales, such as direct contacts through the basal lamina between dermal and epidermal cell processes, and tight contacts between dermal cell processes and basal lamina, the density of which is higher at the base of the feather or scale buds than elsewhere. Other features are specific of scale development, such as the prominent orientation of tubular dermal cell processes.

When appendage morphogenesis is inhibited in hydrocortisone-treated embryos or in the scaleless mutant, the above-mentioned features do not appear. In skin of hydrocortisone-treated embryos, epidermal differentiation is accelerated with early appearance of hemidesmosomes and peridermal granules, and precocious onset of keratinization. By contrast, in the scaleless mutant, these morphological features of epidermal differentiation appear at approximately the same stages as in normal embryos. Synthesis of beta-keratin, which is specifically found in cutaneous appendages, does not take place in hydrocortisone-treated embryos nor in the scaleless mutant, except in the transient subepidermal cells of dorsal skin.

In the dermis of hydrocortisone-treated embryos, collagen fibres accumulate at high density without any orderly organization, while in the scaleless mutant they are laid down in orthogonal plywood fashion.

These ultrastructural features can be related to developmental events and might represent part of the morphogenetic message that the dermis is known to transmit to the epidermis during the formation of cutaneous appendages.

Erroneous deposition of elastin-like material in fibrillar bodies (colloid or civatte bodies) and amyloid I. ANTON-LAMPRECHT, M. L. ARNOLD and V. VOIGTLANDER, Universitäts-Hautklinik Heidelberg, FRG

Fibrillar bodies, amyloid, and elastic globuli are members of a family of cytid bodies subjacent to the dermo-epidermal junction. While the epidermal origin of fibrillar bodies and the keratin nature of their constituting filaments is well established, the origin of amyloid in the various primary and secondary variants of localized and systemic amyloidosis is still under discussion, ranging from epidermal (keratinocyte) origin over in situ formation (aberrant fibroblast synthesis) to deposition of immunoglobulin light chains or their fragments (plasma cells, plasmacytomas). Amyloid thus seems to be heterogeneous in nature, in spite of the ultrastructural similarity of amyloid fibrils. Elastic globuli are composed of elastic material solely, and are considered to result from extensive synthesis of elastic fibre components.

Fibrillar bodies are capable of incorporating foreign substances such as immunoglobulins or serum proteins. Transformation of fibrillar bodies to amyloid has been claimed for cases of localized cutaneous amyloidosis, and intimate spatial interrelationships between amyloid deposits and pre-existing, mostly degenerating, connective tissue fibres (especially elastic fibres) were frequently observed. No such interrelationships are known for fibrillar bodies.

We report here an erroneous deposition of elastin-like material into fibrillar bodies in a case of non-scarring epidermolysis bullosa with intraepidermal blister formation, and into amyloid masses in a severe case of acquired cutis laxa with (plasmacytoma-induced) primary systemic amyloidosis (Annales de Dermatologie et de Vénérologie 1985; 112: 779–780). These observations may shed light on some basic requirements for formation and maturation of elastic fibre material, and on the interrelationships that seem to exist between the normal microfibrils (elastotubules) of the elastic fibre system and some recently recognized proteins associated with both, elastic microfibrils and amyloid (AP/SAP components).

Pseudoxanthoma elasticum is not only an 'elastin disease': an ultrastructural report of two cases P. BOULAC-SAGE, L. DE GENTILE, L. DUBUSSION, M. S. DOUTRE AND C. BEYLOT Laboratoire d'Histopathologie Cutanée, Hôpital du Haut-Leveque, Centre de Microscopic électronique, Université de Bordeaux II, Bordeaux, France

Pseudoxanthoma elasticum (PXE) is a genetic, connective tissue disease in which the more obvious abnormalities concern elastic fibres. However, the other components of the connective tissue are also involved.

Two male patients (34 and 57 years) exhibiting typical clinical signs of PXE were examined. Biopsies were taken for the PXE axillary lesions and processed for light and electron microscopy.

By light microscopy, swollen and irregularly clumped elastic fibres were accumulated in the middle and lower third of the dermis.

Electron microscopy allowed the following observations:
(i) irregularly shaped elastic fibres often exhibited holes of various sizes and shapes and were sometimes calcified;
(ii) an abundant thready material consisted of both compact and electron dense areas, and loose filamentous areas;
(iii) collagen fibres were highly irregular in size with numerous 'collagen flowers';
(iv) the ground substance was abundant with filaments and knobs around collagen and elastin fibres;
(v) fibroblasts presented a prominent RER with dilated cisternae.

