0022-202X/83/8004-0302$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 80:302–382, 1983

Vol. 80, No. 4
Printed in U.S.A.

ABSTRACTS

The Society for Investigative Dermatology, Inc. and European Society for Dermatological Research Joint International Meeting

The Capital Hilton Hotel
Washington, D.C.

April 27–May 1, 1983

Wednesday, April 27, 1983

2:00 PM–8:00 PM	Registration	Upper Lobby
4:00 PM–6:00 PM	All Posters Installed	Upper Terrace
6:00 PM–7:30 PM	Welcoming Reception	Presidential Ballroom

Thursday, April 28, 1983

7:00 AM–5:00 PM	Registration	Upper Lobby
7:00 AM–7:30 AM	Continental Breakfast Residents and Fellows only	Foyer No. 2
7:30 AM–9:00 AM	IRVIN H. BLANK RESIDENT/FELLOW FORUM **Mechanisms of Carcinogenesis** David R. Bickers, M.D., Presiding I. Bernard Weinstein, M.D., Multistage Carcinogenesis: Cellular Targets and Host genes Margaret L. Kripke, Ph.D., Immunobiology of Ultraviolet-Induced Skin Cancer Douglas R. Lowy, M.D., Transforming Genes and Cancer	Federal Room
8:00 AM–9:00 AM	Annual Business Meeting—SID Members Leonard C. Harber, M.D., Presiding Presentation of STEPHEN ROTHMAN AWARD to Richard B. Stoughton, M.D.	Presidential Ballroom
9:00 AM–9:20 AM	Welcoming Remarks Leonard C. Harber, M.D., SID President Gerd Plewig, M.D., ESDR President	Presidential Ballroom
9:20 AM–12:00 PM	PLENARY SESSIONS Ruth K. Freinkel, M.D. and Herbert Hönigsmann, M.D., Presiding	

Two Antigen Presentation Pathways, Only One of Which Requires Langerhans Cells, Lead to the Induction of Contact Hypersensitivity. J. W. STREILEIN, P. R. BERGSTRESSER, Depts. Cell Biol., Int. Med., Dermat., UTHSCD, Dallas, Texas

Evidence indicates that induction of contact hypersensitivity (CH) takes place within skin: (a) timed excision of hapten-injected sites removes the relevant sensitizing depot; (b) *H-2* antigens of mouse skin restrict effector function of T cells induced by haptenated grafts. Since Ia-positive dendritic cells seem to be required for antigen presentation, it has been proposed that epidermal Langerhans cells (LC) assume this function in skin. Supporting evidence includes: (a) sites depleted of LCs do not promote induction of CH; (b) surface devoid of LCs (cornea, tape-stripped body wall skin) fail to immunize to Ia antigens; (c) suspensions of epidermal cells enriched for LCs stimulate allogeneic lymphocytes and present antigen to primed T cells *in vitro*. We have examined the requirement for LCs by employing mouse strains BALB/c, C3H, C57BL/6, which differ at *H-2* and other loci. When shaved abdominal wall skins were exposed to acute, low dose UVB radiation and painted with DNFB, BALB/c, but not C3H or C57BL/6, mice developed CH, suggesting genetic susceptibility to this UVB effect. Similar treatment of F_1 and segregant backcross populations revealed that susceptibility to UVB-impaired induction of CH is a dominant trait governed by 3 separate loci, one of which is *H-2*. We next tape-stripped abdominal wall skin of BALB/c, but not C3H, mice developed CH, suggesting that the former can utilize an antigen presentation pathway independent of LCs. We propose that two, independent pathways of antigen presentation for CH exist, that only one requires LCs, and that genetic control of this pathway is mediated in part by genes within the major histocompatibility complex.

Mechanisms Involved in Epidermal Cell Induced, Con A-Driven T Cell Activation. LAURA A. STINGL, THOMAS A. LUGER, GEORG STINGL, Departments of Dermatology I & II, University of Vienna Medical School, Vienna, Austria.

Con A-driven T cell proliferation is an accessory cell-dependent phenomenon. It is as yet unknown whether the signals derived from these accessory cells are linked to the expression of Ia antigens and/or the capacity to provide immuno-enhancing signals, e.g. Interleukin I. Since epidermal cells consist of both Ia-positive Langerhans cells (LC) and keratinocytes which produce the Interleukin I-like factor ETAF, we attempted to determine whether epidermal cells (EC) could promote Con A induced T cell proliferation and, if so, to define the role of the EC subpopulations in this process.

When highly purified (column adherence, anti-Ia + C′ treatment) T cells from Balb/C and C3H/He mice were cultured for 72 hr in the presence of Con A alone, no proliferation (as measured by ^{3}H-thymidine incorporation) was seen. Untreated, but not anti-Ia + C′ treated, syngeneic or allogeneic EC served to promote Con A-driven T cell responses. Both LC-containing and LC-depleted EC generated equal amounts of ETAF activity as measured in a thymocyte proliferation assay. Continuous presence of anti-Ia antibodies during the culture period significantly reduced the amount of Con A-induced T cell proliferation. When EC were substituted by Ia-negative, ETAF producing PAM 212 cells or semi-purified exogenous ETAF, Con A did not induce T cell proliferation.

Our results demonstrate that LC are required for the generation of EC-induced Con A-driven T cell responses and that keratinocyte-derived cytokines do not suffice to replace their function.

Comparative Epidermotropism (E-tropism) of T Cell Subsets Recruited Nonspecifically into Sites of Contact Hypersensitivity (CH) Reactions to DNFB in Mice. R. E. TIGELAAR, Dermatology Dept., UTHSCD, Dallas, Texas

The factors affecting lymphocyte migration patterns within the skin are incompletely understood. Using a murine model of CH to study the phenotypes of cells nonspecifically recruited into such sites, we have shown previously that T cells exhibit greater migration into and/or retention in the epidermis (i.e. are more E-tropic) than non-T cells (JID 74:245, 1980). To test the hypothesis that different T subsets have distinctive E-tropic capacities, purified T cells (99% Thyl.2+, 99% Lytl+, 27% Lyt2+) were prepared from oxazolone-immune mice. Preincubation with a monoclonal anti-Lyt2 was followed by "panning" on anti-IgG-coated plates, which resulted in two fractions enriched for the two major subsets of murine T cells: nonadherent cells (<3% Lyt2+,

i.e. enriched for helper/inducer phenotype) and adherent cells (86% Lyt2+, i.e. enriched for suppressor/cytotoxic phenotype). After radiolabeling with ^{3}H-Uridine, 2 × 10 cells were injected i.v. into groups of DNFB-immune mice just prior to DNFB ear challenge. 24 hr later the ears were processed for autoradiography: the mean frequencies of labeled cells per cross-section in both the dermis (D) and epidermis (E) are shown below.

Cells Injected	D + E (±S.E.)	E	[% in E]
1) Untreated T cells	52 ± 1.9	5.7 ± 0.5	11.1 ± 1.2
2) anti-Lyt2, not panned	50 ± 10.3	6.2 ± 2.1	11.0 ± 1.7
3) Panned-nonadherent	73 ± 9.4	4.0 ± 1.0	5.1 ± 1.2
4) Panned-adherent	51 ± 6.1	14.0 ± 1.4	27.3 ± 1.3

The data indicate that Lyt2-enriched cells are strikingly more E-tropic than either Lyt2-depleted or unfractionated T cells. These results are consistent with the hypothesis that recruitment into the epidermis of phenotypically distinct T cell subsets is a selective process.

Anti-Leu-1 Therapy for Cutaneous T Cell Lymphoma (CTCL). A. R. Oseroff, R. A. Miller, T. Meeker, P. T. Stratte, I. R. McDougall and R. Levy, Stanford University Medical Center, Stanford, California.

Seven patients with advanced CTCL refractory to conventional therapies have been treated with multiple infusions of monoclonal anti-Leu-1 antibody in doses of 0.25–100 mg with minimal complications. Four patients had 70–90% reduction in cutaneous and lymph node disease, two had a smaller (20–50%) response, and one had no clinical benefit. Four patients made an anti-mouse Ig antibody response; in three this limited the duration of therapy. Although anti-Leu-1 reached cutaneous tumor infiltrates and, in the non-responder, an axillary lymph node, there was no histological or clinical evidence for *in situ* action. In responding patients, anti-Leu-1 caused large transient diseases in the number of circulating T cells. Dosage was critical, as high serum antibody levels induced cell surface modulation of the Leu-1 antigen thereby inhibited the clearance of circulating cells. The distribution of indium-labelled autologous lymphocytes was studied with whole body imaging and blood counts in a patient with a normal white count and differential. Within 24 hr, more than 90% of the labelled cells left the circulation and were exchanged into tumor sites and into the RE system. Subsequent administration of anti-Leu-1 caused a rapid shift of labelled cells into the liver, and to a lesser extent, and with slower kinetics, into the spleen. The return of circulating T cells was due in part to re-equilibration from extracutaneous sites as well as to the release of some modulated cells from the liver. Clinical regression of cutaneous disease may be based on the deposition of circulating cells in the RE system with subsequent mobilization of cells from tissue sites.

Different Papillomavirus Genetic Sequences Are Required for Morphologic Transformation and Extrachromosomal Replication. Douglas R. Lowy, Sisir K. Chattopadhyay, and Yasuharu Nakabayashi, Dermatology Branch, National Cancer Institute, Bethesda, Maryland.

In mouse cells that are morphologically transformed by bovine papillomavirus (BPV) virions, by the molecularly cloned 8 kb full-length viral DNA genome, or by a specific 5.5 kb sub-genomic BPV DNA fragment (called 5.5T), the viral DNA is maintained exclusively as multiple circular extrachromosomal molecules. We have now determined more precisely the BPV genetic elements required for transformation and have separated the transforming function of the BPV DNA from its ability to maintain its extra-chromosomal location. The capacity of certain BPV DNA deletion mutants lacking specific sequences within 5.5T to transform the mouse cells suggested that the gene(s) encoding the transforming protein(s) is (are) located in the rightward 2.3 kb (called 2.3R) of 5.5T. We confirmed this hypothesis by constructing a hybrid DNA molecule composed of a retroviral DNA control element (which contains sequences for activation, promotion, and initiation of RNA transcription) and 2.3R. Neither the retroviral DNA nor 2.3R would themselves transform the mouse cells. However, ligation of these two DNAs to each other resulted in very efficient oncogenic transformation. Further, deletion of sequences from one of the three open reading frames in 2.3R did not inhibit the capacity of the hybrid DNA to induce cellular transformation. In cells transformed by the deletion mutants or by the hybrid DNA, the viral DNA sequences were found to be integrated, rather than extrachromosomal.

These results localize the BPV transforming gene(s) to 2.3R and demonstrate that other viral sequences in 5.5T are necessary for maintenance of the viral DNA in its extrachromosomal state.

Characterization of the Human α_2(I) Collagen Gene. Madeleine Duvic, Jenny Ting, Shingo Tajima, Russel Kaufman, and Sheldon Pinnell, Division of Dermatology, Duke Univ. Med. Ctr., Durham, North Carolina.

Vertebrate collagen genes have a length and complexity which are unmatched by other known genes. The large number of intervening sequences requires extensive RNA processing and adds another level of complexity in the synthesis of collagen. In order to understand the mechanisms of defective collagen synthesis and regulation, we have attempted to isolate and characterize human Type I collagen genes. By screening a human bacteriophage gene library we have isolated and characterized over 30,000 base pairs of DNA which represent most of the human α_2(I) collagen gene. We have confirmed that these recombinants contain α_2(I) sequences by direct sequence analysis and by characterizing m-RNA which specifically anneals to the recombinants. DNA sequence analysis revealed coding blocks of 108 and 54 base pairs which predicted the typical collagen GLY-X-Y amino acid pattern. The amino acid sequence predicted from these coding blocks was compared with bovine α_2(I) sequences and revealed a unique run GLY-GLY-LYS-GLY-GLU-LYS common to both human and bovine α_2(I) collagen. The cloned collagen gene sequences have also been used to select specific RNA species which directed the synthesis of only pro α_2(I) chains in *in vitro* translantion studies.

An extensive restriction endonuclease map of the cloned DNA has been prepared. A variation in an Eco RI site at the 5′ end of the cloned gene may represent an endonuclease polymorphism.

Transfection of Xeroderma Pigmentosum Cells with Cloned DNA. David B. Whyte, Miroslava Protic-Sabljic, John Fagan, and Kenneth H. Kraemer, National Cancer Institute, N.I.H., Bethesda, MD 20205

Cells from patients with xeroderma pigmentosum (XP) are hypersensitive to killing by ultraviolet (UV) radiation. As a first step in attempting to clone the gene(s) responsible for the XP cells' UV hypersensitivity, we chose the model system described by Mulligan and Berg (Proc Natl Acad Sci 78:2072, 1981) utilizing the cloned bacterial gene xanthine-guanine phosphoribosyl transferase (XGPRT) (in a plasmid containing pBR322 and SV_{40} sequences) to assess transfection ability. Reconstruction experiments utilizing a 6-thioguanine (6TG) resistant ($HGPRT^-$) subline of the EB virus transformed lymphoblastoid cell line (LCL) XP20s demonstrated the ability to detect $HGPRT^+$ cells at a frequency of 10^{-5} to 10^{-6} in mass culture using HAT (hypoxanthine, aminopterin, thymidine) selection. Treatment of the $HGPRT^-$ LCL with cloned DNA using modifications of the calcium phosphate precipitation technique of Graham and Van Der Eb (Virology 52:456, 1973) failed to produce a detectable frequency of $HGPRT^+$ cells. Similarly, treatment of XP fibroblast lines XP6Be and XP2Ne with cloned XGPRT DNA using MAAT-X (mycophenolic acid, adenine, aminopterin, thymidine, xanthine) selection failed to produce detectable colonies. In contrast, treatment of the SV_{40} transformed cell lines XP20s(SV_{40}), XP12T703(SV_{40}), XP12Be(SV_{40}), XP6Be(SV_{40}) and XP2Ne(SV_{40}) with cloned DNA, using MAAT-X selection, all produced detectable numbers of colonies. Transfected subclones of XP20s(SV_{40}), and XP12Be(SV_{40}) have been established that grow well in selective or non-selective medium for at least 3 months. SV_{40} transformation, whether by selection or by alteration of cellular properties, appears to facilitate uptake and/or expression of cloned DNA sequences in XP cells.

Characterization of Distinct Species of Human Epidermal Cell Thymocyte Activating Factor. T. A. Luger, M. B. Sztein, J. A. Charon, and J. J. Oppenheim, 2nd Department of Dermatology, Vienna; LMI, NIDR, N.I.H., Bethesda, MD.

Human epidermal cell thymocyte activating factor (ETAF) derived either from normal epidermal cells or a squamous cell carcinoma cell line has recently been described to be a low molecular weight (m.w.) protein which is indistinguishable from human macrophage derived Interleukin I (IL I). Since ETAF like IL I stimulates a wide variety of different target cells including lymphocytes, fibroblasts, neutrophiles, monocytes and hepatocytes, it seemed likely that different species of ETAF rather then one may be responsible for this multiplicity of biological activities. Biochemical studies showed that ETAF exhibits molecular weight (m.w.) heterogeneity:ETAF eluted as 2 peaks off gel filtration chromatography at m.w. 12–20 K. and 40–70 K. However, as

is the case with IL I, the high m.w. form of ETAF appears to be an aggregate and can be recovered within the low m.w. form upon rechromatography. In addition, Isoelectrofocusing of ETAF resulted in 3 isoelectric peaks of thymocyte growth enhancing activity, as well as fibroblast proliferation stimulating capacity at pI 7.2 (α ETAF), pI 6.0 (β ETAF) and pI 4.8 (δ ETAF). ETAF partially purified by gel filtration was chemotactic for human polymorphonuclear leucocytes (PMN) and mononuclear leucocytes (MNL) and if injected into C3H/H3J mice induced the production of serum amyloid A (SAA) by hepatocytes. After separation by IEF into 3 different species the chemotactic activity of PMN dissociated from that for MNL.α and β ETAF were chemotactic for PMN, whereas δ ETAF attracted predominantly MNL. Furthermore α and β ETAF were most active in inducing SAA production. δ ETAF induced minimal levels of SAA. These results provide first evidence that human keratinocytes may release a group of distinct immunopotentiating factors all of which have ETAF activity.

Effect of *In Vitro* and *In Vivo* UV on the Production of ETAF by Human and Murine Keratinocytes. JOHN C. ANSEL, THOMAS A. LUGER, AND IRA GREEN, Lab. Immunol. NIAID and NIDR, NIH, Bethesda, Maryland 20205

There has been recent interest in a keratinocyte derived cytokine called epidermal thymocyte activating factor (ETAF). ETAF is a potent low m.w. peptide secreted constitutively by normal murine and human keratinocytes with a diverse number of interleukin-1 (IL-1) like properties and may have an important immunomodulating role in inflammatory and proliferation skin disorders. Several recent reports have suggested that UV may inhibit the keratinocyte production of ETAF. Therefore, we directly examined the effect of UV on ETAF production of 2 keratinocyte cell lines; PAM 212 a murine transformed cell line, and SCC a human squamous cell carcinoma keratinocyte cell line. The cells were suspended in RPMI (5×10^5 cells/ml) and irradiated (0–100 mJ/cm^2) with a bank of 4 Westinghouse FS20 bulbs. In addition, newborn BALB/c mice were UV suspended (5×10^5 cells/ml) in RPMI media. After UV the PAM 212, SCC, and BALB/c cells were washed with PBS ×2 and resuspended in fresh serum free RPMI media (5×10^5 cells/ml) at 37°C, 5% CO_2 for 24 hr. After incubation period (24 hr post UV), supernatant (extracellular) and lysate (intracellular) ETAF activity was determined using the thymocyte proliferation assay. The results surprisingly demonstrated a dose dependent increase, not inhibition, of both intracellular and extracellular PAM 212, SCC, and BALB/c keratinocyte ETAF activity after sublethal amounts of UV; no increased IL-2 activity was detected. This increased ETAF activity was cyclohexamide sensitive, suggesting that de novo synthesis of ETAF, rather than cell membrane leakage was responsible for the increase in ETAF activity. This increased ETAF production could have important local and systemic effects.

Homologous Nature of Human Leukocytic Pyrogen and ETAF. D. N. SAUDER, N. L. MOUNESSA, S. I. KATZ, C. A. DINARELLO, J. I. GALLIN, NIH, Bethesda, Maryland; McMaster University, Hamilton, Ontario; Tufts University, Boston, Massachusetts.

Leukocytic Pyrogen (LP), the macrophage derived factor that mediates fever in man is similar if not identical to Interleukin-1 (IL-1). IL-1 is thought to play a major role in T cell activation. Epidermal cell derived Thymocyte Activating Factor (ETAF) mediates many of the functions of IL-1. This study investigated the relationship between ETAF and LP. ETAF was prepared from the human keratinocyte cell line A431. LP was prepared from human monocytes. Function was assessed by lectin induced thymocyte proliferation and fever induction. Using gel filtration, both factors eluted as a peak of 15,000 molecular weight. Flat bed isoelectric focusing (IEF) yielded peaks for ETAF at pH 6.8, 5.0, 4.7, and 6.8, 5.7 and 5 for LP. These peaks corresponded to peak fever inducing activity. In addition, since many cutaneous diseases are characterized by inflammatory infiltrates, we investigated chemotactic activity of these factors. Crude ETAF and LP preparation had significant chemotactic activity for both human mononuclear cells and for PMN. However, monocyte chemotactic activity was not seen in IEF purified samples. Chemotactic activity for PMN however, was found in 3 peaks for both LP and ETAF and these peaks corresponded to peak fever inducing activity and IL-1 activity. A rabbit antibody prepared against LP blocked IL-1 activity, fever inducing activity and chemotactic activity. The results of this study suggest that these molecules coming from completely distinct cell populations, have closely related biologic and biochemical characteristics and may play a significant role in normal host defense mechanisms as well as immunopathologic states.

Production of Fibronectin by Human Keratinocyte Cultures. M. KUBO, D. A. NORRIS, S. R. RYAN, P. NAKANE, AND R. A. F. CLARK, Departments of Dermatology & Pathology, Univ. Colorado School of Medicine, Denver, CO.

Fibronectin (Fn) is an important adherence matrix and attractant for monocytes, endothelial cells, and fibroblasts during inflammation and is directly associated with the migrating epidermis during wound healing. Although Fn is not found in normal intact epidermis, it has not been determined whether epidermal cells can produce Fn in response to the appropriate stimulus. We report that human epidermal cells maintained in non-stratified colonies produce and release Fn *in vitro*.

Non-stratified human keratinocyte cultures in defined, serum-free medium showed diffuse and granular Fn localization with perinuclear accentuation by indirect immunofluorescence after paraformaldehyde and acetone fixation, using rabbit F(ab')$_2$ anti-human Fn and FITC-conjugated goat anti-rabbit IgG. Fibrillar Fn deposition of the substratum was also seen. No fluorescence was seen with negative controls of normal rabbit serum, anti-human albumin, or anti-human fibrinogen. In early cultures, all keratinocytes contained Fn. Enzyme immunoassay confirmed that the defined medium initially contained no Fn, but that conditioned medium taken from growing cultures contained Fn in direct proportion to the number of cells in the culture.

By immunoelectronmicroscopy, Fn localized in the endoplasmic reticulum and nuclear membrane. Immunofluorescent staining with anti-keratin antibody and the presence of tonofilaments by electronmicroscopy (EM) confirmed that all of the cells were keratinocytes. No Langerhans cells were detected by EM or by OKT6 staining.

These experiments prove that certain phenotypic states of keratinocytes can produce and secrete the important matrix protein Fn.

The Assembly of Laminin Molecules Is Enhanced by Type IV Collagen. D. WOODLEY, D. KLIEIWER, C. N. RAO, L. LIOTTA, G. R. MARTIN, AND H. K. KLEINMAN, Department of Dermatology, University of North Carolina, Chapel Hill, North Carolina and National Institutes of Health, Bethesda, Maryland.

Laminin (LN), a basement membrane zone (BMZ) glycoprotein ($M_r = 10^6$), is an attachment factor for epithelial cells (ECs). Certain ECs have LN receptors. When skin is separated through the BMZ (by suction blistering or protease activation), LN remains mainly with the dermis but is also found on the ECs. Since LN-to-LN interactions could be important for epidermal-dermal adherence, we studied LN-to-LN binding (1) by affinity chromatography with ^{125}I-labelled-LN run on columns of immobilized LN and (2) by an antigen-to-antigen ELISA assay which assesses affinities between BMZ components. We found a 3-fold increase in the amount of LN bound to the LN affinity column when type IV collagen was present over that seen when either albumin was present or when LN was incubated on control ethanolamine-blocked sepharose columns with or without the presence of type IV collagen. In the ELISA, significant binding (2–3 fold increase) of soluble LN to LN immobilized in polystyrene wells occurred only when the soluble LN was incubated in the wells in the presence of 0.5–2.0 μgs of type IV collagen. No binding was observed with LN alone or in the presence of fibronectin and albumin.

These data suggest that LN molecules self assemble or aggregate poorly, thus making a simple LN-to-LN bond as the mechanism of epidermal-dermal adherence unlikely. Further, the assembly of LN molecules into a matrix structure or the formation of LN-LN bonds is probably mediated via type IV collagen.

Regulation of Skin Calcium-Binding Protein Synthesis in Normal and Malignant Epidermal Cell Cultures. PAMELA HAWLEY-NELSON, JANA PAVLOVITCH, AND STUART H. YUSPA, National Cancer Institute, Bethesda, Maryland, and Hôpital des Enfants-Malades, Paris, France.

Modulation of medium ionic calcium concentration has been shown to regulate terminal differentiation in cultured mouse epidermal cells (Hennings, et. al., Cell 19:245, 1980). This regulation is altered in malignant epidermal cells and cells exposed to carcinogens. Recently a 12.5 kd vitamin D-dependent calcium binding protein (SCaBP) was isolated from rat skin (Laouari, et. al., FEBS Letters 111:285, 1980) and by immunofluorescence was found associated with the basal layer of normal epidermis but in all strata of carcinomas. An antiserum to SCaBP immunoprecipitates 12 kd protein from water soluble extracts of ^{35}S methionine labeled epidermal cells cultured in 0.07 mM calcium medium (which favors basal cell growth). Cultures switched to 1.2 mM calcium (conditions favoring differentiation) for 48 hr do not synthesize

this protein. When cells are labeled in 0.07 mM Ca^{++} and switched to 1.2 mM Ca^{++} for 72 hr, labeled 12 kd protein is immunoprecipitated, while cells held in 0.07 mM Ca^{++} do not retain prelabeled immunoprecipitable material. These results suggest that the synthesis of SCaBP is regulated by extracellular calcium levels, but the protein may be stabilized in the differentiating cells. The tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate, which induces differentiation in low calcium cells, also inhibits synthesis of SCaBP. Tumorigenic mouse and human epidermal cell lines show variable regulation of SCaBP synthesis in response to different levels of extracellular calcium. These results suggest that synthesis of SCaBP is modulated during terminal differentiation of normal keratinocytes but the regulation of synthesis is altered in malignancy.

12:00 PM–2:00 PM LUNCH AND WORKSHOPS

Retinoids Federal Room

Gerd Plewig
Peter Elias

Langerhans Cell South American

Georg Stingl
Paul Bergstresser

Keratinocyte Culture and Differentiation Gallery

Tung-Tien Sun
Barbara Gilchrest

Receptors Pan-American Room

Ervin Epstein, Jr.
Edward O'Keefe

Mast Cell Massachusetts Room

Beate Czarnetzki
Nicholas Soter

New Aspects of Bullous Disease New York Room

Luis Diaz
Ray Gammon
Tadeuz Chorzelski

Melanogenesis Ohio Room

Vincent Hearing
Peter Fritsch
Yutaka Mishima

Melanoma Immunology California Room

Rona MacKie
Jean-Claude Bystryn

2:00 PM–2:45 PM SID GUEST SPEAKER

Presidential Ballroom

Leonard C. Harber, M.D., Presiding

TWENTY-THIRD ANNUAL HERMAN BEERMAN LECTURE

Lawrence M. Lichtenstein, M.D., Ph.D.
"The Function of Human Mast Cells"

3:00 PM–5:30 PM CONCURRENT SCIENTIFIC SESSIONS

SESSION A Presidential Ballroom

John A. Parrish, M.D., Presiding

Inhibition of Lymphocyte Proliferation by Water Soluble Psoralen Derivatives. CAROLE L. BERGER, FRANCIS P. GASPARRO, CHARLES CANTOR, JOHN WELSH, PETER DERVAN, AND RICHARD L. EDELSON, General Clinical Research Center, Department of Genetics, Columbia University, N.Y., N.Y. and Chemistry, Caltech, California.

The therapeutic efficacy of psoralen compounds can be evaluated by testing inhibition of lymphocyte blastogenesis. The proliferative response of lymphocytes to phytohemagglutinin (PHA) is inhibited *in vitro* by exposure to 100 ng/ml 8 methoxypsoralen (8-MOP) and 3 juoles (J)/cm^2 of ultraviolet A light (UVA). High drug concentrations attainable with water soluble psoralens were tested to determine if they might be more effective phototherapeutic agents, requiring lower UVA doses. Lymphocytes isolated from peripheral blood were incubated with 8-MOP, amino-methyltrimethyl psoralen (AMT), hydroxymethyltrimethyl psoralen (HMT) or amino, nitrophenyl or carboxyside chain substituted AMT, or 8-MOP. The cells were irradiated with 3J UVA/cm^2 on a black light source. Tritiated thymidine uptake was assessed 3 days post-PHA stimulation.

All concentrations of photoactivated AMT inhibited lymphocyte proliferation better than 8-MOP, HMT or any side-chain substituted psoralen. At 10 ng/ml AMT (S.I. = 1.09) was 24 × more effective than 8-MOP (S.I. = 26.10). Additional side chains decreased the effectiveness of all psoralens, but even highly substituted psoralens were capable of inhibiting proliferation at 100 ng/ml. A 2 μg/ml AMT solution inhibited lymphocyte responses (S.I. = 0.47) 65 × more effectively than 2 μg/ml 8-MOP (S.I. = 30.60) at 0.1J/cm^2 UVA. One J/cm^2 UVA was required to totally inhibit lymphocyte response with 2 μg/ml 8-MOP.

Photoactivated AMT may provide a better therapeutic modality than 8-MOP, requiring 10 fold lower UVA doses for inhibition of cell division.

Kinetics of 5-Methoxy- and 8-Methoxypsoralen Distribution to Serum and the Lens of Orally Dosed Guinea Pigs. WAYNE WAMER, ALBERT GILES, JR., AND ANDRIJA KORNHAUSER, Food and Drug Administration, Washington, D.C.

We have recently determined that there is a correlation between serum and epidermal levels of 8-MOP in the guinea pig (GP) (Science 217:7733, 1982), a result with implications for both basic and clinical investigations. Since one of the well established risks of PUVA treatment is cataract formation, a result also observed in animal models, we have extended our studies to the determination of 8-MOP levels in the lens. Additionally, since 5-MOP is a possible alternative drug to 8-MOP in PUVA, we included this compound in the present study. A group of 96 albino GP's were used; 64 were orally dosed with either crystalline 8-MOP or 5-MOP at 15 mg/kg in a gelatin capsule. The remaining group served as control. Serum and lens samples were extracted and analyzed by reverse phase HPLC. GP's receiving 5-MOP showed detectable serum levels at 2 hr (216 ng/ml). At other time points the levels were not significant. 5-MOP was not detectable in the lens at any time point. In contrast, 8-MOP was detected after 1, 2, 3, 4, 6 hr in serum at 2365, 2438, 1205, 1064 and 171 ng/ml and in the lens at 168, 360, 790, 570 and 480 ng/g respectively. At 18 hr, 8-MOP was not detectable in serum or lens. We conclude: 1) after equivalent oral doses, 5-MOP lens levels are significantly lower than 8-MOP levels. Extrapolating these results to a clinical situation, the use of 5-MOP in PUVA therapy may result in reduced risks of cataract formation; 2) 8-MOP is eliminated from the lens more slowly than from serum; 3) HPLC is a promising technique for assaying drug levels in the eye, permitting the measurement of the unmetabolized drug which is not easily achieved by the common fluorescence or radiolabel techniques.

Bioactivity of a New Liquid Formulation of 8-Methoxypsoralen (8-MOP): Time Course and Effect of Diet. P. C. LEVINS, R. W. GANGE, K. MOMTAZ-T, M. A. PATHAK, AND J. A. PARRISH, Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114

A new liquid containing capsule and a crystalline preparation of 8-methoxypsoralen were compared in 12 subjects. Each subject took .6 mg/kg body weight of each formulation on different days. Six volunteers ate a low-fat meal before ingestion of drug, and 6 volunteers ate a high-fat meal. Phototoxicity was tested from ½ hr to 6 hr after ingestion of 8-MOP by exposure to 320–400 nm radiation (UVA) from a xenon arc. A series of graduated doses of UVA were administered at each time point to determine the minimum phototoxic dose (MPD). Ingestion of drug and grading of erythema were conducted in a double-blind, bilaterally symmetrical fashion; each volunteer took one preparation of the drug, was exposed on one side of the back and 2 days later took the other preparation and was exposed on the other side of the back. The phototoxic reaction was graded by 2 "blind" observers and peak erythema evaluated at 72 hr.

The mean MPDs after both low- and high-fat meals were 40% lower for the liquid preparation than for the crystalline preparation. The photosensitive state was shorter and more predictable after the liquid preparation. Ingestion of liquid 8-MOP induced photosensitivity in all 12 subjects, while crystalline 8-MOP failed to photosensitize 3 subjects. Liquid 8-MOP induced phototoxicity peaked 2.5 hr earlier than crystalline 8-MOP induced phototoxicity after a low-fat meal (1.67 hr vs. 4.2 hr). and 1 hr earlier after a high-fat meal (2.5 hr vs. 3.5 hr). Incidence of nausea and other side effects were similar following ingestion of both preparations.

A new liquid 8-MOP preparation may thus allow lower doses of UVA to achieve therapeutic results in PUVA, and less waiting time after ingestion of drug.

Persistent Photosensitivity Induced by Topical and Oral 8-Methoxypsoralen (8-MOP) and Suberythemogenic UVA Irradiation. R. W. GANGE, P. C. LEVINS, J. MURRAY, R. R. ANDERSON, AND J. A. PARRISH, Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114

Crosslinking of DNA is probably important in cutaneous psoralen phototoxicity. 8-Methoxypsoralen (8-MOP) + UVA (PUVA) also generates psoralen-DNA monoadducts, which may be later converted to crosslinks by re-exposure to UV. Following suberythemogenic PUVA treatment, if sufficient monoadducts were present at the time of a second UV exposure, sufficient crosslinking to result in erythema might result from re-exposure, even if free psoralen was no longer present. This was tested in humans following topical and oral sensitization. The skin of 10 volunteers was treated with aqueous 8-MOP (0.003%) followed immediately by 0.25 × the minimum phototoxic dose (MPD) of UVA (mean MPD 0.29 J/cm^2). Twenty-four hr later as little as 1.19 ± 1.78 J/cm^2 UVA resulted in delayed erythema in this area, compared with >>40 J/cm^2 in skin treated with 8-MOP but not previously irradiated. Marked sensitivity was also shown after 48 and 72 hr, and from as little as 0.0625 × MPD UVA given initially. Erythema peaked 72 hr after the second exposure. In contrast, after oral 8-MOP sensitization + 0.25 × MPD (mean MPD 10.6 J/cm^2) in 10 subjects, 63 ± 24 J/cm^2 of UVA given 24 hr later was required to induce delayed erythema, compared with 90.4 ± 22 J/cm^2 in sites not exposed to 0.25 × MPD after 8-MOP ingestion. Topical 8-MOP and immediate suberythemogenic UVA irradiation therefore induced marked persistent photosensitivity, while oral 8-MOP and suberythemogenic UVA caused only a minor reduction in UVA erythema dose 24 hr later. This difference may be related to a greater ratio of monoadducts to crosslinks formed by the initial suberythemogenic irradiation after topical sensitization compared with oral sensitization. This finding may explain unpredictable phototoxic reactions after topical PUVA treatment.

Photobiological and Phototherapeutic Properties of New Monofunctional Pyrido Psoralens. DIETRICH AVERBECK°, LOUIS DUBERTRET°°, EMILE BISAGNI°, JACQUELINE MORON°, DORA PAPADOPOULO°, SYLVANO NOCENTINI°, JOCELYNE BLAIS°, AND JACQUES ZAJDELA°, HÔP. H. MONDOR CRÉTEIL°°; INST. CURIE, PARIS°.

In an attempt to decrease the potential genotoxic hazards of PUVA, psoralens only capable of forming mono-adducts with cellular DNA were synthesized. Among the new molecules tested, two pyrido-psoralens appear to be most interesting. They absorbe light in UVA region (λ max 325 and 330 nm). They complex to DNA and, under UVA irradiation, they induce only mono adducts in DNA as demonstrated by fluorescence studies and DNA denaturation-renaturation. In diploid eukaryotic cells (Yeast) they are more efficient than 8-MOP in cell killing, less mutagenic than 3-CPs, but as efficient as 3-CPs in induction of mitochondrial damage. In contrast with 3-CPs they are devoid of oxygen dependent (i.e. photodynamic) action. In mammalian cells they exert a stronger antiproliferative effect than 8-MOP as measured by inhibition of DNA synthesis. In V79 chinese hamster cells they appear to be clearly less mutagenic than 8-MOP, furthermore, in C3H embryonic mouse cells they show a lower cell transforming ability than 8-MOP. Only a low tumor incidence can be observed with pyrido-psoralens using UVA doses and number of irradiations twice as high as those needed to produce a 90% tumor incidence in mice with 8-MOP. In man, after 20 J/cm^2 of UVA two hr after topical application of 10 μg/cm^2 of pyrido-psoralens, a slight delayed erythema was observed. For therapeutic studies, 8-MOP and the pyrido-psoralens were applied on different spots of the same psoriatic plaque, at the same molar concentration, using the same excipient and the same protocol of UVA irradiation. Under these conditions these new monofunctional pyrido psoralens appear to be more efficient than 8-MOP. Thus new pyrido-psoralens could increase the safety and efficiency of PUVA.

Recovery of Delayed Cutaneous Hypersensitivity After PUVA Therapy. CELIA MOSS, PETER FRIEDMANN, SAM SHUSTER, AND JUDY SIMPSON*, Depts. Dermatology and *Medical Statistics, Univ. Newcastle-upon-Tyne, UK.

PUVA therapy for psoriasis suppresses delayed cutaneous hypersensitivity (DCH) to dinitrochlorobenzene (DNCB); the afferent limb of the immune response is impaired more than the efferent,[1] probably by the effect on Langerhans cells (LC). The importance of immune suppression will depend on how long its duration persists. Since LC numbers return to normal by 3 weeks after PUVA therapy, DCH should also have recovered in that time. To see if this is so 11 psoriatic patients were sensitised 2 weeks after completion of PUVA therapy and 9 patients 4 weeks after. 500 μg of DNCB was used for sensitisation and 4 weeks later subjects were challenged with 3.125, 6.25, 12.5 and 25 μg of DNCB. Responses were measured as increase in skinfold thickness at the challenge sites and dose-response curves were compared by analysis of variance. 46 patients sensitised during PUVA and 20 untreated patients served as controls. The groups were comparable for age, sex, extent of psoriasis, dose of PUVA and skin type. The proportions successfully sensitised by 500 μg of DNCB at 2 weeks (91%) and at 4 weeks (89%) were greater than during PUVA (62%) and the same as in the untreated group (90%), showing susceptibility to the induction of sensitivity had returned to normal. Dose-response curves for challenge were shifted in parallel to the right by PUVA ($P < 0.001$ cf untreated controls). During the recovery period the dose-response curves moved progressively back to the left with an increased response at low doses and a decreased slope ($P < 0.001$). This is in keeping with an a simultaneous augmentation of afferent and a diminution of the efferent components of the response which appears to be transient.

[1] Moss C, Friedmann PS, Shuster S (1981) J Invest Dermatol, 76, 432

Psoralen-Containing Sunscreen Is Tumorigenic in Hairless Mice. LYLE E. CARTWRIGHT AND JOSEPH F. WALTER, Division of Dermatology, University of California, School of Medicine, San Diego, California.

Sunscreens containing 5-methoxypsoralen (5-MOP) are currently being marketed to promote tanning by inducing psoralen-mediated ultraviolet (UV) A (320–400 nm) melanogenesis. The rationale is that this may prevent UVB (290–320 nm) radiation-induced skin damage. However, mouse studies have shown that 5-MOP has the same cutaneous photocarcinogenic potential as 8-methoxypsoralen. In addition, the 5-MOP containing sunscreen Sun System III (SS III) when combined with UVA induces epidermal ornithine decarboxylase activity, an enzyme associated with tumor promotion. Therefore, we investigated whether SS III had sufficient psoralen concentration to be tumorigenic in hairless mice exposed to chronic, intermittent UVA radiation. SS III was applied to hairless mice 5 days per week for 20 weeks. After each application the mice were exposed to 2.5 to 10 joules/cm^2 UVA radiation. All test groups developed atypical squamous papillomas in direct proportion to the dosage of UVA radiation received. A shorter latency period for tumor development was seen with larger UVA doses. Test animals followed up to one year developed invasive squamous cell tumors. Control groups (SS III without UVA and UVA without SS III) remained free of tumors. Animals receiving SS III plus UVA developed persistent skin thickening and increased dermal cyst formation similar to that reported with chronic exposure to UVB, a known carcinogenic wavelength. Over-the-counter sunscreens containing 5-MOP do contain sufficient psoralen concentrations to cause cutaneous phototoxicity and photocarcinogenicity in mice and their use in humans should be discouraged in the interest of preventing further UV-induced skin damage and skin cancer.

A Quantitative Study of Histopathological Changes in PUVA-Induced Lentigines. L. KANERVA, K.-M. NIEMI, J. LAUHARANTA, AND A. RANKI, Department of Dermatology, University Central Hospital, Helsinki, Finland

Persistent lentigines develop during long term PUVA treatment. We examined 61 patients who had received more than 1000 J/cm^2 of PUVA. 25 of these (=41%) had PUVA lentigines. One of these patients also had ashen gray maculae and one had stellate shaped larger hyperpigmented maculae. All these 25 patients had received more than 150 treatments of PUVA. The mean total dose was 2070 ± 585 J/cm^2 (mean ± S.D.).

The light and electron microscopic findings of PUVA lentigines from 25 patients were quantitated. The percentages of patients with the appropriate findings are given in parenthesis. Light microscopy: Hyperpigmentation (100%), hyperkeratosis (68%), acanthosis (20%), accentuated granular layer (32%), dyskeratotic cells (36%), enlarged nuclei in keratocytes (48%), giant keratinocytes (20%), increased number (56%) or size (32%) of melanocytes. Thickened PAS-positive basement membrane or homogenization of papillary dermis (44%). Electron microscopy: Vacuolation (80%), or fibrillary degeneration or strong cytolysis (12%) of keratocytes, lipid droplets (48%) or lysosome-melanosome complexes (40%) within melanocytes, melanosomes dispersed predominantly singly in keratincytes (25%), split basal lamina (72%), normal

looking Langerhans' cells (LC) (100%), LC-LC appositions (8%), LC-mononuclear cell appositions (0%). Ashen gray maculae disclosed massive amounts of melanophages in the dermis. Stellate shaped maculae had the same histopathology as the lentigines.

The exceptionally large melanocytes with or without nuclear irregularities are of special concern. The melanocyte and keratinocyte atypia necessitate a close follow-up of patients with prolonged PUVA treatment. LCs seem to return to epidermis during prolonged PUVA therapy.

Effect of PUVA on Smooth Muscle Contraction by Anaphylatoxin Activity of Zymosan Activated Serum (ZAS). NORIO MORI AND NOBUYUKI MIZUNO, Dept. of Dermatol. Nagoya City Univ. Medical School, Nagoya, Japan

PUVA inhibits the chemotactic activity of ZAS. This study was designed to characterize the effect of PUVA on another function of anaphylatoxin with Schultz-Dale test.

ZAS was made by Vallota and Müller-Eberhard's method. Small intestine of guinea pig was used as muscle. In the preliminary study, the dose-dependent inhibition of muscle contraction by ZAS was confirmed with the addition of the mixture of anti-human C3a and C5a rabbit serum.

Four groups of test samples (1 ml) were prepared. Namely (1) Z + R + E (absolute control), (2) Z + R + P (control), (3) Z + R + E + U (control), and (4) Z + R + P + U (PUVA). Where Z, R, E, P, and U represent 300 μl of ZAS, 650 μl of Ringer's solution, 50 μl of 99.5% ethanol, 50 μl of 0.3% 8-methoxypsoralen in E, and 5 J/ml of UVA, respectively. In control groups (1), (2), and (3) muscle contraction was (+++), while in group (4) (PUVA) it was (+).

In place of ZAS, histamine, serotonin, acetylcholine, or prostaglandin F2α were tested in the same system, but PUVA could not inhibit their activity.

In conclusion, PUVA inhibits muscle contraction by anaphylatoxin C3a and/or C5a specifically. And this fact further supports the concept that PUVA inhibits anaphylatoxin activities.

Distribution of 4,5'8-Trimethylpsoralen and Metabolites in Body Fluids and Tissues. SIBA G. CHAKRABARTI, TAPAS K. PRADHAN, PEARL E. GRIMES, HAROLD R. MINUS, MARTINA DIOLULU, AND JOHN A. KENNEY, JR., Department of Dermatology, Howard Univ. College of Medicine, Washington, D.C.

The purpose of this study was to measure 4,5',8-trimethylpsoralen (TMP) in blood of patients treated with this drug for vitiligo. A second objective was to measure the drug levels in blood, tissues and body fluids of the guinea pig and to identify metabolites of the drug from guinea pig organs after oral administration. TMP from vitiligo patients and the drug and its metabolites from guinea pig organs were extracted at pH 9.0 in a 95:5 mixture of hexane:isopropanol. TMP was quantitatively measured by using a reverse-phase HPLC method. Metabolites of TMP from guinea pig organs were identified by using a thin-layer chromatographic (TLC) technique. In vitiligo patients peak blood TMP levels occurred either at 1 hr or 2 hr. Peak blood TMP levels occurred at 2 hr when the patients were fasted 8 hr. In 14 patients the peak blood concentrations (in ng/ml) were: 444, 450, 460, 570, 600, 140, 800, 195, 600, 580, 411, 224, 280 and 242. TMP concentrations in guinea pig body fluids and organs (in ng/ml or ng/g) were: plasma—500 ± 50; whole blood—420 ± 100; small intestine—620 ± 85; RBC—450 ± 63; aqueous humor—240 ± 24; epidermis—225 ± 18; adrenal—122 ± 20; lung—110 ± 16; liver—35 ± 9; kidney—31 ± 11; dermis—25 ± 10; spleen—15 ± 6; heart—12 ± 5. Four metabolites were identified by TLC using a solvent consisting of toluene and acetic acid (8:2). The R_f values of the metabolites were 0.20, 0.41, 0.78 and 0.90. R_f of unmetabolized TMP was 0.65. It appears that the distribution of TMP in body fluids and tissues is uneven and a significant accumulation of the drug occurs in epidermis and in aqueous humor, indicating the need for eye protection after PUVA therapy.

Phototoxicity and Ornithine Decarboxylase Induction with Different Psoralens and UVA. NICHOLAS J. LOWE, AND MICHAEL J. CONNOR, Division of Dermatology, UCLA School of Medicine, Los Angeles, CA 90024.

The biological activities of 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), 3-carbethoxypsoralen (3-CP) and 5-methylisopsoralen (5-MI) administered topically (0.9 mg/cm^2) or orally (25 mg/kg) followed by UVA irradiation (5 J/cm^2) were compared in the hairless mouse. Parameters measured were the ability to induce ornithine decarboxylase (ODC) activity, a potential marker for carcinogenicity, and the induction of skin edema (increase in double-skin-fold thickness) and erythema, both indicators of phototoxicity.

Results:

Psoralen	ODC Activity	Double-Skin Fold Thickness	Erythema
Oral			
8-MOP	10.4 ± 7.9	180%	+++
5-MOP	3.55 ± 1.34	154%	++
3-CP	0.48 ± 0.25	109%	–
5-MI	3.76 ± 2.4	139%	–
Topical			
8-MOP	28.9 ± 19.3	166%	++++
5-MOP	21.8 ± 10.2	152%	++++
3-CP	0.08 ± 0.04	95%	–
5-MI	13.7 ±2.9	163%	++

The reduced phototoxicity of oral 5-MOP + UVA compared to oral 8-MOP + UVA appears to be a pharmacokinetic effect, since topical 5-MOP + UVA was as active as topical 8-MOP + UVA. The skin erythema seen following topical 5-MI + UVA was reduced compared to 8-MOP + UVA and only occurred at 48 hr and later. The ability to crosslink with DNA is not a prerequisite for ODC induction and the induction of skin edema since 5-MI which can only form monoadducts gave a similar response to 8-MOP and 5-MOP.

SESSION B — Congressional Room

Sture Liden, M.D., Presiding

Mitogenic and Immune Suppressive Effects of Minoxidil on Epidermal Cells and Lymphocytes *In Vitro*. R. L. COHEN, B. COOK, V. C. WEISS, A. Y. STINSON, D. P. WEST AND D. A. CHAMBERS, Univ. of Ill., Health Sci. Center, Chicago, Ill.

Minoxidil, an anti-hypertensive agent which promotes vasodilatation also has secondary effects on hair growth. In testing the pharmacological mechanisms through which minoxidil acts, we examined its effect on epidermal cells and splenocytes in culture. Epidermal cultures from neonatal Balb/c mouse skin prepared by trypsinization and ficol gradient fractionation yielded a population of >90% basal cells. Cultures containing 10^6 cells/ml in RPMI 1640 plus 13% FCS were incubated at 33°C. Addition of minoxidil (5–10 μg/ml) produced a second peak of DNA synthesis 8 days after culture initiation, alterations in cell morphology and keratinization which were not seen in controls. Splenocytes from normal and nude (T-cell immunodeficient) animals were incubated in serum-free RPMI 1640 (Cell 3:375, 1974) at 5×10^6 cells/ml at 37°C in a 5% CO_2-humid atmosphere. DNA synthesis was measured by the incorporation of (^{3}H) thymidine (20 Ci/mol) into acid-precipitable DNA, 48 hr after culture initiation. Lymphocyte cultures containing 10–40 μg/ml of minoxidil, were incubated in the presence and absence of the mitogen Concanavalin A (Con A, 1 μg/ml) or lipopolysaccharide (LPS, 20 μg/ml). When Con A was added to normal lymphocytes in the presence of minoxidil, DNA synthesis was inhibited up to 95% with no alteration of cell viability as measured by trypan blue exclusion. Analogous experiments with the B-cell mitogen LPS, revealed no inhibition in either normal or nude mice suggesting a specific effect on T-cells. Parallel experiments with thymocytes showed 95% inhibition consistent with specific T-cell suppression. These results suggest that minoxidil has a mitogenic effect on epidermal cells and immunosuppressive effects on lymphocytes.

Histochemical Identification of Epidermal Cells by Nucleotide Phosphatase Ecto-Enzymes Is Both Substrate and Cation Dependent. B. CHAKER, M. D. THARP, P. R. BERGSTRESSER, University of Texas Health Science Center at Dallas, Dallas, Texas.

The cell surface ecto-enzyme, Ca^{++}/Mg^{++}-dependent adenosine triphosphatase (ATPase), is present on dendritic Langerhans cells (LCs) in mammalian epidermis and has been used for the enumeration of LCs in epidermal whole mounts and cell suspensions. In conjunction with studies addressing the physiologic role of LC nucleotide phosphatases, we examined substrate and cation specificity for nucleotide phosphatases on LCs. Skin specimens from three species, mouse (BALB/c and A/J), rat (Sprague-Dawley) and human cadaver were examined both as EDTA-separated whole mounts and as trypsin-disaggregated single cell suspensions. Skin specimens were incubated with purified ATP, ADP, or AMP. Cell surface ATPase activity was observed to be Na^+/K+ independent and Ca++/Mg++ dependent, although Mg++ and Ca++ were interchangable. Optimal staining occurred with 20 mM

added Mg++ or Ca++, with significant decrease in enzyme activity at higher and lower concentrations. In epidermal whole mounts, ADP was more specific than ATP as a substrate for identifying dendritic LCs. AMP and adenosine failed to stain epidermal dendritic cells, but AMP stained follicular keratinocytes intensely in both mice and rats. In cell suspensions, ADP was again superior to ATP in identifying LCs. However, AMP stained as many as 10% of cells with an intensity equal to that of ATP. We conclude: 1) ADP is a more specific substrate for LC nucleotide phosphatase than ATP, and 2) AMP which may contaminate stock supplies of ATP will label in cell suspensions cells derived from hair follicles rather than epidermal LCs. These characteristics of nucleotide phosphatase specificities may account for variations among laboratories for LC enumeration.

Local Effects of Granuloma Transplantation into the Skin of Nude Mice. M. NISHIMURA, M. HIGUCHI, K. FUKUYAMA, AND W. L. EPSTEIN, Department of Dermatology, University of California, San Francisco, California.

Granulomas which develop in nude, athymic (nu/nu) mice after schistosome infection are small and devoid of eosinophils (eos) or mast cells (mc), while those in thymus intact (nu/+) mice are large and contain eos and mc. To test for local control factors of granulomatous inflammation BALB/c background nu/+ mice were infected with *Schistosoma mansoni* and hepatic egg granulomas which developed were isolated and grafted into the skin of nu/nu mice. Biopsies taken at various times post grafting were examined by light and electron microscopy. In addition intraperitoneal injection of TdR-H^3 was used to detect, by autoradiography, the source of cells populating the homograft sites. One week later granulomas appeared amorphous and pale staining and the graft was surrounded by a leukocyte response. TdR-H^3 injection of nu/+ mice labeled donor cells in the grafts but these cells disappeared within 1 week. After 2 weeks repopulation with macrophages and monocytes began and by 3–5 weeks granulomas were morphologically comparable to those of the nu/+ donors, but differed from hepatic granulomas in nu/nu mice. TdR-H^3 injection of recipient nu/nu mice before grafting resulted in labeling of mononuclear cells, eos and mc in the granulomas suggesting that the cells of nu/nu mice acquired nu/+ type responses to schistosome eggs. Only the leukocyte response without further progression of granulomas occurred if schistosome eggs without nu/+ tissue were grafted into nu/nu mice. Organ cultures of isolated granulomas from nu/+ mice prior to grafting reduced the numbers of developing granulomas in nu/nu mice and no granulomas developed if the grafts were cultured for more than 4 days before implantation. The granulomatous reaction is influenced by a substance in nu/+ granulomas.

Induction of T Suppressor (T_s) and T Effector Lymphocytes (Ly) of Delayed Type Hypersensitivity (T_{DH}) of Contact Allergy *In Vitro*. JÜRGEN KNOP, Universitäts-Hautklinik, von-Esmarch-Str. 56, D-4400 Münster

T_s Ly play an important role in the regulation of the T_{DH} cells of contact allergy. In our study to investigate the effects of cytokines on T_s cells of contact allergy we developed an *in vitro* system in which we were able to induce T_{DH} cells and T_s cells of contact allergy *in vitro* and testing the functional expression of these cells *in vivo*. For primary sensitization of T_s and T_{DH} bone marrow macrophages (BM-MPH) were antigen pulsed with 2,4-dinitrosulfonic acid and cocultured with spleen Ly. After 3 days the Ly were harvested and 5×10 cells were injected intravenously into BALB/c mice. Contact sensitivity (CS) was demonstrated by sensitization and ear painting with 2,4-dinitrofluorobenzene (DNFB) in Balb/c. Ly cultured in the presence of 5 day old antigen pulsed BM macrophages were able to transfer c.s., however no suppression. Using 10 day old BM MPH as antigen-presenting cells Ly were educated which transferred suppression, however, no sensitization. Studying the expression of Ia antigens on the antigen-presenting macrophages using monoclonal anti-Ia antibodies we found strong expression of Ia on 5 day old BM-MPH and much less expression on 10 day old BM MPH. On the other hand, DNP labeled bone marrow macrophages (5 and 10 day old) did not induce significant suppression *in vivo* after i.v. injection—in contrary to DNP labeled spleen cells. It can be concluded from these studies that different macrophage populations may differ in their ability to induce T_{DH} and T_s lymphocytes; this may not only depend on the expression of Ia antigens on the surface but possibly on mediators released by the these cells which may influence differentiation of T_s or T_{DH}.

Induction of Suppressor T Cells in Contact Sensitivity to Fluorescein Isothiocyanate (FITC). MASUTAKA FURUE, KUNIHIKO TAMAKI, Dept. of Dermatology, Univ. of Tokyo, Tokyo, Japan.

Contact Sensitivity (CS) to FITC has been shown to be regulated by B cells or monocytes rather than T cells. In order to further elucidate the regulatory mechanism(s) in CS to FITC, the following experiments were performed. Four hundred μl of 0.5% FITC were applied epicutaneously to the shaved abdomen of BALB/c mice and 6 days later 20 μl of 0.5% FITC were applied to the ear. Increment in ear thickness was assessed 24 hours later. Epidermal cells were conjugated with FITC (FITC-EC) and $1 \sim 2 \times 10^7$ cells were injected to the syngeneic mice via subcutaneous (s.c.) intraperitoneal (i.p.) of intravenous (i.v.) route. Epicutaneous application of FITC, s.c. and i.p. administration of FITC-EC induced marked ear swelling. Administration of FITC-EC via i.v. route didn't show ear swelling. Also mice injected with 1 mg of FITC showed no ear swelling. To determine whether this unresponsiveness represented tolerance, mice injected with FITC were tried to be sensitized with FITC painting or s.c. injection of FITC-EC. Neither showed ear swelling. In attempts to passively transfer suppression, 10×10^7 spleen cells were harvested 7 days later and were injected to syngeneic mice 2 hours before FITC painting. This led to suppression of CS. When these spleen cells were treated with anti Thy1.2 antibody and complement, sensitization was restored. These spleen cells could not suppress CS when injected i.v. 4 days after painting. Thus the hapten specific T cells, which act at the afferent side than the efferent side, are induced in CS to FITC.

Cellular Requirements for Delayed Ear Swelling Following DNFB Challenge of Mice Passively Sensitized with IgE Anti-DNP. M. C. RAY, T. J. SULLIVAN, M. D. THARP, AND R. E. TIGELAAR, Departments of Dermatology, Internal Medicine, & Microbiology, UTHSCD, Dallas, Texas.

We have reported that topical ear challenge with DNFB of BALB/c mice passively sensitized (p.s.) i.v. 48 hr before with monoclonal IgE anti-DNP produces delayed ear swelling which peaks at 24–48 hr (Clin. Res. 30(2):604, 1982). The following studies were initiated to analyze the cellular requirements for these IgE-initiated delayed responses. Genetically mast cell-deficient mice (W/W^v and Sl/Sl^d) have a profound deficiency in mast cells compared to their mast cell-replete (+/+) littermates. We have verified this deficiency by toluidine blue-stained skin sections, by a quantitative assay for tissue histamine, and by testing p.s. mice for passive cutaneous anaphylaxis (PCA) following intradermal antigen challenge. On the other hand, in four experiments, W/W^v or Sl/Sl^d mice p.s. with IgE anti-DNP demonstrated delayed reactions after DNFB challenge which were greater than in unsensitized controls (20.8 ± 1.9 vs $6.6 \pm 0.9 \times 10^{-4}$ in, $p < .001$) and equivalent to reactions seen in p.s. +/+ mice ($18 \pm 1.4 \times 10^{-4}$ in). Nu/nu mice are deficient in mature functional T cells, but have normal numbers of functional mast cells (demonstrated by unimpaired PCA reactivity). However, nu/nu mice challenged with DNFB after p.s. with IgE anti-DNP did not exhibit delayed ear swelling over that seen in unsensitized controls ($p > .5$). We conclude: 1) cutaneous mast cells do not play a critical limiting role in these IgE-initiated, delayed cutaneous inflammatory reactions, and are thus distinct from the mast cell-dependent, "late phase reactions" previously described in man and rats; and 2) these reactions are T cell-dependent. It is conceivable that similar mechanisms may be involved in the dermatitis seen in some patients with the atopic diathesis.

Suppression of Dinitrofluorobenzene (DNFB) Contact Hypersensitivity by *In Vivo* Low Dose Ultraviolet B (UVB) Radiation: Action Spectrum Studies. CRAIG A. ELMETS, MARK J. LEVINE, DAVID R. BICKERS, Dept. of Dermatology, Case Western Reserve Univ. School of Medicine, Cleveland, Ohio.

Antigen-specific unresponsiveness to contact sensitizers can be produced by *in vivo* administration of relatively low doses of UVB radiation followed by epicutaneous application of hapten. This is strictly a local cutaneous effect since the unresponsiveness can be evoked only by hapten at the irradiated site. In this study we examined the wavelengths of UV radiation which most efficiently produce the unresponsiveness observed. Panels of female C3H mice were exposed for 4 consecutive days to either 70 mJ/cm^2 broad band UV from a bank of 4 FS20 fluorescent lamps or to varying fluence rates of narrow band UV radiation from a xenon arc monochromator. Immediately following the final UV exposure DNFB was applied epicutaneously to the irradiated site. Five days later mice were ear challenged and the subsequent ear

swelling response was assayed. When mice were exposed to broad band UV the interposition of a window glass filter abrogated the unresponsiveness that occurred without the filter, thus indicating that the action spectrum for this effect lies within the UVB range. In the narrow band UV experiments mice exposed to 297 nm. radiation exhibited the greatest inhibition of the ear swelling response. Exposure to 40 mJ/cm^2 daily resulted in 60% suppression. Significant inhibition could also be produced with 270 nm. monochromatic radiation, but required 70 mJ/cm^2 daily for equivalent inhibition. The wavelengths tested above 297 nm. were less effective; unresponsiveness could not be produced by doses up to 100 mJ/cm^2 daily. These studies indicate that the action spectrum for UVB erythema and for the production of UVB-induced unresponsiveness to DNFB are quite similar.

Characterization of Plasminogen Activator (PA) and PA Inhibitor Associated with Hypersensitivity Reaction in Murine Leprosy. Seiichi Izaki, Toshihiko Hibino, Yasumasa Isozaki, and Masakatsu Izaki, Department of Dermatology, Iwate Medical University School of Medicine, Morioka, Japan.

A saline soluble fraction of murine lepromas which are developed in an immunogenetical "resistent" strain, C57BL/6, of mice shows activity of PA, as well as regulating inhibitor for PA. In order to determine properties of the PA and the PA inhibitor, we carried out chromatographic and enzymologic experiments. After inoculation of 2×10^8 Mycobacterium lepraemurium into 80 C57BL/6 mice, granulomas were removed weekly and extracted with 0.05 M Tris-HCl + 0.1 M NaCl, pH 7.5. PA activity was measured with pyroglutamyl-glycyl-arginine-p-nitroanilide and fibrin plates. Inhibitor activity for urokinase was measured.

1) Tissue extract with PA inhibitor activity (10.7 ± 2.0 CTA U/mg prot) was prepared from developing subcutaneous nodules 4–6 weeks after infection. Sephacryl S-200 gel chromatography revealed a peak of PA inhibitor activity at 45,000 in m.w. A kinetical analysis with the peptide substrate demonstrated time-dependent, stoichiometrical and irreversible enzyme-inhibitor binding. 2) Tissue extract with PA activity (13.1 ± 4.9 CTA U/mg prot) was prepared from older murine lepromas which showed localizing tissue reaction with central necrosis 8–34 weeks after injection. Sephacryl S-200 gel chromatography showed a peak of PA activity at 23,000 in m.w. The pH optimum was found to be weakly alkaline (pH 8–9). The enzymatic activity was inhibited in the presence of the PA inhibitor fraction extracted from earlier lesions.

The PA, possibly representing a degradation peptide of tissue PA, and the intrinsic irreversible PA inhibitor demonstrated in the immunoreactive murine lepromas are assumed to have roles in the granulomatous tissue reaction against the acid-fast bacilli.

Immunohistochemical Analysis of the Cellular Immune Response in Contact and Irritant Dermatitis in Man. A. Scheynius, T. Fischer, U. Forsum, and L. Klareskog, Institute of Clinical Bacteriology, Departments of Dermatology and Immunology, University of Uppsala, Uppsala, Sweden.

Cellular immune responses in contact and irritant dermatitis were analysed and compared *in situ* using a double immunohistochemical technique.

Allergic test reactions were elicited in 10 patients and irritant reactions in 8 patients using the Finn chamber technique. Skin biopsies were taken after 6 to 72 hr. Frozen sections were studied with a technique that permits the simultaneous detection of HLA-DR expressing cells, using rabbit anti-HLA-DR antibodies, and another cell population using murine monoclonal antibodies. These were Leu 1, Leu 2a, Leu 3a, OKM 1, OKT 6, OKT 9, anti-IgG and anti-IgM antibodies.

The allergic skin reactions usually showed larger cell infiltrates than the irritant skin reactions. There were however no qualitative differences in the cell patterns. In both types of reaction Leu 3a reacting cells ("helper/inducer" phenotype) dominated in perivascular cell infiltrates, while Leu 2a reacting cells ("suppressor/cytotoxic" phenotype) were few and scattered. An increased amount of HLA-DR expressing cells were found beneath the epidermis and among these were OKM 1 reacting cells ("monocyte/macrophage" phenotype) as well as OKT 6 reacting cells (Langerhans cells). Only occasionally cells expressing IgG or IgM were observed.

The basic cell reactions in contact and irritant dermatitis appear to be similar.

Effects of Glucocorticosteroids on Antigen Presenting Cells. Werner Aberer, Laura A. Stingl, Stephanie Pogantsch, and Georg Stingl, Dept. of Dermatol., I., Univ. of Vienna Medical School, Vienna, Austria.

Recent reports indicate that pharmacological doses of glucocorticosteroids induce structural alterations in epidermal Langerhans cells (LC). In this study we asked whether glucocorticosteroid-induced changes in LC surface characteristics are paralleled by alterations in LC-dependent immunological functions of epidermal cells (EC).

Balb/c mice were treated daily with triamcinolone acetonide (TAC), administered either intraperitoneally (i.p.) in doses of 5, 20, and 50 mg/kg or topically to both ears for 5 consecutive days. Ia-positive EC as visualized by immunofluorescence were quantified on epidermal sheet preparations and LC-dependent immunological functions of EC were determined on various time intervals after the last steroid treatment.

Both topically and i.p. administered TAC produced pronounced alterations in LC surface markers and in the capacity of EC to induce antigen-specific, allogeneic, and syngeneic proliferation in T cells from non-steroid-treated animals. When assessed in day 2 after the last TAC-treatment, numbers of Ia-positive EC were greatly reduced in all treatment groups (50%–95%) with the lowest numbers recorded after topical or high-dose (50 mg/kg) i.p. treatment. This numerical decrease was paralleled by a substantial impairment not only of LC-dependent immunological functions of EC but also of the antigen-presenting capacity of peritoneal exudate cells. Over a time course of 7 weeks, LC numbers and LC-dependent *in vitro* functions gradually returned to normal values, the slowest recovery being recorded in animals which had received topical steroids.

Our data suggest that the effects of glucocorticosteroids on immune-responsiveness may be, at least partly, due to the effects of these drugs on antigen presenting cells.

Lymphocyte Blastogenesis Induced by Pentadecylcatechol Incorporated into the Lipid Bilayers of Liposomes. Anthony A. Gaspari, Robert L. Rietschel, and Joyce Klemm, Dept. of Dermatology, Emory Univ. School of Medicine, Atlanta, Georgia.

The ability of liposomes to present antigens to sensitized lymphocytes and cause a mitogenic response was studied. Guinea pigs were exposed topically to Pentadecylcatechol (PDC), and subsequent elicitation patch testing confirmed hypersensitivity to this antigen. Peripheral blood and splenic lymphocytes were obtained from both the sensitized group and an immunologically naive (control) group. Lymphocyte transformation was studied by H^3 thymidine uptake. The PDC antigen, regardless of how it was presented, failed to stimulate lymphocytes from the control group. This antigen did result in significant blastogenesis in the sensitized group's lymphocytes, but the degree of stimulation was dependent upon the manner in which the antigen was presented to them. Unconjugated PDC antigen caused a blastogenic response by the primed lymphocytes but to a lesser degree than membrane conjugated PDC. The degree of enhancement of the mitogenic response to the PDC-antigen can be ranked as follows: PDC conjugated to natural biologic membrane > PDC incorporated into lipid vesicles > unconjugated PDC. This suggests that liposomes have the ability to act as surrogate membranes and can present antigens to immune effector cells in a manner which results in specific immune recognition.

SESSION C — Federal Room

Alberto Gianetti, M.D., Presiding

Diagnosis of IgE Mediated Milk Allergy in Atopic Dermatitis: A Micro-ELISA Technique Bypassing Interference of Antibodies Other Than IgE. Alessandro Plebani[+], Stefania Seidenari[++], Antonia Avanzini[+], and Alberto Giannetti[++], Department of Pediatrics[+] and of Dermatology[++], University of Pavia, Italy

The *in vitro* detection of specific IgE directed against milk proteins by conventional RAST technique may be hampered by competition with IgG, IgA and IgM antibodies directed against the same antigenic determinants. Levels of anti-milk antibodies other than IgE are particularly high in infancy and childhood when the problem of diagnosis of cow's milk allergy is most frequent. To avoid competition between IgE and other antibodies we devised an ELISA microtechnique whereby serum IgE is immunoadsorbed using rabbit anti-IgE previously coupled to microtiter plates. Biotin-labeled milk proteins are then added and attach to specific IgE; the interaction is then revealed by avidin-conjugated peroxidase. Because many molecules of biotin are coupled to the allergen and many molecules of peroxidase are coupled to the allergen and many molecules of peroxidase are coupled to avidin, the color reaction is amplified. IgE antibodies agaist milk proteins were

assessed using this assay in 14 patients (age ranging from 3 to 10 years) with atopic dermatitis whose symptoms improved on a milk-free diet and recurred on milk challenge. We found that 8 were RAST and ELISA positive, 4 RAS negative and ELISA positive and 2 RAST negative and ELISA negative for specific circulating IgE antibodies against milk proteins. These preliminary data suggest that this micro-ELISA technique correlates better than RAST with the clinical data.

Lymphocyte Adenylate Cyclase Stimulation in Atopic Eczema and Psoriasis. J. M. HANSON, C. B. ARCHER*, J. MORLEY, AND D. M. MACDONALD*, Departments of Clinical Pharmacology, Cardiothoracic Institute, Brompton Hospital and Department of Dermatology*, Guy's Hospital, London, England

Impaired beta-adrenergic reactivity has been suggested to be a primary determinant of both atopic eczema and psoriasis. We examined peripheral blood lymphocytes to determine the existence of such a defect and to establish whether this was selective for beta-adrenergic stimulation.

Lymphocyte cAMP responses to isoprenaline (isoproterenol), histamine and PGE2 were measured in 14 patients with atopic eczema, 12 patients with psoriasis and 16 normal volunteers, both in the presence and absence of a potent phosphodiesterase inhibitor (PDEI). Cells were separated using a Ficoll-Paque gradient and stimulated in duplicate, with isoprenaline (10^{-5} M and 10^{-3} M), histamine (10^{-4} M) and PGE2 (1.1×10^{-4} M) for 10 min at 37°C. Total cAMP was estimated, in duplicate, by radioimmunoassay.

There were no significant differences between basal cAMP levels in each group. The atopic group exhibited impaired responses to isoprenaline and PGE2 in the presence of PDEI; differences between atopic and control cAMP responses were exaggerated by the omission of PDEI, when there was also an impaired response in the atopics to histamine. Cyclic AMP responses to all stimulants were normal in psoriasis.

Impaired beta-adrenergic reactivity has been demonstrated in lymphocytes from patients with atopic eczema but this was not found to be selective for the beta-agonist. These findings were not due to prior administration of adrenergic medications. Contrary to some previous studies impairment of adenylate cyclase activation was not found in psoriasis.

Adenylate Cyclase Activity During Growth and Maturation of Keratinocytes: Comparison of Two Methods of Study. DAVID I. WILKINSON AND ELAINE K. ORENBERG, Department of Dermatology, Stanford University School of Medicine, Stanford, California

Keratinocyte (KT) cultures are models for study of adenylate cyclase (ACase) in epidermal growth/differentiation. Its activity may be monitored by assay of cAMP by either RIA or by measuring ^{3}H-cAMP formed in prelabeled cells. These two methods for intact cells were compared.

Guinea pig ear KTs were maintained in primary culture in 35 mm dishes for 27 days using McCoy 5a medium plus FCS (10%). Every 2-3 days, dishes (×3) were exposed to fresh medium (1.5 ml) with ^{3}H-adenine (5 μCi) or an equivalent amount of cold adenine, for 2 hr at 37°C, then rinsed and isoproterenol (10 μM) added in PBS with IBMX (1 mM) and Hepes (15 mM); controls omitted isoproterenol. After 15 min at 37°C, then aspiration, cold 5% TCA was added. Insolubles were collected by centrifugation; TCA was removed from supernates which were lyophilized. Residues from prelabeled cells were assayed for ^{3}H (total ^{3}H:^{3}H-ATP+^{3}H-cAMP) before purification of ^{3}H-cAMP, which was expressed as % ot total ^{3}H. Residues from samples using cold adenine were partially purified, then assayed by RIA for cAMP, expressed as pmoles/dish.

Response of KTs to isoproterenol in terms of cAMP was maximal during culture days 5-12 for both prelabeled (^{3}H-cAMP) and unlabeled cells. During the next 7 d, the response of prelabeled fell by 50% but the response of unlabeled by 19%. The amount of cAMP (as measured by RIA) equivalent to 1% conversion of ^{3}H-ATP increased from 80 pmoles (day 2) to over 300 pmoles (day 20). The conclusion is that ^{3}H-adenine labels the ATP pool(s) in an increasingly inadequate manner with increase in culture age. Thus there are limitations of the prelabling method in studies of ACase in KT cultures of varying age and degree of differentiation.

Elevated Cord Blood Leukocyte Phosphodiesterase Activity in Offspring from Atopic Parents. N. HESKEL, S. C. CHAN, S. R. STEVENS, J. M. HANIFIN, Dept. of Dermatol., Oregon Health Sciences University, Portland, OR.

Children with 1 or 2 atopic parents have a high (50–75%) likelihood of being atopic compared to those with no atopic parents. There are no known predictive biochemical markers for atopy. Cyclic AMP-phosphodiesterase (PDE) activity is elevated in peripheral blood mononuclear leukocytes (MNL) of adults with atopic dermatitis and allergic respiratory disease. We asked whether PDE in cord blood MNL from offspring of atopic parents is elevated and predictive for atopy. We measured PDE activity in MNL from 39 umbilical cord blood samples and from 32 parental peripheral blood samples.

Presence of atopy was determined by carefully defined criteria. We isolated MNL from blood by Ficoll-Hypaque gradient centrifugation. Coded specimens were assayed for PDE activity by radioenzyme technique.

Twenty-five newborns with 1 or 2 atopic parents had a mean PDE activity of 4.19 ± 0.57 units/10^8 cells, significantly higher than 14 babies born to nonatopic parents with average PDE of 2.1 ± 0.37 units/10^8 cells ($< .005$). In the group with 1 or both atopic parents ($n = 22$), mean parental PDE activity was 4.97 ± 0.6 units/10^8 cells, significantly higher than that in the group of 10 nonatopic parents, which was 2.36 ± 0.15 ($p < .005$). We found no significant difference in PDE levels between offspring of atopic fathers and offspring of atopic mothers.

This first-phase study shows elevated cord blood leukocyte PDE in neonates with atopic parents. Elevated leukocyte PDE in atopy may account for abnormal cellular immune regulation and could provide a biochemical indicator of atopy. Projected second phase, prospective studies will determine the reliability of cord blood in predicting childhood atopy.

Influence of Cholinergic Stimulation upon Histamine Releasability in Patients with Atopic Eczema. JOHANNES RING, HANS SEDLMAIR, Dept. of Dermatology, Ludwig-Maximilians-Universität, München, W-Germany

Altered patterns of "releasability" of mediator secreting cells have been reported to occur in atopic diseases. In rat mast cells histamine (H) release can be increased by cholinergic stimuli. Here the cholinergic influence upon *in vitro* H release from peripheral leucocytes was studied in 18 patients with atopic eczema (IgE 67-16200 U/ml) and 15 normal non-atopic controls. Stimuli used were anti-IgE, carbamylcholine (CC) or a combination in varying concentrations. H was measured fluorometrically. At 10^{-3} and 10^{-4} dilutions anti-IgE-induced H release was significantly higher in atopics than in normals ($p < 0.05$). After stimulation with CC alone leucocytes of patients with atopic eczema released small ($3.8 \pm 1.2\%$ at 10^{-8} M), but consistently higher amounts of H than controls ($0.3 \pm 0.1\%$). The addition of CC to anti IgE led to an enhancement of H release at all concentrations tested (10^{-10}-10^{-4} M) with a maximum at 10^{-4} M. The CC (10^{-4} M) mediated increase in anti-IgE-induced H release at a 10^{-4} dilution was significantly higher in atopics (Δ of 10%) than in normals (Δ of 7%). Cholinergic enhancement of H release was most pronounced in patients with high serum IgE levels (>1500 U/ml). These data give further support to the idea that an altered releasability of mediator secreting cells—perhaps in connection with cholinergic mechanisms—might play a role in the pathogenesis of atopic diseases.

Increased Adenylate Cyclase Catalytic Activity and Substrate-Induced Phosphodiesterase in Atopic Leukocytes. L. L. GUERIN, S. C. CHAN, AND J. M. HANIFIN, Department of Dermatology, The Oregon Health Sciences University, Portland, OR

We have previously reported the marked elevation of cAMP-specific phosphodiesterase (PDE) in mononuclear leukocytes (MNL) of patients with atopic dermatitis (AD). We questioned whether increased PDE may be substrate-induced, secondary to increased adenylate cyclase (AC) activity. We have studied phosphodiesterase activity in MNL following treatment with exogenous cAMP and we have assessed baseline and stimulated AC activity in normal and AD cells.

Hypaque-Ficoll isolated MNL were extensively homogenized, then membranes were sonicated and concentrated by 180,000g centrifugation. Following isoproterenol (ISO, 1 mM) or forskolin (FSK, 100 μM) stimulation, AC activity was determined by cAMP generation from ^{32}P-ATP. Data was expressed as units (pmol cAMP/60 minutes/mg protein). Intact normal MNL were incubated with 1–100 μM cAMP or dibutyryl cAMP. PDE activity was measured by radioenzyme assay.

Basal, unstimulated AC activity did not differ in normal (10.13 ± 6.2 U, $n = 9$) and AD (8.28 ± 5.49 U, $n = 10$) preparations. ISO stimulation produced no difference in AC activation in both groups. In contrast, FSK produced a mean 12-fold increase in activity in AD compared with a six-fold increase in normal preparations ($p < 0.005$). PDE activity was consistently and significantly increased by cAMP exposure.

Our data show normal hormone receptor-regulatory unit coupling but accentuated AC catalytic activity in AD cells. These findings suggest possible substrate-induced generation of PDE and may account for our previous findings of normal basal cAMP levels in the presence of elevated PDE activity in MNL from patients with AD.

Elevated Leukocyte Phosphodiesterase As a Mechanism for Impaired Cellular Regulation in Atopic Dogs. S. C. CHAN, C. A. HIRSHMAN, J. E. PETERS, AND J. M. HANIFIN, Depts. of Dermatology and Anesthesiology, The Oregon Health Sciences University, Portland, OR

Basenji-Greyhound (BG) dogs demonstrate multiple atopic features including: 1) atopic-like dermatitis; 2) asthma with reactivity to antigenic and nonspecific stimuli; and 3) blunted leukocyte cAMP responses to β-adrenergic agents. The latter, in humans, is due to elevated leukocyte cAMP-phosphodiesterase (PDE) activity. We assessed β-adrenergic characteristics in 9 sensitized (SBG) and 12 unsensitized (UBG) BG dogs compared with 12 mongrel (MON) dogs.

Mononuclear leukocytes (MNL) were isolated on Ficoll-Hypaque gradients. β-Adrenergic receptors were assessed by ^{125}I-cyanopindolol (ICYP) in the presence or absence of propranolol. Cyclic AMP responses to isoproterenol (ISO) were measured by radioimmunoassay. PDE activity was quantitated by radioenzyme assay.

In mongrels, ISO stimulated cAMP levels increased from 1.27 ± 0.18 pmol to 2.20 ± 0.18 pmol/10^6 cells, while negligible increases occurred in MNL from both UBG and SBG. Basal MNL PDE activity was elevated in SBG (0.223 ± 0.023 U/10^6 cells) and UBG (0.128 ± 0.019 U) compared with MON (0.030 ± 0.006 U; $p < 0.001$). The PDE inhibitor Ro 20-1724 (Ro) restored ISO stimulated cAMP responses in BG dog MNL. Cholera toxin and forskolin, in the presence of Ro, caused equal increases of cAMP in MON and BG dogs, indicating adequate adenylate cyclase activity. MNL from both MON and BG dogs showed similar numbers and affinities of saturable, specific ICYP binding on MNL over a 10–400 pM range.

Inadequate intracellular cAMP due to high PDE activity may relate to biochemical defects underlying reactive airway disease and immune dysfunction in this canine model of atopy.

A Glucocorticoid Receptor in Rat Dermis and Epidermis. KENNETH SMITH AND SAM SHUSTER, Department of Dermatology, University of Newcastle-upon-Tyne, UK

We have previously characterised a cytosolic glucocorticoid receptor in whole rat skin; we now characterise and compare the cytosol receptor protein in dermis and epidermis. Skin was removed from 3-day-old rats. Epidermis was separated from dermis by incubating in phosphate buffer with 10 mM sodium molybdate and 100 mM mercaptoethanol Tissues were homogenised and centrifuged at 100,000g to yield a cytosol fraction which was incubated with 10^{-8} [^{3}H]-triamcinolone acetonide with and without 10^{-6} M triamcinolone acetonide to assess non-specific binding. Binding was measured after dextran-charcoal adsorption and Sephadex G-25 chromatography. The distribution of receptor was 82% in the dermis and 18% in the epidermis; its content for each was also related to DNA and protein content of each tissue. The dissociation constants were 0.21×10^{-9} M ± 0.04×10^{-9} M and 0.28×10^{-9} ± 0.08×10^{-9} M (n = 12) for dermal and epidermal receptors respectively. The receptor-glucocorticoid complexes were inactivated at 37°C ($t_{½}$ dermis = 11.5 min, $t_{½}$ epidermis = 13.0 min) and by increasing concentrations of trypsin, triton X-100 and NaCl. Both receptors showed similar steroid specificity. The ranking in terms of competition for receptor sites was: clobetasol propionate, fluocinolone acetonide, betamethasone valerate, triamcinolone acetonide, Ro 12 2074, dexamethasone, corticosterone progesterone and cortisol, and this approximates to their therapeutic potency. However, the lower-ranked steroids tended to bind with more affinity to the epidermal than to the dermal receptor. Thus, a cytosolic glucocorticoid receptor is present in rat dermis and epidermis; physicochemically the two receptors appear similar, but there may be differences in the extent to which glucocorticoids compete for them.

Localization of β-Adrenergic Receptor Sites in Human Epidermis with a Fluorescent β-Antagonist. ELAINE K. ORENBERG, DAVID I. WILKINSON, AND JONATHAN N. MANSBRIDGE, Dept. of Dermatology, Stanford University School of Medicine and the International Psoriasis Research Foundation, Stanford, California

Adrenergic receptors are responsible for selective recognition and binding of catecholamines and may in turn affect epidermal cell growth via cAMP. β-Adrenergic receptors have been characterized in epidermal tissue homogenates and human keratinocyte (HK) cultures, both of which represent heterogeneous cell populations with regard to cell types and developmental stages. We have adapted the use of a dansyl analog of dl-propranolol (DAPN) to visualize the distribution of β-adrenoreceptor sites in cryostat sections of human skin and HKs *in vitro*. Air dried sections (4 μ) and rinsed cultures were covered with DAPN (10^{-5} M) in 0.08 M NaH_2PO_4 buffer, pH 7.4, 30 min at 24°C, then washed ×3 (10 min) with buffer. Control sections were either pretreated or received together with DAPN one of the following: dl-propranolol (PRP), l-PRP, butoxamine, l-isoproterenol and l-epinephrine; then examined under phase contrast and epi-illumination at 363 nm with a fluorescence microscope. DAPN fluorescence appears as yellow dots arranged in a beaded chain-like manner pericellularly in all epidermal layers except stratum corneum. Staining is most intense in the basal layer and in follicular keratinocytes. While this is a qualitative method, fluorescence intensity is directly dependent on receptor density and binding affinity of DAPN. Pretreatment with β-agonists or antagonists markedly reduced or eliminated staining. In HK cultures fluorescent dots were observed over the cell surface suggesting cell membrane localization. Sections from psoriatic plaques had no staining in parakeratotic areas, but fluorescence was evident in the orthokeratotic regions usually only at the stratum granulosum. This suggests that the decreased sensitivity of adenylate cyclase in psoriatic tissue may be associated with decreased receptor density or binding affinity.

Monoclonal Antibody to Brown Recluse Spider Venom Localizes Asymmetric Membrane Receptor. L. B. NANNEY, R. S. REES, J. B. LYNCH, AND L. E. KING, Depts. of Plastic Surgery, Anatomy, Dermatology, Vanderbilt and V.A. Medical Centers, Nashville, Tennessee

Previous work in our laboratory has suggested that a dermonecrotic factor (DNF) from Brown Recluse Spider Venom (BRSV) binds to human erythrocyte (RBC) membranes and produces typical skin necrosis when injected intradermally. We have developed an immunocytochemical method to ultrastructurally localize binding of the DNF from BRSV. Intact human RBC's were prepared from fresh blood and incubated with this DNF. After multiple washing with PBS, RBC's were incubated with either a monoclonal antibody to the dermonecrotic factor or preimmune sera. Samples were again rinsed and incubated with protein A-gold solution. Isolated ghost RBC membranes were also prepared and incubated in an identical manner. All samples were fixed, thin sectioned, and stained for examination by transmission electron microscopy. No electron dense gold particles were seen in any control specimens. Intact RBC's incubated with DNF from BRSV had protein A-gold particles randomly distributed along the external plasma membrane. Dermonecrotic factor binds to a specific membrane blood receptor which is accessible to the monoclonal antibody. To determine if DNF binding sites were present on both the inner and outer surfaces of the RBC plasmalemma, we examined RBC membranes. Our immunocytochemical marker was localized only on the side of the plasma membrane corresponding to the external surface. Conclusions: The dermonecrotic factor binding sites are accessible to antibody in intact RBC's. The protein A-gold immunocytochemical treatment provides a direct demonstration of the asymmetrical binding of BRSV to human erythrocytes and also serves as an ultrastructural marker for the external surface of human erythrocytes.

Proteolysis of Epidermal Growth Factor (EGF) Receptor by Calcium Activated Protease (CANP). LLOYD E. KING, JR. AND RONALD E. GATES, Dermatology, Vanderbilt and VA Medical Centers, Nashville, TN

The membrane receptor for the mitogenic polypeptide, EGF, is also a tyrosine residue specific, autophosphorylating protein kinase whose activity is increased when EGF binds. *In vitro*, two forms of the receptor with different M_r (180K and 160K) can be identified. We showed that an intracellular endogenous protease converted the larger form into the smaller form (Molec and Cell Endocrinol 27:263, 1982) in A-431 human epidermoid carcinoma cells which were broken in the presence of 1 mM Ca^{++}. However, similar studies using a normal tissue, rat liver, showed no conversion of the EGF receptor-kinase into the smaller form. Either the receptor-kinase from normal liver cannot be degraded by the protease or the proteolytic activity is not expressed in this tissue. Since CANP activity in liver is suppressed by an endogenous inhibitor, CANP was separated from its inhibitor in rat liver cytosol using DEAE-cellulose chromatography. Partially purified CANP was incubated ±Ca^{++} at 20°C for 5 min with membranes prepared from A-431 cells and rat liver. After proteolysis was stopped with leupeptin, the samples were cooled to 0°C and incubated with [γ-^{32}P] ATP, 1 mM

Mn^{++} ± EGF for 2.5 min to label the receptor. Samples were separated by electophoresis on 5% acrylamide-sodium dodecyl sulfate gels and autoradiographs prepared. Once separated from its protein inhibitor and in the presence of Ca^{++}, CANP from rat liver degraded EGF receptor-kinase in both rat liver and A-431 cell membranes. Premixing CANP with either leupeptin or its specific inhibitor (boiled or unboiled) prevented this proteolysis.

Conclusion: CANP could function as part of a highly controlled nonlysosomal mechanism for degrading cytoplasmic proteins and the cytoplasmic portion of membrane proteins such as the EGF receptor-kinase.

SESSION D South American Room
William J. Cunliffe, M.D., Presiding

Interferon Treatment of Warts in Patients with Epidermodysplasia Verruciformis. ELLIOT J. ANDROPHY, ISRAEL DVORETZKY, AND DOUGLAS R. LOWY, Dermatology Branch, National Cancer Institute, Bethesda, MD

Epidermodysplasia Verruciformis (EV) is a rare condition of widespread chronic papillomavirus infection for which there is no satisfactory treatment. We have established a protocol to study the response of warts in EV patients to intralesional and systemic treatment with interferon, based on a preliminary report describing the efficacy of interferon, treatment of laryngeal papillomas (Haglund et al., Arch. Otolaryngol. 107:327 [1981]) and on the observation that interferon treatment of mouse cells transformed by bovine papillomavirus reverts the cells to their normal untransformed phenotype and cures the cells of the viral DNA (Turek et al., Proc. Nat. Acad. Sci., in press). We have thus far treated two patients. Each has had EV for more than thirty years, and one has had several cutaneous carcinomas in association with his EV. Several warts were treated with intralesional human leukocyte interferon (10^5 U/lesion, t.i.w.) for one month by a double-blind protocol. The interferon-treated lesions flattened completely while no change was noted in the saline-treated control lesions. Systemic interferon (0.8×10^5 U/kg i.m., t.i.w.) was given for one month. Side effects were minimal. Within two weeks of beginning systemic therapy, both patients had an unequivocal reduction in the thickness of virtually all warts. After completion of systemic treatment, the lesions on the lower extremities of one patient resolved completely and remained in remission for more than two months of therapy. Both patients' clinical response was correlated with histopathological improvement and with the amount of viral antigen in the lesions. We conclude that interferon may be effective in the treatment of some papillomavirus infections.

Effector Mechanisms in Spontaneous Regression Phenomenon of Numerous Flat Warts: Cytochemical and Functional Characterization of Aggressor Mononuclear Cells. HACHIRO TAGAMI, KEIJI IWATSUKI, AND TOMOZO OKU, Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Involution of flat warts is the only systemic and simultaneous regression phenomenon of numerous tumors in man clinically predictable due to sudden occurrence of inflammation in all the warts. Histologically, such warts show a dense mononuclear cell infiltration which is sharply confined to the wart tissue. We present cytochemical and tissue culture data to demonstrate that cell-mediated immunity plays an important role in this spontaneous tumor rejection phenomenon in man.

Using α-naphthyl acetate esterase staining, main components of the aggressor cells were revealed to be lymphocytes and mononuclear phagocytes. Although their proportion was variable from case to case, the former outnumbered the latter in 11 out of 12 cases. Some aggressor cells surrounding an isolated damaged tumor cell, the feature resembling that of satellite cell necrosis in acute graft-vs-host reaction, showed the morphological marker of T lymphocytes.

In primary culture of inflamed flat warts obtained from 9 cases, we observed wart-derived cell growth in 3 days which proceeded in a centrifugal pattern as noted with those from ordinary flat warts taken from 3 cases. However, in contrast to the latter, mononuclear cells began to come out from the original wart fragments later and to attach to the out-grown wart-derived cells, destroying them in the absence of autologous serum. Most of these mononuclear cells disappeared after treatment with anti-human T lymphocyte serum and complement.

These results strongly indicate the involvement of cell-mediated immunity in the spontaneous regression phenomenon of numerous flat warts.

Bleomycin Therapy for Warts: One Possible Mechanism of Action. DAVID R. BICKERS AND RAKESH DIXIT, Department of Dermatology, Veterans Administration Medical Center and Case Western Reserve University, Cleveland, Ohio

The treatment of warts remains at times a difficult problem and the variety of treatment modalities suggested is seemingly limitless. Several recent reports indicate that the intralesional injection of the anti-tumor agent bleomycin is effective in treating recalcitrant warts. Bleomycin is a glycopeptide antibiotic which is widely used as an anti-tumor agent and is isolated from culture broth of Streptomyces Verticillu Prior studies have shown that bleomycin inhibits mitosis and DNA synthesis and causes DNA chain breakage and that this latter reaction requires microsomal enzymes and NADPH. This study was designed to determine whether epidermal microsomal enzymes are capable of catalyzing bleomycin-mediated chain breakage in DNA isolated from the epidermis. Aerobic incubation of bleomycin with epidermal microsomes, epidermal DNA and NADPH caused substantial chain breakage of the DNA which was dependent upon concentrations of drug, microsomal protein and NADPH. The reactive oxygen scavenger superoxide dismutase, the metal chelator EDTA, and cytochrome C which inhibits microsomal electron transport each inhibited the enzyme-mediated chain breakage reaction, whereas ascorbic acid enhanced it. These studies indicate that oxidation/reduction of adventitious iron bound by bleomycin and accompanying reactive oxygen generation participate in the microsomal-catalyzed DNA damage caused by bleomycin. Since human wart virus is a DNA virus, it is reasonable to suggest that epidermal microsomal enzymes may be capable of enhancing bleomycin-mediated DNA chain breakage and that this reaction is in part responsible for the efficacy of this drug in the treatment of warts.

Characterization of an Acid Protease Produced by *Candida albicans* and Related Species. THOMAS L. RAY AND CANDIA D. PAYNE, Department of Dermatology, Univ. of Iowa College of Medicine, Iowa City, Iowa

In experimental murine cutaneous candidiasis, pathogenic species of *Candida* (C.) invade the epidermis and in some instances the dermis. Since enzymes liberated by C. may facilitate tissue invasion, 6 species of C. were surveyed for proteolytic enzyme activity. C. cultures grown on Remold's dextrose agar with 1% albumin as the sole nitrogen source, produced clearing zones at pH 4.5, but not 7.0, suggesting extracellular liberation of an acid protease.

Culture supernatants of *C. albicans* grown in Remold's albumin broth for 7 to 10 days contained an acid protease for albumin and hemoglobin substrates. No neutral protease activity was detected using BAEE, TAME, and casein as substrates. Partial purification by ultrafiltration, molecular sieve and Affi-Gel blue chromatography yielded a thermolabile enzyme with a pH optimum of 3.5, activity at 5.0, and irreversible inactivation at pH's above 7.5. The acid protease was approximately 42,000 daltons by S200 gel filtration and SDS-PAGE, and had an isoelectric point of 4.5. It was resistant to PMSF and 10 mM EDTA, and was unaltered by ferric and ferrous ions. Pepstatin inhibited the activity in a dose dependent and apparent equimolar fashion. The enzyme did not digest wool keratin.

Enzyme production was quantitated for 6 species under identical conditions. *C. stellatoides*, *albicans*, and *tropicalis* were the greatest producers respectively, while *C. parapsilosis*, *guillermondi* and *krusei* produced little or no activity. Production was unaltered by the addition of iron to the cultures.

C. acid protease is a pepsin-like enzyme which is calcium, magnesium and iron independent, and is associated with pathogenic *Candida* species.

Hydroxamate-Type Siderophore Production by Opportunistic and Pathogenic Fungi. MARK HOLZBERG AND WILLIAM M. ARTIS, Department of Dermatology, Emory University School of Medicine, Atlanta, Georgia

Iron is necessary for microbial growth and is associated with the production of various microbial virulence factors. Iron-chelating siderophores, which are thought to facilitate microbial acquisition of this metal, have been reported to be produced by a number of bacteria and nonpathogenic fungi. It has been suggested that these siderophores may function as virulence factors. There have been few studies on production of siderophore by opportunistic and pathogenic fungi. This investigation examines siderophore production by *Absidia corymbifera*, *Aspergillus niger*, *Rhizopus arrhizus*, *Rhizopus oryzae*, *Blastomyces dermatitidis*, *Candida albicans*, and *Trichophyton mentagrophytes*. Fungi were cultured at 37°C and 27°C in a chemically defined Low-Iron Media (11 ng/ml Fe). Culture supernatants were assayed by two

nonspecific siderophore methods ($FeCl_3$ and $Fe(ClO_4)_3$) and three chemically specific assays (catechol, 2,3-dihydroxybenzoate, and hydroxamate). All fungi secreted siderophore. Only siderophores of the hydroxamate type were found. More siderophore was produced at 27°C. The present study adds eight fungi to the list of known siderophore producers and reconfirms siderophore production by *H. capsulatum*. Current research is directed toward determining whether these siderophores are antagonistic to the fungal growth inhibitory activity mediated by transferrin.

Development of an Optimal Immunoassay for IgE Antibodies to *S. aureus*. STEPHEN J. FRIEDMAN, ARNOLD L. SCHROETER, AND HENRY A. HOMBURGER, Departments of Dermatology and Laboratory Medicine, Mayo Clinic, Rochester, Minnesota

Immunoglobulin E antibodies to *S. aureus* (Staph-IgE Ab) are found in the serum of some patients with atopic dermatitis and the hyperimmunoglobulin E syndrome. The frequency of occurrence and clinical significance of these antibodies is not well documented; but, their presence may indicate those patients with atopic dermatitis who would benefit from antibiotics in their treatment regimen.

To investigate the clinical significance of Staph-IgE Ab we have developed an optimized immunoassay which utilizes purified cell walls (PCW) from *S. aureus* Wood 46, a protein A deficient strain, as an immunosorbent for Staph-IgE Ab in serum. We compared Wood 46 PCW and whole organisms (WO) as immunosorbents for Staph-IgE Ab by performing tests on sera from 200 patients with a variety of dermatoses, including 143 with atopic dermatitis. We used radiolabeled affinity purified, Fc specific anti-IgE (4 ng/tube) as a detection protein. Sera with Staph-IgE Ab demonstrated dose-dependent binding to PCW and WO, but the ratio of specific to nonspecific binding was much greater with PCW. Peak specific binding to the PCW and WO immunosorbents was approximately equal, 29% of total counts. Nonspecific binding to WO was greater than to PCW, 5% versus 2%, respectively; and, WO binding varied directly with the serum concentration of IgE. By the use of PCW, Staph-IgE Ab were detected in a minimum serum volume of 0.5 μL.