These observations illustrate the alterations of all components of connective tissue in PXE. The thready material do not correspond, as previously said, to altered elastin fibres, but to collagenous protein, fibrinogen and glycoprotein (Journal of Cutaneous Pathology 1984; 11: 282–291). In addition, numerous collagen flowers are present in PXE, as in most connective tissue diseases.
François' syndrome: an ultrastructural study R. CAPUTO, S. CAVICCHINI and M. MONTI First Clinic of Dermatology, Milan, Italy

François' syndrome (FS) is a rare, genetically determined dermo-condrocorneal dystrophy characterized by cutaneous fibromata, bilateral corneal dystrophy and enchondral ossification abnormalities. Five cases of FS have been described so far, all with proliferation of specific fibroblastic cells of unknown nature. In histological preparations of skin and mucous lesions, the entire dermis is thickened, due to the newly formed collagen, with disorganization of the connective fibres, large fibroblastic cells with highly vacuolized cytoplasm (spongocytes), and decreased amounts of elastic fibres and the vascular component.

Histologically in our case, the infiltrating cells were strongly positive for lysozyme and negative for protein S 100. An EM study of the proliferative cells was done to clarify their fine structure and their functional role. We observed that most of these cells were large fibroblasts with multilobated and infolded nuclei. Cytoplasm was abundant and rich in strongly dilated, smooth and rough endoplasmic reticulum containing tubules and lipids. These features gave the cells their spongy look. The cytoplasm also contained many lysosomes, coated vesicles and myelinated bodies. Some cells showed macrophage attributes. The dermis showed zones of rarefaction and disorganization of the collagen fibres and degraded elastic material. Mastocytes were also not infrequent. These histochemical and ME findings suggest that FS proliferating cells could be considered as peculiar fibroblasts with macrophagic attributes.

Cutaneous involvement in Pompe disease W. GEIBHART, W. JURECKA, M. MAINITZ and E. PASCHKE*, Department of Dermatology II, University of Vienna, Medical School, Vienna, Austria; Department of Pediatrics, University of Graz, Austria

Light- and electron-microscopic demonstration of specific storage material is a most valuable tool in the establishment of an accurate diagnosis of many metabolic disorders. In addition, the knowledge of the preferentially involved tissue elements gives better insights into the pathomechanics and symptomatology of the individual disease.

In Pompe disease, such a diagnosis is usually based upon biochemical data (alpha-glucosidase-deficiency) from cultured fibroblasts of muscle tissue, combined with the demonstration of glycogen deposits in various cell types. Skin biopsies from patients suffering from infantile (generalized) and adult (muscular) type of Pompe disease were investigated by light and electron microscopy. In both cases, large amounts of lysosomally stored glycogen could be demonstrated in cutaneous arrector pili muscles. In addition, specific inclusions were also present in keratinocytes, melanocytes, endothelial cells and fibroblasts of the infantile, generalized type. These findings indicate that skin biopsies are not only valuable for the confirmation of the diagnosis, but also for the differentiation of special variants of Pompe disease.

Merkel cell mitosis in the hair follicles of mouse embryo skin Y. MEROT, P. CARRAUX and J. H. SAURAT Clinique de Dermatologie Hopital Cantonal Universitaire, Geneva, Switzerland

Merkel cells (MC) are intraepidermal cells presently considered as epithelial cells (they express low molecular weight cytokeratins) with neuroendocrine differentiation. They possess unique and characteristic ultrastructural features: dense-core, membrane-bound (neuro-secretory type) granules 100–200 nm in size, which are the ultrastructural markers of MC. Mitosis of MC has never been observed previously and this suggests either a long life span with very infrequent or even no mitosis, or that mitosis was taking place in a precursor cell devoid of specific granules.

In the course of studying MC development in the mouse embryo skin, skin specimens (heads) were obtained from 12- to 18-day-old mouse embryos, immediately fixed in 3% glutaraldehyde, and processed for standard electron microscopy.

Intraepithelial cells with dense-core, membrane-bound granules, i.e. MC, were exceptional in the hair follicle epithelium of 12-day-old embryos. However, they increased daily in number thereafter, and were already easily recognizable from Day 13. Furthermore, within the hair follicles on 13- and 14-day-old mouse embryos, we observed four mitotic cells (three prometaphases and one telophase) showing several dense-core, membrane-bound granules typical for MC granules. These cells were located in a suprabasal position, and were surrounded by ultrastructurally characteristic keratinocytes. From Day 12 to Day 18, we never observed dermally located cells with MC ultrastructural features.

These observations indicate that: (1) proliferation of MC can take place in the epidermis; (2) mitosis can occur in a cell already showing signs of neuro-endocrine differentiation, i.e. dense-core, membrane-bound granules; and (3) MC mitoses preferentially seem to occur in early embryonic life.

Trabecular carcinoma of the skin W. JURECKA, M. MAINITZ and D. METZE, Second Department of Dermatology, University of Vienna, Medical School, Vienna, Austria

Reflecting on the various names that have been used for putative Merkel cell carcinoma, including neuroendocrine carcinoma of the skin, trabecular carcinoma, primary small cell carcinoma of the skin, and others, the origin of these tumours remains enigmatic. It is now widely accepted that this tumour is derived from, or differentiates towards, dermal neuroendocrine cells, although it remains unclear whether the cutaneous Merkel cell is a specially differentiated epidermal cell or a cell of neural crest origin. Six cases of trabecular carcinoma have been studied by light and electron microscopy. Immunohistochemical staining was performed with S100 cytokeratin, neurofilament and neuron-specific enolase. One case showed lymph node metastases. A second case showed multifocal trabecular carcinoma of the scalp, probably due to lymphogenetic metastases in highly actinically damaged skin together with multiple keratoses and basal cell carcinoma. After 3 months of oral etretinate (75 mg daily) the progress of the trabecular carcinoma had halted, but he had developed squamous cell carcinoma. A third case showed a tumour on the arm, in which Bowen’s carcinoma was diagnosed in the periphery, and trabecular carcinoma in the central exophytic and ulcerated area. Taking into account that epidermal tumours may show neuroendocrine differentiation, a possible relationship of these tumours are trabecular carcinoma of the skin is discussed.

Ultrastructural autoradiography with radiolabelled monoclonal antibodies against melanoma-associated antigens W. TILGEN, I. KAUFMANN, M. ENGSTNER and S. MATZKU*, Universitäts-Hautklinik und *Inst.f.Nuklearmedizin, Deutsches Krebsforschungszentrum, Heidelberg, FRG

The potential diagnostic and therapeutic efficacy of monoclonal
antibodies (mAbs) depends on the fate of the immune complexes after antibody binding. Possible mechanisms are: (1) stable anchorage to the cell surface, (2) internalization, and (3) shedding of the immune complex.

To elucidate the pathways of mAbs directed against melanoma-associated antigens, melanoma cell lines in metabolic active state were incubated with a series of 125I-labelled mAbs for different time intervals. Binding and release of mAbs were then analysed by their ultrastructural localization.

Using different mAbs, all three reaction types could be detected: (1) stable localization of immune complexes in the cell surface was observed after 30 and 120 min, covering the whole cell membrane; (2) internalization became evident by localization of immune complexes within the cytoplasm after 120 min; (3) A shedding process was indicated by dissociation of immune complexes from the cell surface; this was supported by findings in metabolically inactive glutaraldehyde-prefixed cells, which showed stable binding of mAbs to membrane antigen without further dislocation.

Radioantibody binding assays confirmed these ultrastructural findings. Analogous experiments on nude mice were performed to correlate the in vitro data with the rate of tissue accumulation of mAbs in vivo. There is no doubt that internalization favoured high accumulation of mAbs in the tumour, which may be of value, especially for therapeutic purposes, e.g. mAb-mediated transport of cytototoxic drugs.

Radioantibody binding assays confirmed these ultrastructural findings. Analogous experiments on nude mice were performed to correlate the in vitro data with the rate of tissue accumulation of mAbs in vivo. There is no doubt that internalization favoured high accumulation of mAbs in the tumour, which may be of value, especially for therapeutic purposes, e.g. mAb-mediated transport of cytotoxic drugs.

Thus, it was possible to visualize the dynamics of mAb-induced antigen modulation by ultrastructural autoradiography, which by itself is a statical method.