We conclude that the use of PCW from *S. aureus* is required in immunoassays for Staph-IgE Ab to avoid false-positive results caused by high levels of nonspecific binding to WO. Immunoassays which utilize WO are not reliable for clinical studies of Staph-IgE Ab.

Chronic Dermatophyte Infection: Demonstration of Elevated Serum IgA Reactive with Polysaccharide from *T. mentagrophytes*. SHOZO HONBO, WILLIAM M. ARTIS, AND HENRY E. JONES, Department of Dermatology, Emory Univ. School of Medicine, Atlanta, Georgia

There is little information on the Ig class responses to the dermatophyte antigens. We have measured the Ig response within the immunoglobulin classes IgG, M, A, and E to a polysaccharide and a peptide antigen deirved from *T. mentagrophytes*. Sera from 27 patients with chronic dermatophytosis, average duration of infection 21 years, and 47 noninfected control subjects were analyzed using an Enzyme Linked Immunosorbent Assay. The Ig responses were compared as the $\log_2$ of the final reacting dilution or as absorbance values. Chronically infected patients showed significantly elevated IgA response to the polysaccharide antigen ($\log_2$, x = 6.88 against 4.64). The IgG response was slightly elevated, and the IgM equal to control values. IgE reactive against dermatophyte polysaccharide was also elevated in chronically infected patients. The IgG, M, and A reactions with the peptide antigen were much lower than with the polysaccharide antigen. The IgA reaction with the peptide antigen was, however, elevated. The significance of the IgA antibody response to dermatophyte polysaccharide is unknown.

TSS-1, A Staphylococcal Exotoxin Different from Pyrogenic Exotoxin-C, Is Preferentially Synthesized *In Vivo* and Associated with Toxic Shock Syndrome. HIDEKI NAKASHIMA AND KIRK D. WUEPPER, Department of Dermatology, The Oregon Health Sciences University, Portland, OR

Vaginal isolates of *Staphylococcus aureus* were obtained from verified cases of toxic shock syndrome from the Centers for Disease Control and locally. Exoproteins were synthesized and released into RPMI 1640 medium in dialysis casings surgically implanted into a rabbit peritoneum. These proteins contained potent nonspecific mitogenic activity for peripheral blood mononuclear cells which was associated wyth a protein which we purified by DEAE Sepharose CL-6B exchange chromatography and AcA 54 gel permeation chromatography. The M_r is 30K daltons and pI is 7.2. It behaves as a nonspecific mitogen for human and rabbit lymphocytes. Mouse, rat and guinea pig lymphocytes were not stimulated, however. Proliferative activity was neutralized by a precipitation antibody prepared in rabbits.

This proliferative factor (TSS-1) is functionally similar to SPE-A, B, and C, enterotoxin F, and a protein purified from strain #1169 and generously supplied by Dr. R. L. Stone. TSS-1 differs from SPE-C which is neither neutralized nor precipitated by our antiserum and does not co-migrate with TSS-1 in analytical IEF or PAGE. TSS-1 is produced by all of six TSS strains supplied to us (OHSU; CDC #033 and #050; and; from the Miami Valley Laboratories, Harrisburg, #1169 and #587). TSS-1 does not cause hemolysis, intraepidermal or subepidermal separation of the skin.

Growth of *S. aureus in vivo* favors TSS-1 production over SPE-C/enterotoxin F and it may be the major or critical toxin in toxic shock syndrome. Host factors which lead to the preferential synthesis of TSS-1 are presently unknown.

Pathogenesis of Tinea Versicolor I. Skin Occlusion: Effect on *Pityrosporum orbiculare*, Skin PCO_2, pH, Transepidermal Water Loss and Water Content. JAN FAERGEMANN, R. ALY, D. R. WILSON, AND H. I. MAIBACH, Department of Dermatology, University of California, San Francisco, California

Many fungal infections including tinea versicolor can be induced experimentally using plastic occlusion. To define the effect of occlusion on some factors known to predispose to infection, the following experiment was performed.

The effect of 8 days skin occlusion on *Pityrosporum orbiculare*, skin PCO_2, pH, transepidermal water loss (TEWL), and water content (WC) was studied in 10 healthy male volunteers. *P. orbiculare* counts increased from baseline counts of $2.1 \times 10^2/cm^2$ to $2.3 \times 10^3/cm^2$ after 3 days occlusion ($p < 0.01$); bacterial counts increased from $2.9 \times 10^3/cm^2$ to $1.8 \times 10^5/cm^2$ after 8 days occlusion ($p < 0.01$). pH increased during occlusion from 5.6 to a maximum at day 3 of 6.7 ($p < 0.01$); TEWL increased to a maximum of 11.74g m^{-2} h^{-1} after 3 days of occlusion compared to 4.39g m^{-2} h^{-1} before ($p < 0.01$).

P. orbiculare counts, Ph, and TEWL were lower at 8 days than 3 days occlusion. WC and PCO_2 remained high after 8 days; WC was then 57.1% compared to 48.0% before occlusion ($p < 0.01$); PCO_2 was 63.1 mm Hg compared to 53.1 mm Hg before occlusion ($p < 0.01$).

The increased levels of these factors may partially explain the higher risk of infection in occluded compared to non-occluded skin.

Pathogenesis of Tinea Versicolor II. An *In Vitro* Model for Growth and Filament Production of *Pityrosporum Orbiculare* (*Pityrosporum ovale*) on Human Stratum Corneum. J. FAERGEMANN, R. ALY, AND H. I. MAIBACH, Department of Dermatology, University of California, San Francisco, California

In an adherence study with human stratum corneum cells and *Pityrosporum orbiculare* we noted that the yeast cells produced short filaments when incubated with stratum corneum cells. We expanded on this empirical observation in developing this *in vitro* model mimicing tinea versicolor.

When *P. orbiculare* (*P. ovale*) was incubated with stratum corneum cells, suspended in phosphate buffered saline, for 90 minutes at 37°C short filaments were produced in 11–17.5% of the yeast cells.

A successful culture of *P. orbiculare* (*P. ovale*) on human stratum corneum *in vitro* is described. When 10^8 cells ml^{-1} of *P. orbiculare* or *P. ovale* were inoculated on stratum corneum pieces in an environment with 7% CO_2, for 5 days, a picture resembling that seen microscopically in tinea versicolor was observed. Filaments were produced in 39–48% of *P. orbiculare* (*P. ovale*) cells; the longest pseudohyphae, 40–60 μm, were produced by *P. ovale*.

This culture method provides the possibility to study the pathogenic mycelium form of *P. orbiculare* (*P. ovale*) *in vitro*. The influence of antimycotics, other drugs, and interaction with other microorganisms can be studied.

Activation of Human Mononuclear Leukocyte Transglutaminase by Streptococcal Proliferative Factor and A Mitogenic Factor Associated with Toxic Shock Syndrome. JUDY G. ZETTERGREN, HIDEKI NAKASHIMA, MARIAN J. LEIFER, AND KIRK D. WUEPPER, Department of Dermatology, The Oregon Health Sciences University, Portland, Oregon

Cellular transglutaminase (TGase) activity is enhanced in human peripheral lymphocytes after incubation with Concanavalin A or Phytohemagglutinin. We tested two purified "toxins" which cause T lymphocyte blast transformation in order to clarify their mechanism of

action. Streptococcal proliferative factor (SPF) is an exoprotein (M_r 29,000, pI 5.2) purified from culture filtrates of *S. pyogenes*, Strain NY-5. TSS-1 is a toxin (M_r 30,000, pI 7.2) purified from *S. aureus* isolated from vaginal isolates from patients with toxic shock syndrome. TGase activity was measured by incubation of cell lysates with casein, $CaCl_2$, dithiothreitol and ^{3}H-putrescine for 60 min at 37°C C. Aliquots in triplicate were precipitated with TCA on filter paper, washed, dried and counted.

Human mononuclear cell TGase activity was enhanced 3–5 fold within 30 minutes after incubation with either toxin. This occurred only when the toxin was incubated with intact cells; addition of toxin to cell lysates was without effect. TGase was not measureable extracellularly. Histamine, a competitive substrate for TGase, markedly inhibited ^{3}H-putrescine incorporation. Incubation of human lymphocytes with both cycloheximide and toxin did not alter TGase activation.

The bacterial mitogens, SPF and TSS-1, behave similarly to phytomitogens, which bind cell surface glycoproteins and purturb the cellular membrane, possibly by crosslinking of membrane proteins by TGase.

The Role of Eccrine Sweat in Delivery of Ketoconazole to Human Stratum Corneum. RUSSELL HARRIS, H. E. JONES, AND W. M. ARTIS, Dept. of Dermatology, Emory Univ. School of Medicine, Atlanta, Georgia

The purpose of this study was to determine the mechanism(s) of ketoconazole delivery to human stratum corneum. Possible modes of delivery investigated included sweat and sebum. Ten normal healthy human volunteers were given ketoconazole 400 mg p.o. qAM for two weeks. Samples of serum, sebum, sweat, and stratum corneum were quantitatively analyzed by high pressure liquid chromatography (sensitivity 0.005–0.010 mcg/ml). Sebum was also obtained from 3 patients on long-term ketoconazole therapy. *In vitro* studies with ^{3}H-ketonconazole were also performed. Serum levels confirmed adequate absorption in all individuals (mean 7.25 mcg/ml). Ketoconazole was found in thermogenic whole body eccrine sweat one hr following a single oral dose of 400 mg. The concentration of ketoconazole in sweat obtained on days 7 and 14 of oral ingestion were similar (range 0.014 to 0.323 mcg/ml; mean 0.072 mcg/ml). The mean concentration in palmar stratum corneum was 4.89 mcg/gm and did not change from day 7 to day 14. Ketoconazole was not found in the sebum collected after two weeks of oral ingestion. It was present, however, in the sebum of patients on long-term ketoconazole therapy (mean 4.7 mcg/ml). The concentration of ketoconazole was several fold greater in eccrine sweat/sediment (principally desquamated keratinocytes) than in eccrine sweat. *In vitro* studies with ^{3}H-ketoconazole confirmed the preferential binding of ketoconazole to keratinocytes and after admixture and equilibration demonstrated partitioning to lipid-rich sebum. We conclude that eccrine sweat rapidly transports ketoconazole across the blood-skin barrier where it may bind or partition to keratinocytes and surface lipids.

7:00 PM–8:30 PM Reception
National Museum of American History
Constitution Avenue and
14th Street, N.W.

Friday, April 29, 1983

8:00 AM–5:00 PM Registration — Upper Lobby
8:00 AM–8:20 AM SID Members Meeting — Presidential Ballroom
8:20 AM–12:00 PM PLENARY SESSIONS — Presidential Ballroom

Gerd Plewig, M.D. and Kimie Fukuyama, M.D., Presiding

KF-1 Monoclonal Antibody Defines a Specific Basement Membrane Antigenic Defect in Dystrophic Forms of Epidermolysis Bullosa. J. D. FINE, S. M. BREATHNACH, AND S. I. KATZ, Dermatology Branch, NCI, NIH, Bethesda, MD

The monoclonal antibody, KF-1 (formerly called EP-1), identifies a newly recognized noncollagenous constituent of the lamina densa of the basement membrane zone (BMZ) of stratified squamous epithelia. In order to determine whether this BMZ constituent is altered in patients with epidermolysis bullosa (EB), a heterogeneous genetic disease affecting the BMZ, we investigated the distribution of KF-1 antigen in the skin of patients with EB simplex (EBS) and EB dystrophisa (EBD, both dominantly and recessively inherited). Biopsies were taken from clinically normal skin sites and indirect immunofluorescence studies were performed using serial dilutions of KF-1 antibody and antibodies to other constituents of the normal epidermal BMZ (bullous pemphigoid antigen (BPA), laminin and type IV collagen). BPA, laminin and type IV collagen were present in normal amounts in the skin of all patients examined. In contrast, there was no detectable binding of KF-1 to the skin of 5/6 patients with recessive EBD, while one recessive EBD patient showed only faint BMZ staining with high concentrations of KF-1. Moreover, all 7 patients with dominant EBD had diminished skin binding of KF-1 as evidenced by low titers and/or decreased staining intensity. KF-1 binding was normal in the skin of all 51 controls, 7 EBS patients and 4 parents of recessive EBD patients. In addition, KF-1 antigen was detectable in normal fetal skin at 25 weeks but not at 14 weeks gestation. These findings indicate that the KF-1 antibody defines a BMZ defect in dystrophic forms of epidermolysis bullosa which is not seen in control individuals, EBS patients or obligate heterozygote parents of patients with recessive EBD. The identification of KF-1 antigen in normal fetal skin may facilitate the in utero diagnosis of EBD.

Restriction Enzyme Mapping of the Pro$_{\alpha 2}$(I) Gene in a Patient with the Marfan Syndrome. ELIZABETH RILEY, SHINGO TAJIMA, RUSSEL KAUFMAN, AND SHELDON PINNELL, Division of Dermatology, Duke Univ. Med. Ctr., Durham, North Carolina

The Marfan Syndrome has been associated with a defect in type I collagen. An abnormal $_{\alpha 2}$(I) collagen chain with an additional 20 amino acids N-terminal to the collagenase cleavage site has been described by Byers et al. PNAS 78:7745, 1981, in an individual with this syndrome.

We have performed restriction enzyme mapping of this patient's pro$_{\alpha 2}$(I) collagen gene and compared it with the normal gene to determine if any large organizational changes are present. Seven cloned EcoRI fragments representing 20,000 basus of the pro$_{\alpha 2}$(I) human collagen gene were nick translated and hybridized against EcoRI digested genomic DNA. All gave bands that were equal in size to the probe used with the exception of the 2.8^B kb probe which diffusely hybridized to the genomic DNA. This 2.8^B kb fragment, known to be near the collagenase site, was determined to contain Alu I sequences by hybridization with an Alu I probe. When an 850 base segment of the 2.8^B kb fragment, containing no repetitive sequence, was used as a probe the band obtained was of normal size.

We have thus developed probes to map the human pro$_{\alpha 2}$(I) collagen gene and by this technique have found no gross insertions or rearrangements in the pro$_{\alpha 2}$(I) gene of this Marfan patient. On the basis of this data it is likely that the abnormal pro$_{\alpha 2}$(I) gene in this individual resulted from a small duplication or a defect in RNA processing.

Ia Antigen Synthesis by Keratinocytes and Impaired Langerhans Cell Function in Acute Cutaneous Graft-Versus-Host Disease. S. M. BREATHNACH AND S. I. KATZ, Dermatology Branch, NCI, NIH, Bethesda, MD

Keratinocytes express Ia antigen (Ia) during cutaneous graft-versus-host disease (GVHD); it is, however, unclear whether this Ia is synthesized by keratinocytes or merely adsorbed from Ia-bearing Langerhans cells (LC). The functional significance of Ia on keratinocytes in GVHD is also unknown. We therefore sought to determine the origin of keratinocyte Ia in a murine model of GVHD. Lethally (900 rads) X-irradiated (XR) C3H (Ia^k) mice developed acute GVHD 7 days after injection of Balb/c (Ia^d) bone marrow and spleen cells and expressed keratinocyte Ia of host (Ia^K) origin in immunofluorescence studies. In other studies, XR C3H mice were made chimeric for LC expressing both Ia^d and Ia^k by injection of (Balb/cxC3H)F_1 marrow. Keratinocytes of these chimeric mice expressed only Ia^k and not Ia^d during GVHD induced by injection of C57Bl/6 (Ia^b) marrow and spleen cells, suggesting that keratinocyte Ia in GVHD is synthesized by keratinocytes. We next investigated the fate of LC in GVHD and the alloantigen presenting capacity of Ia-bearing keratinocytes. XR alone reduced the number of Ia-positive LC in epidermal sheets by 64 ± 3% after 12 days; no dendritic Ia-positive cells were seen in GVHD epidermis at this time. By contrast, 48% of GVHD epidermal cells (EC) in suspension expressed Ia compared with 3% of controls and 1.5% of XR cells. When EC from normal C3H mice, XR C3H mice or C3H mice undergoing GVHD were cultured with Balb/c responder lymphocytes for 5 to 7 days, GVHD EC produced markedly less T cell stimulation (11 ± 2% of control) than EC from XR animals (70 ± 10% of control) at optimal responder:stimulator ratios. These findings suggest a functional impairment of LC in GVHD and indicate that although host keratinocytes synthesize Ia in GVHD they lack alloantigen presenting capacity.

Ia Expression by Murine Epidermis *In Vivo* Without Evidence of Skin Disease. GERALD G. KRUEGER, MANSOOR EMAM, LEE K. ROBERTS, AND RAYMOND A. DAYNES, Departments of Medicine and Pathology, Univ. of Utah School of Medicine, Salt Lake City, Utah

Ia expression by cells of the immune system are important in cellular communication and antigen presentation. The presence of Ia on keratinocytes in mycosis fungoides, lichen planus, psoriasis and graft vs host disease suggests an association with inflammatory processes. However, Ia on the endothelium and on lactating epithelium suggest the possibility of a broader function. Within three weeks of grafting normal allogeneic skin onto inbred nude mice the keratinocytes of the graft express Ia antigens (I-A and -E/C); only of graft haplotype. Studies were initiated to determine the cellular humoral mechanisms associated with this phenomenon. Parabiosis between a heterozygous nude (nu/+) mouse and a homozygous nude (nu/nu) mouse resulted in Ia expression throughout the epidermis of both animals within one week but not in controls. Following separation, Ia expression persisted for 3 weeks in the nu/nu animal but not in the nu/+ partner. Widespread expression of Ia on keratinocytes also occurs following the injection of 10^7 nu/+ splenocytes or 0.1 ml of a sera (subq or i.v.) from a nu/+ animal. Sera from nu/nu animals has no effect. Induction of Ia on host keratinocytes by sera rules out a graft-vs-host response. In all of these experiments, there is no gross or microscopic pathology. The ease of induction and the manipulation available to control Ia expression in this system suggests that it will help dissect the interworking between the immune system (cells and immune mediators) and "nonimmune tissues" (keratinocytes). Further, it will permit investigations into the necessity of Langerhans cells for antigen presentation as well as the role of the ubiquitous Ia in health and disease.

HLA-DR Antigens on Keratinocytes in Diseased Skin. BEATRIX VOLC-PLATZER, OTTO MAJDIC, KLAUS WOLFF, WALTER KNAPP, AND GEORG STINGL, Department of Dermatology I and Immunology Institute, Univ. of Vienna, Vienna, Austria

HLA-DR antigen expression and synthesis within normal human epidermis is restricted to Langerhans cells (LC). However, using immunohistological techniques, several authors observed HLA-DR determinants on keratinocyte (KC) surfaces in certain disease states, but the mechanisms of acquisition of these antigens by KC remained unclear.

Using monoclonal antibodies with selective reactivity against either membrane incorporated HLA-DR (anti-HLA-DR, Becton/Dickinson, CA) or cytoplasmic antigenic determinants involved in the biosynthesis and assembly of HLA-DR molecules (VIC-Y1, Majdic et al, in press), we attempted to obtain some information as to the site of origin of KC-bound HLA-DR antigens in diseased skin.

By indirect immunofluorescence, skin biopsies from patients with lichen planus chronic graft-versus-host disease and mycosis fungoides displayed a dense lymphocytic infiltrate largely composed of T cells bearing the helper/inducer phenotype. Within the epidermis HLA-DR determinants were found on both LC and KC surfaces. While anti-HLA-DR+, OKT6+ and VIC-Y1+ LC were uniformly distributed over the entire specimen, HLA-DR antigens on KC were mainly observed in epidermal areas overlying dense lymphocytic infiltrates. These anti-HLA-DR+ KC displayed also cytoplasmic fluorescence when tested with VIC-Y1, but were uniformly OKT6 negative. Anti-HLA-DR+ and/or VIC-Y1+ KC were never observed in specimens from epicutaneous patch test reactions or normal human skin.

Reactivity of KC with a monoclonal antibody against the cytoplasmic HLA-DR assembling moiety favors the hypothesis that HLA-DR antigens on KC surfaces in certain skin diseases are actively synthesized by these cells rather than passively absorbed from other HLA-DR+ cells.

Keratin Families: Identification of Constant and Variable Members Using Monoclonal Antibodies. RIVA EICHNER, PAULA BONITZ, AND TUNG-TIEN SUN, Department of Dermatology, Johns Hopkins Univ. School of Medicine, Baltimore, Md. and Departments of Dermatology and Pharmacology, N.Y.U. School of Medicine, New York, N.Y.

Cultured human epidermal cells were used as a model system to study epidermal differentiation. Keratins expressed by epidermal cells grown under nonkeratinizing and keratinizing conditions were compared with keratins of *in vivo* epidermis by one and two-dimensional gel electrophoresis, and by the immunoblot technique using monoclonal anti-keratin antibodies. Two of the antibodies (designated AE1 and AE3), in combination, recognized almost all major epidermal keratins. AE1 recognized a family of acidic (pI < 6) keratins (40K, 48K, 50K and 56.6K), whereas AE3 reacted with a family of more basic keratins (56K, 58K and 65–67K). Within the AE1 family, the 50K keratin was expressed regardless of the course of epidermal differentiation; the 40K and 48K keratins were expressed under nonkeratinizing conditions, whereas the 56.5K keratin was only detected concomitant with keratinization. A parallel situation was observed in the AE3 family: the 58K keratin was always expressed, the 56K keratin was associated with nonkeratinizing cultures, and the 65–67K keratins were synthesized only under conditions permissive for keratinization. These results suggest that human epidermal cells undergoing various types of terminal differentiation express two mutually exclusive keratin families. Furthermore, within each family, there is a constant member (50K and 58K keratins in AE1 and AE3 families, respectively) and variable members. Among variable members, the small keratins (40K, 48K, 56K) are expressed when keratinocytes assume a nonkeratinized morphology, whereas the larger keratins (56.5K, 65–67K) are synthesized only under conditions allowing phenotypic keratinization.

Structural Features of Mammalian Epidermal Keratin Filaments Assembled *In Vitro*. PETER M. STEINERT, Dermatology Branch, NCI; and ALASDAIR C. STEVEN, Laboratory of Physical Biology, NIADDKD, NIH, Bethesda, MD

We have used scanning transmission electron microscopic (STEM) and biochemical methods to study the structure of keratin intermediate filaments (KIF). Frozen unstained specimens were visualized by STEM and the images recorded in digital form. Since the signal recovered from the image is proportional to the mass of material present, such data are useful for measurements of the mass and shape individual KIF and impose stringent constraints on possible models of KIF structure. Bovine KIF have linear masses of about 37 kD/nm. Short KIF and the ends of long KIF, which may represent minimally assembled KIF, had masses of only about 25 kD/nm. In terms of our postulated protofilamentous substructure of KIF, these data indicate that long KIF contain 11–12 protofilaments and minimally assembled ones contain 7–8 protofilaments. Most human KIF average about 24 kD/nm, and as such, are probably incompletely assembled *in vitro*. The same digital STEM images were used for radial density measurements. Surprisingly, the KIF were found to be 15–16 nm wide, rather than the accepted value of 10 nm. The radial distribution of mass indicates that the outer one-third of mass has a density of only one-sixth of the inner two-thirds, which perhaps explains why negatively-stained images of KIF routinely appear to be only 10 nm wide. Limited chymotryptic digestion of mouse or bovine KIF released about one-third of the mass as small glycine- and phosphate-rich peptides without significant apparent alterations in the appearance of the KIF, leaving a residue that was enriched in α-helix. These results all converge on a model of KIF structure in which glycine-rich non-α-helical portions of the subunits protrude from a central core into which the α-helical regions of the subunits are packed.

Isolation and Purification of the Pemphigus Vulgaris Antigen from Human Epidermis. LARRY L. PETERSON AND KIRK D. WUEPPER, Department of Dermatology, The Oregon Health Sciences Univ., Portland, Oregon

Heat separated human epidermis was homogenized and extracted with acetate buffer and 8 M urea to remove soluble proteins and keratins. Various techniques to extract the membrane-rich pellet (Triton X, CHAPS, lithium di-iodosalicylate) were compared, but 1% SDS in Me_2SO 1% was superior for extracting the pemphigus vulgaris (PV) antigen. This antigen and the antigen reactive with stratum corneum antibodies were identified by transfer blotting to nitrocellulose paper, blocking unreacted sites with BSA, incubating with PV serum or 20 control sera and detecting with FITC-anti human IgG.

Since Concanavalin A inhibits the binding of PV antibody to tissue sections, we studied the binding of the extracted proteins to Con A covalently coupled to Sepharose. PV antigen bound to the Con A and was released by 0.02 M methyl alpha-D-mannopyranoside. The proteins thus recovered were subject to gel permeation chromatography on AcA 54, and the PV antigen was detected by the transfer blot technique. PV antigen corresponded to a discrete peak at 66K daltons by gel filtration and gave one homogeneous band at 33K daltons in urea-SDS-PAGE.

A monospecific polyclonal antibody to PV antigen was raised in rabbits, and it stained human epidermis in the same manner as PV autoantibody.

The pemphigus vulgaris antigen has been isolated and purified from adult human epidermis. It is a 66K dalton membrane glycoprotein that is composed of two apparently identical subunits of 33K daltons each.

Bullous Pemphigoid or Pemphigus Antisera Fail to Induce Epidermal Cell Cytotoxicity Using an Improved *In Vitro* Tech-

nique. K. A. FRITZ, S. R. RYAN, S. T. BOYCE, W. L. WESTON, AND D. A. NORRIS, Departments of Dermatology and Cell Biology, University of Colorado School of Medicine, Denver, and Boulder, Colorado

Bullous Pemphigoid (BP) and Pemphigus Vulgaris (PV) are characterized by antibodies which specifically bind to antigens produced by human keratinocytes. *In vitro* and *in vivo* experimentation has implicated these specific antibodies in several immunologic mechanisms of tissue damage in these diseases. We report the use of a new *in vitro* model of epidermal cytotoxicity to determine whether epidermal cell destruction can be produced by BP or PV antisera.

The presence of BP and PV antigens was verified by immunofluorescence on 3 different cultured epidermal cell sources: Normal human (HuK), transformed rabbit (RSC), and transformed mouse (PAM212) epidermal cells. Cytotoxicity of chromium-labeled epithelial targets was determined after 4 hr incubation in microtiter plates with combinations of antisera, human complement, and non-sensitized monocyte, lymphocyte, neutrophil or eosinophil effectors. No significant lysis by antibody, antibody plus complement, or antibody plus effectors (antibody-dependent cellular cytotoxicity or ADCC) was seen with any of the 3 cell types as targets. All 3 targets were shown to be susceptible to ADCC using heteroantisera and other antigen systems.

Although the specific antibodies of PV may directly alter epidermal cells, the specific antibodies of BP and PV do not induce direct lysis by complement or ADCC of epidermal cells, and such lysis cannot be implicated as a significant mechanism of tissue destruction in these diseases. These experiments also show that all antigen-antibody systems are not equally effective in inducing ADCC of epidermal cells.

Dysplastic Nevus Syndrome: Increased Ultraviolet Mutability in Association with Increased Melanoma Susceptibility. MOHAN I. R. PERERA, MARK H. GREENE, AND KENNETH H. KRAEMER, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205

The dysplastic nevus syndrome (DNS) is a pre-neoplastic form of hereditary cutaneous melanoma in which affected individuals have increased numbers of abnormal (dysplastic) nevi and a markedly increased risk of developing cutaneous melanoma. We have carried out a series of quantitative investigations to determine the frequency of ultraviolet (UV)-induced mutations in EB virus-transformed lymphoblastoid cell lines (LCL) derived from 2 patients with the DNS and melanoma (DNS3Be, DNS5Be), a xeroderma pigmentosum (XP) variant (XP30Be), and 2 normal LCL (HH4, GM606). Cell survival was assessed by the ability of cells treated with 254 nm UV (2.25–9.0 J/m^2) to initiate microcultures in 96 well tissue culture plates (Kraemer et al., Mutat Res 72:285, 1980). At the higher UV doses, survival of the DNS LCL was similar to that of the normal LCL. The cell mutation assay was performed by innoculating 4×10^4 cells per well in medium containing 6-thioguanine and estimating the frequency of mutation by counting the number of microculture-containing wells after a 2 week incubation (Furth et al., Anal Biochem 110:1–8, 1981). Mutation frequency in all the untreated LCL was similar. DNS3Be and DNS5Be showed a 2- to 4-fold greater ($p < 0.05$) frequency of induced mutants per surviving cell than the normal LCL following 6.7 and 9.0 J/cm^2 UV. This was nearly as great an increase as was seen with XP30Be. This is the first demonstration of *in vitro* hypermutability in DNS. DNS is thus the second hypermutable human disease and, like XP, may have an increased susceptibility to UV-induced somatic mutations. This abnormality may be etiologically related to the increased melanoma susceptibility in both DNS and XP.

Selective Thermal Neutron Capture Therapy of Melanoma Cells Using Their Specific Functional Differentiation. YUTAKA MISHIMA, MASAMITSU ICHIHASHI, MASAYUKI TSUJI, AND MASATO UEDA, Department of Dermatology, Kobe University School of Medicine, Kobe, Japan

Thermal neutrons are easily absorbed by the non-radioactive isotope ^{10}B, resulting in the emission of α-particles and lithium atoms which together release energy of 2.33 MeV, up to a distance of 14 μ, Their traveling range and also the diameter of melanoma cells. Thus, if we can selectively accumulate ^{10}B in melanoma cells, we can destroy them without serious injury to the surrounding normal tissue. Malignant melanoma, the highly lethal cancerous growth of pigment cells, usually accentuates their specific function of synthesizing melanin. We therefore synthesized seven melanoma-seeking $^{10}B_1$ compounds, of which two, $^{10}B_{12}$-chlorpromazine ($^{10}B_{12}$-CPZ) and $^{10}B_1$-p-boronophenylalanine ($^{10}B_1$-BPA) are found to be highly effective. We then irradiated melanoma which was subcutaneously proliferating in hamster and found that the best and longest suppressive effect was obtained when neutrons and $^{10}B_{12}$-CPZ or $^{10}B_1$-BPA were administered before irradiation, compared to using only neutrons or X-ray treatment. The enhanced melanoma-killing effect of the ^{10}B compounds is also shown by *in vitro* radiobiological analysis. The D_0 value of thermal neutron only is 2.8×10^{12} nvt; $^{10}B_1$-boric acid, 5 μg ^{10}B/ml medium present: 0.9×10^{12} nvt; $^{10}B_{12}$-CPZ, 1.2 μg ^{10}B/ml pre-incubation; 0.9×10^{12} nvt; and $^{10}B_{12}$-BPA, 0.38 μg ^{10}B/ml pre-incubation: 0.9×10^{12} nvt. 28 Hr after administration, a chemical assay of melanoma-bearing hamsters reveals a ^{10}B tumor/blood of 11.5 and a tumor/liver of 15. Preclinical experiments on spontaneous melanoma in Duroc pig skin have shown cure and depigmentation after the combined treatment of $^{10}B_1$-BPA and irradiation without obvious side effects.

Protein Phosphorylation and the Mechanism of Action of MSH. JOHN A. MCLANE AND JOHN M. PAWELEK, Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut

Cloudman melanoma cells in culture respond to MSH with alterations in pigmentation, morphology, and rates of proliferaaion. These responses are preceded by increases in cAMP, and it is likely that the action of MSH is mediated by the activation of a cAMP-dependent protein kinase(s). However, the mechanism by which an activated kinase regulates the various phenotypic changes is unknown. Does the same kinase regulate all the responses? What are the phosphoprotein substrates? Are the actions of MSH mediated solely through the cAMP system? We report here experiments designed to address these questions. Cells were cultured in the presence or absence of MSH for 4 days. Following this period they were lysed in Triton X-100, and the lysates were incubated briefly with ATP^{32} in the presence or absence of cAMP. The lysates were then subjected to SDS-polyacrylamide gel electrophoresis, and the ^{32}P-labelled proteins were detected by autoradiography. Cells which were exposed to MSH showed changes in the labelling patterns for proteins of 105, 90, and 50K daltons. However, phosphorylation of these proteins was not affected when cAMP was added directly to the lysates. In the presence of cAMP, a protein of 30K daltons was phosphorylated whether or not the intact cells had been exposed to MSH. That is, exposure of intact cells to MSH activated a protein phosphorylation system that was not affected by direct addition of cAMP to cell lysates. Conversely, addition of cAMP to lysates activated a protein kinase that was not affected by exposure of cells to MSH. The results indicate that (a) not all cAMP-sensitive protein kinases in Cloudman melanoma cells are activated by MSH, and (b) the cells therefore must have mechanisms for restricting the hormonal signals to particular enzyme systems. This experimental approach should be of value for understanding how MSH regulates multiple functions in melanoma cells.

Epidermal Glycoconjugate Biosynthesis in Organ and Cell Culture: Effect of Retinoids. R. OROZCO-TOPETE, J-C. CHUNG, AND P. M. ELIAS, (intr. by MARY L. WILLIAMS), Dermatology Service, VAMC and Dept. of Dermatology, Univ. of Calif. School of Medicine, San Francisco, CA

Although retinoids exert profound effects on epithelial differentiation, their mechanism of action remains unknown. An effect on post-translational glycosylation of membrane glycoconjugates would explain the retinoids' impact on both cell-substrate and intercellular adhesion. Here, we assessed glycoconjugate biosynthesis from ^3H-D-glucosamine, ^3H-D-galactose, or ^3H-n-acetyl-D-glucosamine by both human second-passage keratinocytes, as well as human and neonatal mouse epidermis exposed to all-trans-retinoic acid, isotretinoin or etretinate (10^{-4} through 10^{-8} M) for 12–96 hr in sugar-free, delipidized media. Glycoproteins (GP) were obtained with 2% SDS, dialysis, and TCA precipitation, glycolipids (GL) by chloroform:methanol extraction, and total glycoconjugates (GP plus glycolipids (GL)) were assessed after aldehyde-fixation, repeated washings, and hydrolysis in 6 N HCl. Whereas retinoids consistently depressed GP plus GL biosynthesis in monolayered keratinocytes, in organ-cultured epidermis they increased GP synthesis by 15–80%, and GL biosynthesis by 200–400%, suggesting that retinoids specifically stimulate GLs more than GPs. Using isolated cell preparations, labelling of glycoconjugates was localized specifically to the basal/spinous layers (granular/cornfield layers and dermis were not stimulated). We conclude that retinoids: (a) exert opposite effects on epidermal glucoconjugate biosynthesis in organ vs. monolayer culture; (b) disproportionately stimulate glycolipid rather than glycoprotein synthesis; and (c) preferentially stimulate the Malpighian layers of epidermis. Stimulation of glycoconjugate biosynthesis in organ culture may provide another convenient bioassay for comparing retinoid potency.

Expression of Keratinocyte EGF Receptor at Reduced Calcium Concentrations Without Protein Synthesis. E. J. O'KEEFE, Department of Dermatology, University of North Carolina, Chapel Hill, North Carolina

Human keratinocytes grown in medium containing reduced calcium concentrations (0.07 mM) have been found to show altered morphology, decreased differentiation, and enhanced proliferative growth in comparison with cells grown in medium with physiologic calcium concentrations (1–2 mM). Since such alterations could be mediated by growth factors, we measured binding of ^{125}I-labelled epidermal growth factor (EGF). Neonatal keratinocytes were subcultured without feeder layers and grown to confluence in 1.1 mM calcium. Medium was changed and cells incubated for various periods in reduced calcium prior to binding assays. Scatchard plots of binding data showed a 5-fold increase in receptor number with no change in affinity ($1–3 \times 10^{-9}$ M) after 4–24 hr at 37°C. Maximal binding occurred at 0.02–0.04 mM calcium and decreased sharply with increasing calcium concentrations. The increase could be prevented by calcium added soon after binding began to increase but was not altered after substantial elevation had occurred, although morphologic changes at reduced calcium concentrations were reversed within several hours. Smaller increases in binding of ^{125}I-somatomedin-C and ^{125}I-concanavalin A were detected, but binding of ^{125}I-hydroxybenzylpindalol, a beta-receptor ligand, was unchanged. The increase in EGF binding was not prevented by puromycin (20 μg/ml), which decreased protein synthesis by 90%. The ability of keratinocytes to display previously synthesized EGF receptors at the cell surface suggests the presence of cryptic or intracellular receptors which can be exposed at low calcium concentrations.

Isolation of Lamellar Bodies from Neonatal Mouse Epidermis by Sequential Selective Filtration. S. GRAYSON, A. D. JOHNSON-WINEGAR, AND P. M. ELIAS, Derm. Serv., VAMC, Dept. of Dermatology, Univ. of Calif. School of Medicine, San Francisco, CA, and Path. Div., USAMRIID, Ft. Detrick, MD

The epidermal lamellar body is the organelle putatively responsible for the deposition of those lipids and hydrolytic enzymes that underlie cutaneous barrier function. Isolation of these organelles has presented a formidable challenge because the pressures required for homogenization and the prolonged isolation procedures result in loss of lamellar bodies and their contents. Since the method for the partial purification of these organelles from fetal epidermis is not applicable to skin *ex utero* (Freinkel, R. K. and Traczyk, T. N., J Invest Dermatol 77:478, 1981), w obtained sheets of stratum corneum and stratum granulosum from neonatal mice with highly purified staphylococcal epidermolytic toxin (Johnson-Winegar, A. D., et al., Infect and Immun 12:1206, 1975) diluted in DME containing 10 mM EDTA and 20 mM Hepes buffer, pH 6.5. The sheets were homogenized in a loose-fitting glass homogenizer, passed through a Stansted cell disruptor at 5,000 p.s.i., and the supernatant passed through an 8 μM Millipore pre-filter followed by a series of nucleopore filters (8 μM → 0.4 μM pore size). The final filtrate comprises a fraction that is highly enriched in intact lamellar bodies with variable amounts of glycogen, and a few other vesicular structures. The success of this novel isolation scheme exploits: (a) the small size of the lamellar body (0.15–0.25 μM); (b) the rapid homogenization and filtering sequence (≃30 min; (c) the use of a non-cytotoxic fraction of epidermolytic toxin; and (d) the controlled pressure delivered by the cell disruptor. Availability of a highly purified lamellar body preparation from post-natal epidermis will help clarify the role of this novel organelle in epidermal function.

The Effect of a Monoclonal Antibody Directed Against Human Basal Cells (VM-1) on the Proliferation of Benign Keratinocytes. VERA B. MORHENN AND GERALD KRUEGER, Department of Dermatology, Stanford University Medical Center, Stanford, CA and Division of Dermatology, University of Utah Medical School, Salt Lake City, UT

We have produced a murine monoclonal antibody directed against human basal cells, termed VM-1 (or W-13), which inhibits the growth of normal human epidermal cells *in vitro*. This antibody also binds to human basal cell and squamous cell carcinomas of the skin and, in the one case tested, cells of the squamous cell carcinomas of a lung tumor but not normal lung tissue.

To determine if the inhibition of growth seen in culture could also be demonstrated *in vivo*, nude mice grafted with human skin were utilized. The grafted nude mice were divided into 4 different groups (n = 2 or 3). Group I, no treatment; Group II, i.v. injection of VM-1 (2 ml); Group III, the human skin was tape stripped (16×) to stimulate basal cell proliferation; Group IV, tape stripping plus injection of VM-1. On days 1, 2, 3 after tape stripping, the human skin was biopsied, incubated in ^{3}H-thymidine for 4 hr, fixed in formalin and cut into histologic sections. Heavily labeled basal cells were quantified by radioautography.

Number of Heavily Labeled Basal Cells/1000 Total Cells (Mean ± SD)			
Group	Day 1	Day 2	Day 3
I	27 ± 9	15 ± 7	108* ± 21
II	28 ± 21	35 ± 21	57 ± 23
III	44 ± 25	73 ± 32	229 ± 116
IV	55 ± 4	40 ± 12	50 ± 10

* The high labeling index on Day 3 is attributed to repeated biopsy.

Staining of frozen sections shows that VM-1 binds only to human skin and not to mouse skin. These results suggest that VM-1 is not toxic to human skin cells and that the antibody prevents basal cell hyperproliferation *in vivo*.

A Monoclonal ab to Merkel Cells (MC). Its Use for the Study of MC Kinetics and Antigenic Properties. J.-H. SAURAT, P. CARRAUX, L. POLLA, L. DIDIERJEAN, AND P. CHAVAZ, Clinique de Dermatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland

A human monoclonal, mu, kappa, cold agglutinin ab of the rare specificity Pr 1h (serum and proper eluates) was used in immunofluorescence (IIF), immunoperoxydase techniques (IIP) and immunoelectron microscopy (IEM) on rabbit lip specimens (38 adults, 6 neonates). We used also (i) autoradiography ($_{3}$HT) coupled to IIP; (ii) IIF double tracing (Rhodamin-FITC) with Pr 1H ab and antiserum to one of the following antigens: Pemphigus vulgaris (PV) Prekeratin (PK) Actin (A) Vimentin (V) skin salcium binding protein (SCaBP).

Pr 1h ab strongly reacted with scattered cells in epidermis which were demonstrated to be MC by EM; no nerve fibers were stained. In IEM, a strong reaction was seen within the cytoplasm and around the granules. This is the first IEM staining of MC so far reported; it demonstrates the expression of a carbohydrate differentiation ag in MC. This ag was similarly expressed in both the definitive and the so called immature transitional MC in rabbit neonates. The coupling of IIP and autoradiography allowed study of 1526 MC in adult rabbit lips; no MC in S phase was seen. In rabbit neonates, in which numerous transitional cells were observed by EM, 8 out of 310 (2.5%) cells appeared positive for both Pr 1h ag and $_{3}$HT; this may support the concept of transitional cell mitosis. IF double tracing showed that MC were PV (−), PK (−), V (−) and SCaBP (−) but A (+). Although more antigenic mapping of MC is being done, the present results show that common keratinocyte ags (PV, PK, SCaBP) are not expressed by MC, not supporting the concept of MC derivation from keratinocytes.

Molecular Aspects of Psoralen Photosensitivity Reactions. M. A. PATHAK AND P. C. JOSHI, Dept. of Dermatology, Harvard Medical School, Boston, MA

Until recently, photosensitization by psoralens (PS) was reported to be an oxygen-independent type-I reaction in which ^{3}PS, the reactive triplet state of PS, photoreacted with DNA to form monofunctional (MF) cyclobutane and bifunctional (BF) interstrand cross-linking adducts. Oxygen-dependent photodynamic reactions of type II, in which ^{3}PS reacted with molecular oxygen ($^{3}O_2$) to produce reactive singlet oxygen ($^{1}O_2$), were believed not to be involved. Twelve linear and angular PS capable of forming MF or BF adducts with DNA were examined with a view to elucidate the role of $^{1}O_2$ in skin photosensitization reactions. Production of $^{1}O_2$ in presence of PS (5×10^{-5} M) and UVA irradiation (320–400 nm, 0–10 J/cm^2) was examined by observing the bleaching of p-nitrosodimethylaniline at 440 nm using histidine as a selective acceptor. Additional evidence for $^{1}O_2$ involvement was obtained from quenching studies with azide ions and 1,4-diazocyclo-[2,2,2]-octane (DABCO) or by carrying out reactions with O_2, N_2, C_2H_5OH, and $^{1}O_2$-generating compounds such as hematoporphyrin, anthracene, and methylene blue. The results showed that both linear PS (interstrand cross-linking) and isopsoralens (angular MF) produced $^{1}O_2$, although at varying degrees. The linear PS produced $^{1}O_2$ greater than MF isopsoralens. Nonphotosensitizing 5-methylangelicin, 4,8-dimethyl,5′-carboxypsoralen, and 5-diethylaminobutoxypsoralen produced little or no $^{1}O_2$. Our data elucidate a new role of $^{1}O_2$ in skin photosensitization and suggest three modes of PS reactions: 1) The nuclear damage involving a type-I reaction independent of $^{3}O_2$ and $^{1}O_2$. 2) The sensitized cell membrane damage of epidermal, dermal, and endothelial cells involving a type-II reaction ($^{3}O_2$ and $^{1}O_2$). 3) Damage of cytoplasmic constituents (enzymes, RNA, lysosomes, etc.) mediated by type-I and type-II reactions.

12:00 PM-2:00 PM LUNCH AND WORKSHOPS

Photomedicine — Presidential Ballroom
Herbert Hönigsmann
John Parrish

Viral Diseases: Clinical and Molecular Aspects — Federal Room
Douglas Lowy
Jean Thivolet

Monoclonal Antibodies in Dermatology — South American Room
D. M. MacDonald
Richard Edelson

Epidermal Cell-T Cell Interactions — Gallery
T. Luger
Daniel Sauder

Biology of Connective Tissue — Pan-American Room
Thomas Krieg
Sheldon Pinnell

Cutaneous Microbiology — Massachusetts Room
W. C. Noble
David Feingold

Proteases — New York Room
V. Hopsu-Havu
Gerald Lazarus

Sweat Glands — Ohio Room
Erhard Holzle
Kenzo Sato
Kenneth Kraning

2:00 PM-2:45 PM ESDR GUEST SPEAKER
Presidential Ballroom
Gerd Plewig, M.D., Presiding
Prof. Dr. Holger Kirchner
"Interferon"

3:00 PM-5:30 PM CONCURRENT SCIENTIFIC SESSIONS

SESSION A — Congressional Room
David R. Bickers, M.D., Presiding

Permeation of Lysosomal Membranes in the Course of Photosensitization with Methylene Blue and Hematoporphyrin: Study by Cellular Microspectrofluorometry. RENÉ SANTUS', CHAHIDE KOHEN°, ELLI KOHEN°, JEAN-PH. REYFTMANN', PATRICE MORLIÈRE°°, LOUIS DUBERTRET°°, AND PAUL TOCCI°. Papanicoalou Canc. Res. Inst. Miami, USA°; ERA CNRS 951'; Hôp. H. Mondor, Paris, France°°.

Lysosomal enzymes are believed to trigger the inflammatory response. There is some controversy on whether the lysosomal membranes can be primary photochemical targets in phototoxic reactions because no results have been obtained under "*in vivo*" conditions, i.e. *with a single irradiated cell and during the photochemical reaction.* The photodynamically induced liberation of lysosomal enzymes using β-galactosidase and N-acetyl-β-D-glucosaminidase as markers for the lysosomal enzymes has been studied by microspectrofluorometry on mouse L cells and human fibroblasts. The high sensitivity of the fluorescence detection makes it possible to use 4-methylumbelliferyl substrates for the enzymes contained in a single cell. As a matter of fact, these substrates do not fluoresce upon excitation at 365 nm while the split substrate does. Hematoporphyrin and methylene blue readily incorporate into both cells and, upon excitation at 365 nm and at wavelengths greater than 600 nm, sensitize lysosomal membrane damages. The kinetics of the enzyme liberation can be followed by the increase in the fluorescence at 460 nm. It strongly depends on the photosensitizer. Photosensitization with methylene blue which binds to negatively charged macromolecules and to the outside of the plasma membrane provokes major morphological changes before lysosomal membranes damages, while both processes cannot be easily separated with the lipophilic hematoporphyrin. Thus the involvement of lysosomal enzymes in the phototoxicity of photodynamic agents may depend on the sensitizer.

Benoxaprofen Phototoxicity: Mechanisms for Membrane Photodamage. I. E. KOCHEVAR, K. WUJEK-HOOVER, AND M. J. YOON. Dept. of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, MA

The phototoxic response to benoxaprofen (BXP), a non-steroidal anti-inflammatory drug, occurs a few minutes after exposure of skin to UVA (320–400 nm) and appears to involve degranulation of mast cells. BXP-photosensitized membrane damage is a possible mechanism because, *in vitro*, BXP photoinitiates disruption of RBC membranes. The purpose of this study was to characterize the photochemistry of BXP and the photoreactions which may produce membrane damage. BXP (50 μg/ml) was irradiated in pH 7 PBS, in ethanol and in mixtures of these solvents using 290–380 nm radiation. In aqueous solution BXP photodecarboxylated to yield decarboxybenoxaprofen, DBXP, (quantum yield = 0.75) as detected by TLC and HPLC. As the ethanol content increased, the quantum yield decreased. DBXP has the same absorption spectrum as BXP but is more hydrophobic so that it may dissolve in cell membranes and photosensitize damage. When BXP (100 μg/ml) was irradiated in the presence of RBC (3×10^7/ml) the rate of formation of DBXP paralleled that of RBC photohemolysis. Addition of human serum albumin (HSA) to BXP shifted the BXP fluorescence maximum from 380 nm to 338 nm and inhibited the photohemolysis. The minimum HSA concentration (0.8%) which completely shifted the spectrum also completely inhibited photohemolysis. Irradiation of BXP or DBXP with isolated RBC membranes caused crosslinking of the membrane proteins as determined by SDS-PAGE.