A study of melanosome shape in a case of complex dyschromia, and in control caucasoid and negroid individuals C. Foldeş*, R. Trouvé†, F. Cottenot* *Dept Dermatology, Hop St Louis, 75010 Paris, France; †INSERM U.26, Hop Fornand Widal 75010 Paris, France

In addition to the study of melanosome size, and melanosome distribution patterns in keratinocytes as single or as complexed, it seems to be interesting to consider the shape of melanosomes. Since, on prints, melanosomes appear as sections of general ellipsoid, we use the technique of Franklin (Journal of Cell Biology 1977; 74: 485–491).

It allows the estimate of three-dimensional parameters from cytological two-dimensional preparations, and the study of the profile/axis ratio distributions. The long and short axes of melanosomes are measured, and their ratio (t) is determined. The distribution of (t) is characteristic of the shape of melanosomes. It has been determined in hypopigmented and hyperpigmented skin of a Caribbean patient with complex dyschromia, and in control Caucasian and Negroid skin. Melanosomes from the hypopigmented skin of our patient exhibit an intermediate shape between control Negroid and Caucasian skins. Melanosomes from the hyperpigmented skin exhibit an abnormal (t) distribution: it appears to be due either to a combination of melanosomes of various shapes, or to an abnormal non-ellipsoidal shape of the melanosomes.

The estimation of the melanosome shape appears to be an interesting tool in the study of pigmentary disease. However, other normally and pathologically pigmented individuals must be investigated before valid conclusions may be obtained.

Atypical PUVA lentigo: a histological and ultrastructural study O. Gauthier* J. F. Surleve-Bazille†, Y. Gauthier* and L. Texier†, *Department of Dermatology, Hop R. Boulin, Libourne, France; †Electron Microscopy, University of Bordeaux I, Talence; ‡Dermatology Hop St André, 33000 Bordeaux, France

Widespread lentigines occurred following PUVA therapy in a 60-year-old woman presenting a Sezary-like syndrome. The patient had received PUVA therapy (6–7 J/cm²) weekly for 4 years.

Light microscopy study of PUVA lentigines showed a dermal infiltrate and exocytosis, a little amount of melanin in the epidermis, and pigment incontinence.

The split-dopa reaction revealed an increased number of melanocytes. TEM study showed scarce presence of melanin in the keratinocytes. Numerous melanocytes containing lipid droplets were observed. In addition to lymphо-histiocytic dermal infiltrate, pigment-laden melanophages were seen in the papillary dermis.

Contrary to previous studies of PUVA lentigines, no melanocytic atypia such as binucleated melanocytes or giant melanosomes were observed.

The basal lamina was constantly found to be multilayered and protruding deeply into the papillary dermis.

These findings indicate that the unusual aspect of this PUVA lentigo (exclusive melanin overloading of dermal macrophages) might be linked to the initial clinical syndrome associated with an alteration of the basal lamina.

Features for the recognition of malignant melanocytic cells using high-resolution image analysis (comparison between semi-thin and thin sections) W. Stolz*, W. Abmayr†, C. Schmoeckel* and O. Braun-Falco• *Department of Dermatology, University of Munich, FRG; †Gesellschaft für Strahlen und Umweltforschung mbH Munich, FRG

For the evaluation of criteria differentiating between benign and malignant intraepidermal melanocytic cells, 400 nuclei of malignant melanomas and benign nevi were investigated in semi-thin (LM) and thin sections (EM), using quantitative methods by means of digital image processing. Different kinds of caryometric and chromatic textural features were selected and multivariate analysis was performed.

In addition to caryometric parameters, the chromatin texture was found to be important for the recognition of malignant cells in EM and LM and was included in the discrimination function. With linear discriminant analysis, about 90% (EM) and 85% (LM) of the nuclei were classified correctly, using nuclear area and different chromatic textural features.

We conclude that high-resolution image analysis methods allowed not only a differentiation between malignant and benign lesions, but can be used also to differentiate melanocytic nuclei as malignant.

Isolation of epithelial appendages N. Martinet, and T. Nigra, National Institute of Dental Research, NIH, Bethesda, MD 20892 and Dermatology Department, Washington Hospital Center, Washington, D.C. 20010, USA

We have designed a technique for isolation of epithelial appendages, having first worked with mice in order to optimize the isolation
conditions, and have been able to isolate large quantities of mouse hair follicles. We have compared the keratins extracted from hair shaft, skin and hair follicle by electrophoresis and immunoblotting. Our results show that a keratin of 57 KD is common to hair follicle and skin, and that other keratins are shared by hair follicle and hair shaft but do not appear in skin. Culturing hair follicles embedded in methyl cellulose with radiolabelled arginine allowed us to localize a 200 KD insoluble protein by fluorography, very likely to be mouse trichohyalin. We have extended this technique to human skin appendages, using skin from optimal locations. Sweat glands were isolated from soles, sebaceous glands from nipples, and hair follicles from hairy skin. Such isolated epithelial appendages will be useful for numerous and varied studies.

**Buschke–Ollendorf syndrome (BOS): ultrastructural study of two cases**

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Ultrastructural studies of BOS are rare and emphasize on elastic fibres abnormalities with increase of elastin. Only Uitto (Journal of Investigative Dermatology 1981; 76: 284-287) has noted highly irregular collagen fibrils, with collagen flowers. A 58-year-old woman and her 56-year-old brother were examined for numerous typical dermal papules. These patients had also osteopoikilosis. Biopsies were performed on the skin involved, and investigated by light and electron microscopy. Light microscopy showed an accumulation of abnormal, broad and interlacing elastic fibres in the dermis. By electron microscopy, the elastic fibres were usually large, with a branching appearance. The periphery of these fibres was incised by deep recesses, isolating globular and anastomotic lumps. In contrast with abundant electron-lucent elastin matrix, the microfibrils were very decreased. Collagen fibrils were highly irregular in cross section, with numerous collagen flowers, particularly near the abnormal elastic fibres. The fibroblasts were scarce, with marked dilated rough endoplasmic reticulum. The scanning electron microscopy confirmed the elastin and collagen fibre abnormalities. Our findings, as did Uitto’s study, demonstrate once more that the nature, aetiology and pathogenesis of the so-called dorsal cyst are still unknown, as is reflected by the variable synonyms such as mucous, synovial, myxoid, or mucinous degeneration cyst. To our knowledge, transmission electron microscopic investigations have not yet been performed. Six lesions extirpated under regional block anaesthesia were fixed in buffered glutaraldehyde and processed for TEM. Within the connective tissue, areas of massive increase in mucoid ground substance were found which possibly represent precursors of the true cystic space. As suggested from light microscopy of paraffin and epoxy resin semithin sections, no synovial lining of the cystic space was found. The lumen contained finely granular mucinous substances, some erythrocytes, fibrin and occasional macrophages resembling mucoplaques as seen in mucous granulomas (mucocoeles) of the oral mucosa. The lumen wall consisted mainly of collagen fibres, but myofibroblast-like cells were sometimes found in between the connective tissue fibres and rarely at the luminal border, thus resembling a ganglion wall. Some cells contained cross-striated collagen fibres. Abundant secretory fibroblasts, cells with many lysosomes of variable size and shape, and large amounts of cellular debris were found in the connective tissue. The results suggest that the cysts studied are not synovial cysts but may be due to degenerative changes in the proximal nail fold, which may later become ganglion-like.

**A glucagonoma syndrome: an histological, immunocytochemical and ultrastructural study**

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We report a typical case of necrolytic migratory erythema occurring in a 36-year-old woman, with patent diabetes mellitus but without weight loss or basal hyperglucagonemia. Removal of the pancreatic tumour induced complete regression of cutaneous lesions. The Pancreatic tumour was composed of nests, islands of relatively uniform enlarged cells, with amphophilic cytoplasm, nucleocytoplasmic atypia, and showed vascular invasion. Grimelius’ argyrophilic reaction was strongly positive in some cells. Immunocytochemistry for various hormones was performed: the tumour was immunonegative for insulin, gastrin, somatostatin, VIP, HGH and ACTH. Immunocytochemical stains for glucagon (DAKO)-specific neurone enolase, keratin (KL1, Immunotech) showed rare but strongly reactive cells. Electron microscopy confirmed the diagnosis of an islet cell neoplasm: the tumour was composed of a uniform population of epithelial cells with secreting granules measuring 150, 230 nm, and characterized by the presence of an eccentric electron-dense peripheral nucleoid, a typical feature of glucagon-producing cells. Cutaneous pathology showed eosinophilic necrosis of the upper third of the stratum malpighium, spongiosis with exocytosis of mononuclear cells, and eosinophils together with a moderate lymphohistiocytosis infiltrate of the superficial dermis.