These results indicate that photochemistry of BXP molecules which are free in solution or bound to HSA is not responsible for membrane photodamage and that BXP or DBXP located in membranes may cause membrane damage by photosensitizing crosslinking of membrane proteins.

The Pathogenesis of Amiodarone Photosensitivity and Pigmentation. C. B. ZACHARY*, G. C. STOREY, D. W. HOLT, W. J. MCKENNA°, AND D. M. MACDONALD*, Laboratory of Applied Dermatopathology* and Poisons Unit, Guy's Hospital and Cardiology Department°, Hammersmith Hospital, London, England.

Amiodarone (A) therapy, valuable in control of cardiac dysrhythmias, is often complicated by photosensitivity of unknown cause.

Skin biopsies from patients with photo-induced pigmentation were submitted to light and electron microscopy and electron dispersive x-ray microanalysis. Tissue was also subjected to selective high performance liquid chromatography to quantify levels of A and its major metabolite desethylamiodarone (D).

Light microscopy revealed dermal deposition of pigment characteristic of lipofuscin while ultrastructurally lysosomal dense bodies with granular concentric lamellae and vacuolation were seen in macrophages. X-ray microanalysis showed large quantities of iodine with some sulphur and iron in the phagocytosed dermal pigment. Mean concentrations (mgm/kg wet weight) of A/D in light exposed skin (177/826.5) were 10 times those in non-exposed areas (18.6/84) and correlated with plasma levels of 2.5/2.3 mgm/1 in one patient.

Monochromator studies revealed abnormal acute responses at 307.5, 320 and 400 nm, consistent with a phototoxic reaction to A/D with possible amplification by fixation of A/D in dermal histiocytes.

The electron dense lysosomal deposits are rich in iodine and show positive staining for lipofuscin. Thus these may represent aggregates of the abundant lipid soluble A and degraded cell membranes to which the A has become bound after exposure to UV-irradiation.

Effect of Captopril on Vascular Permeability Changes in Demethylchlortetracycline (DMCT)-Induced Phototoxicity. RYOICHI KAMIDE, HENRY W. LIM, AND IRMA GIGLI, Division of Dermatology, University of California, San Diego, California.

Captopril, an inhibitor of angiotensin-I converting enzyme, was recently reported to inhibit vascular permeability changes caused by various vasoactive mediators, including histamine and compound 48/80 (J Clin Invest 59:1207, 1982). We demonstrated previously that in guinea pigs (GPs), the development of DMCT-induced phototoxic lesions was associated with an increase in vascular permeability, which was abolished in complement-depleted GPs (Clin Res 30:552A, 1982). Captopril was utilized in this study to dissect the relative roles of complement and mast cell-derived mediators in phototoxicity. Hartley strain albino GPs were treated orally with three doses of captopril, 50 mg/kg/dose, while control animals received phosphate buffered saline. They were then injected intradermally with DMCT (0.025–0.05 mg), followed by ultraviolet-A (UV-A) irradiation (8 J/cm^2). After completion of the irradiation, Evans blue dye was injected intravenously, and

the increase in vascular permeability was evaluated by the degree of localized bluing. For comparison, bluing induced by compound 48/80, a mast cell degranulator, and trypsin-treated C5 (as a source of C5a anaphylatoxin) were assessed. Captopril treatment partially suppressed the bluing induced by compound 48/80. In contrast, it did not alter the bluing induced by UV-A irradiation of the DMCT-injected sites, nor that induced by trypsinized-C5. These data offer additional evidence for the active participation of the complement system in the development of phototoxicity, and suggest that the role of the mediators released by degranulation of mast cells in phototoxicity is not a primary one.

Effects of Various Wavebands of Nonionizing Radiation on Langerhans Cells and Contact Hypersensitivity in the Mouse. WARWICK L. MORISON, AND CORA BUCANA, Basic Research Program-LBI, Frederick Cancer Research Facility, Frederick, MD 21701

In mice, UVB radiation suppresses contact hypersensitivity (CHS) to chemicals that are applied subsequently to unirradiated skin. UVB also decreases the number and alters the morphology of Langerhans cells (LC) at the site of irradiation as shown by ATPase staining and EM. We wished to determine whether these effects on LC are related to the UVB-induced systemic suppression of CHS and to investigate the effect of other wavebands of radiation on the development of CHS. The plucked dorsal skin of BALB/c and C3H/HeN mice was exposed to 6.9×10^6 J/m^2 UVA radiation. On day 3, mice were sensitized [trinitrochlorobenzene (TNCB) applied to abdomen] and on day 9, challenged with TNCB on the ears, which had been covered during irradiation. UVA reduced the LC at the site of irradiation to a number that was undetectable by ATPase staining or EM. However, this was not associated with a decrease in CHS; in fact, UVA treatment enhanced the CHS reaction. Subsequent exposure of the dorsal skin of these UVA-irradiated mice to UVB radiation resulted in the usual degree of suppression of CHS (64–83% reduction of ear swelling). This suggests that the morphological alterations in LC observed after irradiation are insufficient to produce systemic suppression of CHS and that such suppression can be produced in the apparent absence of LC. In contrast, exposure of mice to high doses of visible radiation had no detectable effect on the density or morphology of LC but produced a small amount (30%) of systemic suppression. Sunlight, a composite of these 3 wavebands, suppressed the CHS reaction by 55–77%. Research sponsored by the NCI, DHHS, under contract No. NO1-CO-23909 with Litton Bionetics, Inc.

The Action Spectrum for Erythema in Tetrachlorsalicylanilide-Induced Contact Photosensitivity in the Guinea Pig. COLIN A. RAMSAY AND FAWNDA PELLETT, Division of Dermatology, University of Toronto, Canada.

This study aimed to determine whether contact photosensitivity leads to a change in UVB or UVA reactivity of the skin. Fourteen female albino guinea pigs were successfully contact photosensitized with 2% tetrachlorsalicylanilide (TCSA), UVB (90 mJ/cm^2) and UVA (4.5 J/cm^2). Twelve non-sensitized animals formed the control group. Skin not previously exposed to TCSA in the photosensitized group and normal skin in the controls was employed for determination of the action spectrum for erythema. A grating monochromator was used with a 1000W Xenon-Mercury arc lamp. Skin sites were irradiated at different doses at wavelengths between 250 nm and 360 nm. The minimal erythema dose was the lowest dose required to produce erythema (MED) at each wavelength 24 hr after irradiation. In the control group the mean MED was significantly lower on the cranial half of the body than the MED on the caudal half at 302 nm (< 0.001) and 313 nm ($p < 0.05$). Contact photosensitized animals required *higher* doses of UVR than the controls to produce erythema at 250 nm ($p < 0.01$) and 280 nm ($p < 0.01$). These differences existed when tests were performed on cranial or caudal parts of the body. At all other wavelengths tested to 320 nm, there were no differences between the control and the sensitized animals. Erythema did not develop at a dose of 10 J/cm^2 at 365 nm in either group. Thus care must be exercised in the choice of body site used for UV erythema tests in the guinea pig. Contact photosensitized animals appear to be less sensitive at 250 nm and 280 nm than controls and in these photosensitized animals skin that has not been in contact with TCSA does not react to 365 nm at a dose of 10 J/cm^2.

Tetracycline Phototoxicity: Correlation with *In Vitro* Test Systems. T. HASAN, I. E. KOCHEVAR, D. J. MCAULIFFE AND B. S. COOPERMAN. Dept. of Dermatology, Harvard Med. School, Massachusetts General Hosp., Boston, MA, and Dept. of Chemistry, Univ. of Pennsylvania, Philadelphia, PA

The ability of tetracyclines to cause phototoxicity in man varies with their structure: demethylchlorotetracycline (DMCT) ≃ chlorotetracycline (CTC) > tetracycline (TC) > minocycline (MC). The mechanism for tetracycline phototoxicity is unknown. To investigate the mechanism we evaluated three *in vitro* test systems, compared the results with *in vivo* phototoxicity and made initial mechanistic observations. 1) Irradiation of the drugs (75 μM) in pH 7 PBS with 290–380 nm caused their photodestruction. Photolysis rate constants obtained from the decrease in the drug absorption maximum at 375 nm were: (DMCT) 0.42, (CTC) 0.39, (TC) 0.30, and (MC) 0.18 h^{-1}. Bovine serum albumin (2%) increased these rates 1.2- to 4-fold. 2) *In vitro* phototoxicity to lymphoid cells was assessed by irradiating with UVA (320–400 nm) using 5 doses, $0.5 \rightarrow 8 \times 10^4$ J/m^2, with and without 40 μM drug. Phototoxicity was measured as the decrease in ability to stimulate cells with PHA (as determined by ^{3}H-thymidine incorporation into DNA). The dose of radiation (J/m$^2 \times 10^{-4}$) required to reduce the response to PHA by 50% was 0.5 (DMCT); 0.5 (CTC); 4.5 (TC); >8.0 (MC). 3) Neither DMCT nor TC (up to 300 μM) initiated photohemolysis of RBC, even at UVA doses which caused >50% destruction of the drugs.

In summary, the relative rates of photodestruction of the tetracyclines and their relative phototoxicity to lymphocytes parallel their relative photosensitizing ability in man. The primary phototoxic event does not appear to involve membrane photodisruption, but may involve photoreaction of the drugs with proteins.

Further Evidence That the Photoreceptor Mediating UV-Induced Systemic Immune Suppression is Urocanic Acid. E. DE FABO, F. NOONAN, M. FISHER, J. BURNS AND H. KACSER. Dermatology Departments, George Washington Univ., Washington, DC, USA, Univ. of Vienna, Vienna, Austria, and Institute of Animal

The mechanism of systemic immune alteration by UV irradiation is critical in UV carcinogenesis and possibly also in UV-precipitated diseases. Our previous studies established an action spectrum (relative wavelength effectiveness) for UV-induced systemic suppression of contact hypersensitivity (CHS) to trinitrochlorobenzene (TNCB). We found suppression was mediated via a specific photoreceptor in mouse skin, and suggested the photoreceptor was urocanic acid (UCA), a stratum corneum component, a conclusion supported by our finding that removal of the stratum corneum by tape stripping prior to irradiation prevented suppression. The following experiments were designed to test this hypothesis further.

Mice genetically deficient in histidase (EC 4.3.1.3.) and thus in skin UCA (<10% of normal) and corresponding wild-type mice were used. Both mutant and wild-type mice gave a CHS response to TNCB. Irradiation on the shaved back with FS40 sunlamps caused more skin damage in mutant than in wild-type mice. Thus, doses of UV which gave minimal observable skin damage were used. A dose-response curve for UV-induced systemic suppression of CHS in wild-type mice was comparable to that obtained previously in BALB/c mice. No suppression of CHS, however, was found at the same UV doses in the mutant mice. These results support our original hypothesis (De Fabo and Noonan, 1980, Abstr. 8th Intl. Congr. Photobiol., P345) that skin UCA is the photoreceptor for UV-induced systemic suppression of CHS. Thus, UCA may have an important role in photocarcinogenesis.

Serological Evaluation of Polymorphous Light Eruption. HERMAN S. MOGAVERO, JR., JEFFREY D. BERNHARD, JAN E. MUHLBAUER AND WARWICK L. MORISON, Department of Dermatology Johns Hopkins and Harvard Medical Schools, Baltimore, MD and Boston, MA

Polymorphous light eruption (PMLE) is an idiopathic delayed response to sunlight. It shares a number of clinical and histological characteristics with lupus erythematous (LE). The exclusion of LE is an important consideration in the evaluation of PMLE patients. The purpose of the present study was to evaluate a group of PMLE patients for a series of serological markers that have been described in LE patients including a subset who fail to demonstrate antinuclear antibodies (ANA) on routine substrates.

Sera from 15 patients with PMLE were assayed for ANA on mouse liver, human spleen and human epithelial tumor cell line. Antibodies to native DNA and single stranded DNA were determined by indirect immunofluorescence of Crithida luciliae and a Farr radioimmunoassay respectively. Antibodies against nRNP, SM and La antigens were determined by immunodiffusion (ID) against a rabbit thymus extract (RTE). Antibodies against Ro antigen were determined by both ID and counterimmunoelectrophoresis (CIE) against a human spleen extract (HSE).

One patient had a + borderline ANA on mouse liver at a titer of 1:20;

two different patients had a borderline + ANA on HEp-2 cells at 1:80 and no patients were positive on human spleen. All patients lacked antibodies to native DNA but 4 patients had low levels anti single stranded DNA antibodies (up to 28.2% with 25% the upper limit of normal). No patients demonstrated Ro antibodies by either ID or CIE against HSE.

The data demonstrates the absence of Ro antibodies in the PMLE population. This is significant since the exclusion of the ANA −/Ro + subset of LE would be missed by routine ANA determinations. We conclude that this study serves to further support the differentiation of PMLE from LE.

The Significance of DNA Interstrand Cross-Links and Singlet Oxygen in Goeckerman Therapy. P. C. JOSHI AND M. A. PATHAK, Dept. of Dermatology, Harvard Med. School and Mass. General Hospital, Boston, MA

The therapeutic effects of crude coal tar (CCT) in the Goeckerman regimen for treatment of psoriasis are related to cytotoxic and photosensitizing action of certain agents (anthracene, fluoranthrene, pyrene, acridine, etc.) present in CCT that cause inhibition in the synthesis of DNA and cell proliferation. We present new evidence to suggest that therapeutic effects of CCT or its photoreactive ingredients are related to: 1) their ability to photoconjugate with DNA to form interstrand cross-links (ICL), and 2) the production of reactive cytotoxic species of oxygen referred to as singlet oxygen ($^1O_2$). The ability of CCT and its ingredients to produce ICL was examined by hydroxyapatite column chromatographic techniques using calf thymus DNA (A_{260} 1-1.2), CCT (1–20 μg), and irradiation with UVA (320–400 nm, 0–16 J/cm^2). The production of $^1O_2$ with CCT and its photoreactive ingredients (0.1–10 μg/ml) was examined by monitoring the bleaching of paranitrosodimethylaniline at 440 nm induced by $^1O_2$ reaction with histidine as an acceptor. Additional evidence for $^1O_2$ involvement was obtained from $^1O_2$ quenching studies with azide ions, DABCO, and by carrying out reactions under O_2, D_2O, and C_2H_5OH. The results showed that CCT produced ICL in DNA very similar to that produced by 8-methoxypsoralen except that the rate of ICL was much faster with psoralens. The rate of $^1O_2$ production was dependent on the concentration of CCT and irradiation dose. The efficiency of $^1O_2$ production was of the following order: hematoporphyrin > phenanthrene > acridine > CCT > fluoranthrene > anthracene > 8-MOP. In conclusion, CCT effects in Goeckerman regimen involve both the production of cytotoxic $^1O_2$ and ICL reaction with DNA. This inhibits the synthesis of DNA and proliferation of cells.

Ocular and Cutaneous Manifestations of Allopurinol Therapy. SIDNEY LERMAN, LYNN A. DRAKE*, AND JUDITH MEGAW, Departments of Ophthalmology and * Dermatology, Emory University, and * Veterans Administration Hospital, Atlanta, Georgia

Allopurinol is a commonly used antihyperuricemic agent in treating gout. Toxic epidermal necrolysis, particularly in patients with renal decompensation, is well documented, as is mucous membrane involvement. We have demonstrated a relationship between chronic allopurinol therapy and cataract formation. Ambient UV photosensitization may play a role in photobinding allopurinol within ocular tissues, particularly the lens (thereby becoming permanently retained there). Although allopurinol absorbs mainly in the UVC region, and only UV radiation longer than 300 nm can penetrate through the cornea, the latter wavelengths can still exert a photic effect on the lens since allopurinol does absorb slightly in this region. Energy transfer may also be involved in allopurinol photoexcitation within the lens. Eleven cataractous lenses derived from patients who had been on chronic allopurinol therapy for more than 2 years were obtained from the National Registry of Drug Induced Ocular Side Effects, Portland, Oregon. Phosphorescence spectroscopy clearly demonstrated the presence of allopurinol triplet in these lenses. (None could be found in cataracts obtained from patients of similar age who did not receive allopurinol.) Skin and blood samples on several patients who developed cutaneous reactions to this drug were also analyzed. These data demonstrate the feasibility of elucidating photochemical, ocular, and cutaneous reactions with drugs suspected of being potential photosensitizers.

SESSION B — Federal Room

David Norris, M.D., Presiding

Indium^{111}-Labelling of Peripheral Blood PMN's: A New *In Vivo* Method for the Quantitation of Polymorph Accumulation in Rabbit Skin. A. V. WAHBA AND G. S. LAZARUS, Dept. of Dermatology, Univ. of Penna., Philadelphia, Pennsylvania

Rabbit PMN's were purified from heparinized blood by dextran sedimentation, hypotonic lysis and separation on Ficol-Hypaque. The PMN's were labelled with 3–5 microcuries per 10^6 cells of In^{111}-oxime for 30 min at room temperature. The cells were spun, resuspended in PBS and reinfused into the rabbit through the ear vein. At the same time, the rabbits were anesthetized and different concentrations of different chemotactic and proinflammatory materials were injected intradermally into the back which had been clipped 4 days earlier. In some experiments, varying concentrations of acetic acid were applied topically. Four to eighteen hours later, the rabbits were sacrificed. 8 mm punch biopsies were obtained from the injection sites and counted in a gamma counter. They were then stained with hematoxylin and eosin and the number of PMN's infiltrating the dermis was quantitated in 100 randomly chosen high power fields. A significant correlation was found between the percent increase in radioactivity and the percent increase in PMN accumulation morphologically. Dose-response curves were generated using such proinflammatory materials as F-Met-Leu-Phe, lipopolysaccharide, activated serum, trypsin, glycogen and acetic acid. These curves were highly reproducible from animal to animal. Using this assay, we found that as little as one μg of trypsin induced detectable PMN accumulation. This is 2–3 logs more sensitive than injecting mice intraperitoneally with trypsin. DFP-inactivation of trypsin inhibited PMN accumulation. This new, extremely sensitive and quantitative bioassay of PMN accumulation permits evaluation of multiple agents in the same animal which decreases animal to animal variation.

13-cis-Retinoic Acid Has Major Anti-inflammatory Activity *In Vivo*. D. A. NORRIS, M. G. TONNESEN, L. A. LEE, W. R. ROBINSON, AND R. J. JOHNSTON, Departments of Dermatology and Medicine, University of Colorado and Department of Pediatrics, National Jewish Hospital, Denver, Colorado

To understand the therapeutic effect of 13-Cis-Retinoic Acid (RA) in cystic acne, it is crucial to know whether RA inhibits the mobilization and function of the leukocytes which produce tissue inflammation. We report highly significant anti-inflammatory effect of RA *in vivo* using a quantitative microhemotaxis technique which is dependent on the ability of monocytes (MONO) and neutrophils (PMN) to adhere to vessel walls, to orient and migrate, and to cross tissue barriers in response to epicutaneous complement attractants.

Seven cystic acne patients were treated with RA for 4 months, and MONO and PMN migration *in vivo* and *in vitro* were sequentially followed. 98% inhibition of PMN and MONO migration *in vivo* were seen during RA treatment:

	Pre-Treatment	2 months	4 months	2 months Post-Treatment
MONO	39 ± 5 (10^3 cells)	.8 ± .5	0	34 ± 17
PMN	250 ± 70 (10^3 cells)	6 ± 3	6 ± 3	186 ± 59

In contrast, local corticosteroids produce only 50% inhibition of MONO and PMN migration.

This RA effect was not due to decreases in PMN or MONO number, or to inhibition of attractant generation. The *in vitro* chemotactic responses during RA treatment did not reflect the degree of inhibition seen *in vivo*, suggesting that inhibition of other functions such as endothelial cell adherence or proteolytic enzyme release might contribute to the profound *in vivo* effect of RA.

We describe a reversible anti-inflammatory effect of RA which may be useful in treating neutrophil-dependent diseases.

Generation of Chemotactic Activity in Normal Serum by Treatment with Acid. ECKHARD KOWNATZKI AND SIBYLLE UHRICH, Division of Experimental Dermatology, Universitaets-Hautklinik, Frieburg, Germany.

Chemotactic factors are potent mediators of inflammatory reactions. They not only induce directed migration of cells, but also increase their adhesiveness, stimulate superoxide anion production, and contribute to increased vascular permeability. The major serum-derived chemotactic factor is the complement split product C5a, which is generated after activation of the complement cascade by either the classical or the alternative pathway. We present evidence that a chemotactic component resembling C5a was generated in normal serum at low pH. Normal guinea pig or human serum was acidified by the addition of 1 N HCl to pH 3.8 and incubated for 1 hr at 37°C. When added to the lower compartment of a Boyden chamber at a dilution of 1:20 in Tris buffered

Hanks' balanced salt solution, it attracted guinea pig peritoneal and human peripheral blood neutrophilic granulocytes to a similar extent as did yeast-activated normal serum. The appearance of the chemotactic agent was time and temperature dependent suggesting the involvement of enzymes. After heating the serum at 56°C for 1 hr no chemotactic activity could be generated by acid treatment, while EDTA, EGTA, hydrazine, and the enzyme inhibitors ϵ-amino caproic acid, PMSF, and benzamidine, present in the serum during the incubation with acid, were without effect. From a column of Sephacryl S-200 the acid-generated chemotactic component eluted with molecules around 15,000 molecular weight. It was stable when kept at low pH, but loss its activity within a day when the pH was raised to neutral. The findings suggest that chemotactic activity is generated in normal serum in an acidic environment as it may be present at sites of inflammation. (Supported by the Deutsche Forschungsgemeinschaft.)

Neutrophiles Produce an Inhibitor of Fibroblast Chemotaxis. HARTWIG MENSING AND BEATE CZARNETZKI, Univ.-Hautklinik, Hamburg, Univ.-Hautklinik, Münster, West Germany

Neutrophiles (PMN) stimulated by the ionophore A 23187 produce eosinophil chemotactic factor (ECF), a lipid generated from arachidonic acid by lipoxygenase, which consists primarily of LTB_4. In previous studies we have proven, that ECF derived from monocytes and pured LTB_4 is not only chemotactic for eosinophils but also for fibroblasts. When testing the crude supernatant derived from ionophore stimulated PMNs we found complete depression of fibroblasts chemotaxis, which was not present in supernatants of stimulated monocytes. Since ECF and LTB_4 are heat-stable, we heated the supernatant from PMNs for 30 minutes at 100° and totally restored the chemotactic response of fibroblasts. Strong chemoattractants for fibroblasts like fibronectin and conditioned medium derived from confluent monolayer cultures of fibroblasts lost their chemotactic ability when mixed with the supernatant from PMNs in dependence from its concentration. Dialysis did not exclude the inhibitor indicating a molecular weight of this factor of more than 10,000. Chemotaxis of both, human embryo fibroblasts and tumor cells were inhibited by this factor. Preincubation of both cell types did not disturb the attachment of the cells (incubation time with the inhibitor: 1 hr).

The data suggest that PMN-derived inhibitor plays an important role in regulation of fibroblasts influx at sites of inflammation.

Selective Inhibition of Neutrophil Chemotaxis by Sulfones. LIANA HARVATH, KIM B. YANCEY AND STEPHEN I. KATZ, Dermatology Branch, NCI, NIH and Division of Blood and Blood Products, National Center for Drugs and Biologics, Bethesda, Maryland

Although the sulfones, 4,4′-diaminodiphenylsulfone (dapsone) and 4,4′-diaminodiphenylsulfone disodium formaldehyde sulfoxylate (sulfoxone), are very effective therapeutic agents for dermatitis herpetiformis and for certain other neutrophilic dermatoses, their mechanism of action is unknown. In this study we determined whether neutrophil (PMN) chemotactic migration to various attractants could be inhibited by sulfones *in vitro*. Human PMNs were isolated from heparinized blood of healthy donors and chemotaxis through polycarbonate filters was assayed in a multiple microwell chemotaxis chamber. The total number of PMNs migrating to the chemoattractants, N-formyl-methionyl-leucyl-phenylalanine (FMLP), human leukocyte-derived chemotactic factor (LDCF) and human complement-derived C5a, was quantified by image analysis and density scanning. In the continuous presence of 10–100 μg/ml of dapsone or sulfoxone, PMN chemotaxis to LDCF or C5a was unaffected, but chemotaxis to FMLP was inhibited by 50–90%. Inhibition was reversed by a single washing of the cells before the assay. Dapsone and sulfoxone competitively inhibited the binding of radiolabeled FMLP to PMNs at concentrations which inhibited chemotaxis. In identical experiments with human monocytes, sulfones did not alter chemotaxis to any of the attractants. These data demonstrate that dapsone and sulfoxone interact reversibly with human PMNs and selectively inhibit their chemotactic response to FMLP. These data suggest that sulfones may selectively inhibit PMN but not monocyte migration to specific attractants generated in the skin of patients with certain neutrophilic dermatoses.

Lectin-Induced Membrane Stabilization in Human Polymorphonuclear Leukocytes: Ultrastructural Analysis, Superoxid Anion Release, Chemotaxis. CHRISTOPH SCHUBERT, HEINZ-E. SCHLAAK, AND ENNO CHRISTOPHERS, Dept. of Dermatology, HORST VÖLCKER, Dept. of Microbiology, Univ. of Kiel, W.-Germany

The effects of wheat germ agglutinin (WGA), and the complement split product C5a, as well as WGA plus C5a on isolated human polymorphonuclear leukocytes (PMN) of normal donors was explored by electron microscopy. For visualization of the WGA binding sites, ferritin labelled lectin was used. Chemotactic activity (Boyden chamber) as well as O_2^- ion release (cytochrom c reduction) were determined before the cells were subjected to electron microscopy. As a result striking ultrastructural alterations (e.g. numerous membrane protrusions and atypical invaginations) were seen in C5a treated PMN. In contrast incubation with WGA revealed an entirely smoothened cellular outline with complete lack of invaginations and protrusions. Cytoskeletal components were particularly obvious in lectin treated cells, radiating from the centriolar area. Granules of the ferritin-WGA complex were located on the cell membrane and on the luminal surfaces of intracellular vesicles. These findings provide evidence that the plasma membrane is rapidly internalized and possibly processed in the Golgi area. Following incubation of ferritin labelled WGA plus C5a, the PMN show a similar ultrastructure as cells incubated with the lectin alone. C5a treated PMN showed greatly increased release of O_2^- ions. When the lectin was used in addition to C5a, O_2^- ion release was still increased, whereas chemotactic migration was absent. These findings provide evidence, that the PMN plasma membrane is rapidly stabilized by WGA. This excludes cellular migration, whereas the capacity to respond to the chemotactic stimulator C5a in releasing O_2^- ions is fully retained.

Specific Non-Responsiveness of Polymorphonuclear Leukocytes Against the Complement Split Product C5a. JENS-M. SCHRÖDER AND ENNO CHRISTOPHERS, Department of Dermatology, Univ. of Kiel, W.-Germany

In patients with various forms of psoriasis (n = 72) we noted an increased chemotactic responsiveness of polymorphonuclear neutrophil leucocytes (PMN) against various chemotaxins with the exception of three patients with severe psoriasis pustulosa. These demonstrated a complete absence of chemotactic response against the partially purified complement split product C5a. No alteration of this cell function was seen using the synthetic chemotactic peptide FMetLeuPhe in addition to leukotrien B_4 or casein as chemoattractants. Not only chemotactic migration (Boyden chamber assay) but also specific degranulation (β-glucuronidase-assay) and superoxide-release (cytochrom c-reduction-assay) were absent when various concentrations of C5a were used as stimulus. In addition to psoriasis, this specific defect was noted in patients with severe bacterial infections of the skin such as erysipelas (n = 8/12), disseminated furunulosis (n = 6/13) and acne conglobata (n = 4/9). This phenomenon was seen to persist for several days and correlated with the acuity of infection. Responsiveness of PMN against C5a was fully restored thereafter. This transient C5a defect was never seen in healthy controls (n = 38). Incubation of patients PMN with fresh serum restored the capacity of C5a-non-responsive neutrophils. Furthermore, when patients sera were assayed in Boyden chambers no increased chemotactic activity was seen and also normal PMN did not become non-responsive against C5a by incubation with patients sera. These observations demonstrate a C5a specific defect present in circulating neutrophils. It is predominantly linked with infectious diseases and differs from C5a mediated neutrophil deactivation or known examples of DMN down regulation.

***In Vivo* Studies of Human C5a As a Mediator of Cutaneous Inflammation in Man.** K. B. YANCEY, C. H. HAMMER, L. HARVATH, L. RENFER, M. M. FRANK AND T. J. LAWLEY, Dermatology Branch, NCI, NIAID, NIH, Bethesda, MD

C5a is an 11,000 dalton fragment of the fifth component of complement (C5) with anaphylatoxic and leukocyte chemotactic activities. In this study we examined the effects of intradermal injections of human C5a in normal volunteers. C5a was prepared by interacting highly purified human C5 with zymosan bound alternative pathway C5 convertase. Under conditions resulting in consumption of 90% of the C5 substrate, the C5a preparation was chemotactic *in vitro* for human monocytes and polymorphonuclear leukocytes (PMN's) at concentrations as low as 10^{-8} and 10^{-9} M respectively This C5a preparation also caused human PMN's to aggregate and to release myeloperoxidase and lysozyme *in vitro*. To examine *in vivo* reactivity of human C5a, this reagent was tested intradermally in 5 normal volunteers. C5a produced immediate wheal and flare reactions with doses as low as 1–2 ng. Twenty minutes after injection of 50 ng, wheals averaged 12 mm and flares averaged 48 mm. Skin reactions demonstrated dose response effects between 1 and 300 ng. Reactions lasted 30–60 minutes and were accompanied by marked pruritus in some subjects. No late phase reactions were seen. There was no tachyphylaxis to repeated C5a injections at 1, 4, or 24 hr. Differential counts on 2 μm plastic sections of skin biopsies 30 minutes after injection revealed perivascular accumulations of PMN's (63%), mononuclear cells (33%), and eosinophils

(4%). Mast cell degranulation was observed on both light and electron microscopy. Wheal and flare were reduced by pretreatment with an H1 blocker but cellular infiltrates were unaffected. All reactivity was abolished by immunoabsorption of the reagent with anti-human C5. This study provides the first comprehensive assessment of the effect of human C5a on human skin.

Chemotactic Factor-Stimulated Neutrophil Adherence to Endothelium—A Neutrophil Effect. MARCIA G. TONNESEN, LEE SMEDLY, AND PETER M. HENSON, Dept. of Dermatology, Univ. of Colorado Sch. of Medicine; Dept. of Pediatrics, Nat'l. Jewish Hosp. and Research Ctr./Nat'l. Asthma Ctr., Denver, CO

Interaction of the polymorphonuclear leukocyte (PMN) with the vascular endothelial cell (EC) is the initial event in the influx of phagocytes to sites of inflammation in the skin. The mechanisms by which leukocytes adhere to and migrate through the endothelium are not understood. *In vivo* and *in vitro* evidence implicates chemotactic factors as participants in neutrophil adherence and transmigration. To investigate possible mechanisms by which chemotactic factors enhance the PMN-EC adhesive interaction, we have developed a sensitive, reproducible, human *in vitro* adherence assay using isolated ^{51}Cr labelled human PMN added to monolayers of human umbilical vein EC in microtiter tissue culture wells. Mean percent adherence of PMN to EC monolayers was 35.0 ± 1.1% (±SEM) in 63 assays with different donor neutrophils. PMN exhibited preferential adherence for human EC compared to tissue culture plastic (30.2 ± 1.0%), human fibroblasts (26.1 ± 2.1%), and human smooth muscle cells (25.8 ± 1.5%). PMN adherence to human EC could be increased from 35 to 57% by the addition of either fMetLeuPhe (FMLP) or partially purified C5 fragments (C5fr). Both FMLP and C5fr increased PMN adherence in a dose response manner. We propose that chemotactic factors act primarily on the PMN, based on 3 lines of evidence: 1) when stimulated, neutrophils lost their preferential interaction with human EC and adhered equally well to all cell types and to plastic; 2) FMLP-stimulated PMN adherence to plastic was essentially identical to stimulated adherence to EC over the entire dose response rate; 3) pretreatment of EC with chemotactic factors failed to increase subsequent neutrophil adherence. Chemotactic factors derived from tissue sites appear to act directly on the neutrophil in the circulation to induce enhanced interaction with the endothelium and thereby may contribute to cell localization at sites of cutaneous inflammation.

Lithium Increases Neutrophil Myeloperoxidase-Mediated Iodination Activity. JOHN A. KAZMIEROWSKI, DAVID S. PEIZNER, AND KIRK D. WUEPPER, Department of Dermatology, The Oregon Health Sciences University, Portland, Oregon

The use of lithium carbonate has been associated with worsening of several cutaneous disorders, including acne and psoriasis. Because the histopathology of each of these disorders is characterized by inflammatory infiltrates of polymorphonuclear leukocytes (PMN) primarily, we investigated whether lithium affects the myeloperoxidase (MPO)-H_2O_2-halide system of intact PMN by using a $Na^{125}I$ iodinization assay. Iodination activity of the PMN MPO-H_2O_2-halide system was measured by the method of Klebanoff and Clark using normal PMN activated by (a) opsonized zymosan or (b) phorbol myristate acetate (PMA) in the presence of $Na^{125}I$, and bovine serum albumin (BSA). In concentrations comparable to normal therapeutic levels (0.5–1.0 mEq/l), lithium caused a significant increase in iodination activity as compared with control values. This increase was dose-dependent.

In a cell-free system using purified canine MPO, H_2O_2 (generated by glucose oxidase and glucose), $Na^{125}I$, and BSA, lithium in comparable concentrations had no effect on iodination activity, suggesting that lithium did not interact with one of the components of this system.

Quantification of MPO in stimulated cells or in the incubation medium showed that most of the increased activity of the lithium-stimulated system occurred in the extracellular fraction. Moreover, extracellular beta-glucuronidase was also increased in a dose-dependent manner from PMN incubated with lithium. The findings establish that increased extracellular release of MPO and beta glucuronidase from PMN is a consequence of lithium exposure.

Enhanced Release of Lysosomal Enzymes from Lithium-Stimulated Neutrophils in Psoriasis. MARJORIE M. YOUNG AND F. J. BLOOMFIELD. Department of Dermatology, The Adelaide Hospital, Dublin, Ireland

We have previously shown that lithium salts cause degranulation of neutrophils *in vitro*. In this study, we compared degranulation of neutrophils from 30 patients with chronic, relapsing psoriasis who were not receiving intensive treatment to 30 healthy controls. Lithium was added to whole blood immediately after venepuncture for 30 minutes at 37°C after which neutrophils were isolated using a Hypaque-Ficoll gradient and enzymes assayed by fluorometric methods. B-glucoronidase was used as a marker for primary granules and Vitamin B12 binding protein as a marker for secondary granules. The results clearly showed increased degranulation from primary and secondary granules in psoriatic neutrophils compared to controls ($P = <0.001$). As the basal levels of enzymes in both groups did not differ, the results indicate enhanced release of lysosomal enzymes. However, measurement of other enzymes associated with neutrophil function, myelopexoxidase and catalase were significantly raised in the psoriatic group ($P = <0.001$). This study shows that the psoriatic neutrophils have a greater capacity to release inflammatory mediators and thereby contribute towards epidermal hyperproliferation. Moreover, this system of studying degranulation of whole blood *in vitro* allows assessment in conditions closely similar to *in vivo* and we propose that the well documented reports of lithium salts exacerbating psoriasis can be explained in part by enhanced release of lysosomal enzymes. We are currently investigating if increased degranulation in psoriasis is due to an intrinsic property of psoriatic neutrophils or due to interaction with a serum factor.

SESSION C — South American Room
Marvin Karasek, Ph.D., Presiding

Grafting of Skin Ulcers with Cultured Autologous Epidermal Cells. J. M. HEFTON, M. WEKSLER, A. PARRIS, D. CALDWELL, A. K. BALIN, AND D. M. CARTER, Cornell Medical College and The Rockefeller University, New York, N.Y.

We are investigating whether cultured human epidermal cells used as grafts accelerate wound-healing in patients with chronic skin ulcers. Epidermal cells were cultivated from split-thickness shave biopsies (9 cm^2) of normal skin from three patients with chronic skin ulcers (15–25 cm^2) of scalp and legs. Epidermis from each specimen was separated from the dermis (37°, 0.5% trypsin). Single-cell suspensions of epidermal cells (2×10^6) were inoculated into 25 cm^2 flasks (MEM, p 7, 20% fetal calf serum) and incubated (37°, 5% CO_2 in air). Stratified sheets developed within 14 to 21 days.

Sheets were washed; removed from culture vessels by adherence to vaseline-impregnated gauze (VIG); and grafted autologously onto clean, granulating ulcers that had previously been debrided and treated with antiseptics. Wounds were covered with water-impermeable, gas-permeable membranes. Cells attached to granulation tissue in 48 to 72 hr; and the VIG was removed. Grafted ulcers were 50% re-epithelialized by 7 days and 75% by 14 days, when epidermis did stain for keratin with vasoflavin. Grafted ulcers were completely re-epithelialized in 21 to 28 days. Control ulcers on the contralateral limb received conventional treatment but no cultured cells. These ulcers were not re-epithelialized after 14 days.

Autografts of cultured epidermal cells thus accelerate the rate of healing of full-thickness skin ulcerations when compared to standard treatments. The strength of such healed wounds is now under study.

Contribution of Specific Medium Components to the Growth and Serial Cultivation of Human Epidermal Melanocytes *In Vitro*. B. A. GILCHREST, I. M. WILKINS, M. A. VRABEL, R. A. WEINSTEIN, AND T. MACIAG, Depts. of Dermatology and Pathology, Beth Israel Hospital, Harvard Medical School, Boston, MA

Requirements for melanocyte growth and serial cultivation were studied by plating disaggregated single cell suspensions of newborn foreskin epidermis on uncoated plastic dishes in medium M199 containing insulin, transferrin, hydrocortisone, epidermal growth factor, nerve growth factor, triiodothyronine, cholera toxin (CT), 2% fetal bovine serum (FBS) and a bovine hypothalamic extract (BHE) dialyzed against a 8000 m.w. sieve to remove its growth promoting activity for keratinocytes. Within 2 weeks, cultures consisted exclusively of melanocytes by phase microscopy and dopa-staining. Melanocytes were subcultured at 4 weeks and grew progressively for up to 12 weeks, 4 passages and 16 post-primary cumulative population doublings, with doubling times of 4–6 days at early passage and 8–12 days at late passage. In the absence of BHE, no significant growth occurred despite FBS supplements to 20%. Removal of either FBS or CT reduced growth ~50% over 10–14 days; removal of other medium constituents individually had no detectable effect. Melanocyte growth was enhanced by conditioned medium (CM) from cultures of either other epidermal melanocytes or human melanoma: 2× in cultures with 20% melanocyte CM and >5× in cultures with 20% melanoma CM. FBS concentration

>2% increased cell yield in all cultures, but led to fibroblast overgrowth at late passage. These data establish that growth-promoting activity for human melanocytes is present in serum, in hypothalamic extracts, and in medium conditioned by either normal or malignant melanocytes. These mitogens in appropriate nutrient medium are sufficient to maintain newborn melanocytes in a proliferative state for up to 3 months *in vitro*.

Effects of Mitotic Arrest on Metabolism of Damaged DNA in Cultured Skin Cells. P. M. Ross, S. I. Wayne, and D. M. Carter, Lab. for Invest. Dermatol., The Rockefeller University, New York, N.Y.

We have studied the effects of the mitotic inhibitor colchicine on the response of cultured normal human fibroblasts to DNA damage produced by X-rays or psoralen plus UVA.

Treatment with 4,5′,8-trimethylpsoralen plus UVA caused formation of crosslinks, detected by increased sedimentation velocity of the cellular DNA in alkali. In control cells the crosslinked DNA was incised and regained normal sedimentation velocity within 24 hr post-irradiation. Colchicine-blocked cells did not incise DNA containing crosslinks within 72 hr additional incubation. Removal of the colchicine at 24 or 48 hr after psoralen treatment permitted the cells to incise the crosslinked DNA.

In an alternative approach, cells treated with colchicine for 2–4 hr were fractionated into mitotic (10%) and nonmitotic (90%) populations by brief trypsinization on ice. These cells and also untreated control cells were exposed to X-rays at 3.60 krads/min for 0–12 min. X-irradiation produced strand breaks and alkali-labile sites in the cellular DNA, lowering sedimentation velocity of cellular DNA in alkaline sucrose. The increase in sedimentation velocity due to post-irradiation incubation was greater for the control and the nonmitotic cells than for the mitotic cells (1.83, 1.8, and 1.2, respectively). The colchicine treatment was not responsible for the effect, because the nonmitotic cells held in colchicine repaired the damages as well as did the untreated cells.

The results indicate that cells blocked in mitosis are less able to execute normal patterns of chromosomal DNA metabolism than are cycling cells.

Oxygen Toxicity Contributes to the Seeding Density Dependence for Growth of Normal Human Fibroblasts. A. K. Balin and D. M. Carter, The Rockefeller University, New York, N.Y.

We have examined the growth of human diploid fibroblasts (WI38 and IMR90 cells) as a function of initial seeding density and oxygen tension. Cells at young and mid-passage levels were subcultivated in DMEM with 10% fetal bovine serum at 0.01, 0.03, 0.1, 1 and 2×10^4 cells/cm^2. Flasks were equilibrated before and after seeding with one of ten gas mixtures containing the desired oxygen tension (9-591 mm Hg) and placed in incubators which measure and maintain a present oxygen tension. Partial pressure of oxygen (PO_2) in media of all flasks was determined at harvest. Cells were shielded from light of wavelength less than 500 nm.

Our results showed that growth varied inversely with oxygen tension and seeding density. For example, at 10^2 cells/cm^2, population doubling time (PDT) decreased from 30 hr (PO_2s 9 and 15 mm Hg); to 45 hr (PO_2 40); 60 hr (PO_2 72) and 150 hr (PO_2 140). At 10^3 cells/cm^2, PDT decreased from 20 hr (PO_2 15); to 25 hr (PO_2 32); 30 hr (PO_2 67); 70 hr (PO_2 140); 110 hr (PO_2 195); and 250 hr (PO_2 280). At greater seeding density (10^4 cells/cm^2) differences in growth below 140 mm Hg were minimized, but oxygen-induced inhibition of cell growth was evident at PO_2s greater than 140 mm Hg, as we previously reported.

Thus, oxygen modulates cell growth under physiologic partial pressures; and oxygen toxicity contributes to the seeding density dependence, commonly seen in cultures of fibroblasts.

Epibolin is the Component in Human Serum Supporting the *In Vitro* Spreading of a Human Squamous Carcinoma Cell Line. Kurt S. Stenn and Hansruedi Federer, Department of Dermatology, Yale Univ. School of Medicine, New Haven, CT and Abt. Krebsforsch, Inst. Path., Univ. Zurich, Zurich, Switzerland

A glycoprotein, collected from human serum, which supports the motility of epidermal cells and named epibolin, has been found to be a single-chained α_2 globulin with a molecular weight of 60–65 Kdal.

Important to the malignancy of cancer cells is their movement into normal tissues. In this study the role of serum in the spreading of one cancer cell line was studied. Using various different protein-containing media and the dissociated human squamous carcinoma cell line (L1CR Lond HN-1) *in vitro* cell spreading was studied. The percent of spread cells on plastic tissue culture substratum was measured microscopically. In defined medium without serum or in the presence of serum albumin cancer cell spreading was minimal. In the presence of whole serum or purified epibolin maximal cell spreading occurred. Antibody to epibolin blocked the spreading activity of whole serum.

The results suggest that these cancer cells have the same serum dependence for spreading as normal epidermal cells and that the important serum component for the spreading of these cancer cells is epibolin.

Induction of Epidermal Transglutaminase (ETG) Activity by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) in Cultured Mouse Keratinocytes. D. C. Ertl and S. M. Puhvel, Division of Dermatology, UCLA School of Medicine, Los Angeles, California

Topical application of TCDD induces hyperkeratosis and squamous metaplasia in hairless mouse epidermis (Puhvel et al., Toxicol Appl Pharmacol 64:492, 1982). This is accompanied by elevated levels of ETG (unpublished observations), the enzyme thought to be the critical regulator of the terminal step of epidermal differentiation. In the present study we have examined the effect of TCDD on ETG activity in neonatal mouse keratinocytes *in vitro*.

Epidermal cells were isolated from newborn mouse skin as previously described (Marcelo et al., J Cell Biol 79:356, 1978), and cultured in low calcium Medium 199 (Ca^{++} 0.07 mM), supplemented by fetal bovine serum. After growth was established (48 hr) and at every medium change thereafter, 10^{-9}M TCDD dissolved in DMSO was added to half the cultures. Other cultures received DMSO alone. Cells were harvested at 48 hr intervals up to the 12th day and the cell pellets frozen. ETG activity was determined in lysates of frozen cells using ^{3}H-putrescine as described by Yuspa et al. (N.Y. Acad Sci 349:251, 1981).

In the presence of TCDD in low Ca^{++} medium, keratinocytes failed to differentiate and continued to grow as basal cells in monolayers. Quantitation of fully cornified envelopes in cultures verified these morphologic observations. However, the ETG activity was elevated to 300% the activity of cells grown in low Ca^{++} without TCDD.

These results indicate that TCDD induces ETG in keratinocyte cultures.

A Chemically Defined Medium for the Growth of Adult Human Keratinocytes. Yannick Jacques, Michelle Grangeret, Catherine Dalbiez, and Uwe Reichert, Dept. of Cell Biology, C.I.R.D., Sophia Antipolis, 06565 Valbonne, France

Chemically defined conditions are of crucial importance when dealing with cell pharmacology. Such conditions have been established for the culture of neonatal human keratinocytes but not for keratinocytes from adult skin. We report here the use of a defined medium for the growth and passage of adult human keratinocytes.

Freshly trypsinized cells were seeded at a density of 30,000 cells/cm^2 on collagen coated dishes in HEPES buffered Dulbecco's MEM/Ham's F12 (1:1) medium containing 10% FCS*. On day 4, the cells were rinsed and refed with the DMEM/F12 medium containing or not the following factors: insulin 10 μg/ml, transferrin 10 μg/ml, selenium ions 10 ng/ml, EGF 50 ng/ml, hydrocortisone 0.4 μg/ml. Media were renewed every 2 days. Mitotic activity, DNA and protein synthesis were evaluated 3 days after switching the cells from the serum containing medium:

	M.I.	DNA	Protein	^{3}H-Hist incorp^n
		(μg/dish)	*(μg/dish)*	*(dpm/dish × 10^{-3})*
Dead cells (air dried)	0.0	6.1 ± 0.9	238 ± 22	27 ± 9
Spent medium (10% FCS)	3.3	6.7 ± 0.4	329 ± 30	78 ± 11
Fresh medium (no FCS/facts)	1.1	6.6 ± 0.4	256 ± 23	55 ± 4
Fresh medium (10% FCS)	7.2	7.6 ± 0.7	343 ± 11	98 ± 8
Fresh medium (5 factors)	17.9	9.2 ± 0.3	386 ± 13	218 ± 9

Cells in DMEM/F12 stopped growing and differentiated within 10 days. On the contrary, cells in the defined medium could be grown for more than 3 weeks with obvious signs of mitotic activity and they could be successfully subcultured in the same medium. We are currently investigating which factors are responsible for the mitogenic effect observed.

* FCS = Fetal Calf Serum

Human Keratinocytes in Culture Use Amino Acids As the Major Source of Metabolic Energy. Uwe Reichert, Bernd Schaarschmidt, Yannick Jacques, and Joseph Gazith, Dept. of Cell Biology, C.I.R.D., Sophia Antipolis, 06565 Valbonne, France

Passaged human keratinocytes from infant and adult skin were grown

to a density of about 50,000 cells/cm^2 on collagen coated surfaces in HEPES buffered Dulbecco's MEM containing 5% or 10% fetal calf serum. Using Warburg manometry, an exchange of (0.50 ± 0.03 S.E.M.) pmol O_2 against (0.47 ± 0.03) pmol CO_2 per cell and hour has been measured over the first 48 hr. The respiratory quotient was RQ = 0.94 ± 0.06. Enzymatic lactate determination revealed the production of (0.86 ± 0.09) pmol lactate/cell/hr during that time.