**Visualizing percutaneous drug transport; an analytical electron microscopic study**

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Analytical electron microscopy (AEM) was used to study _in vitro_ skin penetration of tri-iodothyronine. Penetration experiments took place in a diffusion cell with human skin as a membrane; the donor compartment contained a drug solution with or without an added agent. Diffusion times ranged from 3 to 24 h. After completion of a penetration experiment, skin samples were prepared for TEM using standard methods. The topographic distribution of the iodinated drug inside the skin sections was measured _in situ_ by x-ray micro-analysis using a solid state detector. The quality of the fixation was verified from TLC analyses and mass-spectrometry on fixatives and dehydration fluids, as well as from
model experiments using gelatin. The results are explained in terms of possible mechanisms of penetration.

They furthermore suggest that AEM will be particularly useful for studying the penetration of peptides through the skin and other epithelial tissues of interest.

Ultrastructural findings in early large plaque parapsoriasis

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Large plaque parapsoriasis is a chronic dermatosis which is clinically characterized by polymorphous skin lesions, ranging from erythematous to poikilodermatous macules. The peculiarity of the disease lies in the possible progression to malignant lymphoma. Histologically, early large plaque parapsoriasis shows a non-specific dermatitis, and the differentiation from small plaque parapsoriasis is difficult.

Ultrastructurally, early large plaque parapsoriasis reveals an interstitial oedema in the squamous and basal cell layer. There are also signs of a discrete liquefaction degeneration of the basal cells. The cellular infiltrate lying in the papillary body and the upper corium consists of lymphocytes and macrophages. Exocytotic lymphocytes show a cerebriform nucleus contour and scanty cytoplasm often situated in contact with Langerhans cells. On the ultrastructural level, early large plaque parapsoriasis can be characterized and differentiated from small plaque parapsoriasis, which shows less epidermal oedema and only little tendency of the sparse cellular infiltrate to invade the epidermis. These ultrastructural findings, on the other hand, do not allow an interpretation of the possible pathogenesis of early large plaque parapsoriasis or of small plaque parapsoriasis. For differentiation, clinical features are of greater value, for example the typical onset, which is always solitary in large plaque parapsoriasis and multiple in the small plaque type.

Ultrastructural observations on pseudokaposi M. Fimiani*, M. de Santis†, C. Miracco1 and L. Andreausi*, †Istituto di Clinica Dermosifilopatica, Universita di Siena; †Istituto di Anatomia ed Istologia Patologica, Universita di Siena, Italy

Kaposi sarcoma (KS) and pseudokaposi sarcoma (PKS) were reported to be ultrastructurally similar.

In order to obtain further information about the fine morphology of PKS, we studied a case of Bluefarb–Stewart Syndrome (BSS) in a 21-year-old male. Punch biopsies for electron microscopy (EM) were obtained from nodular lesions and processed routinely. EM examination revealed an abundant proliferation of mainly hypertrophic endothelial and fibroblast-like cells, organized in vascular channels, often without appreciable lumen, surrounded by actively phagocytic pericytes. Sometimes, however, the endothelial cells clearly delimited a vascular lumen and protruded thereinto. The basal membrane was consistently altered, non-homogeneous in thickness, and polysaturated. It was only occasionally possible to discern erythropagocytosis phenomena. In the endothelial cell cytoplasm, variable quantities of Weibel–Palade and multivesicular bodies and numerous pinocytotic vesicles could be seen. Endothelial cell junctions were of varying complexity, but were most commonly tight junctions. The fibroblast-like cells were usually situated at the periphery of the vessels. They were elongated with abundant cytoplasm and large amounts of filaments, which were densest about the cell periphery where there were also numerous pinocytotic vesicles and dense bodies. These cells have been identified as myofibroblasts. In the cytoplasm of the endothelial cells, there were some crystalline formations of periodic structure, probably of proteic material. They were non-viral in character but otherwise difficult to interpret.

Histopathology of the skin in homozygous variegate prophyria

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The patient was born 5 weeks prematurely in June 1980. During the first two months of life, he had severe skin disease: large, partly haemorrhagic vesicles in the face and body, and the skin abraded easily. The vesicles healed leaving cheloid scars. During the subsequent years, the patient has had a moderate increased fragility of the skin on the exposed areas.

Porphyrin analyses were made when the patient was 2 years old: urinary porphyrins and their precursors normal, fecal protoporphyrin 500 nmol/g (normal < 100) and coproporphyrin 130 nmol/g dry weight (normal < 30), and erythrocyte protoporphyrin 4200 nmol/l (normal < 600). Lymphocyte protoporphyrinogen oxidase activity was 0-4 in the patient and in the parents (who were first cousins) 2-3 and 2.7 nmol/h/mg protein, respectively (normal 3-6-6-0). Other enzymes of the haeme-biosynthesis were normal. The findings suggest homozygous variegate prophyria. The biopsy of the involved skin showed subepidermal cleavage with homogenous granular material and basal lamina reduplication. The superficial vessels were also surrounded by granular material and by basal lamina layers.

Immunohistochemistry and electron microscopy of long-lasting allergic patch tests

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Allergic patch tests usually disappear within 5–10 d, but in rare cases persist for weeks or months. Five cases of these long-lasting allergic patch tests have been observed by us during the last 3 years, and biopsies were taken at 15–75 days. Lymphocytes and Langerhans cells were analysed using immunohistochemistry and conventional transmission electron microscopy. The epidermis showed focal parakeratosis and mild acanthosis. A moderate to strong infiltrate of inflammatory cells was seen in upper and mid dermis. As in normal patch tests, most inflammatory cells were pan-T-lymphocytes (OKT1-+). 50–75% were OKT4+ and 25–50% were OKT8+, the average ratio of OKT4/OKT8 cells being 2:1. Only few, usually OKT8+, lymphocytes were encountered in the epidermis. OKT6+ Langerhans cells (LCs) were normal or increased in number in the epidermis, while very few cells displayed OKIal antigen in the epidermis. Most epidermis dendritic cells were thus OKT6+/OKIal-negative, indicating loss of OKIal-staining of LCs OKT6+ cells were seen in high amounts in the dermis, especially around hair follicles.

Using electron microscopy, LCs were seen to be surrounded by mononuclear cells, both in the epidermis and the dermis, sometimes in a rosette-like fashion. Langerhans cells and mononuclear cells were seen to cross the basal lamina, indicating persistence of increased activity of these cells. Mononuclear cells resembling LCs were abundant in the dermis, but only few contained Birbeck granules, indicating that not all OKT6+ cells contain these granules in the dermis.
A defect in down-regulation of the contact hypersensitivity response, or a constant antigen stimulation, could be responsible for the long-lasting allergic patch tests. Currently it is not known whether re-testing these patients would result in recurrence of the long-lasting patch test.

**Neurogenic sarcomas in Von Recklinghausen's neurofibromatosis**

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Neurogenic sarcomas are a major and relatively frequent complication in the course of Von Recklinghausen’s neurofibromatosis. The present study of five cases was aimed at investigating the histological aspect of tumour proliferation, which can be atypical. Detection of S100 protein or NSE by the immunoperoxidase technique are in some cases negative. Ultrastructural studies revealed variable degrees of Schwannian differentiation: cellular basal membrane, neurosecretory granules, neurofilaments, sparse junctions.

**Accessory cells in the cutaneous infiltrate of B-cell lymphomas**

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The micro-environment of non-Hodgkin lymphomas is supposed to reproduce the features of corresponding normal lymphoid tissue, either in the lymph node or in the skin. In fact, accessory cells of B and T zones of the lymph node have been described in cutaneous B- and T-cell lymphomas. We performed an ultrastructural study of the cutaneous infiltrate of five patients with B-cell lymphoma, to ascertain whether the accessory cells of B-zones, dendritic reticulum cells (DRC), were consistently present. The infiltrates were chiefly formed by lymphocytes and peculiar non-lymphoid cells. These cells had an irregular shape, the number and length of cytoplasmic projections varying greatly among the patients. The cytoplasm contained flat cisternae of rough endoplasmic reticulum, smooth vesicles and tubules (more numerous close to the Golgi apparatus), a well-developed Golgi apparatus and a variable number of round, membrane-bound bodies, presumably primary lysosomes; secondary lysosomes or residual bodies were exceptional. The nucleus was indented and had a well-recognizable fibrous lamina, a thin peripheral rim of condensed chromatin, and a usually small nucleolus. These cells and their cytoplasmic projections always contacted several lymphocytes. Desmosome-like junctions between these cells, or small processes coated with electron-dense material were not seen. In conclusion, the non-lymphoid cells observed in our cases resembled less mature forms of DRC, like those described in lymph nodal neoplastic follicles.
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