In order to gain more information on the substrates oxidized, D-glucose, L-glutamine and palmitic acid in the medium have been separately traced by the addition of the corresponding (U)^{14}C-labeled compounds using multiwell cultures. The ^{14}C-CO_2 evolved was trapped on glass fiber filters which were glued on the cover above each well and soaked with 2 N KOH. The results of a kinetic study show that about 45% of the CO_2 produced is originated from glutamine (which contributes 37 mol-% to the free amino acids in the medium), 8.5% from glucose, and 2% from palmitic acid. After 48 hr, 16% of the initial amount of glutamine (C_0 = 3.9 ± 0.4 mM) have been oxidized but only 2% of glucose (C_0 = 5.4 ± 0.5 mM) which is mainly found converted to lactic acid. Experiments with isoproterenol and cholera toxin provided no evidence for qualitative or quantitative changes in the glycolytic or respiratory pathways due to compounds elevating the intracellular level of cAMP.

We conclude that human keratinocytes in culture use amino acids as the main substrate for respiration, whereas glucose is predominantly glycolised to lactic acid.

Plasminogen Activator in Cultured Human Epidermal Cells. K. Hashimoto, K. Singer, S. Morioka, and G. Lazarus, Department of Dermatology, Osaka Univ. Hosp., Osaka, Japan; Department of Medicine, Division of Dermatology, Duke University Medical Center, Durham, NC; and Department of Dermatology, University of Pennsylvania, Philadelphia, PA

Plasminogen activator (PA) is synthesized in a highly regulated fashion by a wide variety of cell types. In addition to its role in the fibrinolytic cascade, PA is important in the regulation of extracellular proteolysis and plays a significant role in tissue remodeling and cell migration. Although PA has been suggested to be involved in epidermal differentiation and recent reports have correlated PA with disease activity in psoriasis and suggested a role for PA in the pathophysiology of pemphigus, little is known about PA metabolism in human epidermis or cultured human epidermal cells (HEC). We report that primary cultures of HEC produce PA as demonstrated by the ability of conditioned medium or cell lysates to hydrolyze fibrin in the presence of plasminogen, and to cleave ^{125}I-plasminogen to characteristic fragments. The major molecular species of PA in HEC was inhibited by DFP and comigrated in SDS-PAGE with the high m.w. band of human urokinase (M_r 55,000). Production of PA by HEC was inhibited >80% by 10 μg/ml cycloheximide, stimulated >4-fold by 10^{-6} M colchicine, and not affected by cytochalasin B or the tumor promoter TPA. Both cholera toxin and epidermal growth factor stimulated PA activity in HEC at concentrations which best support *in vitro* growth of HEC. These data characterise characterize PA metabolism in HEC and provide a framework for the study of PA as a regulatory molecule in human epidermis.

Biphasic Entry of Glucocorticoids into Cultured Human Skin Keratinocytes and Fibroblasts. Johanna A. Kempenaar and M. Ponec, Department of Dermatology, University Hospital, Leiden, The Netherlands

The uptake of various glucocorticoids (glc's) by confluent cultures is a rapid temperature-sensitive process. All glc's tested accumulate in the cells, since the ratio between the intracellular and extracellular concentration is higher than 1.

The nature of glc uptake by the cells is most probably a simple passive diffusion, since the amount of glc taken up by the cells increases linearly with increasing extracellular glc concentration. Furthermore, no inhibition of the uptake is observed in the presence of an excess of the same or another glc or in countertransport experiments by preincubation of the cells with the same or another glc.

A good correlation is found between the amount of glc taken up by the cells and glc lipophilicity (as a measure of lipophilicity partition coefficient between octanol and water was taken). Decreasing cellular volume (up to 20%) affects little the total amount of glc associated with the cells, what suggests that a large portion of glc is attached to the cell membrane and the glc uptake is probably driven by distribution of glc between lipid-rich phase and water. The fraction of glc attached to the membrane can easily be released by repeated washing of cells with steroid-free medium. The fraction which stays associated with the cells is very small when preloading of cells with glc was short and became significantly larger with prolongation of the preloading period. Since the glc uptake is not significantly affected by pretreating the cells with various enzymes, no strong interaction of glc with the membrane components might be expected. Results suggest that the uptake of glc is a biphasic process in which a rapid, non-specific, high-capacity association to the cell membrane is followed by a slower internalization process.

Factor XIII Inhibits Epidermal Cell Migration *In Vitro*. T. Hashimoto and R. Marks, Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN

Factor XIII (fibrin stabilizing factor) is an essential component of the clotting cascade that ultimately results in a stable thrombus composed of polymerized fibrin. It is normally present in the blood as a profactor which when converted into the active molecule is responsible for the polymerization of monomeric fibrin to the stable polymeric form by the formation of cross links. It is used in the congenital deficiency state in which there is a bleeding tendency and to promote wound strength after surgery. Factor XIII has other actions including the crosslinking of fibronectin to itself and to fibrin and the stimulation of fibroblastic activity. Because of the complex interactions between clot formation and re-epithelialisation this study is aimed at characterizing the effect of Factor XIII on active epidermal cell movement *in vitro*. Human skin explants obtained from skin sheets removed by keratotome from normal healthy volunteer subjects were incubated for 48 hr (free floating) or 7 days (attached to bottom of Petri dishes) in Eagles MEM either unsupplemented or supplemented with either foetal calf serum or human fibrinogen. Factor XIII was added to the culture dishes in each of the following concentrations: 0.0, 0.01, 0.05, 0.1, 0.5, 1.0, 5, 10 and 50 iμ/ml. In some experiments calcium was also added to the medium. After incubation the explants were assessed for the degree of epidermal cell migration that had taken place from the cut edges. The assessments were either made histologically or on whole explants planimetrically. The results (Table) indicate that Factor XIII caused inhibition of epidermal cell migration. This effect appeared to be dose dependent but was not calcium or fibrinogen dependent. These findings indicate that Factor XIII has a direct effect on epidermal cell migration unrelated to any action on fibrin.

SESSION D — Pan-American Room
Thomas Krieg, M.D., Presiding

The Collagen Lattice: A Physiological Model for Studying Fibroblasts. Bernard Coulomb°, Louis Dubertret°, Charlotte Merrill', René Touraine°, and Eugene Bell', Hôp. H. Mondor, Créteil, France°; M.I.T., Cambridge, U.S.A.'

Human fibroblasts grown as a monolayer or in a tissue equivalent model were compared with respect to endogenous peroxidase activity, membrane permeability and collagenolytic activity. Peroxidase activity was also assayed in fibroblasts of fresh human skin biopsies.

Incubation of cells and tissues in Diaminobenzidine (DAB) prior to fixation allowed to detect a peroxidase activity associated with the perinuclear envelope in dermal fibroblasts "*in vivo*', in fibroblasts in collagen lattices, i.e., the tissue equivalent model, but not in fibroblasts cultivated in monolayer. Moreover on the plastic substrate, monolayered fibroblasts are impermeable to DAB, as shown by the absence of staining of mitochondrial cytochrome c oxidase, suggesting a key difference in the permeability properties of monolayered cells as compared with cells in the model or "*in vivo*". However DAB was found to enter cells after they were treated with trypsin, while "*in vivo*" or in collagen lattices no trypsinization was required for entry of the reagent.

Cells in collagen lattices and monolayered cells differ also in the expression of a collagenolytic activity as detected by indirect measurements of collagen degradation and by measurements of dialysable labelled hydroxy-proline after labelling with ^{3}H proline.

These comparisons between fibroblasts in monolayer, in the collagen lattices and "*in vivo*" suggest that the lattice model will be a useful system for studying dermal fibroblasts "*in vitro*" since cells in the model appear to exist in a state more like that of cells "*in vivo*".

Collagen $_{\alpha 1}$(I)-like Gene Defects in Ehlers-Danlos Syndrome Type II and Severe Lethal Osteogenesis Imperfecta. F. M. Pope, Elizabeth Weiss*, and A. C. Nicholls, Dermatology Research Group, MRC Clinical Research Centre, Harrow, Middlesex, U.K. and NIMR, Mill Hill, London

A number of inherited disorders of connective tissue are now recognised as caused by abnormalities of collagen. Diseases such as Ehlers-

Danlos Syndrome, Osteogenesis Imperfecta and the Marfan Syndrome have been associated with biochemical abnormalities which include chain deletions, amino acid substitutions and changes in the enzyme regulating various post translational modifications of procollagens.

Using a genomic probe to a Cosmid packaged $_{\alpha 1}$(I)-like gene containing at least 37Kb of the whole collagen gene, we have identified abnormalities in the 3′ and fragment of Eco R1 and Bam HI digests of DNA from a father and son with typical EDS II and at least one patient with lethal OI Congenita. These findings imply abnormalities within the procollagen C terminal extension peptide and should allow accurate genetic counselling and possible intrauterine diagnosis of these two diseases within these particular families.

Elastofibroma Dorsi: Biochemical Characterization of Connective Tissue Components and Morphometric Analyses of the Elastic Fibers. JOUNI UITTO, P. A. ABRAHAM, DANIEL J. SANTA CRUZ, FRANK HIROSE, J. L. PAUL AND R. H. PEARCE, Harbor-UCLA Med. Ctr., Torrance, CA, Washington Univ. Sch. of Med., St. Louis, MO, and Univ. of British Columbia, Vancouver, BC.

Nine cases of elastofibroma dorsi (EFD) were studied. Histologically, the lesions demonstrated abundant connective tissue consisting predominantly of collagen. Embedded in the collageneous matrix were characteristic globular structures which could be visualized by elastin-specific stains. The area fraction (AF) occupied by the elastic structures was determined by computerized morphometric analyses. Systemic analyses of the entire sections, using computer-selected, non-overlapping fields indicated an AF of 0.096 ± 0.046 (mean ± S.D.) for EFD. When the same sections were analyzed by using operator-selected fields to be representative of histopathologic changes in EFD, the corresponding AF was 0.172 ± 0.090; this value is significantly different from AF of the elastic fibers in control dermis, the latter value being 0.070 ± 0.022 (mean ± S.D.; n = 10; p ~ 0.02). Two cases were analyzed by biochemical means. Assay of 4-hydroxyproline indicated that these tumors contained 48.7 ± 8.5 and 70.1 ± 4.6 mg collagen per 100 mg dry weight. The collagen proved to be exclusively of type I, as determined by SDS-polyacrylamide gel electrophoresis and amino acid analyses. Elastic fibers, isolated by solubilization of non-elastic structures, comprised 5.0 ± 0.4 and 1.1 ± 0.1 mg/100 mg of tissue. Further extraction with 0.1 M NaOH at 99°C indicated that insoluble elastin consisted of 0.81 and 0.23 mg/100 mg of tissue. Thus, EFD is a connective tissue tumor predominantly containing type I collagen. A small percentage of the total tissue is occupied by unusual elastic structures which are the histopathologic hallmark of EFD.

Collagen Deposition in Skin Wound Healing. LEONARDO CELLENO°, FERDINANDO SERRI°, WILLIAM MONTAGNA°°, AND PETER BENTLEY°°°, Dept. of Dermatology, Catholic Univ. Rome°; Dept. of Dermatology°°, Dept. of Biochemistry°°°, Oregon Health Sciences University, Portland

Wound healing has been used as a model to investigate the synthesis and deposition of new collagen in cutaneous repair. Linear incisions through the dermis to the adipose tissue were made on the back skin of 3 pigtailed macaques. Biopsy specimens were removed 36 hr, 4, 7, 10, 12, 17, days later and processed for EM, histochemistry and immunofluorescence (IF).

At 36 hr we found active fibroblasts in the deep dermis with a well developed RER and an enlarged Golgi complex in which fine parallel filaments, packed together and crossed by a number of periodical vertical bands (every 2800A°) were present. IF performed at the same time with antisera against Type I and III collagen was positive only for production of Type III collagen. In the subsequent specimens more active fibroblasts with the EM aspects described above were visible. IF at 4 days and afterwards was positive only for production of Type I collagen. The leucine aminopeptidase reaction indicated that the activity and presence of the fibroblasts in wound reached the maximum at 12 days.

In conclusion it appears that collagen production after skin injuries starts earlier than believed so far. The first collagen synthesized is Type III, which as has been found in culture, is catabolized early and then replaced by Type I. The filaments within the Golgi vacuoles are in our belief procollagen molecules, as shown by others with ImmunoEM.

Occlusive Dressings Influence Dermal Collagen Synthesis and Epidermal Resurfacing in Superficial Wounds. O. M. ALVAREZ, K. D. LEVENDORF, P. M. MERTZ, AND W. H. EAGLSTEIN, Department of Dermatology, University of Pittsburgh School of Medicine, Pittsburgh, PA. Wound Healing Research Laboratories, Department of Surgery and Medicine (Dermatology), Cornell University Medical College, New York, N.Y.

The effects of an occlusive, O_2-permeable polyurethane film (PUF), an occlusive, O_2-impermeable hydrocolloid dressing (HCD), and wet to dry gauze dressings (WDG) on dermal repair and epidermal resurfacing of partial-thickness wounds were studied in swine. Keratome-induced wounds were covered with either PUF, HCD, WDG or left uncovered (air exposed). Wounds were excised on days 1–7 after wounding and the epidermis was trypsinized away from the dermis. The epidermal sheet was visually evaluated for re-epithelialization and analyzed for keratin production by SDS-PAGE. The dermis was assayed for collagen biosynthesis by measuring isotope incorporation into collagenase sensitive protein and collagen types I and III by gel filtration. Relative collagen synthesis was significantly greater ($p < 0.05$) in the wounded dermis of both PUF and HCD treated wounds when compared to air exposed and WDG-treated wounds. Collagen synthesis was similar in wounds treated with either the O_2-permeable (PUF) or O_2-impermeable (HCD) dressings. The relative proportion of collagen types I and III remained unaltered as a result of the treatments. Both HCD and PUF significantly accelerated re-epithelialization by 36% and 21% respectively. WDG dressings delayed resurfacing by 5%. These dressings did not alter the proportion of epidermal keratin components. These results suggest that 1) atmospheric O_2 is of little importance in the healing of normal superficial wounds and 2) occlusive dressings alter the rate of collagen production during wound repair.

Inhibition of Collagen Biosynthesis in Cultures of Scleroderma Skin Fibroblasts by a Purified Fraction of Structural Glycoprotein Extracted from Normal Rabbit Dermis. +FRANÇOIS X. MAQUART, FRANCE HUI SZE KWONG, ALAIN RANDOUX, JACQUES P. BOREL, ++BERNARD KALIS, RAOUL TRILLER, AND HÉLÈNE RICHARD-DELHOMME. + Laboratoire de Biochimie, ERA CNRS n° 959, Faculté de Médecine 51095 Reims Cedex, France. ++Service de Dermatologie, Hôpital Sébastopol, CHU 51092 Reims Cedex, France

A homogeneous fraction of structural glycoproteins (SGP) with a molecular weight of about 16,000 d has been prepared from normal rabbit dermis by a 8 M urea extraction followed by a Sepharose 4 B chromatography. The effects of this fraction on the incorporation of 14 C-proline into the proteins synthesized by scleroderma and normal human dermis fibroblasts have been studied.

When added to the culture medium of scleroderma cells, the SGP inhibits the secretion of collagen and non-collagenous proteins (NCP) in a linearly concentration-dependent manner, ranging from 0.7 to 4.10^{-6} M SGP. The inhibition is about twice more effective on collagen than on NCP secretion and the percentage of the incorporation of ^{14}C-proline into collagen decreases progressively when the concentration of SGP added to the culture medium increases. By contrast, in normal fibroblasts, the inhibition is as effective on collagen as on NCP secretion, up to the concentration of 2.10^{-6} M SGP, and the percentage of collagen secreted is not modified. Very little effect is found on the incorporation of ^{14}C-proline into the proteins of cell layer, either in normal or scleroderma fibroblasts.

Such results demonstrate that the increased secretion of collagen by scleroderma fibroblast cultures may be significantly reduced by a glycopeptide extracted from normal dermis. Further studies are in progress to determine if the inhibiting factor is lacking in sclerodermal skin.

Glucocorticoid Receptors in Human Skin Fibroblasts in Culture: Correlation of Receptor Density with Changes in Collagen Metabolism. AARNE I. OIKARINEN, CHERYL MEEKER, HELJA OIKARINEN, ELAINE TAN, AND JOUNI UITTO, Harbor-UCLA Medical Center, Torrance, California

Specific, high-affinity glucocorticosteroid receptors were assayed in skin fibroblast cultures derived from 11 control subjects of varying ages and four patients with progressive systemic sclerosis (PSS). The receptors were assayed by the binding of [^{3}H]dexamethasone, and the receptor density and the dissociation constant (K_D) were estimated from Scatchard plots. The receptor densities varied in controls from 2.8 to 25.4 pmol/mg DNA, and no apparent correlation between the receptor density and the age of the donors was noted. The receptor densities in 4 patients with PSS were 2.1, 2.2, 4.8, and 18.4 pmol/mg DNA; the values were not different statistically from the controls. The K_D values in all cell lines varied from 6.3 to 25.5×10^{-9} M. In further studies, connective tissue metabolism was studied in detail in 3 cell lines, demonstrating low, medium, or high levels of receptor density. In the cell lines with low and medium receptor density, dexamethasone,

in the range of 10^{-5}– 10^{-9} M, had no effect on collagen production, as assayed by the synthesis of [^{3}H]hydroxyproline, while in the cells with highest receptor density, a slight stimulation of collagen synthesis was noted with concentrations of 10^{-6}–10^{-9} M. In contrast, the production of collagenase, assayed by degradation of ^{3}H-labelled type I collagen substrate following a brief trypsin activation, was reduced in a dose dependent manner in all 3 cell lines, the inhibition with 10^{-5} dexamethasone being up to 56% of the control. The results indicate that high-affinity glucocorticoid receptors are present in cultured fibroblasts, but no clear correlation between the receptor density and glucocorticoid-mediated changes in collagen metabolism could be demonstrated.

Purification and Characterization of a Metallo-Protease from Human Skin Fibroblasts: *In Vitro* Study of Its Capacity to Degrade Elastic Fiber Systems. C. FRANCES, G. MEIMON, M. SZENDROI, AND G. GODEAU, Laboratoire du Tissu Conjonctif, Université Paris Val de Marne, Creteil, France

An elastase type protease was isolated from human vulvar fibroblasts. The aim of this work was to purify and to characterize a similar neutral protease from Triton X extracts of human skin fibroblasts. The purification was performed by ion exchange and affinity chromatographies. Its inhibitory profile was studied using the blockers of the serine, metallo and sulfhydryl-proteases. In order to assess its potential catabolic effect, it was tested on structural glycoprotein microfibrils isolated from porcine aortas and on insoluble radiolabelled elastins from bovine ligamentum nuchae, human and porcine aortas. Its potential to degrade human skin elastic fibers was studied by measuring the length of these fibers, on appropriately stained tissue sections of human skin, prior to and after enzyme action. Buffer and pancreatic elastase were used as controls. Results were quantified by morphometry.

This neutral protease is a metallo-protease. It was found to be inactive against ^{3}H ligamentum nuchae insoluble elastin. In contrast, it was able to degrade structural glycoproteins and to a lesser extent elastin from human or porcine aortas. Analysis of the data obtained by morphometry indicated that the enzyme attacked rapidly both elaunin and oxytalan fibers and only slowly the mature elastic fibers.

These results suggested that this protease may play a role in the fragmentation and progressive degradation of elaunin and oxytalan fibers observed during aging of human skin.

Age-Related Changes in Dermal Collagen. C. R. LOVELL*, K. A. SMOLENSKI+, AND N. D. LIGHT+, * St. John's Hospital for Diseases of the Skin, London. + ARC Meat Research Institute, Langford, U.K.

Previous reports have suggested that the dermis of young normal adults contains 85% type I and 15% type III collagen, whereas the latter is predominant in fetal skin. However, little is known about the changes in dermal collagen which occur during aging.

We obtained abdominal skin at operation or post mortem of subjects with no evidence of inflammatory skin disease. Defatted samples were subjected to cyanogen bromide digestion and the identities of the resulting peptides were determined using SDS polyacrylamide gel electrophoresis. The ratio of type I to III collagen was calculated from densitometric analysis of the marker peptides $_{\alpha 1}$(I)CB8 and $_{\alpha 1}$(III)CB8 and H.P.L.C. analysis of denatured collagen chains.

Although there is some variation between samples, the proportions of type III collagen remained constant (approx. 10–15%) in young and middle-aged adults. However, in older subjects (60 and over), the proportion of type III was found to be over 20%. These results suggest either preferential synthesis of type III collagen or preferential degradation of type I fibres in the elderly.

Collagen Fibril Formation During Embryogenesis. RAUL FLEISCHMAJER, BJORN OLSEN, RUPERT TIMPL, JEROME S. PERLISH, AND OLIVIA LOVELACE, Dept. of Dermatology, Mt. Sinai School of Medicine, New York, N.Y., Dept. of Biochemistry, UMDNJ-Rutgers Medical School, Piscataway, N.J. and Max-Planck Institut fur Biochemie, Munich, West Germany

Recent immunoelectron microscopy (IEM) studies showed that the aminopropeptide (AP) of types I and III procollagen are incorporated into fine fibrils at 60 nm intervals. The purpose of the present study is to further define the role of the AP in collagen fibrillogenesis. Chick embryo skins (10, 12, 14, 15, 16, 18, 21 days and six months) were studied by IEM with antibodies directed against the AP of type I collagen. Fibril diameter was correlated with age by measuring 9,812 fibrils. The AP was also demonstrated by SDS-acrylamide gel electrophoresis, electroblot transfer and immunoautoradiography and by examining SLS-crystallites from different age groups. Early type I collagen fibril formation takes place at the cell surface and involves polymerization of pN-collagen (collagen plus AP). Orthogonal arrangement of fibrils was also noted. When the fibril reaches about 35 nm in diameter, the AP is removed. Thus, fibril growth from 35 to 125 nm involves a second mechanism, perhaps fibril fusion since fibril distribution at various age groups revealed selective fibril diameter populations at 50, 75, 100, 125 nm. Active fibrillogenesis correlated well with the presence of AP as demonstrated by SDS-acrylamide gel electrophoresis with the pNα1(I)/α1(I) ratio, ranging from 0.320 (10 days) to 0.010 (6 months). The AP was also demonstrated during active fibrillogenesis by immunoblotting and by SLS-crystallites. The initiation of collagen fibril formation takes place at the cell surface and involves polymerization of pN-collagen. Further growth may involve fibril fusion although other mechanisms cannot be ruled out.

Investigation of Macromolecules Involved in Vulvar Lichen Sclerosus. G. MEIMON, C. FRANCES, J. WECHSLER, AND J. HEWITT, Laboratoire de Biochimie du Tissu Conjonctif. Université Paris Val de Marne, Creteil, France

In Lichen Sclerosus (L.S.), histological changes of the upper dermis seem to be the primary event of the disease. The aim of this work was to characterize these connective tissue alterations. Mucosal vulvar biopsies from 10 patients with histologically proved L.S. were compared with 10 control samples from age-matched women. Each biopsy was cut into 3 pieces. One fragment was used for electron microscopy; the second for indirect immunofluorescence, using anticollagen type I, III, IV and antifibronectin sera. Successive extractions with calcium chloride, trichloracetic acid and guanidium chloride were performed with the third fragment to yield a final residue containing elastin.

Collagen degeneration and regeneration, observed ultrastructurally in diseased dermis with increased amounts of ground substance, appeared to alter the affinity of collagen fibers for the anticollagen type I, III, IV sera. A decrease in elastin tissue content was observed by electron microscopy and quantified by biochemical techniques. A loss of fibronectin expression was observed in the pathological dermis and at the dermo-epidermal junction which looked normal ultrastructurally.

These results suggested an enzymatical process in the pathogenesis of L.S. No elastase activity on insoluble ligamentum nuchae elastin could be extracted from 6 normal and 6 pathological biopsies. In contrast, an elastase type activity on N succinyl trialanine nitroanilide was detected in both groups. This activity was higher in pathological samples, ($p < 0.01$). This enzyme activity appears to be related to the elastase type protease previously isolated from human vulvar fibroblasts.

6:00 PM–7:30 PM Presidential Ballroom and Upper Terrace

WINE AND CHEESE RECEPTION
Authors at Posters

Saturday, April 30, 1983

8:00 AM–5:00 PM Registration Upper Lobby
8:00 AM–12:00 PM CONCURRENT SCIENTIFIC SESSIONS

SESSION A Presidential Ballroom
Jean H. Saurat, M.D. Presiding

Localization of Soluble, Preformed Immune Complexes (IC) in Murine Skin. STEVE A. JOSELOW AND MART MANNIK, Division of Rheumatology, University of Washington School of Medicine, Seattle, Washington

We have studied the localization of soluble, preformed IC in the skin of mice, to define physiochemical requirements for deposition and to contrast this with other vascular beds. C57B1/6J mice were injected with IC made from human serum albumin (HSA) and rabbit anti-HSA at 5 times antigen excess (5×AgAb), 50 times excess (50×AgAb), and with reduced and alkylated antibody (5×AgAb-RA). The lattice was characterized on sucrose density gradient and they were injected as 5 mg antibody in 0.5 ml. The mice were sacrificed at varying intervals and tissues (skin from various sites, heart, liver, kidney) were studied by immunofluorescence with anti-rabbit IgG. Deposition in the skin occurred early and remained for at least 8 hr, presenting predominantly in vessels with some diffusion into the interstitium. Dermo-epidermal junctional staining was infrequently seen. The extraglomerular kidney staining seen with 5×AgAb-RA paralleled that seen in the heart and skin. Small-latticed IC (50×AgAb) did not deposit. We conclude that

deposition in the skin is transient, occurs mostly in a vascular pattern with early extravasation, and requires a lattice $>Ag_2Ab_2$.

	1 hr	4 hr	8 hr	12 hr	48 hr
Skin					
5×AgAb	3+	3+	1.5+	±	0
50×AgAb	±	±	±	±	0
5×AgAb-RA	3+	3+	1.5+	±	0
Kidney 5×AgAb-RA (glomerular/extra-glomerular)	2+/4+	3+/3+	3+/2.5+	4+/2+	4+/±
Heart 5×AgAb-RA	3+	2.5+	2+	1+	0

(Scale: 4+ = dense deposits in lpf; 3+ = dense deposits in most hpf. 2+ = dense deposits in some hpf; 1+ = rare clean deposits; ± = rare faint deposits)

T-Lymphocyte Subsets in Blood and Tissues of Patients with Lepromatous Leprosy and Erythema Nodosum Leprosum. T. H. Rea, R. L. Modlin, A. C. Bakke, D. A. Horwitz, and C. R. Taylor, Sections of Dermatology and Clinical Immunology and Rheumatic Disease and Department of Pathology, University of Southern California, Los Angeles, CA

Erythema nodosum leprosum (ENL), a common source of morbidity in lepromatous leprosy (LL), is of uncertain pathogenesis, but clearly represents an acute inflammatory reaction superimposed on the histiocytic granuloma of LL. To further study the immunopathology of LL and ENL, we have studied T lymphocyte subsets in LL patients with (c) or without (s) ENL and controls. Monoclonal antibodies were used, in conjunction with flow cytometry to examine blood or a modified immunoperoxidase technique to evaluate frozen tissue sections. Specificities sought included Pan T (Leu 1, OKT3), T helper/inducer (TH, Leu 3, OKT4) and T suppressor/cytotoxic (T_S, Leu 2, OKT8).

In blood 16 LLsENL patients had a selective deficiency in the T_H cell (mean 522 ± 324/mm^3) as compared to 20 controls (965 ± 218), $p < .001$, with a secondary lymphopenia, $p < .01$, pan T cytopenia, $p < .001$, and low helper:suppressor ratio, $p < .001$. In contrast, the T lymphocyte subsets in 15 LLcENL patients did not differ significantly from the controls.

In tissues of LLc or LLs ENL the T_H and the T_S phenotypes were both admixed with the histiocytes. The seven LLsENL tissues showed a predominance of the T_S phenotype and a mean T_H:T_S ratio of 0.8 ± .4. In contrast, the nine LLcENL tissues showed a predominance of the T_H cells and a mean T_H:T_S ratio of 2.1 ± .4, significantly greater than in LLsENL, $p < .001$.

Our data indicate that active cell-mediated immune mechanisms may be important in the pathogenesis of LLsENL, where the T_S lymphocyte predominates, and of ENL, where the T_H lymphocyte predominates.

Peripheral Blood T Cell Subsets in Leprosy. T. H. Rea, A. C. Bakke, and D. A. Horwitz, Sections of Dermatology and Clinical Immunology and Rheumatic Diseases, University of Southern California, Los Angeles, CA

To further study immunological phenomena in leprosy, peripheral blood T cell subsets were measured in 53 patients with active leprosy, 18 patients with inactive leprosy, 27 patients with SLE and 20 normal controls. Flow cytometry with an Ortho Spectrum III cytofluorograph was used. The specificities sought were pan T (OKT3), helper/inducer (T_H, OKT4) and suppressor/cytotoxic (T_S, OKT8).

The results are summarized in the table.

Group (N)	Mean/mm^3 ± SD				Mean T_H/T_S
	Lymphs	Pan T	T_H	T_S	
Control (20)	2300	1513	965 ± 218	437 ± 100	2.1
SLE (27)	1475*	1040**	470 ± 295**	510 ± 365	1.2**
Inactive Leprosy (18)	2064	1285	823 ± 390	429 ± 228	2.3
Active Leprosy (53)	1976†	1292	728 ± 373*	516 ± 278	1.6*
LL no reaction (16)	1602*	968**	522 ± 324**	394 ± 184	1.4**
LL with reaction, ENL or Lucio (17)	2017	1340	807 ± 338	502 ± 274	1.8
Tuberculoid (12)	2317	1577	936 ± 481	645 ± 229*	1.5†
Reversal reaction (6)	2291	1457	608 ± 173**	662 ± 395	1.3

† = $p < .05$; * = $p < .01$; LL = lepromatous; ENL = erythema nodosum leprosum

The specific helper cell deficiency found in SLE and LL not in reaction suggests that the immunologic abnormalities common to SLE and leprosy may have similar cellular origins. Normal levels of helper cells in ENL and Lucio suggest a role for cell-mediated immunity in these reactions. High levels of suppressor cells in tuberculoid patients may reflect a compensatory response to potentially destructive granulomas.

Behçet's Syndrome: Immune Complex-Mediated Injury, Enhanced Leukocyte Movement, and Colchicine. Joseph L. Jorizzo, R. Donald Hudson, Frank C. Schmalstieg, John C. Henry, Emilio B. Gonzalez, Jerry C. Daniels, Prapand Apisarnthanarax, Yukinobu Ichikawa, and Tito Cavallo, Departments of Dermatology, Pediatrics, Internal Medicine and Pathology, University of Texas Medical Branch, Galveston, Texas

Current major questions regarding the pathomechanism of Behçet's syndrome relate to: immune regulatory dysfunction, circulating immune complexes (CIC), enhanced neutrophil chemotaxis, and the effects of colchicine therapy. Six patients met rigorous requirements for the diagnosis of Behçet's syndrome including exclusion of inflammatory bowel disease and of other autoimmune diseases. Only 1/5 patients showed evidence of immune regulatory dysfunction (increased T-helper/suppressor ratio) as assessed by T4/T8 monoclonal antibody technique. While only 1/5 patients had CIC as detected by standard *in vitro* techniques (Clq binding and Raji cell assays), all five patients tested had evidence of CIC by the *in vivo* histamine trap test (6/6 controls normal). Sera from 6/6 patients enhanced chemotaxis of neutrophils to zymosan activated serum (5.07 ± 1.99 enhanced to 7.45 ± 2.56, $p = 0.01$) when compared to seven controls. Colchicine therapy resulted in inhibition of the ability of patients' serum to enhance the movement of 4/4 patients' cells (3.97 ± 3.0 not increased to 3.97 ± 2.16) and 7/7 control patients' cells. Clinically in a longitudinal assessment of colchicine therapy using each patient as his own control, colchicine effected size and duration of oral and genital aphthae, but not uveitis or arthritis. Immune complex-mediated injury followed by excessive accumulation of neutrophils are possibly involved in the pathogenesis of cutaneous pathergy lesions, aphthae, and other lesions of Behçet's syndrome. Further evaluation of colchicine therapy is warranted.

An Autoantibody Against a Fragment of C4 Found in the Serum of a Patient with C2-Deficiency. Michael Meurer, Otto Braun-Falco, and Irma Gigli, Depts. of Dermatology, Univ. of Munich and Univ. of California, San Diego

The C3 nephritic factor and the recently described C4 nephritic factor are unusual human gammaglobulins with antibody activity against the complement enzymes C3bBb and C4b2a respectively. We now describe a new autoantibody against activated C4. This antibody was detected in the serum of a patient with a deficiency of C2 who presented with a lupus-like syndrome. The serum, as well as the IgG and IgM fractions purified from it, strongly agglutinated human red cells (RBC) coated with complement proteins using sucrose-activated normal human serum (NHS) as the source of complement, while cells coated with heat-inactivated NHS or with C4-deficient serum (C4DS) were not. However, agglutination did not occur when purified C4 was added to the C4DS used as source of complement. Using different conditions to activate the complement system, it was shown that the antibody reacted with erythrocytes coated with C4b and C4c but not with C4d. Addition of the antibody to NHS during sucrose activation in the absence of RBC resulted in the neutralization of the capacity of the antibody to agglutinate complement coated RBC. This inhibition was not observed when EDTA-treated NHS or C4DS was used indicating that activation of C4 in the serum was required for the neutralization of the antibody. The agglutinin activity was not inhibited by preincubation of the antibody with purified C4, C4b, or C4c, however, significant inhibition was noted when the antibody was present during the proteolytic cleavage of C4 by $\overline{C1s}$. These results show that the antibody may recognize a fragment of the C4 molecule that is constantly exposed on cell bound C4b or C4c, but is only transiently present during fluid phase activation of C4 by $\overline{C1}$.

Heterogeneity of Epidermal Cells Detected by Expression of Differentiation Antigens in Normal and Nude Balb/c Mice. D. A. Chambers, R. L. Cohen, M. Heiss, and M. E. A. F. Alves, Univ. of Illinois Health Sciences Center, Chicago, Illinois

The mechanisms by which epithelial homeostasis is maintained are still undefined. Previous experiments in this and other laboratories have suggested that subpopulations of basal epidermal cells exist. In the immune system, specific populations of cells are often delineated by unique surface antigens. Recent associations between skin, lympho-

cytes and macrophages, e.g., the physiology of Langerhans cells, and the ability of epidermal cells to secrete lymphokine-like molecules; led us to examine epidermal cells for the presence of classical T-lymphocyte markers. Adult Balb/c mouse skin, dissociated by trypsin was passed through a ficol gradient to yield a population of basal cells (>90%). Monoclonal anti-Thy1.2 antibody was incubated with cells and detected by the presence of an FITC goat anti-mouse IgG on the cell surface by fluorescence microscopy. Mouse epidermal cells contained a subpopulation of Thy1+ cells amounting to 12 ± 6% postfractionation. Parallel experiments with frozen sections from skin and lip showed the clustering of Thy1+ cells. Neonatal Balb/c epidermal cultures contained Thy1+ cells throughout 16 days of culture. Athymic mice have been characterized as Thy1 deficient. However, nude mouse epidermal cells contained 27 ± 5% Thy1+ cells. The presence of increased numbers of Thy1+ cells in nude epidermis was unexpected. Control experiments revealed the presence of Thy1+ cells in nude and normal brain, normal spleen and thymus but less than 2% in nude spleen. These results suggest that a specific subpopulation of epidermal cells exists whose function remains to be elucidated.

Induction of Nuclear Antigen Expression in Human Keratinocytes by Ultraviolet Light (UVL). W. P. LeFeber, D. A. Norris, J. C. Huff, M. Kubo, S. R. Ryan, and W. L. Weston, Department of Dermatology, University of Colorado School of Medicine, Denver, Colorado

UVL is strongly associated with the development of cutaneous lupus erythematosus (LE) and has been shown to alter DNA, to promote the development of antibodies to DNA, and to induce DNA release from epidermis *in vivo*. We used antisera to nuclear antigens as probes to study the effect of UVL on nuclear antigen expression in epidermal cell cultures.

Human keratinocytes cultured in defined, serum-free medium were irradiated with 0.2 or 2.0 mJ/cm^2 UVB, and the location of different nuclear antigens was determined by incubation with anti-RNP, SSA, ssDNA, or dsDNA antisera, or with monoclonal antihistone antibody followed by fluorescein-labeled anti-IgG conjugates. Cells were incubated with antisera either before or after fixation with acetone.

In prefixed cultures, very weak speckled nuclear (ANA) and cytoplasmic staining were seen with multiple antisera prior to irradiation. Following irradiation, very strong cytoplasmic staining and ANA's were seen by 8 hr, diminishing by 24 hr. Cells fixed after incubation with antisera showed less ANA but more cytoplasmic and cell membrane staining 8 hr post-UVL. No significant fluorescence was seen with control sera before or after UVL.

Using a propidium iodide viability assay, we confirmed that post-UVL cytoplasmic expression of nuclear antigens was seen in living cells; cycloheximide did not inhibit the UVL-induced nuclear antigen changes.

We have demonstrated that UV irradiation of cultured human keratinocytes causes expression of nuclear antigens in the cell cytoplasm and on cell membranes resulting in binding of specific LE antisera.

Distribution of Major Histocompatibility Antigens in Normal Skin. Terence J. Harrist, Dirk J. Ruiter, Atul K. Bhan, and Martin C. Mihm, Jr., Departments of Dermatology and Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA

The distribution of HLA-A,B,C antigens (HLA), β2-microglobulin (β2m), and Ia-like antigens (Ia) were studied in normal skin from various sites of 18 subjects (1 each) using an immunoperoxidase method and monoclonal antibodies. All specimens were similar except for the number of Ia-positive intraepithelial dendritic cells which varied with the anatomic sites. HLA and β2m were present on the epidermal basal and spinous keratinocytes, on the outer root sheath epithelium of the infundibulum and upper isthmus of follicles, on the excretory sebaceous duct epithelium and on the generative epithelium of sebaceous glands. Ia-positive intraepithelial dendritic cells were more frequent in the follicular infundibulum and isthmus than in its inferior portion or in the epidermis. In the straight eccrine duct, HLA and β2-positivity was most striking in its lower portion with decreasing staining intensity on the ductal epithelium superficially. In contrast, the acrosyringial epithelium was markedly Ia-positive with decreasing intensity of staining as the duct penetrated the dermis. Only slight HLA and β2m staining was present in the eccrine and apocrine glands, but intense staining was present in the intradermal apocrine ducts. In the dermis, HLA, β2m and Ia were observed on fibrohistiocytic cells, dendritic cells, endothelium, and pericytes. Ia-positivity of the acrosyringium implies that Langerhans/indeterminate cells are not the only intraepidermal Ia-positive cells. The relative absence of HLA and β2m as well as the presence of Ia in the acrosyringium is unique because Ia distribution is much more restricted than that of HLA. Moreover, the degree of expression of HLA and β2m appears to be variable on nucleated cells.

Scleroderma-Like Changes in Mouse Chronic Graft-vs.-Host Disease. Henry N. Claman, Bruce D. Jaffee, and J. Clark Huff, Departments of Medicine and Dermatology, University of Colorado School of Medicine, Denver, CO

We studied the development of scleroderma-like changes in the skin of mice with chronic graft-vs.-host disease (GVHD) in an attempt to understand idiopathic scleroderma and human chronic GVHD. The most consistent changes occur across minor histocompatibility barriers, using 5×10^7 B10.D2 spleen cells given to irradiated BALB/c recipients (600r). These strains are identical at the H-2^d and minor lymphocyte stimulating (mls) loci and their cells do not stimulate each other in a mixed leukocyte reaction. (This mimics the situation in human bone marrow transplantation.) Mice were monitored by body weight and serial skin biopsies. Skin was processed for H and E sections and for immunofluorescence for IgG, IgA, IgM, C3, fibrin and fibronectin. Skin changes in GVHD (but not control) mice included thickening of the dermis, alignment of collagen, a mononuclear infiltrate deep in the staining for IgM, IgA, C3, fibrin and fibronectin were present around the basement membrane zone of hair follicles of GVHD animals, but not controls, by day 11 and increased in prominence by day 42. In GVHD animals, granular staining for Ig's, C3 and fibrin was present along the basement membrane zone of the epidermis at day 20 and increased in frequency and strength by day 42. This model appears to provide a suitable model for investigating the immunological consequences of GVHD, and perhaps of the pathogenesis of scleroderma.

Suppression of Epidermal Graft-Versus-Host Disease (GVHD) with Ultraviolet B Radiation. Arnold Glazier, Warwick L. Morison, Cora Bucana, Allan D. Hess, William E. Beschorner, and Peter J. Tutschka, Bone Marrow Transplantation Program, The Johns Hopkins Univ. School of Medicine, Baltimore, MD and the Cancer Biology Program, Frederick Cancer Research Facility, Frederick, MD

Fulminant GVHD develops in Lewis rats transplanted with RT1 incompatible ACI bone marrow and spleen cells. Ultraviolet B irradiation (UVB) of Lewis flank skin for 2.5 hr/day at an irradiance of 6W/M^2 for three consecutive days depletes exposed skin of interfollicular but not follicular Langerhans cells as determined by both adenosine triphosphatase staining and electron microscopy. Lewis rats treated unilaterally with UVB radiation prior to lethal ionizing total body irradiation and allogeneic ACI marrow and spleen cell transplantation develop fulminant acute GVHD by day 11. Extensive grade II acute interfollicular and follicular GVHD is seen in the unexpected skin. In striking contrast, UVB exposed skin from symmetrical biopsy sites on the same rats have only rare focal lesions of interfollicular epidermal GVHD. Follicular GVHD although present is less severe in UVB exposed skin. These results suggest that Langerhans cells may be an important initial target in major histocompatibility complex mismatched acute GVHD and that UVB phototherapy deserves further assessment in the prevention of epidermal GVHD.

Ultraviolet B Exposure Converts Murine Lichenoid Graft-vs-Host Skin Disease (GVHSD) into Sclerotic GVHSD. M. R. Charley, J. N. Gilliam, and R. D. Sontheimer, University of Texas Health Science Center at Dallas, Dallas, TX

Chimeras produced by reconstitution of irradiated C67Bl/6J (Bl/6) mice with LP/J bone marrow and spleen cells (LP/J → Bl/6) show a persistent lichenoid tissue reaction involving the epidermis and follicular epithelium. There is only minimal dermal sclerosis in some mice between days 21–25, which resolves and does not recur during the remainder of the life of the chimera (40 days). When these same LP/J → Bl/6 chimeras were exposed to three consecutive daily doses of 20 milli-Joules/day of UVB on one-half of their shaved abdomens starting on the day of transplantation, they consistently developed a dense and hypocellular dermal sclerosis limited to the area of UVB exposure by day 25. Control mice that were syngeneically transplanted and exposed to UVB were normal on biopsy by day 25. To test the hypothesis that the UVB induced sclerosis was mast cell dependent, chimeras were prepared using irradiated, mast cell deficient mice (WBB6F-W/W^v) reconstituted with Bl/6 cells. These chimeras develop histologic evi-

dence of acute GVHSD by 2 to 3 weeks but do not have detectable dermal mast cells until 2 mos. post transplant. When these mast cell deficient chimeras were exposed to UVB as above, they also developed dense dermal sclerosis limited to the site of UVB by day 25. Thus, mast cells are not necessary for the development of the sclerosing reaction.

Because chronic human GVHSD in some patients has appeared selectively in areas of sun exposure (Shulman et al., AJP:91, 545–564, 1978), this mouse model offers the opportunity to study the mechanisms involved in UVB induced dermal sclerosis in GVHSD.

A Comparison of Two Histologic Patterns of Murine Graft-vs-Host Skin Disease (GVHSD). J. Bangert, M. Charley, J. N. Gilliam, and R. D. Sontheimer, UTHSCD, Dallas, Texas

We have observed that chimeras, produced by reconstitution of irradiated C57Bl/6J (Bl/6) mice with bone marrow and spleen cells from LP/J mice (LP/J→Bl/6), develop alopecia, but that the reciprocal chimeras (Bl/6→LP/J) do not. In order to detect any histopathologic differences between these two chimeras that might explain this discrepancy in expression of clinical disease, we examined in a blinded fashion 57 skin biopsies from LP/J→Bl/6 and Bl/6→LP/J chimeras, syngeneically transplanted controls and non-transplanted normal mice. Each of 27 pathological criteria was graded on a 0 to 4+ scale of increasing severity. Striking differences were noted between the two chimeras. LP/J→Bl/6 chimeras showed almost no pathologic change before 12 days. At 12–15 days, a lichenoid interface dermatitis developed with liquefaction degeneration of the epidermis and the pilosebaceous unit in all mice. It was intense (3–4+) in 5 out of 7 mice (71%). Dermal inflammation was moderate to marked (2–4+), while subcutaneous inflammation was mild (1–2+). Only minimal sclerosis was seen. In contrast, the Bl/6→LP/J chimeras had a biphasic response. Between 8 and 16 days, a lichenoid interface dermatitis was seen (2+) which, starting at 17 days evolved into a hypocellular, dermal and subcuticular sclerosis (3+) in 8 of 10 (80%). The alopecia seen in the LP/J→Bl/6 chimeras could have resulted from the more prolonged lichenoid injury to the follicles that occurred in these animals (30 days vs. 8 days in the Bl/6→LP/J chimeras).

In summary, these two chimeras clearly show different histopathologic patterns of GVHSD and may serve as useful animal models for studying the lichenoid and sclerosing reaction patterns of cutaneous injury.

IL-2 Substantially Corrects a Combined Gamma/UVB Radiation Induced Defect in Murine Antigen Presenting Cells. R. D. Granstein, A. Tominaga, J. A. Parrish, and M. I. Greene, Departments of Dermatology and Pathology, Harvard Medical School, Boston, MA

Exposure of animals *in vivo* or antigen-presenting cells (APC) *in vitro* to UVB radiation (280–320 nm) results in severe impairment of normal antigen-presenting function. It has recently been shown that Interleukin 1 (IL-1) can substantially correct this defect *in vitro* and *in vivo* (submitted for publication). We have now examined the ability of IL-1 and Interleukin 2 (IL-2) to correct a combined gamma ray (GR)/UVB-induced defect in APC. Briefly, low density APC (LD) were obtained from BALB/c mouse spleens by flotation on bovine serum albumin. The LD were haptenated with azobenzenearsonate (ABA), exposed to 1500 rads GR, 0.33 KJ/m^2 UVB or combined GR/UVB, and cultured for 5 days with T-cells isolated from BALB/c mice primed with ABA 7 days earlier. The generation of cytotoxic lymphocyte effector cells was measured by the ability of effectors to specifically lyse ABA-coupled P815 target cells in a ^{51}Cr release assay. The results show that IL-1 (in a dose that corrects the UVB-induced defect) can not correct the GR/UVB defect [% killing (E/T 100:1): ABA-LD-GR = 49.1; ABA-LD-GR-UVB = 3.7; ABA-LD-GR-UVB-IL-1 = 3.4] but that IL-2 substantially and significantly corrects this defect [% killing (E/T 100:1): ABA-LD-GR = 22.3; ABA-LD-GR-UVB = 1.0; ABA-LD-GR-UVB-IL-2 = 24.9]. Thus, UVB plus gamma irradiation induces a different or additional defect in APC function, compared to UVB alone, that is bypassable by IL-2.

Studies on the Relationship Between Murine Epidermal Cell Thymocyte Activating Factor (ETAF) and Ultraviolet Radiation (UVR). Raymond A. Daynes and Lorise C. Gahring, Dept. of Pathology, Univ. of Utah, Salt Lake City, UT

Keratinocytes exposed to UVR *in vitro* exhibit a dosage dependent decrease in ETAF (IL-1) production which appears to correlate with a loss in cell viability over a 24 hr period. Skin biopsies from chronically UVR exposed mice, exhibiting a marked epidermal hyperplasia, were analyzed for ETAF activity. We found that both whole skin biopsies as well as enzymatically dissociated epidermal cells from UVR exposed animals produced greater (2–3×) levels of ETAF than biopsies of equal size taken from normal mice. However, equal amounts of ETAF were produced when the analysis was made on a per cell basis. IL-1-like activity could also be demonstrated in the serum of chronically UVR-exposed mice. An analysis of tumors induced by UVR (both squamous cell carcinomas and fibrosarcomas) established that the majority tested were capable of constitutive ETAF production. Serum from mice bearing ETAF-producing tumors were found to contain demonstrable levels of IL-1-like activity. Due to the many possible biologic activities of ETAF (IL-1), we hypothesize that this mediator serves important functions in the pathogenesis of UVR-induced inflammatory responses. Constitutive IL-1 production by tumors may also provide for a novel tumor escape mechanism which employs liver-induced acute phase reactants for the inhibition of anti-tumor immune responses.

Isolation and Characterization of Human Upper Epidermal Cytoplasmic Antigens. H. Hintner, P. M. Steinert, and T. J. Lawley, Dermatology Branch, NIH, Bethesda, MD

Upper cytoplasmic (U-Cyt) antibodies, directed against cytoplasmic antigens in keratinocytes in the upper layers of the epidermis, have been defined by indirect immunofluorescence (IIF). The absence of these antigens in the basal cell-layer, cultured epidermal cells and certain skin tumors suggests that they may be differentiation antigens, perhaps keratin intermediate filaments (KIF). KIF were extracted from normal human epidermis, separated by polyacrylamide gel electrophoresis, transblotted to nitrocellulose strips and used as substrates for antibody binding. Twelve normal human sera (NHS) were tested for anti-KIF antibodies and for U-Cyt antibodies. Five of 12 NHS had U-Cyte antibodies by IIF, and the same 5 NHS had antibodies against high molecular weight (HMW; 65K, 63K, 61.5K) KIF proteins. The antibody titers were 25 to 125× higher by transblotting than by IIF. Absorption of test sera with purified KIF removed U-Cyt and anti-KIF antibodies. Absorption with fibroblast IF, vimentin, did not remove U-Cyt or anti-KIF antibodies. Absorbed U-Cyt or anti-KIF antibodies were both eluted from the same KIF preparation and bound to U-Cyt antigens as shown by IIF and HMW KIF proteins by transblotting. Absorption of a serum containing U-Cyt antibodies, antibasement membrane zone antibodies and antinuclear antibodies with purified KIF removed U-Cyt antibodies but not the other types of antibody. In addition all test sera, even those which lacked U-Cyt antibodies were found to have low titer antibodies against low molecular weight KIF proteins by transblotting. These data indicate HMW KIF proteins are antigens which U-Cyt antibodies are directed against and that low titer antibodies against KIF proteins may be much more common than previously appreciated.

Immune Responses Induced by Feeding a Liposome Incorporated Hapten. Jerome R. Pomeranz, Bryan R. Davis, Ni Ankrah, and Barbara P. Barna, Department of Dermatology, Cleveland Metropolitan General Hospital, Department of Chemistry, Cleveland State Univ. and Department of Immunopathology, Cleveland Clinic, Cleveland, Ohio

Randomly bred, Hartley strain, starved guinea pigs were fed a water soluble hapten, picryl sulfonic acid (PSA), incorporated into multilamellar liposomes prepared from phosphatidyl choline:cholesterol (1:1). Experimental groups were fed 236 mg (600 mg lipid) in 10 ml phosphate buffered saline, pH 7.5 (PBS) and 39.5 mg PSA (300 mg lipid) in 10 ml PBS. An additional group was fed 236 mg PSA in 10 ml PBS emulsified with corn oil 3:1. Controls were fed multilamellar liposomes (600 mg lipid) in 10 ml PBS and 10 ml PBS alone. At two weeks contact tests to 1, 0.3 and 0.1% picryl chloride (PCl) in acetone-olive oil (3:1) were negative. Passive cutaneous anaphylaxis (PCA) tests to picryl protein conjugates were positive on serum from 5/7 animals fed PSA in PBS corn oil emulsion and from 1/10 fed 236 mg PSA in liposomes. One week later they were immunized with 80 μg PCl in complete Freund's adjuvant. Contact and PCA tests two weeks later revealed 5/7 animals fed PSA in corn oil-PBS emulsion were tolerant to contact sensitization. Of those fed 236 mg PSA in liposomes, only 2/10 were tolerant, but the remainder of this group was not as strongly sensitized to PCl as the groups fed 39.5 mg PSA in liposomes or the liposome and PBS controls. PCA tests in all groups remained essentially unchanged.

This study shows that feeding a water soluble hapten in liposomes induces contact tolerance in a few animals and lowers the intensity of sensitization in most of the others However, it is not as effective in inducing contact tolerance as feeding the hapten in a corn oil-saline emulsion.

Thy 1 Antigen-Bearing, Dendritic Epidermal Cells Are Separable from Langerhans Cells in Murine Epidermal Cell Suspensions. P. R. BERGSTRESSER, R. E. TIGELAAR, AND J. W. STREILEIN, Departments of Dermatology, Internal Medicine, and Cell Biology, UTHSCD, Dallas, Texas

Employing immunofluorescence microscopy and monoclonal antibodies against Thy 1.2 and Ia antigens, we have identified in murine epidermis a dendritic cell population which differs from both Langerhans cells and melanocytes. Thy 1 antigen-bearing epidermal cells are primarily dendritic; they are distributed relatively evenly across skin surfaces, although densities vary greatly from site to site and among various inbred murine strains. Surface densities were highest in ear skin from the pigmented strain B10.A (580 cells/mm^2), a value approaching that of epidermal Langerhans cells, and they were lowest in abdominal wall skin from the albino strain BALB/c (5 cells/mm^2). Thy 1 antigen-bearing epidermal cells possess neither melanin nor Ia antigens and their distributions within skin are disparate from those of both melanocytes and Langerhans cells. A "panning" method was used to separate Thy 1-positive from Ia-positive cells. Disaggregated epidermal cells (4% Thy 1-positive; 6% Ia-positive) were first incubated with monoclonal anti-Thy 1.2 followed by adherence to plastic dishes coated with goat anti-mouse IgG. 64% of recovered adherent cells were Thy 1-positive, whereas the percentage of Ia-positive cells remained unchanged (8%). These studies demonstrate the anatomic and physical uniqueness of these two separate populations of dendritic epidermal cells. We propose that Thy 1 antigen-bearing cells are T-lymphocytes that reside normally in murine epidermis.

Thy-1 Antigen Expression on Murine Epidermal Cells. ERWIN TSCHACHLER, HEINZ LEIBL, GEROLD SCHULER, JUDITH HUTTERER, KLAUS WOLFF, AND GEORG STINGL, Department of Dermatology I., University of Vienna; Department of Dermatology, University of Innsbruck, Austria

Thy-1 antigen is a glycoprotein which is mainly expressed on thymus-derived lymphocytes and certain cell types of neuroectodermal origin. In this report, we describe the occurrence of Thy-1+ cells within normal murine epidermis.

A population of brightly fluorescent, highly dendritic, evenly distributed cells was seen when sheet preparations of C3H/He, C3H/He nu/nu, Balb/c and C57Bl/6 ear epidermis were reacted with either FITC-IgG_{2_b} rat anti-mouse Thy-1.2 monoclonal antibody (Becton/Dickinson Sunnyvale, CA) or IgM mouse anti-mouse Thy-1.2 monoclonal antibody (NEN, Boston, MA) followed by FITC-rat IgG_{2_b} anti-mouse IgM monoclonal antibody (Becton/Dickinson). Appropriate controls for either direct (preincubation with unconjugated reagent) or indirect (control ascites) immunofluorescence assays were consistently negative. Double labeling experiments on both sheet preparations and epidermal cell suspensions demonstrated that Ia-positive epidermal cells and Thy-1.2 reactive cells represent mutually exclusive populations. Immunoelectronmicroscopy revealed that Thy-1+ displayed abundant intermediate-sized filaments and were neither keratinocytes nor Langerhans cells. Since some of them contained singly-dispersed melanosomes they were considered to belong to the melanocyte lineage.

Thy-1 may represent a useful differentiation and functional marker for melanocytes.

Basal Cells from Human Epidermis Induce Thymocyte Maturation. RONA M. MACKIE AND MARIE E. HUGHES, Department of Dermatology, Glasgow University, Glasgow

We have investigated the possible production by normal human epidermal cells of a thymocyte maturation factor similar to that reported found in a murine keratinocyte cell line (PAM 212). We have cultured normal human thymocytes in Eagle's medium conditioned by previous culture for 24 hr of normal human epidermal cells. Medium was conditioned by casal cells, suprabasal cells or total epidermal cells. Langerhans cells were destroyed by incubation of epidermal cells. Langerhans cells were destroyed by incubation of epidermal cells with NAI 34 antibody (Dr. A. J. McMichael) and human complement. After culturing thymocytes for 24 hr in epidermal cell conditioned medium, medium conditioned by HeLa cells or fresh medium, they were then examined using monoclonal antibodies NAI 34 and OKT3. 85% of normal human thymocytes express human thymocyte antigen (HTA) as defined by NAI 34 and this is lost in the process of intrathymic maturation to T lymphocytes. In contrast, 100% of peripheral blood T lymphocytes express T cell antigen identified by monoclonal antibody OKT3. By culturing thymocytes with epidermal cell conditioned medium we have demonstrated a loss of the HTA marker and concomitant acquisition of the OKT3 cell surface antigen. This phenomenon is not observed when conditioned medium from other cell types is used or when thymocytes are cultured in fresh medium alone. Studies with medium conditioned by basal and suprabasal cell types show that the thymocyte maturation effect is most marked with medium from the basal cell enriched fraction.

Facteur Thymique Serique-Producing Cells Studied by Monoclonal Antibodies: Absence in Normal and Psoriatic Skin. MONIQUE ANDARY, MIREILLE DARDENNE, COLETTE BLANCHARD, J. J. GUILHOU, AND J. CLOT, Department of Cellular Immunology, Hôpital Saint-Eloi, Montpellier, INSERM U25, Hôpital Necker, Paris, and Clinic of Dermatology, Hôpital Saint-Charles, Montpellier, France

An increase of Facteur Thymique Sérique (FTS) levels was previously demonstrated in psoriatic sera (Brit. J. Derm., 1976, 95:295). As recent works reported the presence of thymic hormone-like substances in epidermal cells (J. Invest. Dermatol., 1982, 78:330; Cell., 1981, 24:885), the possible production of FTS by psoriatic skin should be investigated. We used IgG1 and IgG2a monoclonal antibodies (MoAb) produced by immunization of mice with synthetic nonapeptide FTS coupled to BSA (Immunology Letters, 1982, 4:79). We studied 12 psoriatic patients and 4 healthy volunteers. Unfixed cryostatic sections (3 μm thick) of involved and uninvolved psoriatic skin and normal skin were stained by indirect immunofluorescence techniques using MoAb anti-FTS and specific fluorescent goat anti-mouse Ig. As positive controls in each experiment, thymus sections from mice aged 4–6 weeks were processed. Negative controls were carried out by incubating skin and thymus sections with phosphate buffered saline and fluorescent goat anti-mouse Ig. FTS positive cells were always detected in thymus sections, whereas no positive staining could be demonstrated neither in psoriatic nor in normal skin. Thus the high levels of FTS in Psoriasis could not be explained by a skin production. Moreover, the negative data herein observed in normal skin with highly specific anti-FTS MoAb questionned the presence of thymic hormones detected by heterologous antisera in skin.

SESSION B — Federal Room

Willem van Vloten, M.D., Presiding

The Carcinogenic Effect of Artificial Sunlight and UVA Irradiation. BENT STABERG, HANS C. WULF, THOMAS POULSEN, PER KLEMP, AND HOLGER BRODTHAGEN. Departments of Clinical Physiology, Dermatology, and Pathology, The Finsen Institute, Copenhagen, Denmark

The carcinogenic effect of artificial UV-sunlight followed by unfiltered or filtered UVA irradiation (Philips TL 40W/09) in human "solaria doses" was studied in 9 groups of 25 lightly pigmented hairless mice. Artificial sunlight for 3 months induced only a moderate tumor incidence (proportion of surviving animals with at least one tumor) of 0.15 after one year, whereas unfiltered UVA irradiation for 9 months alone gave rise to no tumors. The combination of artificial sunlight for 3 months followed by unfiltered UVA irradiation for 2, 4, or 6 months, significantly increased the tumor incidence after one year to 0.28, 0.68, and 0.72, respectively. When the UVA radiation was filtered through a 2 mm glass plate thus eliminating the radiation below 320 nm, and the UVA doses were equivalent to those used in the unfiltered trial, these figures were significantly reduced to 0.18, 0.35, and 0.45, respectively.

It is concluded that UVA irradiation in "solaria doses" does not in itself evoke skin cancer, but enhances the carcinogenic effect of artificial UV-sunlight in hairless mice. This photoaugmentative carcinogenic effect can be reduced but not eliminated by attenuating the shorter wavelengths of the UVA radiation.

A Defective DNA Endonuclease in Melanotic but Not Amelanotic Mouse Melanomas. MURIEL W. LAMBERT AND GENE K. POTTER, Department of Pathology, UMDNJ-New Jersey Medical School, Newark, New Jersey 07103

Chromatin-associated DNA endonucleases (Endos) from melanotic and amelanotic tumors derived from a Cloudman mouse cell line (S91 NCTC 3960, CCL 53), and propagated by serial subcutaneous injection into DBA/2J mice, were examined and compared. Histological, histochemical, and electron microscopic examination of these two types of tumors showed that the amelanotic neoplasms had very low levels of tyrosinase activity and that mainly stage I and some stage II melanosomes were present, whereas in the melanotic tumors all cells showed moderately high levels of tyrosinase activity and all stages of melanosome development were found, with stage III melanosomes predomi-

nating. Chromatin-associated non-histone proteins were extracted from isolated nuclei of both types of tumor cells and subjected to isoelectric focusing (IF). Each IF fraction was assayed for Endo activity using calf thymus DNA as substrate. Seven clearly distinguishable and very similar peaks of Endo activity (pIs 3.9–9.2) were found in both the melanotic and amelanotic tumor nuclei. In addition, a chromatin-associated Endo activity at pI 6.6, which we have previously reported to be present in nuclei of non-pigmented human lymphoblastoid cells but absent in melanotic mouse melanoma cells, was again found to be absent in melanotic cells but was present in amelanotic mouse melanoma nuclei. These results suggest that the presence or absence of melanin in mammalian cells may have a profound effect on nuclear endonucleases active on DNA, or alternatively, that abnormalities in these DNA endonucleases may have an important influence on melanization.

A New Chromosome Rearrangement Syndrome: Werner's Adult Progeria. Susi Scappaticci*, Decio Cerimele**, and Francesca Cottoni**, Chair of Biology* and Institute of Dermatology**, Univ. of Sassari, Italy

Increased frequency of pseudodiploidy in Werner's syndrome involving variable clonal structural rearrangements in fibroblasts strains has been observed; it has been called "variegated translocation mosaicism" (VTM). Lymphocyte cultures from blood obtained from six patients affected by Werner's syndrome and fibroblast cultures from skin explants obtained from five patients were set up.

In lymphocytes a mean of 0.15 abnormalities per cell (a/c) were observed, against 0.04 for the controls; the mean of aneuploid metaphases was 0.07 for the patients and 0.01 for the controls. In fibroblast cultures the incidence of structural rearrangements was higher than in cultured lymphocytes; the a/c ratio ranged from 0.09 to 0.40 against a range of 0.09 to 0.018 in the controls. The detailed analysis with Q-bands of fibroblast cultures demonstrated evidence of clonal evolution, defined as the presence of at least two metaphases with the same structural changes in halos. Four clones were demonstrated: two had involvement of chromosome n.1 and two of the X chromosome. Two clones showed premature centromere disjunction of the X chromosome.

The observation of VTM in fibroblasts analyzed *in situ* in the outgrowth halos from primary skin explants and in cultured lymphocytes demonstrate that Werner's syndrome is indeed a further example of chromosome rearrangement syndrome.

A New Form of Xeroderma Pigmentosum: Reduced DNA Repair Without Neoplasia. Kenneth H. Kraemer[a], Hanoch Slor[b], and Alan Andrews[c], [a] N.I.H., Bethesda, MD 20205, [b] Tel Aviv Univ., Tel Aviv, Israel, and [c] Columbia Univ., New York, NY 10032

Xeroderma pigmentosum (XP) is an autosomal recessive disease with sun-sensitivity, multiple cutaneous neoplasms, and defective DNA repair. We have found a family of Israeli Arabs where the DNA repair defect occurs without sun-sensitivity or cutaneous neoplasia. The father (XP4TA) was 57 years old, had similar pigmentation to his neighbors and no cutaneous neoplasms. A 28 year old son (XP3TA) worked outdoors as a house painter for many years. He had pigmentation on his face which was typical of the population, moderate conjunctival injection and no acute sun-sensitivity or cutaneous neoplasms. A 30 year old son (XP1TA) had marked sun-sensitivity at 4 mo. of age. He began developing cutaneous neoplasms at age 10 and has had about 10 neoplasms removed from his face. He has an unusual mottled hyperpigmentation with marked telangiectasia of the face. There is moderate conjunctivitis. None of the patients had neurological abnormalities. Ultraviolet (UV)-induced unscheduled DNA synthesis (UDS), a measure of DNA repair, was reduced to 35%, 43%, and 52% of normal in fibroblast cultures from XP1TA, XP3TA, and XP4TA respectively. Cell fusion studies, measuring UV-induced UDS in heterokaryons, showed that XP1TA and XP2TA were not in XP complementation groups A, C, or D. This family thus demonstrates an apparent dominant inheritance of a DNA repair with neoplasia while 2 other family members have defective DNA repair without neoplasia. Thus other (presently unmeasured) defects may interact with defective DNA repair in producing cutaneous neoplasia in XP.

DNA Repair Protects Against Cutaneous and Internal Neoplasia: Evidence from Studies of Xeroderma Pigmentosum. Kenneth H. Kraemer, Muyng M. Lee, and Joseph Scotto, National Cancer Institute, Bethesda, MD 20205

Xeroderma pigmentosum (XP), is a rare, autosomal recessive disease with sun sensitivity and multiple neoplasms in association with defective DNA repair. Cultured cells from XP patients are hypersensitive to killing and to induction of mutations by ultraviolet (UV) radiation and by a number of DNA-damaging chemical carcinogens. By reflecting the clinical consequences of deficient DNA repair, XP serves as a model disease for determining the effects of proficient DNA repair. Using a standard form, we abstracted reports of 726 XP patients (from 41 countries) published from 1874 to 1982. Estimates of the frequency of development of malignant neoplasms among XP patients were derived. Expected neoplasm rates were calculated from data published for the U.S. population. The XP patients under age 20 years had an estimated 2000-fold increase in prevalence of skin cancer (basal cell plus squamous cell, or melanoma), of cancer of the anterior eye (conjunctiva or cornea), and of cancer of the anterior tongue. These sites are all potentially exposed to UV, a potent carcinogen which produces DNA damage that is poorly repaired by XP cells. XP patients under age 20 years had an estimated 12-fold increase in prevalence of neoplasms in sites shielded from UV. Among XP patients under age 40 years with internal cancer, there was a disproportionate representation of neoplasms of the brain, oral cavity (excluding tongue), and leukemia. These internal neoplasms may be related to exposure to environmental chemical carcinogens that cause DNA damage which, like UV-induced damage, is poorly repaired by XP cells. This study suggests that DNA repair plays a major role in protection against UV-induced neoplasia and a smaller role in protection against internal neoplasms in the general population.

A New Patient with Both Xeroderma Pigmentosum and Cockayne Syndrome is in a New Xeroderma Pigmentosum Complementation Group. J. H. Robbins, A. N. Moshell, M. A. Lutzner, M. B. Ganges, and J.-M. Dupuy, Dermatology Branch, NCI, NIH, Bethesda, MD, Institute Pasteur, Paris, France, and Institute Armand-Frappier, Univ. of Quebec, Quebec, Canada

The first patient (XP11E) reported to have both xeroderma pigmentosum (XP) and Cockayne syndrome (CS) had the unique XP DNA excision repair defect of XP complementation group B (Ann Intern Med 80:221, 1974). We have confirmed the diagnosis of a second patient (XP-SC-8) reported (Arch Fr Pediatr 35:65, 1978) to have both XP and CS whose cells we have previously shown not to be in XP complementation groups A-E or G (Clin Res 30:582A, 1982). We have now determined if skin fibroblasts (GM 3248) from XP-SC-8 are in the remaining XP complementation group F. Cultures were prepared of XP-SC-8 cells alone, of group F cells (GM 3542; XP3Y0) alone, and of XP-SC-8 cells fused with inactivated Sendai virus to the XP3Y0 cells. The cultures were then irradiated with 254-nm ultraviolet (UV) light and incubated at 37° with tritiated thymidine. Autoradiographs were then prepared. The XP-SC-8 cells had 30%, and the XP3Y0 cells had <10%, of the normal rate of DNA excision repair as determined by their UV-induced unscheduled DNA synthesis. The multinucleate cells obtained by fusing XP-SC-8 cells with the XP3Y0 cells resulted in complementation, since there was more UV-induced unscheduled DNA synthesis per nucleus in the fused cells than in the strains' unfused cells. Therefore, the XP-SC-8 strain is not in XP complementation group F. Since the XP-SC-8 strain complements all the currently known XP complementation groups (A–G), patient XP-SC-8 is in a new XP complementation group which we designate group H.

Different Anticellular Effects of Interferon on Non-Malignant and Malignant Epidermal Cell Lines. Miriam M. Brysk, Richard C. Newton, W. Robert Fleischmann, Jr., and Christina M. Fleischmann, Departments of Dermatology and Microbiology, University of Texas Medical Branch, Galveston, Texas

Recently a synergistic antitumor action was observed when virus type (IFN α/β) and immune (IFN-γ) interferons were used in combination. This potentiation was observed both *in vivo* and *in vitro*. Two paired sets of non-malignant and malignant carcinogen-exposed epidermal cell lines (JB-1 and JB-8; D-1 and D-11a) were exposed to IFN γ (50 μ/ml) and IFN α/β (500 μ/ml) used alone or in combination. Cell growth was measured in 3 day growth kinetic experiments. Treatment of non-malignant cell with each interferon alone had little or no effect on cell growth. Combination interferon treatment had a slight effect on the cells (about two fold inhibition). Treatment of malignant cells with IFN-γ or IFN α/β used separately had a small effect on cell growth (1.6–3.2 fold inhibition). In contrast, treatment of the malignant cells with combined interferons showed a dramatic inhibition of cell growth (20–30 fold) and even killed the malignant cells. The anticellular potentiating activity of the combined interferons appears to sharply differentiate between non-malignant and malignant epidermal cells.

The Loss of Beta-2-Microglobulin as a Marker of Malignant Epithelial Tumors. Maureen Olivier, Cary Goldsman, and

DEBA SARMA, Department of Dermatology, Tulane University School of Medicine, Department of Pathology, VA Medical Center, New Orleans, Louisiana

We examined twenty-eight formalin-fixed, paraffin-embedded skin samples to determine the usefulness of the loss of B_2 microglobulin as a marker of malignant epithelial tumors.

Specimens were de-paraffinized and stained for B_2 microglobulin by the use of the immunoperoxidase technique. Brown to black intercellular deposits in the adjacent normal epidermis of the biopsied specimens served as controls.

Three seborrheic keratoses, four keratoacanthomas, four actinic keratoses, four Bowenoid lesions, four squamous cell carcinomas, five basal cell carcinomas and three malignant melanomas were examined. All seborrheic keratoses were positive. All squamous cell and basal cell epitheliomas and malignant melanomas were negative. The remainder of the lesions demonstrated positive staining in only 50% of the specimens.

Our results confirm that there is loss of B_2 microglobulin from the cell surface of certain malignant lesions and there is positive staining of the clearly benign seborrheic keratoses. Variable staining in the low grade malignant and premalignant categories renders B_2 microglobulin as an unreliable method for differentiating borderline lesions.

Clinicopathological Correlation of Malignancy with Loss of Membrane B2 Microglobulin in Eccrine Porocarcinoma. C. A. HOLDEN[+], MARCIA SHAW°, P. MCKEE*, AND D. M. MACDONALD[+], [+] Dermatology Department, Guy's Hospital, ° Dermatology Department, St. Thomas' Hospital, * Histopathology Department, St. Thomas' Hospital Medical School, London, England

The absence of cell surface B2 microglobulin (B2M) has been shown to be a criterion of malignancy in epidermal neoplasms. Although rare, benign eccrine poroma may be difficult to distinguish histologically from its malignant counterpart. We have used a new chicken antibody against B2M to label fifteen tumours in a three step immunoperoxidase reaction on paraffin sections and correlated the degree of positive labelling with the histopathology and clinical behaviour of the tumour in the majority of cases. Two independent observers, unaware of the clinical history and histology, assessed coded sections for percentage positive labelling. A histopathologist examined blind the haematoxylin and eosin sections for evidence of malignancy. At the conclusion of the series the immunological, histological and clinical details were correlated. Three groups emerged on the basis of B2M positivity; 1 = 90% positive, 2 = approx 50% positive, 3 = 90% negative. Group 1 comprised five tumours, clinically and histologically benign. Group 3 contained six tumors all histologically malignant of which three cases had clinical evidence of malignant behaviour. The four tumours in group 2 were intermediate immunologically, but histologically were classified as malignant although there was no definite evidence of malignancy on clinical grounds.

Thus surface B2M was absent in those groups in which clinical evidence of malignancy was available and its loss correlated well with the histological criteria of malignancy.

T Lymphocyte Subsets in Skin, Blood and Lymph Nodes from Homosexual Men with Kaposi's Sarcoma. R. L. MODLIN, M. A. CONANT, C. R. TAYLOR, P. R. MEYER, F. M. HOFMAN, R. J. LUKES, J. W. PARKER, A. J. AMMANN, AND T. H. REA, Departments of Dermatology, Pathology and Pediatrics, University of Southern California, Los Angeles, and University of California, San Francisco

In order to evaluate the number and distribution of T lymphocyte subsets in skin, blood and lymph nodes from homosexual men with Kaposi's sarcoma (KS), monoclonal antibodies directed against T-helper/inducer (T_H) and T-suppressor/cytotoxic (T_S) cells were used with cytofluorometry and immunoperoxidase techniques. Greater than 90% of the infiltrating cells in nine KS skin specimens stained for factor VIII, indicating a vascular endothelial origin. The remaining cells were equally T_H and T_S, the total number of lymphocytes correlating inversely with disease extent. T_H:T_S ratio in blood and interfollicular areas of lymph nodes were determined:

Mean ± S.D. Helper/Suppressor Ratios:	Blood	Lymph Node
Controls (N = 21 blood, 7 lymphoid tissue)	2.1 ± 0.3	3.0 ± 1.2
Homosexuals with lymphadenopathy (N = 7)	0.6 ± 0.3*	0.7 ± 0.3*
Homosexuals with Kaposi's sarcoma (N = 8)	0.6 ± 0.4*	0.9 ± 0.3*

* $p < .001$

T_S cells were more numerous in germinal centers, mantle and interfollicular areas in lymph nodes from homosexuals with KS or lymphadenopathy as compared to controls. Furthermore OKT6+ and OKT10+ cells were more numerous in lymph nodes from these homosexuals as compared to controls. Thus the immune alterations in homosexual men with KS are associated with abnormal numbers and localization of T cell subsets in the core of the immune system—the lymph node.

Histogenetic Origin of Large Cell Undifferentiated Tumours in Lymph Nodes Using Leukocyte Common and Keratin Antibodies. W. J. CUNLIFFE, I. LAUDER, D. B. HOLLAND, AND G. GOWLAND, University Depts. of Dermatology, Pathology and Immunology, Leeds, U.K.

A not infrequent clinical-pathological problem is the erythrodermic patient with lymphadenopathy especially when the nodal histology reveals a large cell tumour of uncertain origin. It is not uncommon for there to be difficulty in distinguishing between an undifferentiated carcinoma and lymphoma.

To resolve this difficulty 43 cases with large cell undifferentiated tumours arising in such nodes have been studied by the immunoperoxidase technique using two antisera; one a monoclonal antibody to a leukocyte common antigen; the other of epithelial specificity raised against human plantar callus keratins.

A definite diagnosis with positive staining of tumour cells was obtained in 36 cases. The intensity of staining with the keratin antiserum showed 11 cases diagnosed as metastatic carcinomas were of squamous cell origin. In 15 patients (original diagnosis considered uncertain) a definite diagnosis was possible using a combination of the two antisera. In 2 cases a potentially major diagnostic error was detected. In the 7 cases with no staining of the tumour cells, strong contrasting staining of the residual lymphoid cells, inferred the tumours were not lymphoid in origin.

The two antisera provided a rapid accurate technique for differential diagnosis of large cell tumours presenting in lymph nodes thus ensuring the correct treatment was commenced quickly.

Oxy Radical Scavenging Enzymes in Human Epidermal Tumors. FERDINANDO SERRI, SILVIA BORRELLO, ANTONIO SECCIA, GIANNA M. BARTOLI, AND TOMMASO GALEOTTI, Inst. Dermatology and General Pathology, Catholic Univ. Roma, Italy

Aerobic cells possess protective mechanisms against the toxic effect of reactive oxygen and lipid peroxidation compounds which include enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). The study of oxidant- and radical-induced aging and cancer appears particularly suitable in the skin where intracellular generation of toxic species may be enhanced by radiation exposure. CuZnSOD and GSH-Px activities have been measured in human epidermis, in three epidermal tumors (basal-cell, intermediate and squamous-cell epitheliomas) and, for comparison, in a noncancerous epidermal hyperplasia, i.e. psoriasis. Epidermis exhibits low values (SOD: 23.0 ± 0.8 (10) μg/g wet weight; GSH-Px: 16.0 ± 2.6 (7) nmol/min/mg prot.) with respect to non-proliferating tissues. Tumor transformation seems to induce an additional reduction in SOD activity, as indicated by the lower values in squamous-cell (14.5 ± 2.2 (6) μg/g) and intermediate (16.2 ± 1.4 (5) μg/g) epitheliomas, whereas GSH-Px remains unchanged. SOD appears to be also lowered in psoriasis, although contamination of specimens by keratin squames may cause underestimation of the true content. The low level of scavenging enzymes in epidermis may establish a condition of pronounced vulnerability to life-span shortening and carcinogenic agents acting through oxy radical formation. Moreover, the SOD decline in epidermal tumors is likely correlated to the degree of malignancy, as suggested by the normal values of the enzyme in the more benign and non-metastasizing basal-cell epithelioma (24.7 ± 2.0 (3) μg/g).

The Sézary Cell—A Morphologically Distinct but Nonspecific Cell Type. A. C. CHU[+], D. ROBINSON[+], N. P. SMITH*, M. F. SPITTLE*, J. L. M. HAWK*, AND D. CATOVSKY[+], * St. John's Hospital for Diseases of the Skin and [+] Royal Postgraduate Medical School, London

Circulating Sézary cells were identified and investigated in 2 groups of patients: 1) 6 patients with erythrodermic cutaneous T cell lymphoma (CTCL) 2) 4 patients with chronic actinic dermatitis (CAD) of the actinic reticuloid varient. Sézary cells were enumerated on light microscopic examination of Wrights stained blood smears. T cell subpopulations were examined using the monoclonal antibodies OKT3 (pan T cell) OKT4 (helper) OKT8 (suppressor) in an indirect immunofluorescence technique. The ultrastructural morphology of the cells labelling with OKT4 and OKT8 was finally examined using an indirect immunogold technique. Sézary cells were identified in all patients, 37

± 15% in the CTCL and 30 ± 18% in the CAD groups. Major differences were found in the circulating T cell sub-populations in the 2 groups; CTCL group showed a high percentage of $OKT4^+$ cells (OKT4 71 ± 12.5%, OKT8 8.8 ± 5.7%, helper/suppressor 9.9 ± 3.9: 1) and the reverse was found in the CAD patients, with a high $OKT8^+$ population (OKT4 25 ± 4.7%, OKT8 73 ± 14%, helper/suppressor 0.36 ± 0.13:1). Immunoelectron microscopy confirmed that morphologically typical Sézary cells labelled with OKT4 in CTCL patients and OKT8 in CAD patients. The study demonstrates that although Sézary cells are morphologically distinctive, they are not specific for a single disease being a feature of benign (CAD) and malignant (CTCL) dermatoses, and are not restricted to a particular T cell sub-population.

Cutaneous T Cell Lymphoma—Diagnosis Using Monoclonal Antibodies Against Normal and Malignant T Cells. A. C. Chu*, C. L. Berger', R. L. Edelson', M. F. Spittle*, and N. P. Smith*, * St. John's Hospital for Diseases of the Skin, London, U.K. ' Dept. Dermatology, Columbia Presbyterian Hospital, New York, U.S.A.

The aim of the study was to examine the circulating T cells in patients with cutaneous T cell lymphoma (CTCL) for abnormalities that would be of diagnostic value. The T cell phenotype was assessed using the monoclonal antibodies OKT1, OKT3 (pan T cell), OKT4 (helper), OKT6 (cortical thymocyte, OKT8 (suppressor), OKT10 (common thymocyte), and BE1 and BE2 (reactive with tumour associated antigens on the surface of CTCL cells), in an indirect immunofluorescence technique. 13 patients with CTCL: 4 Sézary Syndrome, 3 advanced CTCL with organ involvement, 5 plaque stage and 1 early patch stage CTCL; 14 patients with various benign dermatoses: 10 chronic actinic dermatitis, 3 widespread psoriasis, 1 erythrodermic pityriasis rubra pilaris (PRP); and 15 normal adult controls were studied. The helper/suppressor T cell ratio (control 2:1) was elevated in patients with Sézary Syndrome (6:1), plaque stage CTCL (3:1), and PRP (6.2:1). T cells reactive with OKT6 and OKT10 (control 1 ± 1.5%; 5 ± 3.5%) were found in advanced CTCL (25 ± 24%; 48 ± 22%) and early CTCL (16%; 17%). In 12 of the 13 patients with CTCL, reactivity with BE1 (29 ± 23%) or BE2 (39 ± 14.5%) was observed, a feature not seen in the benign or control groups.

In this study, helper/suppressor ratio was found to be of little diagnostic value in CTCL. Abnormalities of T cell phenotype which allowed differentiation of CTCL from benign dermatoses were the presence of immature T cells reactive with BE1 or BE2.

The Intracutaneous Growth of Murine Lymphomas: Epidermal Invasion Is Characteristic of Multiple Tumor Phenotypes. M. Piepkorn and R. Tigelaar, Departments of Medicine, University of Utah, Salt Lake City, UT, and Dermatology, University of Texas Health Sciences Center, Dallas, TX

The affinity of lymphoid cells for epidermis known as epidermotropism is an important feature of mycosis fungoides (MF). Implied by studies indicating that MF may be a neoplasm of helper T lymphocytes is the possibility that epidermotropism is a phenotypic hallmark of select T cell subsets. We tested this proposal using 8 murine lymphoma lines of BALB/c origin: 3 histiocytic (FcR+, C3R+, Ig−, Thy 1−), 1 B cell (IgM+, FcR+, Thy 1−) and 4 T cell lines (Ig−, Thy 1+), including 1 with "helper" (Ly 1+23−), 1 with uncommitted (Ly 1+23+), and 2 with "suppressor" (Ly 1±23+) markers. The intracutaneous growth pattern of these sublines was studied on H&E-stained sections from samples through the centers of each tumor obtained at times after intradermal injection into parallel groups of mice. With the exception of a histiocytic line having a fibroblast-like growth pattern, all of the lymphomas manifested variable epidermotropism which followed a typical sequence. Following dermal growth and spread to the dermal-epidermal junction, tumor cells appeared within the stratum spinosum. Subsequently, collections of cells appeared in spaces within the epidermis (Pautrier-like abscesses) in tumors ≥2 cm in diameter, and eventually the epidermis necrosed. Tumors multiply passed in either skin or ascites fluid were equally epidermotropic. In this *in vivo* system, it is clear that intraepidermal growth so characteristic of MF does not correlate with cell surface phenotype. Although this model imperfectly represents the human disease, our observations are nonetheless consistent with recent studies using monoclonal antibodies to cell surface antigens which have demonstrated mixtures of lymphocyte subsets within the epidermis of MF.

Identification of Herpes Simplex Virus Antigens and DNA in Lesions of Mycosis Fungoides. L. A. Lee, J. C. Huff, B. J. Edmond, W. L. Weston, and D. A. Norris, Department of Dermatology, University of Colorado School of Medicine, Denver, CO

Chronic stimulation of the immune system by viral antigens has been postulated to be important in the pathogenesis of certain lymphomas, including the cutaneous lymphoma mycosis fungoides (MF). Because of the localization of herpes simplex virus (HSV) in the epidermis and reports of an increased incidence of HSV infections in MF patients, we examined lesional and uninvolved skin from 6 MF patients with early, untreated disease for evidence of HSV. Evidence of exposure to HSV was obtained by enzyme immunoassay (EIA) for herpes antibodies. Presence of HSV glycoprotein B (gB) in lesional skin was documented by focal specific staining within epithelial cells of epidermis or adnexa with anti-gB monoclonal antibody using an indirect immunofluorescent technique (IIF). The presence of HSV DNA in homogenates of skin biopsies was confirmed in preliminary studies by nucleic acid hybridization using a P^{32}-labeled HSV DNA probe. Results were as follows:

	EIA	IIF		Hybridization	
		Lesion	Uninvolved Skin	Lesion	Uninvolved Skin
# Positive/# Tested	5/5	6/6	0/5	3/5	0/5

HSV antigen and DNA have been found in lesional skin of MF patients with serologically-demonstrated prior exposure to HSV. We propose that HSV may be involved in the development of MF lesions.

Low Natural Killer Cell Activity and Insensitivity to Interferon in Cutaneous T Cell Lymphoma Patients. L. Laroche and D. Kaiserlian, INSERM U 25, Hôpital Necker, Paris, France

Natural killer (NK) cell activity is considered a major function of the host defense mechanism against tumors. Using the human myeloid cell line K 562 as the target cell, we determined the NK activity at different effector/target cell ratios of peripheral blood lymphocytes (PBL) from 23 cutaneous T cell lymphoma (CTCL) patients and controls. The ability of human leukocyte interferon (IFNα) to augment the cytotoxicity of NK cells was tested after an *in vitro* preincubation of the effector cells with IFNα. At the same time, the distribution of T cell subsets was studied using monoclonal antibodies of the OKT series in an indirect immunofluorescent technique.

NK activity was strikingly reduced in CTCL patients at all effector to target cell ratios in comparison with normal subjects. No difference was observed between leukemic (L) (having more than 10% circulating Sézary cells) and non L groups. This NK defect was insensitive to IFN action. In both groups, a T cell imbalance was observed consisting of an augmentation of the OKT4/OKT8 ratio which was more pronounced in the L group. The NK level was not correlated to the corresponding numbers of OKT4+ "helper" and OKT8+ "suppressor" lymphocytes.

These data indicate a similar dysfunction of the immune system involving non T, non B lymphocytes in L and non L CTCL patients. This NK defect could be related to the presence of a newly described type C retrovirus (HTLV) or the increased number of unrelated intercurrent infections. Whether this anomaly has a predictive significance in premycotic stages has yet to be tested.

Circulating Atypical Lymphocytes in Patients with Erythroderma. R. Willemze, J. Hermans*, W. A. van Vloten, and C. J. L. M. Meijer**, Departments of Dermatology, Medical Statistics* and Pathology**, University Hospital Leiden, The Netherlands

Demonstration of atypical cells in peripheral blood smears which is used to differentiate Sézary's syndrome (SS) from other forms of erythroderma, is neither an objective nor reliable method, since these cells have also been found in the peripheral blood of patients with inflammatory dermatoses. It was attempted to find more objective criteria which permit early diagnosis of SS. Therefore the absolute and relative numbers of lymphocytes, T and B cells, and the distribution of T-cell subpopulations as defined by Fc-receptors (Tμ, Tγ) and monoclonal antibodies (OKT3, 4, 8, HLA-DR) were investigated in 31 patients with erythroderma including 9 patients with SS, 4 with mycosis fungoides (MF), 5 with atopic dermatitis (AD), 8 with chronic eczema and 5 with psoriasis. In addition the degree of nuclear indentation (nuclear contour index; NCl) of at least 75 lymphoid cells was measured on EM-photographs. The mean NCl and the percentages of cerebriform mononuclear cells (CMC, defined by NCL ≥ 6.5) were calculated. Non-erythrodermic patients with MF (n = 8), AD (n = 5) and psoriasis (n = 5) and 12 healthy individuals served as controls.

Statistical analysis showed that the OKT4/OKT8 ratios, the mean NCl values and the percentages of CMC were significantly increased in the SS group as compared to all other groups. Since percentages of

CMC up to 19% were found in erythroderma on the basis of AD, chronic eczema and psoriasis, the presence of 20% CMC or more and/or the presence of cells with NCL ≥ 11.5, which were exclusively found in SS and some cases of MF, are proposed as differential diagnostic criteria. Characterization of the numbers of T, B, Tμ and Tγ cells had limited differential diagnostic significance.

Peripheral Blood Involvement in Skin Limited T Cell Malignancy. R. H. KELLER, S. SWARTZ, M. LYONS, J. TROY, AND R. E. JORDON, Research Service, Wood VAMC, Sections of Immunology and Dermatology; Dept. of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin

Mycosis Fungoides (MF) is considered a skin limited T cell malignancy. Although some patients with MF relapse after therapy and others progress to a leukemic phase, the factors associated with recurrent disease remain poorly defined. We therefore, examined the relationship of the DNA content and the percentages of T cell subsets from skin biopsies (S) and peripheral blood (PB) in this disorder using an EPICs5 flow cytometer (FCM). Eleven patients with histologically diagnosed MF and four patients with Parapsoriasis en Plaque (P) were studied and compared to normal controls (PB). In all MF patients studied, disease was limited to the skin by laboratory criteria including lymphangiogram and a Sézary cell screen of bone marrow and peripheral blood. In addition, the percentages and ratios of T helper and suppressor cells in the PB of all patients studied were within normal limits. Nonetheless, FCM analysis of the DNA/RNA content of PB lymphocytes demonstrated aneuploidy in 5/11 MF patients, 1/4 P patients but no evidence of aneuploidy in unstimulated or mitogen (PHA) stimulated control PB lymphocytes. In 3 of 5 MF patients standard karyotypic analysis confirmed the presence of aneuploidy but in 2 MF patients aneuploidy could not be confirmed by karyotypic analysis. Skin mononuclear cells in 2 MF patients and 1 P patient were also examined and each revealed an aneuploid DNA content. Finally one MF patient with PB aneuploid DNA content treated with Electron Beam (EB) therapy continues to demonstrate a PB aneuploid DNA and one patient who demonstrated aneuploidy after EB has progressed to a leukemic phase. These data suggest that PB involvement can be detected in MF patients by FCM and that PB aneuploidy correlates with prognosis.

Immature T Cell Lymphoma with Specific Cutaneous Involvement. E. FERRIER, L. LAROCHE, C. FRANCES, M. RAPHAEL, A. DELCOURT, F. GRAY, AND C. PENIT, INSERM U 25, Hôpital Necker 75015 Paris, France

Specific skin involvement is generally described in cutaneous T cell lymphomas (CTCL), Japanese adult T cell leukemias (ATL) and recently in peripheral T cell lymphomas (PTCL). We report unusual immature phenotypes observed in 3 mixed non Hodgkin's lymphomas with specific macular rashes. Two patients had limb pains related to lymphomatous infiltrations of nerve endings. Hemograms were normal in all cases.

Immunological studies were performed on peripheral blood lymphocytes (PBL) or lymph node cells, using monoclonal antibodies (Ab) of the OKT series and anti-terminal deoxynucleotidyl transferase (TdT) IgG in indirect immunofluorescent tests.

PBL from all patients reacted with OKT11 Ab (Ab to E receptors). In contrast, they bound very little to OKT3 (Ab to peripheral mature lymphocytes). TdT, negative in PBL but present in 80% of thymocytes, was identified in the two cases tested. Two patients exhibited the "helper" cell phenotype (OKT4+), none the "suppressor" one (OKT8+). Early thymic antigens, T6, T9, T10, were not expressed. Two cases bore Ia-like antigens. Similar results were obtained with a lymph node cell suspension in the one case tested.

These findings indicate that the 3 lymphomas were of thymic origin with leukemic phases. The phenotype of these lymphoma cells is less mature than those of CTCL and PTCL, corresponds to an intra-thymic differentiation level and brings to mind that described in ATL. Whether these T immature lymphocytes have a specific capacity to colonize the skin has yet to be tested.

SESSION C — South American Room
Howard P. Baden, M.D., Presiding

Precursor-Product Relationship Between Prekeratin and Keratin in Mouse and Human Epidermis. D. BREITKREUTZ, P. E. BOWDEN, R. QUINLAN, W. W. FRANKE, AND N. E. FUSENIG, German Cancer Research Centre, D-6900 Heidelberg, FRG

The relationship between prekeratin, the tonofilament protein of living epidermis and keratin, the filament protein of the stratum corneum is still a matter of debate. For clarification we compared the size, charge and peptide maps of individual prekeratin and keratin polypeptides from human and newborn mouse epidermis. Citric acid extractable prekeratin and urea-mercaptoethanol solubilised keratin were analysed by SDS-PAGE and 2D-electrophoresis. Single polypeptides were digested with V8 protease or trypsin and analysed in one- and two-dimensions. Two basic (68 K, 59 K; pI 6.5-8) and two acidic (57 K, 52 K; pI 5.2–5.6) prekeratin polypeptides (HPk) were identified in human arm, back and scalp epidermis while heel contained two extra acidic chains (65 K, 50 K). Human stratum corneum extracts contained three acidic keratin (HK) polypeptides (64 K, 57 K, 55 K) and again extra chains were found in the heel. The four HPk chains had unique V8 protease and tryptic peptide maps as did two of the HK chains. The same was true for the individual prekeratin (MPk: 67 K; pI 6.5–8 and 57 K; pI 5.2–5.6) and keratin (MK: 63 K, 61 K; pI 6–7 and 57 K, 56 K; pI 5–5.5) chains from mouse back skin. However, a high incidence of identity was found between certain prekeratin and keratin chains (eg HPk68/HK64, HPk57/HK55; MPk67/MK63, MPk57/MK56). In addition mouse and human chains of similar size and charge had similar peptide maps (HPk68/MPk67; HK64/MK63 etc.). It is concluded that prekeratin chains synthesized in the lower living epidermis are modified later in differentiation to smaller more acidic chains. This mechanism is conserved, being similar in mice and men, and may allow tighter packing of filaments in the horny cell.

Keratin Phosphorylation by Cyclic AMP-Dependent Protein Kinase. HITOSHI KOBAYASHI, OSAMU NEMOTO, KATSUKO UMEDA, TAKASHI AOYAGI, AND YUSHO MIURA, Department of Dermatology, Hokkaido Univ. School of Medicine, Sapporo, Japan

The phosphorylating system by protein kinases in living cells has been emphasized of its importance especially on the biological quick responses of cells to environmental changes via humoral transmitters. In the epidermal cells, only phosphorylase was clarified to be activated in the phosphorylating system by cyclic AMP-dependent protein kinases (cAMP-PK) through the adenylate cyclase-cAMP system. In the present study, keratin, which is the most abundant and important constituent of the skin, was focussed upon the investigation whether keratin could be a substrate protein of phosphorylation by protein kinases.

Firstly, living pig epidermal cells were incubated with the presence of ^{32}P-orthophosphate, and phosphorylated proteins were analized by SDS-polyacrylamide gel electrophoresis and autoradiography. In addition, dephosphorylation was monitored after the stop of the reaction. It was confirmed not only that keratin polypeptides were strongly phosphorylated but also that dephosphorylation process was present in living epidermal cells. Secondly, keratin phosphorylation was confirmed by cell free system with the presences of [γ-^{32}P]ATP, cAMP, partially purified epidermal cAMP-PK, and keratin extracted from pig epidermal cells. Furthermore, it was noticed that there existed the difference of phosphorylation potential among various strata of the pig epidermis, confirmed by both cell suspension and cell free system.

Keratin phosphorylation was confirmed both *in vivo* and in vitro. The different phosphorylation potential among strata might be related to epidermal cell differentiation.

Glycine- and Serine-Rich Regions of Epidermal Prekeratin Chains Are Major Phosphorylation Sites. P. E. BOWDEN AND R. QUINLAN, German Cancer Research Centre, D-6900 Heidelberg, FRG

Prekeratin, the citric acid soluble intermediate filament protein from epidermis, has a high content of glycine and serine. The component polypeptides vary in size (40–70 K) and charge (larger, pI 6–8 and smaller pI 5–5.6), and are phosphorylated at serine residues. In order to further characterise the site of phosphorylation, we have examined the proteolytic digestion products of the major chains of mouse and human epidermal prekeratin labelled *in vitro* with either ^{35}S-methionine, ^{14}C-glycine, ^{14}C-serine or ^{32}P-orthophosphate. Mouse and human epidermal prekeratin were isolated by acid extraction and fractionated by SDS-PAGE. Excised labelled polypeptides were digested with V8-protease and the peptides analysed by SDS-PAGE and visualized by silver staining and fluorography. Additionally, ^{32}P-labelled tryptic peptides were produced and analysed in two dimensions. The 68 K polypeptide was easily digested by V8 protease and rapidly gave four major peptides (10–15 K). The three smaller peptides were labelled with methionine while the larger was not. This peptide (15 K) was rich in glycine and serine and accounted for the majority of the bound ^{32}P-phosphate. After tryptic digestion several phosphorylated peptides were found.

The smaller acidic polypeptides were more resistant to V8-protease and gave rise to larger peptides (24–28 K). One of these (25 K) contained the majority of the glycine, serine and phosphate. Again several tryptic peptides were phosphorylated. The results were similar for both human and mouse prekeratin and demonstrate that the major polypeptides of this intermediate filament protein are phosphorylated in discrete regions which contain little or no methionine but are rich in glycine and serine.

Different Phosphorylated Sites of Pig Epidermal Keratin by Epidermal Growth Factor and Cyclic AMP. Takashi Aoyagi, Katsuko Umeda, Osamu Nemoto, Naoko Kato, Hitoshi Kobayashi and Yusho Miura, Department of Dermatology, Hokkaido Univ. School of Medicine, Sapporo, Japan

Epidermal growth factor (EGF) stimulate phosphorylation of several pig epidermal proteins one of which was pig epidermal keratin. Cyclic-AMP-dependent protein kinase also stimulated phosphorylation of pig epidermal keratin. In order to elucidate the role of EGF- or cyclic-AMP-stimulated phosphorylation of pig epidermal keratin, phosphoaminoacids of pig epidermal keratin were characterized by two-dimensional electrophoresis and autoradiography. Pig epidermal slices were ^{32}Pi-labeled in the presence or absence of EGF, and pig epidermal keratin was extracted by the method of Steinert. For cyclic AMP-dependent phosphorylation, pig epidermal keratin and cyclic AMP-dependent protein kinase were independently extracted by the methods of Steinert and Corbin, respectively. Pig epidermal keratin was ^{32}Pi-labeled in the presence or absence of cyclic AMP-dependent protein kinase. Pig epidermal keratin phosphorylated by EGF or cyclic AMP was hydrolyzed and analyzed by two-dimensional electrophoresis and autoradiography. EGF stimulated phhosphotyrosine ^{32}Pi-labeling while no phosphotyrosine was detected in the control. Cyclic AMP stimulated phosphoserine ^{32}Pi-labeling, but no phosphotyrosine ^{32}Pi-labeling was detected in the cyclic AMP-treated pig epidermal keratin. The overall results indicate that although both EGF and cyclic AMP stimulated phosphorylation of pig epidermal keratin, EGF stimulated phosphorylation at the tyrosine residue of pig epidermal keratin, and cyclic AMP stimulated phosphorylation at the serine residue of pig epidermal keratin.

Epidermal Keratinocyte Calmodulin: Shifts in Hyperproliferative Systems. J.A. Fairley, V.A. Hogan, C.L. Marcelo, Univ. Mich. Med. Schl., Ann Arbor, Michigan

We studied the presence and production of the calcium binding protein calmodulin (MW 16.7 K), in hyperproliferative systems in vitro (0.06 mM Ca^{++} culture conditions) and *in vivo* (psoriasis). Neonatal mouse keratinocytes were grown in medium containing 0.06 mM Ca^{++} to d7. At d7 1 μCi/ml of [^{3}H]-leucine was added to the cultures and half were switched to normal (1.2 mM) Ca^{++} levels. 24 hr later the cells were harvested. Cells were scraped into a final volume of 500 λ/Petri of 20 mM Tris HCl pH 7.5. Cells from 4–5 35 mm^2 Petri dishes were pooled. Calmodulin was extracted by a modification of the method of Iizuka (JID, 1982). The cells were sonicated then spun at 3000 g. The supernatant was boiled at 95°C for 5 min. and then spun at 10,000 g for 30 min. The resulting supernatant containing calmodulin was lyophilized and resuspended in 60 λddH_2O/Petri. The amount of protein present was measured by a modified Lowry protein assay. One dimensional polyacrylamide gel electrophoresis (PAGE) was done on each sample. PAGE verified the presence of a 16.7 K band. Isoelectic focusing showed a protein of the appropriate pl (≃4.0). The 16.7 K band was cut from the gels, dissolved in Protosol and counted in a scintillation counter. The 1.2 mM Ca^{++} cells showed a 87% increase in [^{3}H]-leucine labelling of the 16.7 K band compared to the hyperproliferative, low Ca^{++} cells. Involved and uninvolved skin was obtained from psoriatics by keratome. The cm^2 of skin removed and wet weight was measured. After snap freezing the skin was ground, then extracted for calmodulin similar to the cultures. After lyophilization the samples were resuspended in ddH_2O proportional to wet weights. PAGE showed the 16.7 K band much more prominent in the involved skin. Quantitation of these differences may show that calmodulin is involved in regulation of proliferation of the epidermis.

Decreases in Keratins and Cell Envelopes, and Increases in Keratohyalin Granule Proteins Occur in Low Calcium Regulated Keratinocytes. Cynthia L. Marcelo and Robert C. Gold, Univ. Mich. Med. Schl., Ann Arbor, Michigan

Neonatal mouse keratinocytes cultured in low (0.06 mM) calcium (Ca^{++}) show rapid growth and littler stratification when compared to cells grown in normal 1.2 mM Ca^{++} levels. The effect of low Ca^{++} on the amount and synthesis of noncovalently cross-linked and disulfide cross-linked keratins, of cell envelopes and of viable cell and keratohyalin granule (KG)-related proteins was studied. Primary cultures were prepared and grown at 32°C in low Ca^{++} M-199 + 10% FBS. Autoradiography of [^{3}H]-Tdr labeled cultures showed that by day 7, the labeling index was 45–50%; after 2 weeks, 16–20% of the cells were labeled. 3 μg/ml of retinoic acid and of the aromatic retinoid Ro 10-9359, and 10^{-8} M triamcinolone acetonide inhibited the low Ca^{++}-induced hyperproliferation. Cultures grown in low Ca^{++} M-199 (day 7) were pulse labeled with 10 μCi/ml [^{3}H]-leucine (2h) and left in low Ca^{++} M-199 or "switched" to normal Ca^{++} M-199 for 24 hr. The cultures were then serially extracted with a series of buffers (1M KPO_4; 1% SDS in PBS; 4 M urea + reducing agents) to yield 6 protein fractions. Comparison of the low Ca^{++} and "switched" cultures to normal Ca^{++} grown cells showed that: 1) the low Ca^{++} cells had approximately 25–30% of the normal Ca^{++} culture amounts of non-covalently (4M urea) and disulfide cross-linked (8M urea + reducing agents) keratins, and of cell envelopes while having 30% more KG-related protein and two-fold more viable cell proteins. [^{3}H]-leu labeling of the keratins was greatly decreased (10–20% that seen in controls), and was 70% higher in the viable cell, and 150% greater in the KG-related proteins. The switch to normal Ca^{++} for 24 hr had no effect. We conclude that 1) intracellular Ca^{++} levels can regulate cell envelope, keratin and KG-protein synthesis 2) KG-protein synthesis can be separately controlled from keratin and cell envelope formation.

Intermediate Filaments of Human Melanocytes *In Vitro* Contain Vimentin. Joseph Goodhouse, Fred Martin, Leonard M. Milstone, and Sidney N. Klaus, VA Medical Center, Dermatology Service, West Haven, CT.

Intermediate filaments, 9–10 nm in diameter, are characteristic ultrastructural features of mammalian melanocytes and are thought to be involved in dendrite formation, cell motility, and the intracellular movement of melanin granules.

The purpose of this study was to investigate the nature of these filaments in human and guinea pig melanocytes grown *in vitro*. Cell cultures from human foreskin and guinea pig ear skin were established on glass cover slips using Eagle's minimal essential media to which cholera toxin (1×10^{-10}M) was added. One to three weeks after culture, the cells were fixed *in situ* and incubated with either anti-vimentin antibodies raised in guinea pigs or anti-keratin antibodies raised in rabbits. The cells then were rinsed and incubated with fluorescein-conjugated goat anti-guinea pig IgG or sheep anti-rabbit IgG, respectively, and examined using a Leitz fluorescent microscope.

Human melanocytes were vimentin-positive and keratin-negative; keratinocytes were keratin-positive and vimentin-negative. Vimentin-positive filaments were identified in human fibroblasts and in Cloudman melanoma cells; however, guinea pig melanocytes were negative for both vimentin and keratin.

Vimentin-positive filaments in human melanocytes were distributed in the peri-nuclear region and extended into dendritic processes.

Protein Composition and Synthesis in Basal and Suprabasal Cells of Epidermal Keratinocyte Cultures. Philip S. L. Tong and Cynthia L. Marcelo. Dept. of Dermatology, Univ. of Michigan Medical School, Ann Arbor, Michigan

Proteins extracted from basal and suprabasal keratinocytes obtained from neonatal mouse epidermal cultures were analyzed for type, quantity and ^{3}H-leucine incorporation. Multilayered, stratifying (Day 6) and fully differentiated (Day 14) cultures were pulse-labeled with ^{3}H-leu (10μ Ci/ml) for 4 h, and cleanly separately into basal and suprabasal cells by incubation and agitation in a solution of 0.24M NH_4Cl, 0.1M 2-mercaptoethanol, 0.25M sucrose, pH 8.5 for 15 min at 4°C. Kreyberg staining showed that a full monolayer of basal cells free of differentiating cells remained firmly attached to the plastic dish. Both basal and suprabasal cells were analyzed for keratohyalin proteins, viable cell proteins (SDS soluble), keratin proteins (urea + reducing agents soluble) and envelope proteins. 5% (stratifying; D = 6) and 2% (differentiated; D = 14) of the total protein/culture was found in the basal cells. The major proteins in D = 6 and D = 14 basal cells were keratins and viable cell proteins. No significant amounts of keratohyalin and envelope proteins were detected in the basal cells at any time. The suprabasal cell protein contents were identical in both cultures: keratohyalin (2.6%), viable cell proteins (6%), keratin (82%), envelope proteins (9%). ^{3}H-leu incorporation data correlated with the protein data. In both cultures 6% of the total incorporated ^{3}H was found in the basal cells. The synthesis of keratin in the D = 14 basal cells was very curtailed

(60%↓) but was maintained at the same level as the D = 6 cultures in the suprabasal cells. SDS PAGE and fluorography showed that 4 keratins (47 Kd to 58 Kd) were present and synthesized in the basal cells, while the suprabasal cells had 2 additional bands at 64 Kd and 67 Kd. Similar studies will be useful in elucidating the site of drug action in the epidermis.

TCDD Stimulates Growth of Human Keratinocytes in Culture. LEONARD M. MILSTONE AND JANE LAVIGNE, Department of Dermatology, VA Medical Center West Haven, CT.

Epithelial hyperplasia and metaplasia are among the most characteristic responses to the toxic effects of TCDD (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin), the most toxic component of Agent Orange. However, these effects are both species-and organ-specific, making extrapolation from animal models to human toxicity questionable. Epidermal hyperplasia and chloracne are two known results of human exposure to TCDD. We, therefore, examined the effects of TCDD on morphology and growth kinetics in confluent cultures of human foreskin keratinocytes, cultivated and analyzed as previously described (Milstone et al., J Inv Dermatol 79:253–260, 1982). TCDD caused a dose-dependent (10^{-7}–10^{-11} M) increase in the intensity of staining with rhodonile blue and in the formation of swirled ridges. It caused an increase in the thickness of the multilayered epithelial sheet, an increase in ^{3}H-TdR incorporation, and, after one week of treatment, an increase in the rate of desquamation. During the first week of treatment, it prolonged the transit time of cells through the epithelium. We conclude that epidermal hyperplasia, one of the most obvious toxic effects of TCDD in humans, can be reproduced and studied in cultures of human keratinocytes.

Cell Membrane Receptors in Cultured Human Keratinocytes. J. GAZITH, M. T. CAVEY, B. SHROOT, D. CAVEY AND U. REICHERT, Centre International de Recherches Dermatologiques (CIRD), Valbonne, France.

Previously we have shown by binding measurements the existence of β-adrenergic receptors in isolated membranes from passaged human epidermal keratinocytes in culture. In this report we present evidence for the functionality of these receptors, based on the observation that β-adrenergic agonists activate stereospecifically membrane-bound adenylate cyclase in the presence of GTP or 5′-guanylylimido-diphosphate. This activation can be completely reversed by the addition of propranolol. The activation constants (K_a, the agonist concentration required to obtain 50% activation) were found to be closely correlated with the dissociation constants (K_d) from binding experiments (Table):

	L-Isoproterenol	L-Epinephrine	L-Norepinephrine
K_a (activation)	0.4 μM	1.7 μM	57.6 μM
K_d (binding)	0.6 μM	5.7 μM	70.7 μM

Using subtype specific antagonists, the β-adrenoceptors have been subclassified as a homogenous β_2-adrenergic receptor population.

The study has been expanded to other membrane receptors. Up to now, no specific binding could be measured with either α-adrenergic (α_1 or α_2 specific) or with histamine (H_1) antagonists. In the adenylate cyclase assay, no activation of the enzyme was found with histamine or with the prostaglandins $F_{2\alpha}$ and E_2. Phentolamine had no measurable effect on the activation of adenylate cyclase by norepinephrine.

We conclude from these experiments that plasma membranes of cultured human keratinocytes possess functional β_2-adrenergic receptors but do not have either histamine, prostaglandin or α-adrenergic receptors.

Kinetics of Cellular Proliferation and Differentiation in Exponentially Reproducing Cultures of Normal Human Keratinocytes. M. R. PITTELKOW, J. J. WILLE, JR., G. D. SHIPLEY AND R. E. SCOTT, Department of Dermatology and Cell Biology, Mayo Clinic/Foundation, Rochester, MN.

Utilizing an improved medium (MCDB 153) supplemented with hormones and bovine pituitary extract and some modifications of the techniques previously described[1] for the cultivation of normal human keratinocytes (HK), we have studied the long-term growth and population growth kinetics of primary and early-passage HK cultures derived from single specimens of both neonatal foreskins and adult mammary skin. Proliferative cultures of undifferentiated basal epidermal cells could be maintained in the absence of terminal differentiation for at least 20–30 population doublings by propagation in low calcium (100 μM) containing medium. In this medium both neonatal and adult skin basal cells exhibited an approximately 24 hr average generation time, independent of initial cell density, but reached a common limiting saturation density (3–4 × 10^4 cells/cm^2). Autoradiographic studies confirmed that >95% of the cells double their DNA content at the cell doubling rate. Cell cycle durations for the G_1, S and G_2 + M phases computed from flow microfluorimetric data on cells in steady state of exponential growth are: 12 hr, 8 hr and 4 hr, respectively. Induction of mature stratifying HK colonies could be achieved at any stage of batch culture growth by elevating calcium in the medium to 1.2 mM. (Supported in part by J. W. Kieckhefer Foundation, Dermatology Foundation and NIH grants AM 26783 and CA 28240.)

[1] Tsao et al., J Cell Physiol 110:219, 1982.

Ultrastructural Aspects of Human Keratinocyte Differentiation *In Vitro*. P.M. OLMSTEAD AND B.A. GILCHREST, Dept. of Pathology, University of Nebraska, Omaha, NE, and Dept. of Dermatology, Harvard Medical School, Boston, MA

To investigate the contributions of attachment surface and hormonal milieu to epidermal structure and differentiation, human foreskin keratinocytes from single primary culture plates were passaged and maintained both on a lethally irradiated 3T3 feeder layer in the presence of 20% fetal calf serum (FCS) and on human fibronectin (HFN) in serum free medium (SFM) supplemented only with hydrocortisone, epidermal growth factor, triiodothyronine, insulin, transferrin, and a bovine hypothalamic extract. On days 5, 9, and 14 paired plates were fixed and stained to determine colony size, counted to determine total cell number, and examined by transmission electron microscopy for ultrastructural markers of differentiation. In the presence of FCS and a feeder layer, keratinocytes plated at <10% efficiency, then grew progressively to near confluence with a 20–25 fold net increase in cell number by day 14. Colonies containing 3–7 cell layers and a few keratohyalin granules (KHG) in the superficial layers. In the presence of FSM and HFN, keratinocytes plated at 30–35% efficiency, attained near confluence by day 9 with a 15–20 fold net increase in cell number, and declined slightly in number by day 14. Compared to controls either from the same day or a similar degree of confluence, colonies of SFM were up to 4× thicker in cross-section, contained approximately twice as many cell layers, more extensive interdigitation of cell membranes, and more abundant KHG. Number and distribution of tonofilaments, mitochondria, and desmosomes were similar in both sets of cultures. These data establish that keratinocyte colony formation and ultrastructural differentiation proceed at least as well as in the SFM-HFN system as in the FCS-3T3 system and therefore do not depend on any unidentified serum or 3T3-derived component.

Disulfide Bonds in Amyloids of Skin-Limited and Systemic Amyloidoses. HIDEKI MUKAI, TAMOTSU KANZAKI, AND SHIGEO NISHIYAMA, Department of Dermatology, Kitasato Univ. School of Medicine, Sagamihara, Japan

Amyloids of skin-limited amyloidoses may contain a large amount of disulfide bonds (S-S), because it has been suggested to be derived from epidermal keratinocytes. We examined S-S and sulfhydryl groups (-SH) in skin-limited and systemic amyloidoses in frozen and paraffin embedded sections with a thiol reagent, N-(7-dimethylamino-4-methylcoumarinyl) maleimide (DACM). In frozen sections, amyloids of skin-limited amyloidoses contained a large amount of S-S in all cases (14/14) but no -SH (0/14) [macular (9), lichen (4) and skin tumor-associated (1) amyloidoses]. In contrast, amyloids of systemic amyloidoses contained no S-S or -SH (0/2) [primary and myeloma-associated amyloidoses (1 each)]. The identical results were obtained in paraffin sections, i.e., S-S were rich in all skin-limited amyloidoses (53/53), but no S-S in systemic amyloidoses (0/7) and amyloid tumor of tongue (0/2). Furthermore, intraepidermal amyloid-like deposits, observed by Congo-red and Thioflavin-T stains, were found in 27 of 67 cases of skin-limited amyloidoses but none in systemic amyloidoses (0/9). These intraepidermal amyloid-like deposits contained S-S in all cases (27/27) and -SH in 10 of 27 cases.

Thus we have shown that skin-limited amyloidoses can be differentiated from systemic amyloidoses by DACM methods and this appears to depend upon the quantitative difference of sulfur-containing amino acids in both types of amyloids, and that amyloid deposits were present in the epidermis in 40% cases of skin-limited amyloidoses and they contained -SH and/or S-S. Also, we have found that paraffin sections could be usable for DACM methods.

Present study further supports that amyloids in skin-limited amyloidoses are, at least in part, derived from epidermal keratinocytes.

Keratin Expression in Various Epithelia with a Monoclonal Antibody. JACQUELINE VIAC (1), ALAIN REANO (1), MARIE-JEANNE STAQUET (1), JEAN BROCHIER (2), JEAN THIVOLET (1). (1) U. INSERM 209, Pavillon, R, (2) U. INSERM 80, Pavillon P—Hôpital E. Herriot, Lyon, France

A monoclonal antibody against keratins (KL1) from normal human stratum corneum was characterized by its immunohistochemical staining of various epithelia and by its recognition of large keratin polypeptides (57 Kd and 63–67 Kd) using the immunoblot technique. Frozen and deparaffinized sections of normal human epidermis, mucosa and esophagus were stained by this antibody only in the upper cell layer as demonstrated by both indirect immunoperoxidase and immunofluorescence techniques. Approximately 70% of normal keratinocytes isolated after trypsinization were labelled by KL1 whereas most negative cells showed basement membrane zone antigens. All epithelial cells from other human epithelia (thymus, thyroid, bronchial mucosa, stomach, intestines) were positive with KL1. Tumoral cells derived from these epithelia were also positive with KL1. In numerous skin diseases (psoriasis, lupus, lichen planus, . . .) the reactivity pattern of KL1 was modified (e.g. staining of basal cell layer in epidermis; positive Civatte bodies in lichen planus). Epidermal tumors (warts, basal cell carcinoma, squamous cell carcinoma) showed modified reactions when compared with normal epidermis (weak staining pattern, reorganization of keratin filaments with granular aggregates). The reactivity pattern of KL1 was reduced on animal tissues. The epithelia of esophagus and buccal mucosa were negative except in the basal cell layer, no labelling was detected in thymus and intestines. However in most of the epidermis, all cell layers were positive. These results confirmed differences in the expression of high keratins (1) during the course of human normal and pathological differentiation; (2) between epithelial cells from different tissues.

A 48 K and 56 K Keratin As Molecular Markers of Epidermal Hyperproliferation: Monoclonal Antibody Analysis. ROBERT A. WEISS AND TUNG-TIEN SUN, Department of Dermatology, Johns Hopkins Univ. School of Medicine, Baltimore, Md., and Departments of Dermatology and Pharmacology, N.Y.U. School of Medicine, New York, N.Y.

Using 3 monoclonal anti-keratin antibodies, AE1, AE2 and AE3, we have shown that specific keratin classes can be correlated with different types and stages of epidermal differentiation. We have also shown that the AE1 antibody, which selectively stains the basal cells of normal human epidermis, produces a suprabasal staining pattern in psoriasis and a number of other epidermal disorders.

In order to better understand the changes of keratin expression in psoriasis, we prepared keratins from psoriatic epidermis and analyzed them by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and the immunoblot technique. Results from SDS-PAGE analysis confirmed earlier reports that in psoriasis the high molecular weight keratins (65–67 K) are diminished. Furthermore, 2 additional keratins (48 K and 56 K), which were not detected in normal epidermis, were readily detected in psoriasis. Immunoblot analysis showed that the 48 K and 56 K keratins were recognized by AE1 and AE3 antibodies, respectively. These two keratins were not specific to psoriasis but were detected in a wide variety of epidermal diseases including verruca, seborrheic keratosis, basal cell carcinoma and Bowen's disease. Furthermore, the same two keratins were detected in normal human epidermal keratinocytes grown in cell culture.

Our results suggest that the 48 K and 56 K keratins are expressed in a wide variety of epidermal proliferative processes and these two keratins may be regarded as molecular markers of epidermal hyperproliferation.

A Monoclonal Anti-Hair Keratin Antibody Recognizes Keratinocyte Cell Membrane Substance. HIKARU ETO, MITSUHIRO MATSUMOTO, TOSHIO TAZAWA, MASAAKI ITO, AND KEN HASHIMOTO, Dept. of Dermatology, Wayne State Univ. School of Medicine, Detroit, MI, VA Medical Center, Allen Park, MI and Dept. of Dermatology, Niigata Univ., Niigata, Japan

A murine hybridoma secreting IgG_1 monoclonal antibody, HK1, is produced by cell fusion between mouse myeloma cells with spleen cells from mice immunized with low-sulfur hair proteins. By Ouchterlony gel diffusion test, HK1 reacted with low-sulfur hair extract but did not react with epidermal keratin. In tissue survey, HK1 stained the cytoplasm and cell membrane of immature hair cortex cells, inner root sheath cells and immature nail cells. In normal human and mouse skin, HK1 unexpectedly stained cell membranes of epidermal keratinocytes. The staining was often dotted, suggesting that the desmosomes were heavily stained. Basal cells were not stained on the side facing the basement membrane. The staining was strongest at the upper spinous cell layers and there was no staining in the stratum corneum. Same staining pattern was observed in epithelial cells of oral mucosa and esophagus. Pre-incubation of normal skin with lectins (Con A, SBA, WGA, DBA, UEA, PNA, RCA and Osege orange seed lectin) or pemphigus IgG did not block HK1 staining. However, trypsin treatment (0.025%, 20 min.) abolished epidermal staining completely. A high-dose injection of HK1 to newborn mice did not produce pemphigus-like lesions. Immunoelectron microscopic finding suggests antigenic site is located on the keratinocyte cell membrane predominantly in desmosomal areas. These results indicate that HK1 recognizes trypsin-sensitive protein which is associated with cell membrane and particularly heavily located in desmosomes of stratified epithelium. It is interesting that HK1, anti-hair keratin antibody, does not react with epidermal keratin but with keratinocyte membrane substance.

Cytokeratin Polypeptides in Prenatal and Postnatal Epidermis. INGRID MOLL AND ROLAND MOLL, Department of Dermatology, Mannheim Medical School, University of Heidelberg and Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, FRG

Differentiation of epithelial cells is linked with specific patterns of cytokeratin polypeptides, the constituents of the tonofilaments. Cytokeratin patterns of microdissected epidermis from human fetal and adult skin have been analysed by two-dimensional gel electrophoresis of cytoskeletal proteins. Postnatal epidermis displays, irrespective of the stage of life, several cytokeratins (M_r 50,000–68,000) typical of stratified epithelia. In contrast, two-layered epidermis of 10 week fetuses contains major amounts of other cytokeratin polypeptides (M_r 40,000–52,500) typical of simple epithelia. These cytokeratins are gradually reduced and eventually disappear in multilayered epidermis of later fetal stages. Instead, cytokeratins characteristic of adult epidermis are detected and finally predominate. These include one large and basic epidermal cytokeratin (M_r 68,000) which has already been found in three-layered epidermis of 13 week fetuses. Different staining patterns obtained by indirect immunofluorescence microscopy using monoclonal cytokeratin antibodies with different specificities suggest that, in three-layered epidermis, different cytokeratin patterns might exist in the basal, intermediate and periderm layer.

Hormone-Induced Expression of Keratin Genes in Vaginal Epithelium. DENNIS R. ROOP, Laboratory of Cellular Carcinogenesis and Tumor Promotion, NCI, NIH, Bethesda, MD. 20205. MARK KRONENBERG AND JAMES H. CLARK, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

Using cDNA clones corresponding to the major keratins synthesized in newborn mouse epidermis of 55, 59 and 67 kilodaltons (kd), we have examined the expression of these keratin genes in rat vaginal epithelium. It was of interest to investigate the expression of keratin genes in vaginal epithelium since the degree of keratinization in this tissue is hormonally regulated. Total RNA was isolated from vaginal epithelium obtained from ovariectomized rats and at 24, 48 and 72 hr after exposure to estradiol benzoate. Northern blot analysis was performed on these RNAs using cDNA clones for the 55, 59 and 67 kd epidermal keratins as probes. On the basis of size and degree of homology, the mRNAs coding for these keratins in the internal keratinizing epithelium of the vagina appear to be similar if not identical to those synthesized in mouse epidermis. The concentration of the mRNAs coding for these keratins is very low in vaginal epithelium obtained from ovariectomized animals. However, 24 hr after exposure to estradiol, the concentration of the mRNA coding for the 55 kd keratin is dramatically increased to maximal levels. A moderate increase in the concentration in the mRNA coding for the 59 kd keratin is observed at this time with maximal induction occurring at 48 hr. The mRNA coding for the 67 kd keratin only becomes apparent 72 hr after exposure to hormone. The induced expression of these keratin genes after exposure to estradiol correlates with the observed morphological changes in this epithelium of stratification and keratinization.

Extrinsic and Intrinsic Regulation of Keratin Expression in Heterotransplants of Internal and External Epithelia of the Mouse. JÜRGEN SCHWEIZER, IAN C. MACKENZIE+, HERMELITA WINTER, AND MURRAY W. HILL, German Cancer Research Center, Inst. Exp. Pathology, Heidelberg. +Dows Inst. for Dental Research, Univ. of Iowa, Iowa City, Iowa.

Epithelial-mesenchymal interactions were studied considering keratin polypeptide expression in heterotypic recombinants of mouse epi-

thelia. Two series of heterotransplants using epithelia with distinctly different keratin patterns were investigated. a) footpad epidermis/ear dermis and ear epidermis/footpad dermis (external epithelia); b) cheek epithelium/palate dermis and palate epithelium/cheek dermis (internal epithelia). After separation with EDTA, the tissues were both homo- and heterotypically recombined and grown in vivo in subcutaneously prepared protected chambers. 30 days after transplantation, keratins were analyzed by one-and two-dimensional electrophoresis and by peptide mapping. In both series homotypic recombination had no influence on keratin expression. Likewise, the heterotransplants of external epithelia did not reveal any inductive stimulus on keratin polypeptide expression. In contrast, the keratin polypeptide synthesis of internal epithelia was clearly under mesenchymal control. Since these findings were accordingly reflected on the morphological level, the differentiation of certain epithelia can apparently be mediated by extrinsic mesenchymal factors.

Fibrous Protein of Hoof Matrix. HOWARD P. BADEN AND JOSEPH KUBILUS, Department of Dermatology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts.

Considerable data have been reported on the fibrous polypeptides of viable epidermis, but there are few such studies with hard keratins. This report describes the citrate buffer-soluble fibrous protein (FP) of hoof matrix cells and compares it with that of viable epidermis (prekeratin). Bovine hoof matrix was identified histologically, dissected out, and separated from the dermis by incubation in NH_4Cl. The tissue was extracted with citrate buffer, pH 2.65, and the FP isolated by precipitation at pH 7.0. Filaments prepared from this FP had a typical α x-ray diffraction pattern. SDS PAGE of matrix FP showed a number of bands and these were different from perihoof epidermal prekeratin. The matrix FP gave a precipitin line with an antibody to hair FP but not with one to epidermal FP. An antibody to matrix FP reacted with hair and hoof FP but not with epidermal FP. Amino acid analysis of matrix FP was similar to that of cornified hoof FP. When the matrix FP was converted to the S-carboxymethyl (SCM) derivative it gave a urea PAGE pattern identical to that of cornified hoof and hair SCM FP and different from that of epidermal SCM FP. Cultured matrix cells resembled cultured epidermal cells and the SDS PAGE pattern of the FP of both were identical. The FP of cultured matrix cells reacted only with an antibody to epidermal FP. These results indicate that the citrate-soluble FP in the viable layers of hoof shares some properties with epidermal prekeratins but demonstrates the unique characteristics of hard keratins. Matrix cells revert to typical epidermal keratinocytes in culture.

SESSION D — Pan-American Room
Ken Hashimoto, M.D., Presiding

D47: A New Monoclonal Antibody Reacting with the Secretory Cells of Human Eccrine Sweat Glands. JEAN KANITAKIS (1), DANIEL SCHMITT (1), ALAIN BERNARD (2), LAURENCE BOUMSELL (3), AND JEAN THIVOLET (1), (1) U. INSERM 209, Pav. R, Hôpital E. Herriot, Lyon. (2) Laboratoire d'Immunologie, Institut G. Roussy, PARIS. (3) U. INSERM 93, Hôpital St Louis, Paris, France

D47 is a new monoclonal antibody reacting with a surface antigen of human cortical thymocytes, different from the T6 antigen. It was obtained by immunization of mice with human cortical thymocytes. The known crossreactivity of monoclonal antibodies prepared against thymic cells (e.g. OKT6) with skin components prompted us to test D47 on human skin as well as mouse skin and thymus gland, using an indirect immunofluorescence technique. No labelling was observed on animal tissue. On human skin, D47 was found to react with the secretory portion of eccrine sweat glands, i.e. with a cytoplasmic antigen of the secretory cells lining the distal portion of the eccrine sweat gland glomeruli. No labelling was observed on apocrine sweat glands or on the excretory portion of eccrine sweat glands. D47 was also tested on an histologically proven extramammary (vulvar) Paget's disease; no staining of the malignant intraepidermal cells was observed. D47 appears to be an immunologic marker of the secretory cells of human eccrine sweat glands. In performing this function it appears to be of significant help in the investigation of human sweat gland-related pathology, with special reference to the origin of adnexal skin tumors showing glandular differentiation.

Effects of Direct Wounding on the Hair Follicle: Production of Increased Fiber Length. COLIN A. B. JAHODA, AND ROY F. OLIVER, Laboratoire de Zoologie, Université de Grenoble I, France and Department of Biological Sciences, University of Dundee, Scotland

A comprehensive investigation into the consequences of physical damage to the lower region of the rat vibrissa follicle was undertaken. Facial symmetry of the whisker pattern permits quantitative comparison with mirror image counterpart hairs. Terminal length measurements of unoperated vibrissa pairs revealed differences very rarely greater than 2 mm, or about 4%. Using microsurgical techniques, follicular damage was induced with sharpened tungsten needles. Experimental protocol involved 4 different wounding procedures on a total of 150 follicles. Whisker lengths were recorded for up to 300 days.

Where no follicular material was displaced operatively, 50% of experimental hairs were 4 mm or more longer than controls, with an average increase of around 15%. One individual produced a whisker 15 mm, or 30% longer than its counterpart. Removal of the epidermal matrix by hair plucking just prior to operations did not affect the proportion of longer than expected hairs, indicating that damage to the dermal papilla component was largely responsible for elongated fiber production. Histological studies revealed that fiber growth always required the presence of a renewed dermal papilla, however follicles which has produced long hairs did not display increased papilla cell numbers. Graphical representation of the data showed that longer whiskers were produced over extended growth periods but with unchanged or reduced rates of production. This work provides a rare demonstration of experimentally induced extension of hair length and growth period, while strongly implicating the dermal papilla in the regulation of these parameters.

Comparative Studies of Hair Dermal Papilla Cells and Skin Fibroblasts *In Vitro*. COLIN A. B. JAHODA, AND ROY F. OLIVER. Laboratoire de Zoologie, Université de Grenoble I, France and Department of Biological Sciences, University of Dundee, Scotland

The large size of the rat vibrissa follicle permits isolation of individual hair follicle components. This property was utilized to explant and serially cultivate vibrissa dermal papilla (DP) cells in vitro, after which their morphological, behavioural and proliferative characteristics were compared with rat skin fibroblasts. Cells were obtained from rats of the same age and sex, and cultivated at 37°C in MEM supplemented with 20% fetal calf serum. Comparative experiments were carried out at the same initial inoculum densities, and at matched passage numbers. The DP cells were found to have markedly slower replication rates, fewer cell numbers at confluency and less (^{3}H) thymidine incorporation. Moreover the two fibroblast populations could be distinguished morphologically and behaviourally, with the DP cells revealing a tendency to form clusters. These findings confirmed earlier observations on DP explant cultures.

This work reinforces the argument that skin is composed of heterogeneous fibroblast populations, and more specifically the behavioural characteristics of the DP cells relates to their specialized function in the hair follicle. The importance of this cell population is enhanced by the fact that cultured cells can be reimplanted into vibrissa follicles to test their capacity to induce hair growth.

Eccrine Gland Development in Fetal Skin: An Immunohistochemical Study with Carcinoembryonic Antigen (CEA) and S100 Protein. NEAL S. PENNEYS, RECIA KOTT, AND BILL BUCK, Departments of Dermatology and Pathology, University of Miami School of Medicine, Miami, Florida

CEA has been found in mature eccrine and apocrine gland acrosyringium, duct, coil and in eccrine sweat. S100 protein, an astroglial marker of uncertain function, has been found by immunohistochemical means only in scattered cells of the eccrine coil. Using these immunohistochemical markers, we studied the development of eccrine gland in fetal skin.

3 micron sections from formalin-fixed paraffin embedded histologic material from abortuses of varying gestational age were stained for CEA and S100 protein using the peroxidase-antiperoxidase method and rabbit antibodies to CEA and S100 protein. Positive controls were included in each experiment. The antibody control consisted of an omission of primary antibody and its replacement by nonimmune serum.

The detection of CEA in isolated cells in the epidermis is the earliest sign of eccrine gland differentiation and precedes morphologic changes. Small aggregates of CEA-containing cells form a nascent acrosyringium which grows downward to form a duct. CEA-positive material is present in the duct. S100 protein, on the other hand, is not detected in fetal epidermis, acrosyringium or duct. S100 protein-containing cells do appear in the primitive eccrine coil; the development of S100 protein-positivity does not appear to be related to the proximity of cutaneous nerves. We conclude that the biosyntheses of CEA and S100 protein in fetal skin is an integral part of eccrine gland development; the roles

that these substances play in this development are not defined. The immunoperoxidase technique is a powerful probe for investigating the development of adnexal structures in the skin.

Sub-picogram Analysis of Sweat Proteins Using Two-Dimensional Polyacrylamide Gel Electrophoresis. ROBERT W. RUBIN, AND NEAL S. PENNEYS, Departments of Anatomy and Dermatology, University of Miami School of Medicine, Miami, Florida

We analysed sweat collected from six normal individuals to determine if reproducible protein patterns could be obtained using two-dimensional polyacrylamide gel electrophoresis of ^{125}I-labelled sweat proteins. This method, coupled with fluorography and rare earth screen radioautography can reveal sub-picogram quantities of polypeptide.

Sweat samples, collected by droplet in a dry heat sauna at 100 C, were dialysed, concentrated, radio-iodinated, solubilized in 0.1% sodium dodecyl sulfate, and electrophoresed in two-dimensions in polyacrylamide gels. The polypeptide patterns were revealed by silver staining or by fluorography and rare earth screen radioautography of dried two-dimensional gels.

In our best preparations, 90 spots were revealed, seven of which were multiple charge isomers. For the most part, samples from different individuals showed little variation in the most prominent components. Relative amounts of each protein varied considerably from one individual to another. Very little high molecular weight material was present. There were no evidences of cytoskeletal proteins, indicating that the proteins in sweat have not leaked from lysing cells or come from whole cell contaminants in our preparations. Analysis of sweat protein patterns in samples obtained from two patients with cystic fibrosis revealed a different pattern from those seen in sweat of controls. This method of protein analysis will permit further assessment of sweat obtained from individuals with conditions such as cystic fibrosis for the presence of abnormal substances.

Eccrine Gland Differentiation of Trichilemmoma Cells *In Vitro*. TAMOTSU KANZAKI, HIKARU ETO, KOTARO TSUKAMOTO, KEN HASHIMOTO, AND SHIGEO NISHIYAMA, Departments of Dermatology, Kitasato Univ. School of Med., Sagamihara, Japan and Wayne State Univ. School of Medicine, Detroit, MI

Glandular cells occasionally differentiate to produce "domes" *in vitro*. Trichilemmoma, a pluripotent tumor, cell line cells produced ducts as well as "domes" *in vitro*. We investigated this organized differentiation of trichilemmoma cells *in vitro* morphologically, immunologically and biologically.

Light-microscopically, dome wall was composed of a 1 to 3 cell-layer, making a large cavity in the dome. Parts of some domes elongated to produce ducts. Electron-microscopically, dome wall was sealed with tight-junctions at the outermost ends of cells and secretory vesicles were observed in cytoplasm against the cavity. Thus morphologically these domes resembled eccrine glands or syringoma. Histochemically, phosphorylase-positive cells accumulated specifically in the dome wall. Immunologically, monoclonal antibody against eccrine glands was obtained from this cell line. Biologically, various chemical agents induced or suppressed dome formation. Epinephrine (2.5×10^{-5} M), Bt_2c-AMP (1×10^{-3} M), vasopressin (1 unit/ml) and oxytocin (0.2 unit/ml) induced domes 4 to 10-fold (from 2 to 8 to 20 domes/4 cm^2) in 12 hr. In contrast, ouabain (an inhibitor of sodium transport) (5.1×10^{-6} M) suppressed domes (from 4 to 0). Acetylcholine (1.4×10^{-4} M), pilocarpine (2.4×10^{-3} M) or atropine (3.7×10^{-5} M) did not make any effect on domes. Furosemide (Lasix) did not either.

These results suggest that trichilemmoma cells differentiated toward eccrine glands *in vitro*, these cells have receptors to epinephrine and pituitary hormones but not to cholinergic or anticholinergic agents, presumably sweat production is not regulated directly through these agents, and the differentiation is mediated by c-AMP. The key cells for this organized differentiation may be phosphorylase-positive cells.

Male-Female Difference in Sweat Rate: An Irreversible Effect of Androgen on Gene Expression. SHERNAZ WALTON, SAM SHUSTER, L. CHADWICK, PAT KENDALL-TAYLOR*, AND HENRY FIELD**, Depts. Dermatology and *Medicine, Univ. of Newcastle-upon-Tyne and **Dept. Psychiatry, St. Leonards Hospital, London, UK

Maximum sweat rate (MSR) is greater in men than women due to an increase in males at puberty[1]. If this is due to androgen MSR will be at female levels in men with prepubertal eunuchoidism and increase after testosterone (T). MSR was induced by iontophoresis of 0.2% pilocarpine and collected for 15 min on 3 cm paper discs[1] in airtight capsules from 10 men with prepubertal hypogonadism. 5 were untreated and 5 were receiving T. 4 of the former were then treated with T and MSR measured again after 1–12 months. MSR in the untreated eunuchs was 11.7 ± 1.5 mg/sq cm/15 min and no different from prepubertal boys and girls (corrected for surface area) and women[1] but after T they were 27.4 ± 3.0 as in normal males (27.7 ± 1.8). The MSR response to T occurred in the first few months of treatment and was related to pretreatment plasma T and its increase after treatment ($p < 0.01$). Thus the pubertal sex difference in MSR is due to androgen action in the male. If this action is by hormone modulation it should be reversed by removal of androgen. We therefore measured MSR in 8 sexual offenders with medical castration (100 mg cyproterone acetate daily for 1–8 years). Their MSR was 28.8 ± 4.0 which is no different from normal males (27.7 ± 1.8). We conclude that the pubertal action of androgen on sweat glands is by an irreversible switch action on gene expression. MSR is increased in cutaneous virilism (hirsutes)[2] and irreversible gene expression probably explains its poor response to potent antiandrogens.

1. Rees J and Shuster S. (1981) Clinical Science, 60, 689.
2. Shuster S. (1972) British Medical Journal, 2, 285–286.

Eccrine Gland Associated Antigens. A Demonstration by Monoclonal Antibodies. HIKARU ETO, MITSUHIRO MATSUMOTO, HITOSHI KOBAYASHI, AMIR MEHREGAN AND KEN HASHIMOTO, Department of Dermatology, Wayne State Univ. School of Medicine, Detroit, MI and VA Medical Center, Allen Park, MI.

Because of the difficulty to isolate human eccrine glands, production of antibodies against eccrine gland has been unsuccessful. Out of many hybridomas produced with trichilemmoma cell line (Cancer Res. 41, 2468, 1981) as antigens, hybridomas which secrete monoclonal antibodies, EKH5 and EKH6, were selected for this study. Both were found to recognize eccrine structures. EKH5 recognizes eccrine secretory portion in the normal skin and there is no staining in any other tissue components including apocrine structures. EKH6 recognizes eccrine secretory portion and coiled duct structures. No other structures were stained in the normal dermis except a weak staining of hair follicles and myoepithelial cells of apocrine gland. Immunoelectron microscopy revealed that both EKH5 and EKH6 antigens are located in the cytoplasm of secretory cells, predominantly distributed on the luminal side of the gland. When applied to syringoma lesions (2 cases), EKH6 stained syringoma and acrosyringium positively by indirect immunofluorescence and immunoperoxidase methods. This finding supports eccrine origin of syringoma. In sections of 6 eccrine carcinomas (4 porocarcinomas and 2 syringoid carcinomas), tumor cells were stained with both EKH5 and EKH6 antibodies by indirect immunoperoxidase method. These anti-eccrine gland monoclonal antibodies seem to be useful in the study of histogenesis and diagnosis of sweat gland tumors. Eccrine gland search in anhidrotic ectodermal defect may be another area of application.

The Extent of Epidermal Contamination on the Composition of Human Sweat As Studied Using a New Sweat Collector. THOMAS C. BOYSEN, SHIGERU YANAGAWA, FUSAKO SATO, AND KENZO SATO, Marshall Dermatology Research Laboratories, University of Iowa College of Medicine, Iowa City, Iowa.

In studying sweat gland function in diseases, it is imperative to minimize epidermal contamination of sweat. We modified the anaerobic sweat collection method to be applicable to thermally-induced sweat (A) and compare it with the conventional methods of sweat collection, i.e., plastic bag (B) and scraping (C) methods. Briefly, a plastic film, 15 cm × 17 cm, was glued to the back and the skin underneath the film was covered with vaseline and mineral oil (sweat collector). Sweating was induced in 6 men in a sauna at 95%RH and sweat samples collected from the sweat collector every 5 min for 40 min. Sweat protein was measured by the Lowry's method, calcium by cresophthalein method, cAMP by radioimmunoassay, urea by fluorimetric method, and cholesterol by the calorimetric method. Mean values for the first 10 min sweat samples are:

	Protein	Calcium	cAMP	Urea	Cholesterol
	(mg/dl)	*(mg/dl)*	*(pmoles/ml)*	*(mg/dl)*	*(mg/dl)*
Sweat A (bag c̄ oil)	25.3	1.04	0.19	31.0	1.00
Sweat B (bag s̄ oil)	40.4	2.76	0.53	41.4	0.92
Sweat C (scraped)	67.0	6.37	1.32	64.1	5.75

Sweat samples were further studied by immunodiffusion, immunoelectrophoresis and polyacrylamide gel electrophoresis. IgG was present only in scraped sweat. Since mineral oil itself does not affect composi-

tion of sweat and since evaporative water loss in sweat C is less than 30%, we conclude that IgG in sweat is an epidermal contaminant and that other components described above are also profoundly affected by epidermal contamination. The novelty of the new sweat collector will be a useful addition to the methodology of sweat gland research in disease.

Effects of Glucocorticoids on Dermal Proteoglycans. B. SÄRNSTRAND, R. BRATTSAND, A. MALMSTRÖM* AND T. KOBAYASI**, * University of Lund, Sweden; ** University of Copenhagen, Denmark

Glucocorticoids are known to suppress the formation of connective tissue components, especially proteoglycans. This study intends to evaluate the effects of 16α, 17α-Butylidenedioxy 11β,21-dihydroxy-pregna-1,4-diene-3,20-dione, budesonide, a potent glucocorticoid, on proteoglycan synthesis and distribution.

Fibroblast-like cells from human skin were treated with the steroid and then radioactive precursors ($^{35}SO_4^{2-}$ and ^{3}H-glycosamine) were added. One large and one small dermatan sulphate proteoglycan were isolated from the medium. The glucocorticoid induced a decreased content of both proteoglycans. Furthermore, the large glucuronic acid rich proteoglycan M$_r$250000) was more decreased than the small iduronic acid rich one (M$_r$100000).

Young rats (40 g) were treated topically with the steroid for two weeks. Decreased contents of water, fat, hyaluronic acid and sulphated glycosaminoglycans were found in the treated skin, whereas the collagen content was scarcely changed. For electron microscopy, the skin specimens, fixed in glutaraldehyde with cuprolinic blue, revealed compact bundles of collagen fibrils with sparse glycosaminoglycan figures. The collagen fibrils of the treated animals showed cuprolinic blue positive double bands with 73 nm intervals, compared with single bands with 67 nm intervals in the control animals.

In conclusion, proteoglycan contents in skin and cultures of human fibroblast-like cells decreased after glucocorticoid treatment. Proteoglycan constructions on collagen fibril surfaces were changed as found in the double cross-bands. It is likely that these changes influence the properties of the tissue.

Glycoprotein and Glycosaminoglycan Synthesis in the Human Epidermis. GLYN P. ROBERTS AND RONALD MARKS, Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN

Human epidermal glycoconjugates synthesized in organ culture have been characterized with respect to the radioactive sugars they incorporate, their lectin-binding properties and the effect of tunicamycin on their synthesis. Electrophoresis of labelled epidermal extracts showed that whereas D-(1-^{14}C) glucosamine was incorporated into glycolipids, glycosaminoglycans and glycoproteins, L-(1-^{3}H) fucose was incorporated almost exclusively (96%) into glycoproteins. Lectin-binding glycoproteins were detected by overlaying electrophoretic gels with ^{125}I-labelled lectins and by lectin affinity chromatography. Only 63% of the L-(1-^{3}H) fucose incorporated into glycoproteins was retained by ricin-Sepharose 4B and although the metabolically-labelled glycoproteins covered a wide molecular weight range (<14,300->200,000) the main lectin-binding glycoproteins occurred in the molecular weight range 70,000–160,000. Addition of tunicamycin (2 μg/ml) to the incubation medium inhibited the incorporation of D-(1-^{14}C) glucosamine into epidermal glycoproteins but did not decrease the incorporation into hyaluronic acid. Autoradiography of skin incubated with D-(1-^{3}H) glucosamine in the presence of tunicamycin was used to show that hyaluronic acid synthesis decreased in the upper layers of the living epidermis. In contrast autoradiography of skin incubated in the presence of L-(1-^{3}H) fucose showed that the incorporation of this sugar was increased in the granular layer. In conclusion, whereas most epidermal glycoproteins possess N-asparagine-linked oligosaccharide chains into which glucosamine, fucose and small amounts of sialic acid are incorporated, not all of these glycoproteins bind lectins. Glycoprotein synthesis occurs throughout the living epidermis and is increased in the granular layer but glycosaminoglycan synthesis is reduced in the upper layers.

Glycokeratins—Potential Extracellular Components of the Intracellular Cytoskeleton. A. VIDRICH, M. GILMARTIN, J. ZIMMERMAN AND I. M. FREEDBERG, Dept. of Dermatology, NYU Medical Center, New York, N.Y.

The epithelial cell line ME180 synthesizes at least two classes of keratin subunits. The major ME180 keratins, MEK-1 and MEK-2, are distinguishable immunologically and biochemically. Both are phosphorylated, though by different mechanisms. However, phosphorylation is not sufficient to account for the isoelectric variants of these keratins observed on two-dimensional electrophoretograms. Our data suggested that additional post-translational modification was occurring.

When ME180 cells were incubated in the presence of ^{14}C- or ^{3}H-labelled sugars, we noted incorporation of label into keratin subunits. By SDS-PAGE, MEK-1 was found to incorporate galactose, mannose and N-acetylglucosamine while MEK-2 incorporated glucosamine and galactosamine. Two-dimensional electrophoresis and peptide mapping confirmed that the ^{14}C-glucosamine was associated with the isoelectric variant pattern of MEK-2 and specifically with one of the characteristic peptides of this protein. The presence of carbohydrate moieties on keratin subunits implies a potential association of these proteins with the plasma membrane. To test this hypothesis, we subjected intact ME180 cells and a cytoskeletal preparation from ME180 to (^{125}I)-lactoperoxidase-catalyzed iodination. MEK-1 and MEK-2 were iodinated in both preparations. Two dimensional analysis of preparations from intact cells showed that all three isoelectric variants of MEK-2 were iodinated while only the most acidic variants of MEK-1 were accessible to the label.

Each result supports our conclusion that a specific subset of keratins is associated with the cell membrane, possibly penetrating it to reach the extracellular environment. This raises the possibility of the participation of keratin filaments in transmembrane signalling.

The Source of Fibronectin in Basal Cell Epithelioma. R. E. GRIMWOOD, J. C. HUFF, J. HARBELL, AND R. A. F. CLARK, Department of Dermatology, University of Colorado School of Medicine; Department of Clinical Investigation, Fitzsimmons Army Medical Center; Department of Medicine, National Jewish Hospital, Denver, CO

Basal cell epitheliomas (BCE) contain fibronectin both within tumor nodules and at the nodule-stroma interface (basement membrane zone). Fibronectin within or at the periphery of tumor nodules could be derived from the tumor cells, from entrapped or adjacent stroma or from plasma. The present study was designed to elucidate the source(s) of fibronectin within BCE nodules.

If stromal entrapment occurred to any great extent, vonWillebrand factor VIII stained blood vessels within tumor nodules should be evident by immunofluorescence techniques. Likewise, if plasma proteins were deposited in BCE, the tumor nodules should stain with fluorescein-conjugated anti-fibrinogen antibodies. Therefore, six basal cell epitheliomas were double labeled with rhodamine-conjugated anti-human fibronectin and fluorescein-conjugated anti-human factor VIII or fluorescein-labeled anti-human fibrinogen.

Fibronectin was present in a linear disposition along the margin of tumor lobules and as a fine filamentous deposit in the central portions of tumor tissue. There was no evidence of fibrinogen or factor VIII in any of the tumor lobules. Factor VIII was present in a granular pattern within blood vessel walls that coursed between tumor nests. An indirect immunoperoxidase technique using rabbit anti-human fibronectin and peroxidase labeled goat anti-rabbit IgE demonstrated that fibronectin within the central portion of the tumor lobules was closely associated with the tumor cells.

The absence of fibrinogen and factor VIII within the tumor tissue indicates that the fibronectin is probably not plasma or stroma derived while immunoperoxidase data suggest that fibronectin is a product of BCE cells.

Fibronectin Beneath Re-epithelializing Epidermis *In Vivo*: Sources and Significance. RICHARD A. F. CLARK, HENRY J. WINN, HAROLD F. DVORAK, AND ROBERT B. COLVIN, Departments of Medicine and Dermatology, National Jewish Hospital, University of Colorado Medical School, Denver, Colorado, Departments of Surgery and Pathology, Massachusetts General Hospital, and Department of Pathology, Beth Israel Hospital, Harvard School of Medicine, Boston, Massachusetts

Fibronectin and fibrinogen occur under the migrating epidermal tongue during re-epithelialization of an excisional wound, and fibronectin increases in conjunction with capillary and fibroblast ingrowth during wound healing. Although we have previously shown that fibronectin is produced by proliferatiang blood vessels, the source of fibronectin associated with re-epithelialization and fibroblast ingrowth has not been determined. In this report we demonstrate that subepidermal fibronectin derives from plasma early in re-epithelialization of an excisional wound while it comes from both plasma and *in situ* production

late in re-epithelialization, concomitant with the ingrowth of fibroblasts. This finding was established by extirpating 3 mm of skin from the center of a well healed rat xenograph on the flanks of immunosuppressed mice; harvesting the open wound sites at 2, 4, 7 and 10 days after injury; and staining the specimens with reciprocal species specific anti-fibronectin antibodies conjugated with fluorescein. In the first four days after wounding newly forming rat epidermis migrated over mouse fibronectin. In contrast, by 7 days after excision, the rat epidermis transits over a matrix containing both mouse and rat fibronectin indicating that a component of the fibronectin is produced *in situ*. Although the biologic significance of these observations has not been fully elucidated, fibronectin may be part of a provisional matrix that functions to support if not actively participate in cell recruitment to sites of inflammation or wound healing.

Fibronectin and Laminin Synthesis and Distribution in Prenatal and Neonate Rat Skin. J. R. Couchman, and W. T. Gibson, Unilever Research, Colworth Laboratory, Sharnbrook, Bedford MK44 1LQ, U.K.

Much *in vitro* data indicate the importance of extracellular matrix components, in particular fibronectin and laminin, in the control of cellular behaviour and organisation. We have, therefore, examined by immunohistochemical and biochemical methods, the synthesis and distribution of these glycoproteins in skin of late stage foetal and neonate rats. In the dermis, fibronectin staining is widespread, initially associated with mesenchymal cell surfaces. From day 19 of embryonic development onwards, however, a marked change to a fibrillar distribution of dermal fibronectin is apparent. Electron immunohistochemistry shows this to be collagen fibres periodically decorated by fibronectin. At the same time a population of laminin distinct from that of basement membrane zones (BMZ) appears in the upper dermis as a connective tissue component. This population of laminin is transient and persists only until approximately 2 weeks after birth. Histochemical and biochemical techniques confirm that neonate dermal fibroblasts synthesise laminin and have cell surface receptors for it; this being reflected in its ultrastructural distribution *in vivo*. It is apparent that although widespread in BMZ, laminin may also modulate dermal cell-matrix interactions at a time when rapid changes in skin structure occur. The roles of fibronectin, moreover, are not restricted to mesenchymal cells, for there is a population of this glycoprotein in the lamina lucida of BMZ including the dermal/epidermal junction. Cultured keratinocytes synthesise fibronectin indicating both a possible source of this protein in BMZ and important functions in epithelial cell behaviour.

Degradation of the Epidermal-Dermal Junction by Proteinases. Robert A. Briggaman, Norman M. Schechter, Jorma E. Fraki, and Gerald S. Lazarus, Depts. of Dermatology, Univ. of North Carolina, Chapel Hill, N.C., Univ. of Pennsylvania, Philadelphia, Pa., Univ. of Kuopio, Kuopio, Finland

The degradation of normal human skin was studied using the purified human polymorphonuclear leukocyte proteolytic enzymes cathepsin G (Cat G) and elastase (EL) and a chymotrypsin-like proteinase (HSChy) isolated from human skin. The latter proteinase appears to be from dermal mast cells. Fresh, split-thickness human skin (0.4 mm) was cut into 2–4 mm pieces and incubated with enzyme solutions (15 to 70 nM enzyme in Hanks' solution) for varying periods of time up to eight hours, after which specimens were examined in the electronmicroscope. In all cases, the primary damage observed was at the epidermal-dermal junction. EL produced complete destruction of the lamina densa (LD), leaving anchoring fibrils, dermal elastic microfibril bundles, hemidesmosomes and the basal cell plasma membrane essentially unaltered. Separation occurred in the plane of the destroyed LD. The chymotrypsin-like proteinases, HSChy and Cat G, produced epidermal-dermal separation in the lamina lucida, leaving the LD and other structures essentially intact. HSChy produced separation at lower concentrations than Cat G. All changes were time and enzyme concentration dependent, suggesting that they were the direct result of proteolysis. Controls containing proteinases inhibited by phenylmethylsulfonylfluoride showed normal morphology. The skin damage observed may have disease consequences since PMN and mast cells have major roles in skin pathology. These enzymes may also be important probes which help define the structure and function of human skin basement membrane.

***In Vitro* Synthesis of Basal Lamina by Epidermal Cells.** Takae Hirone and Shigeru Taniguchi, Department of Dermatology, Kanazawa University School of Medicine, Kanazawa, Japan.

In a previous paper (Biochemistry of Normal and Abnormal Epidermal Differentiation, University of Tokyo Press, 1980, pp. 159–169), we described that epidermal cells have the capacity to form basal lamina when grown on collagen gel *in vitro*. The present study was undertaken to see if epidermal cells could produce the basal lamina material *in vitro*. Human epidermal cell suspension was prepared by cold trypsinization, and plated into Falcon plastic dishes coated with type I collagen gel. Thin sections of resin-embedded cultures were examined by electron microscopy. Frozen sections of cultures were examined by indirect immunofluorescence using rabbit anti-type IV collagen anti-serum that was obtained from rabbits immunized by subcutaneous injection of purified type IV collagen emulsified with Freund's complete adjuvant. Electron microscopy of the cultures showed a newly-formed basal lamina along the basal surface of the cells. Indirect immunofluorescence with anti-type IV collagen antibody revealed a linear pattern beneath the cells. The evidence strongly suggests that the basal lamina containing type IV collagen is newly synthesized by epidermal cells.

Characterization of a Basement Membrane Matrix Synthesized by Cultured Human Microvascular Endothelial Cells. R. Kramer, P. Davison and M. Karasek, Depts. of Anatomy and Oral Medicine, Univ. of Calif., San Francisco, CA and Dept. of Dermatology, Stanford Univ., Stanford, CA

The basement membrane underlying vascular endothelium plays an important role in vessel integrity and permeability. Human microvascular cells in cell culture deposit a subendothelial matrix. We have studied the synthesis and the biochemical composition of the proteins in this basement membrane-like matrix.

Human microvascular endothelial cells isolated from adult human facial skin by trypsinization of split thickness skin were seeded at confluence on fibronectin-coated dishes and maintained for 3 weeks. Cells were fed with MEM containing 50% human serum, cholera enterotoxin, isobutyl methylxanthine, and ascorbic acid. Cell free matrices were isolated by extraction of the cell monolayters with hypotonic detergent.

Enzyme-linked immunosorbent assay (ELISA) detected the presence of basement membrane specific antigens including laminin, type IV collagen and proteoglycan in the isolated cell-free MEC matrices. SDS-polyacrylamide gel electrophoresis of solubilized matrices isolated from ^{35}S-methionine labeled MEC cultures demonstrated a number of high molecular weight radiolabeled proteins associated with the subendothelial matrix; a major 220,000 dalton, gelatin-binding polypeptide was identified as fibronectin.

These findings demonstrate that cultured human microvascular endothelial cells synthesize and elaborate four of the major constituents present in basement membranes *in vivo*. This model may prove to be useful in studies of the factors that control the synthesis of each of these proteins under conditions of normal and abnormal endothelial cell metabolism.

Proteoglycan Synthesis in Differentiated and Nondifferentiated Keratinocytes. Stanford I. Lamberg, Vincent C. Hascall, and Stuart H. Yuspa, Dept. of Dermatology, The Johns Hopkins Med. Inst., Baltimore, MD, and the NIDR and NCI, National Institutes of Health, Bethesda, MD

Proteoglycans (PG), which appear early in development and in healing wounds, may play a role in epidermal differentiation. Isolated, cultured keratinocytes stop dividing, stratify, and terminally differentiate in Eagle's medium with >1 mM Ca^{++} (HiCa), or they continue to divide, do not stratify, and may be subcultured in medium with 0.05–0.1 mM Ca^{++} (LoCa). By day 3 after plating, primary mouse epidermal cell cultures grown in HiCa accumulate 3 times more Sephadex G-50 excluded, [^{3}H]glucosamine-labeled macromolecules in the cells and 2 times more in the medium than do cultures grown in LoCa; after 24 hr of labelling, 75% of the newly synthesized macromolecules are cell- and 25% are medium-associated. Anion exchange chromatography (DEAE-Sephacel), Separose CL-4B elution profiles, and enzyme and nitrous acid susceptibilities reveal that 80–85% of the labeled macromolecules are glycoproteins (GP), and the rest are hyaluronic acid (HA) and chondroitin and heparin sulfate (CS/HS). The increase in incorporation of label in the HiCa cultures, compared to the LoCa cultures, takes place in both the GP, and the HA and CS/HS fractions. The change is most marked in the HA fraction. Cells plated and grown for 6 days in LoCa media and then switched into HiCa media also show increases in incorporation of label in all fractions, but again predominantly in HA. Increased synthesis of HA appears to be associated with differentiating epidermal cells, but GP(s) or PG(s) also may be involved.

Dystrophic Epidermolysis Bullosa: Ultrastructural Morphometry of the Dermo-Epidermal Junction. M. J. TIDMAN AND R. A. J. EADY, Dept. of Electron Microscopy, Institute of Dermatology, Univ. of London, London E9 6BX

A numerical and structural abnormality of anchoring fibrils (AF) is considered to be the basic defect in the dystrophic forms of epidermolysis bullosa (EB), although quantitative data are limited. Since AF are irregularly distributed in normal skin, a controlled morphometric study of keratinocyte-associated dermo-epidermal junction (DEJ) was undertaken in 7 patients with dominant dystrophic EB (DDEB) and 7 with mutilating recessive dystrophic EB (RDEB). Samples from non-scarred, non-blistered, unrubbed skin from the inner aspects of the thigh and lower leg were processed for TEM. A M.O.P. image analysis system was used to count hemidesmosomes (HD) and plasmalemmal vesicles (PV) along 40 μm lengths of basal plasma membrane, and AF along 40 μm of lamina densa (LD). To define AF, union with the LD was mandatory, and either fanning or banding was also required, thus excluding wisp-like structures normally seen beneath the LD. Compared with site-matched samples from healthy adults, no differences in HD and PV numbers were found in either DDEB or RDEB. No AF were detected in up to 200 μm lengths of DEJ from 9 technically suitable samples from RDEB patients, in the presence of a variable degree of collagenolysis. In 2 of the DDEB patients no AF were seen in either site. In the other 5, AF counts were markedly reduced, mean 11.7 ± 12.9 SD, compared with the normal of 71.8 ± 23.7 SD, although the AF appeared normal, arguing against a primary abnormality of AF structure. Analysis of variance showed no difference between predilection (lower leg) and non-predilection (thigh) sites. Further work is necessary to determine whether DDEB and RDEB can be reliably distinguished on ultrastructural criteria.

12:00 PM–2:00 PM ESDR Members Meeting Federal Room
No Formal Luncheon Planned

2:00 PM–5:30 PM CONCURRENT SCIENTIFIC SESSIONS

SESSION A Presidential Ballroom
Stephen I. Katz, M.D., Presiding

Comparison of the Mixed Lymphocyte Reaction (MLR) Stimulating Capacity of Human Epidermal Langerhans Cells (ELC) and Peripheral Blood Dendritic Cells (PBDC). R. D. SONTHEIMER, Department of Dermatology, University of Texas Health Sciences Center at Dallas, Dallas, Texas

We have reported that human epidermal cells (EC) are more efficient than autologous peripheral blood mononuclear cells (PBMC) in functioning as stimulators of the primary allogeneic MLR and that the ELC is the EC type that is responsible for this difference. Van Voorhis et al. (JEM 155:1172, 1982) have recently reported that 0.1–0.5% of human PBMC display a dendritic morphology on poly-L-lysine coated surfaces and that these HLA-DR positive PBDC are 10–30 times more active than are other leukocytes as MLR stimulators. In order to fully interpret our earlier observations, we have now compared the MLR stimulating capacity of ELC and PBDC. Stimulator cell populations consisted of EC suspensions prepared by trypsin disaggregation of suction blister tops from flexor forearm skin and autologous PBDC enriched fractions prepared from PBMC by the technique of Van Voorhis. The allogeneic PBMC blastogeneic response that resulted from co-culture with these two stimulator cell populatioins was measured at six days by tritiated thymidine incorporation. The PBMC enriched fraction which contained a percentage of dendritic cells that was similar to the percentage of ELC present in the EC suspensions (≅4%) produced a response that was equivalent to that produced by EC. Those fractions which contained a greater percentage of PBDC produced proportionately greater responses. These findings confirm the work of Van Voorhis and suggest that the enhanced MLR stimulating capacity of EC that we have previously observed can be explained by the fact that EC suspensions contain a greater percentage of HLA-DR antigen bearing dendritic alloantigen presenting cells than do unfractionated PBMC.

An Analysis of Ia^+ Antigen Presenting Cell and Accessory Cell Function in Cytotoxic T Lymphocyte Induction *In Vitro* Using Epidermal Langerhans Cells. M. IIJIMA, T. TSUCHIDA, H. FUJIWARA, H. PEHAMBERGER, S. I. KATZ, NCI, NIH, Bethesda, MD

Controversy exists as to the precise identification of cells subserving antigen presenting and accessory cell functions in many immune responses. Antigen presenting cells (APC) can be defined as cells which contain foreign antigen on their surface and thereby activate T lymphocyte responses, whereas accessory cells (ACC) are defined as cells which contain no foreign cell surface antigen but can restore the cytotoxic T lymphocyte (CTL) response in an Ia^+ adherent cell depleted culture system. Because most immunocompetent organs contain heterogenous populations of cells which are difficult to purify, we have utilized Langerhans cells (LC), which reside in the epidermis, which is devoid of classical macrophages or other lymphoid cells, in a study of APC and ACC functions. We determined whether murine LC can function as both APC and ACC in alloreactive and syngeneic TNP-modified CTL induction *in vitro*. LC exhibited potent Ia^+ APC function, comparable to spleen cells, in both allo-CTL induction and TNP-modified CTL induction assays. However, in contrast to spleen cells, when LC were syngeneic to responders in an Ia^+ adherent cell depleted CTL culture, they could not restore the CTL response in either assay. This was due, in part, to the suppression of CTL induction via secretion of prostaglandin E by epidermal cells. Even when prostaglandin synthesis was inhibited by indomethacin, Langerhans cells were unable to exhibit ACC function. Thus, LC can clearly act as Ia^+ APC but not as ACC in CTL induction, whereas splenic dendritic cells may subserve both functions. These data suggest a functional heterogeneity of Ia^+ APC in spleen and epidermis.

Role of Langerhans Cells and Indeterminate Cells in the *In Vitro* Generation of Alloreactive Cytotoxic T-Lymphocytes by Human Epidermal Cells. MICHEL FAURE, ANNE FRAPPAZ, DANIEL SCHMITT, COLETTE DEZUTTER-DAMBUYANT, JEAN THIVOLET, U. INSERM 209, Pavillon R, Hôpital E. Herriot, Lyon, France

OKT6 and BL2-anti-HLA-DR monoclonal antibodies (MCA) were used to determine which cells among normal human epidermal cells (EC) participate in the *in vitro* stimulation of human normal peripheral blood lymphocytes (PBL) in the Mixed-Skin cell-Lymphocyte culture reaction (MSLR) and in the *in vitro* generation of cytotoxic PBL (CTL). MSLR were conducted using stimulator EC (SEC) and either autologous or allogeneic responder PBL; PBL proliferation was measured by uptake of 3H-Thymidine over 18 hr after 5 days in culture. CTL responses were then tested in 4 hr 51Cr assays against autologous or allogeneic PBL targets (targets either autologous or allogeneic to SEC). MSLR and CTL assays were conducted with either untreated SEC, or complement (C′), OKT6 + C′ or anti-HLA-DR + C′ treated SEC and with SEC depleted in either OKT6+ or DR + EC after panning. Immunoelectron microscopy (IEM) of SEC after panning were used to identify OKT6+ and DR + EC. Data show that: (a) allogeneic, but not autologous MSLR lead to the generation of CTL; alloreactive CTL are only observed when SEC and responder PBL are, respectively, autologous and allogeneic to PBL targets; no CTL activity occurs without previous MSLR; (b) responses in MSLR and generation of alloreactive CTL are abolished after SEC treatment with anti-HLA-DR + C′, only significantly reduced after OKT6 + C′ treatment; results are similar when DR+ or T6+ depleted SEC are used; (c) by IEM, only Langerhans cells (LC) and indeterminate cells (IC) express HLA-DR, while LC but not IC are OKT6+. They indicate that generation of human alloreactive CTL occurs after MSLR and depends upon LC and IC among SEC.

Non-Langerhans Cells in the Keratinocyte Band in Culture Bear HLA-DR. W. D. GEOGHEGAN, R. E. JORDON AND R. H. KELLER, Research Service, Wood VAMC, Dept. of Medicine, The Medical College of Wisconsin, Milwaukee, WI, and the University of Texas Health Science Center, Houston, TX

A controversy exists over the presence of HLA-DR+ antigens on keratinocytes. Using FCM and cell sorting (JID 79:277, 1982) keratinocytes were sorted as positive cells together with Langerhans cells and subsequently found to be negative by fluorescence microscopy. We also examined epidermal cells for HLA-DR+ antigens using both fluorescence microscopy and FCM analysis (EPICS 5). Human foreskin epidermal cells were separated on Percoll. Bands 1.06 and 1.09 (1.04/1.06, 1.07/1.09 interfaces) were cultured 24 hr to 45 days in medium 199 without Ca plus 10% FBS. Cells on slides or in suspension were labeled with fluoresceinated mouse monoclonal anti HLA-DR (Becton Dickenson Clone L243). After 24 hr of culture 3% of the cells from the 1.06 band were mobil with morphology consistent with cultured macrophages. Of the remaining cells, 15–30% fluoresced faintly at 1250x but appeared negative at 500x. Cells (1.09 band) cultured for 9 days on slides exhibited speckeled and rim fluorescence at 500x. At 1250x more than 50% of these keratinocyte-like cells fluoresced. Pre-incubation with normal mouse serum or incubation of L243 with protein A did not

block fluorescence. This suggests specificity and non-involvement of Fc receptors. Quantitation of L243 binding to band 1.09 using FCM after suspension culture (24 hr to 8 days) revealed 13–81% HLA-DR+ cells with a log fluorescence modal channel range of 28–113, blocking with unlabeled L243 reduced modal channel values by 9–78%, preliminary cell cycle analysis using acridine orange reveals no correlation of cycling cells (SG_2M) with percent HLA-DR+ cells. Immunoelectron microscopy of HLA-DR+ sorted cells is in progress.

Evidence That Suspensions of TNP-Derivatized Langerhans Cells Promote, While Keratinocytes Impair the Induction of Contact Hypersensitivity in Syrian Hamsters. S. SULLIVAN, P. R. BERGSTRESSER, AND J. W. STREILEIN, Depts. of Cell Biol., Dermatology, and Int. Med., UTHSCD, Dallas, Texas

Based on circumstantial evidence with intact skin, it has been proposed that Langerhans cells (LCs) alone among epidermal cells present epicutaneously applied haptens in an obligatorily immunogenic fashion and thereby prevent tolerance. In an effort to provide confirmatory evidence, we have prepared single cell suspensioins from abdominal wall skin of inbred LSH Syrian hamsters. These epidermal cells were then derivatized with TNBS and subjected to isolation procedures using ficoll gradients. Unseparated derivatized epidermal cells (TNP-EC) contained 4–12% LCs as judged by ATPase staining. LC-depleted suspensions contained 1–6% LCs, and LC-enriched suspensions contained 38–44% LCs. Panels of adult LSH hamsters received intravenous inocula of crude, LC-enriched, or LC-depleted TNP-EC. Upon ear challenge with 1% TNCB 5 days later, only the LC-depleted recipients had failed to develop contact hypersensitivity (CH). When each panel was subsequently reimmunized with abdominal applications of 7% TNCB, recipients of crude and LC-enriched TNP-EC responded vigorously, but recipients of LC-depleted suspensions proved to be unresponsive. These data confirm that epidermal cells stand alone among cell types in their ability, when derivatized with haptens, to induce hypersensitivity rather than unresponsiveness upon intravenous inoculation into normal hamsters. Moreover, since this capacity copurifies with LCs, we conclude that it is this cell type which accounts for this activity. By implication, hapten-derivatized keratinocytes alone must be tolerogenic when injected intravenously.

***In Vitro* Effect of UV Light on Immune Function and Markers of Human Langerhans Cells.** JANUSZ CZERNIELEWSKI, MICHEL PRUNIERAS, Dept. of Cell Biology, C.I.R.D., Sophia Antipolis, 06565 Valbonne, France

In the Mixed Skin Cell Lymphocyte Culture Reaction (MSLR) epidermal cells (EC) induce the proliferation of peripheral blood lymphocytes (PBL). It was also shown that this EC stimulating ability depends on HLA-DR and T-6 antigens bearing cells. We studied here in an *in vitro* system the presence of DR and T-6 antigens and alloantigen presaenting capacity of Langerhans cells (LC) after UV irradiation. EC were obtained from surgical biopsis by trypsin digestion and cocultured either directly with allogeneic PBL (controls) or after irradiation with 10 or 20 mJ/cm2 UV light (wave length 280–400 nm). Responder (R) PBL and stimulator (S) EC were cocultured (R:S ratio 1:1) for 5 days: PBL proliferation was measured by 3H-thymidin uptake. EC from fresh EC suspensions as well as 3 and 5 day-cultures were studied. In some cases EC were UV irradiated and then cultured for 3 days, after which they were used in MSLR. In each case an indirect immunofluorescence staining using monoclonal antibodies (MCAB) anti-HLA-DR and OKT-6 was performed.

	OKT-6	DR	PBL 1	PBL 2	PBL 3
non irrad. EC*	++	+++	47,172 ± 6,276**	55,656 ± 3,279	83,721 ± 9,721
10 mJ/cm2 irrad. EC	++	+++	32,791 ± 3,337	24,279 ± 5,123	43,429 ± 4,853
20 mJ/cm2 irrad. EC	++	+++	29,469 ± 2,511	28,157 ± 3,927	44,857 ± 7,156

** mean ± SEM of cpm; *EC epidermal cell suspension.

No changes were observed after UV irradiation in the number of stained cells. On the contrary we demonstrated the decreased lymphocyte stimulating ability of previously irradiated EC (30–50%). However, this immunologic function was restored after 3 days of EC culture following irradiation. These results suggest that under these conditions the UV induced defect was essentially functional and did not change membrane antigen expression (T-6 and DR).

Cell Membrane Damage of Langerhans Cells by Ultraviolet Light. DAVIDE IACOBELLI, SHOZO TAKAHASHI, AND KEN HASHIMOTO, Department of Dermatology, Wayne State University, Detroit, and VA Medical Center, Allen Park, MI.

Controversy exists in the literature on the effect of ultraviolet light (UVL) on epidermal Langerhans cells (ELC). Some investigators claim that UVL diminishes ELC population or volume, while others believe that it only depletes ELC surface markers. These discrepancies may be due to differences in wave length and dosage used and also to inadequate ultrastructural examination of membrane damage. In an attempt to clarify this issue we assessed the minimal erythema doses (MED) in the UVL-B and UVL-A spectra on albino guinea pigs (GP) and exposed them to increasing doses (1 MED to 6 MED) of λ310, selected for its maximal erythemogenicity. Biopsies were obtained after 24h from the irradiated areas and control sites. Dermo-epidermal separation using EDTA and ATP staining were done for the light microscopy (LM) specimens, whereas whole skin was processed for electron microscopy (EM). LM studies showed that one MED reduced the stained ELC to approximately 45% of the control population. Gradual increase of irradiation up to 3 MED further decreased the ELC (65%). Morphologically the ELC became round and lost their dendrites. These changes progressed up to 3 MED, then reached a plateau. The EM studies showed that the ELC from irradiated areas had high degrees (60–70%) of cytomembrane damage when compared to the control counterpart. Since ATP positivity is due to the functioning of the ATP-ase loci located on the surface of the ELC membrane, we suggest that the decrease in number of ATP positive cells and their morphological changes as observed by LM studies are due to the UVL induced disruption and fragmentation of the ELC cytomembrane.

Timing and Dosage of Ultraviolet Radiation Determines Whether Human Epidermal Langerhans Cells Allostimulatory Capacity Is Enhanced or Inhibited. K. D. COOPER, P. A. FOX, AND S. I. KATZ, Dermatology Branch, NCI, NIH, Bethesda, Md

The potential for modulating cutaneous immune responses by ultraviolet radiation (UVR) has been demonstrated in several animal systems. In order to determine, in humans, the effects of UVB on the alloantigen presenting capacity of Langerhans cells (LC), skin was irradiated *in vivo* (volunteers) with 1–4 minimal erythema doses (MED) or *in vitro* (using foreskins) with the equivalent of 1–8 MED. Epidermal cell suspensions (EC) were prepared either immediately after UVR or 24 hours later and incubated for 7 days with allogeneic mononuclear leukocytes (MNL) or with HLA-Dr, OKM1 marker depleted MNL. The resulting epidermal lymphocyte reaction (ELR) was assessed by ^{3}HTdR incorporation. Pretreatment of EC with a combination of monoclonal anti HLA-Dr and Leu-6 antibodies plus complement totally abolished the ELR, confirming the essential role of LC in the ELR. When EC were prepared immediately after 2 MED *in vivo* or after 2, 4 or 8 MED *in vitro* and used in the ELR, we found statistically significant decreases over a broad range of EC concentrations (42 ± 11% ↓ with 2 MED *in vivo*; 50 ± 2% ↓ with 2 MED *in vitro*; 63 ± 16 ↓ with 4 MED *in vitro*). By contrast, in the in vivo system, EC prepared 24 hours after 2 or 4 MED resulted in statistically significant enhancement of the ELR at low (126 ± 58% ↑ with 2 MED; 72 ± 32% ↑ with 4 MED) and high (176 ± 114% ↑ with 2 MED; 135 ± 43% ↑ with 4 MED) EC concentrations. The enhancement of the ELR did not correlate with the percentage of LC in the EC. The finding of both enhancement and inhibition of the ELR show that UVR modulation of cutaneous immune responsiveness requires a precise understanding of the effects of dosage and timing of the UVR.

An Analysis of Murine and Human Epidermal Cell Suspensions by Means of Immunoperoxidase Staining of Lymphocyte and Langerhans cell Antigenic Markers. GEOFFREY ROWDEN, BISHNUPRIYA MISRA, DANIEL MIKOL, HOWARD HIGLEY, Department of Pathology, Loyola Medical Center, Maywood, IL., USA

Information concerning the numbers of immunocytes in mammalian epidermis is fragmentary. Langerhans cell (LC) numbers have been determined by ATPase and Ia staining of separated epithelial sheets. No reliable estimate of the lymphocytic component of normal epidermis exists. We carried out an investigation of normal epidermal specimens from a series of mouse strains. A comparison was made to human skins using equivalent antibody markers. Epidermal cell suspensions were prepared by means of trypsin separation from the dermis and dispension into single cells. The viability of the suspension was assessed and found to be in excess of 95%. Antigenic markers for Langerhans cells such as Ia antigen and TL antigen (HLA-DR and T-6) as well as S-100

protein were stained by indirect immunoperoxidase on fixed and unfixed cells. Similarly, the lymphocytes were stained for surface immunoglobulin (B cells) and T cell markers such as Thy-1, Lyt-1, Lyt-2, (T-3, T-4, T-5). Cell counts were carried out to determine the percentages of all cell types present in the normal epidermis. Langerhans cells were found to comprise between 4–6.5% of the epidermal population in both murine and human situations. The lymphocytes were almost exclusively of the T lineage with between 3 and 6% of the epidermal suspension stained with a discrete speckled appearance. Redistribution and some capping of stain was noted in fresh preparations. Double staining with fluorescein and rhodamine-labeled reagents demonstrated that separate populations of Ia and T cell marker-positive cells existed.

Isolation of Trypsin-Resistant Mouse Epidermal Cells with Characteristics of Langerhans Cells. G. B. SCHULLER-LEVIS, J. L. ROE, A. K. FOWLER, AND K. B. HELLMAN, N.Y. State Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y and NCDRH, FDA, Rockville, MD

Studies on the characterization and function of Langerhans cells (LC) have been hindered by the inability to purify and separate them from other cells in the epidermis. We have isolated a homogeneous population of trypsin-resistant epidermal cells (EC) from newborn mice. The cells express LC-like characteristics which are similar to those found in other cells of the monocyte/macrophage series. These characteristics include adherence, receptors for Fc-IgG, ATPase activity and phagocytosis of both latex particles and opsonized sheep erythrocytes. In addition, we have shown that the trypsin-resistant epidermal RE-like cells secrete lysozyme and interferon. The ultrastructure of these cells shows striking similarities with the reported ultrastructure of LC with the exception of Birbeck granule. Ultrastructural features which the EC, LC in suspension, and cultured peritoneal macrophages have in common, include numerous mitochondria and lysosomes and a ruffled plasma membrane, characteristic of phagocytic cells. Ultrastructural analysis indicated that the trypsin-resistant epidermal cells did not show desmosomes or tonofilaments, which are characteristic of keratinocytes and, furthermore, they lacked melanosomes, which are characteristic of melanocytes. We conclude that these EC represent a homogeneous population of epidermally derived macrophages. The relationships between subpopulations of macrophages, dendritic cells and LC may be elucidated by further characterization and functional studies of epidermally derived homogeneous populations.

Anatomical Mapping of Epidermal Langerhans Cell Densities in Adults. BRIAN BERMAN, DENNIS S. FRANCE, VIRGINIA CHEN, WARREN I. DOTZ AND GINA PETRONI, Department of Dermatology, Mount Sinai School of Medicine, N.Y., N.Y.

The densities of T6 antigen-bearing Langerhans cells (LC) in 112 biopsies of human skin from 16 surgical outpatients and 4 cadavers were determined with respect to 8 anatomical regions. After 30 minutes incubation in 1N NaBr at 37°C, resultant epidermal sheets were stained with FITC-conjugated mouse monoclonal anti-human T6 antigen IgG1, and dendritic cells enumerated in at least 20 1000x fields under epifluorescence. The regional mean densities of epidermal LC/mm^2 (SEM) were: Head and Neck, 489 (27); Chest, 466 (22); Back, 466 (11); Arms, 458 (25); Legs, 431 (30); Buttocks, 411 (11); Genitalia, 298 (45); Soles, 58 (12). No statistical differences were found between any of these LC densities except for that of the soles which was significantly lower than those of all other regions ($p < 0.002$). No significant differences were detected between the mean body LC densities of patients and cadavers, of 11 Caucasians and 9 Hispanics or of 9 males and 11 females. Bisected epidermal sheets from the backs of 10 additional healthy female adults were examined in parallel for T6 and Ia antigenicity. Anti-Ia immunofluorescence detected significantly fewer LC than were detected by the anti-T6 method ($p < 0.001$), with the mean density of Ia$^+$ LC being 46.4% (2.0) that of the density of T6$^+$ LC. These control studies may aid in the interpretation of possible regional alterations in LC densities in disease states.

Assessment of Langerhans Cell Quantification in Tissue Sections. J. J. HORTON, M. H. ALLEN, D. M. MACDONALD, Laboratory of Applied Dermatopathology, Guy's Hospital, London, England

Numerous reports of altered Langerhans cell (LC) numbers in cutaneous disease have been based on cell counting in histological sections. However little is known of variation of LC numbers according to sex, age, anatomical site or between individuals.

The validity of cell counting on sections was assessed by examining the variability of counts in differing anatomical sites and in different individuals at the same site and the reproducibility of two of the most commonly employed markers of LC was also examined.

From six age and sex matched volunteers, elliptical biopsies were obtained from trunk, arm, leg, dorsum of hand and foot. LCs were identified in three stepped sections from each biopsy by ATPase reactivity and by reactivity with the monoclonal antibody OKT6. The sections were subjected to Quantimetric analysis to give an accurate count of LC per millimetre of basement membrane.

OKT6 labelling proved to be more consistent than the ATPase reaction in LC identification and was employed for statistical evaluation. Significant anatomical variation and significant variation between subjects at the same site was found on counting. However significant differences existed between stepped sections from the same biopsy.

The variation of LC counts between individuals and different anatomical sites, renders invalid, random enumeration in tissue sections of cutaneous disorders. Furthermore, although the cell identification techniques, which are well established in our laboratory, are reliable in terms of visible labelling, reproducibility of LC counting by these methods in histological sections cannot be supported.

Human Musocal Langerhans Cells—Post Mortem Identification of Regional Variations Within the Oral Mucosa, TROY E. DANIELS, University of California, School of Dentistry, San Francisco

Langerhans cells (LC) within human oral mucosa may have biological roles similar to those in skin, but identification of LC in mucosae—which are up to 10 times thicker than skin—poses technical problems and, their frequency is unknown. Therefore, ATPase histochemistry was modified to identify mucosal LC and its specificity confirmed with indirect immunofluorescence using monoclonal antibodies OKT6 and anti-human Ia. Specimens from 8 intraoral sites were obtained from 9 autopsies, 8–20 hours post mortem, excluding cases that had received antemortem immunosuppressive therapy. Mucosa from clinical oral surgical procedures provided controls. Epithelial sheets were prepared after incubation in EDTA, and cryosections were cut from intact mucosa. LC morphology in sections stained by ATPase, OKT6 and anti-Ia techniques was the same in postmortem and clinical specimens, and the 3 methods stained the same cell population. Average LC counts on ATPase-stained sheets from the 8 oral mucosal sites ranged from 160–550 LC/mm^2. Cell counts of 160–220/mm^2 were from thick epithelia with the greatest keratinization (gingiva and hard palate), while counts of 470–550/mm^2 were from the thinnest, least keratinized epithelia (soft palate, ventral tongue, lip, and floor of mouth). In 3 epithelia, the frequency of LC was highly variable: in the dorsal tongue LC occurred on only one side of the filiform papillae; in the hard palate there were regions without LC; and in the buccal mucosa, epithelial LC clustered around connective tissue papillae and their numbers ranged from 130–650/mm^2, apparently depending on the degree of keratinization. LC can be identified in oral mucosa up to 20 hours post mortem. The number of LC in nonkeratinized oral mucosa is approximately the same as in skin but keratinized oral mucosa has fewer LC. The frequency of oral mucosal LC varies inversely with the degree of keratinization. There are regions of the oral mucosa which do not have LC.

Ultrastructural Immuno-Gold Staining of Langerhans Cells. DANIEL SCHMITT, MICHEL FAURE, COLETTE DEZUTTER-DAMBUYANT, AND JEAN THIVOLET, U. INSERM 209, Pavillon R, Hôpital E. Herriot, Lyon, France

Colloidal gold particles are well suited as marker in electron microscopy. Indirect immuno-gold staining was used to identify cell membrane antigens defined by monoclonal antibodies (MCA) on lymphocytes and Langerhans cells (LC): OKT3 (specific for all circulating T cells) OKT6 (which stains LC) and BL2 (monomorphic MCA specific for HLA-DR Ag) were used. Lymphocytes were isolated from blood of healthy donors. LC were obtained from normal human epidermis through trypsinization (trypsin 1:250, 1 hr 30′, 37°C) and subsequent panning (enrichment in LC of OKT6 or BL2 preincubated cells on goat anti-mouse Ig coated dishes). After fixation in 2% periodate-lysine paraformaldehyde (30 mn, +4°C), cells were incubated with MCA (1 hr, 37°C, working dilution 1:5). After washing in PBS (15′ × 2), cell were incubated with the colloidal gold labeled secondary antibodies (1 hr, 37°C, working dilution: 1:3, goat anti-mouse Ig labeled with 20 nm size gold granules: GAM 20, Jansen Pharma., Beerse, Belgium). After washing in PBS (15′ × 2) cells were fixed for 30 mn in 1% OSO4, dehydrated and embedded in epoxy medium. Thin sections were contrasted with uranyl and lead. By this method, the plasma membrane of all circulating T cells shows a specific labeling with OKT3 and a few percent with BL2, recognized by the presence of small evenly distributed gold granules. LC show a regular distribution of T6 antigenic sites revealed

by the gold labeling. Birbeck granules were not stained. This immunogold reaction is also suitable for double marking experiments using homogeneous populations of gold granules having different sizes. For example T6 and DR antigens of LC can be simultaneously located with 20 and 40 nm gold granules respectively in TEM.

Derivation of Langerhans Cell Granules from Cytomembrane. SHOZO TAKAHASHI AND KEN HASHIMOTO, Department of Dermatology, Wayne State Univ. School of Medicine, Detroit and VA Medical Center, Allen Park, MI

The origin and function of Langerhans Cell (L-cell) granules have not yet been clarified. There are two conflicting theories; one claiming cytomembrane derivation and the other Golgi origin. OKT-6 monoclonal antibody has previously been shown to react selectively with L-cell cytomembrane. In this study we used OKT-6 and immunoperoxidase method to stain L-cell granules. The epidermis was separated with 0.02 M EDTA in PBS and incubated in MEM containing OKT-6. After incubation and rinse the tissue was fixed in 4% paraformaldehyde (PFA) containing 0.05% saponin for 5 minutes. Saponin was used to make the membrane permeable to large molecules of IgG-peroxidase conjugate. The tissue was further fixed in 4% PFA. Frozen sections (6–10 μm) were cut and reacted with anti-mouse IgG conjugated with peroxidase. After peroxidase staining 6 μm and 10 μm sections were processed for light microscopy and electron microscopy respectively. Controls were run in the same manner, without OKT-6. L-cell cytomembrane were specifically stained: other cell types were not. In intact cells L-cell granules attached to cytomembrane and adjacent to it were stained. When the membrane of the L-cell was broken by saponin, L-cell granules in the cytoplasm were also stained. Serial sections revealed that OKT-6-positive intracytoplasmic granules were not connected to the cytomembrane. The granules were stained only on the delimiting membranes and not in the center of the granule. Golgi apparatus and related membrane structures were not stained. In control specimens L-cell granules were not stained. These findings suggest that L-cell granules are derived from invaginations of L-cell membrane and retain the antigen to react with OKT-6 even after moving into the cytoplasm.

SESSION B — Federal Room

Malcolm Greaves, M.D., Presiding

Activation of Phospholipase A_2 and C in Murine Keratinocytes by Phorbol Ester 12-O-Tetradecanoylphorbol-13-acetate. C. I. GALEY, V. A. ZIBOH, C. L. MARCELO AND J. J. VOORHEES, Departments of Dermatology, University of California, School of Medicine, Davis, CA and University of Michigan Medical School, Ann Arbor, MI

Although the production of prostaglandins (PGs) after the topical application of membrane perturbing tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) has been described, the source of phospholipids that are hydrolyzed to provide arachidonic acid (AA) for PG-biosynthesis has remained unknown. To characterize the phospholipases and to determine the specific phospholipids (PLs) which release AA we labeled Balb C mouse keratinocytes (1.6×10^8 cells) in serum-free Medium 199 with both [^{3}H]AA (5.0×10^6 cpm) and [^{14}C] stearic (3.3×10^6 cpm). Following a 60 min. incubation at 37°C the cells were centrifuged for 5 min. ($250 \times$ g), washed twice and resuspended in serum-free medium. The labeled cells (1×10^6 cells) were incubated with TPA (100 ng) for 60 min. at 37°C, followed by immediate centrifugation for 5 min. at $250 \times$ g. Labeled lipids were extracted from the cell pellet and the alteration in cellular phospholipids evaluated by TLC chromatography and by counting the ^{14}C in the scintillation counter.

Our data showed (i) that more ^{3}H-AA (90%) than ^{14}C-ST (10%) was incorporated into the major keratinocyte PLs (PC, PE, PI/PS) although the percentage distribution of ^{3}H-AA and ^{14}C-stearic (ST) in PLs) were similar; (ii) a challenge of the [^{3}H]AA labeled cells with TPA resulted in a marked decrease in ^{3}H from PI/PS fraction (83%). Decreases were also observed in PE (55%) and PC (33%) PL fractions. These data indicate that TPA induces the hydrolysis of AA from keratinocyte PLs via the activation of membrane phospholipase A_2 and C. The role of PI/PS turnover in TPA function in particular deserves further studies.

Epidermal Arachidonate Lipoxygenase. THOMAS RUZICKA, EDDA TÖPFER-PETERSEN, MORTON P. PRINTZ, Department of Dermatology, Univ. of Munich, FRG, and Division of Pharmacology, Univ. of San Diego, La Jolla, California, USA

Arachidonic acid (AA) derived products are believed to play a central role in cutaneous inflammation. Therefore, we investigated AA metabolism in guinea pig skin. Homogenates of epidermis and dermis were incubated with 14C-AA for 20 min at 37°C, the products extracted, separated by thin layer chromatography (TLC) and measured on a TLC scanner. The main product of AA comigrated on TLC with 12-hydroxyeicosatetraenoic acid (HETE), and its formation was completely suppressed by ETYA and slightly enhanced by indomethacin. Reverse phase high pressure liuqid chromatography confirmed its identity as 12-HETE. The lipoxygenase was localized in the epidermal layer. Susceptibility to inactivation by sulfhydryl reagents indicates that an essential SH-group is present in a hydrophobic region of the molecule. The enzyme shows a broad pH optimum and a K_m of 2.48×10^{-5} M. The cytosolic enzyme has been partly purified by ammonium sulfate precipitation, Sephadex G-150 and DEAE-Sephadex gel chromatography. It exhibited an apparent high molecular weight, confirmed by electrophoresis in native polyacrylamide gel, and a requirement for an essential divalent cation. The lipoxygenase and hydroperoxidase activities were resolved by anion exchange chromatography. The epidermal 12-lipoxygenase differs from the platelet enzyme in its different susceptibility to SH-reagents and the higher molecular weight. Its role in cutaneous physiology and pathophysiology remains to be elucidated.

Lipoxygenase Products of Arachidonic Acid in Inflamed Skin. R. M. BARR, S. D. BRAIN, A. K. BLACK, R. D. CAMP, M. W. GREAVES, A. I. MALLET, AND E. WONG, Institute of Dermatology, London, England, C. N. HENSBY, CIRD, Valbonne, France

Previously we found increased levels of arachidonic acid and prostaglandins (PG) in inflamed human skin. In this study we measured the monohydroxyeicosatetraenoic acids (HETES) and leukotriene B_4 metabolites of arachidonic acid in normal skin after UV-B irradiation and in the uninvolved skin of psoriatics after topical anthralin treatment. Exudate was collected from suction bullae on control and inflamed abdominal skin and analysed for HETE and PGE_2 by GC-MS and LTB_4 by bioassay.

12-HETE and PGE_2 were raised at 24 hr but not at 72 hr after UV-B irradiation: control and 24 hr values were 14 & 42 ng/ml ($p < .05$, $n = 6$) for 12-HETE respectively, and 5 & 30 ng/ml ($p < .001$, $n = 6$) for PGE_2. 5-HETE levels were below the detection limit of 2 ng/0.1 ml exudate for all samples. Anthralin treatment raised PGE_2 levels from 23 ng/ml in control exudate to 62 ng/ml ($p < .02$, $n = 6$) at 24 hr before declining to base levels at 72 hr. However, 12-HETE was raised at 72 hr (192 ng/ml, $p < .05$, $n = 5$) but not at 24 hr (85 ng/ml) compared with control levels of 50 ng/ml. Six exudate samples (0.6 ml total) at each time point were combined but 5-HETE was still below the limit of detection. Levels of LTB_4 in control and inflamed skin exudates were low ($\leq$100 pg/ml) and close to the limits of the assay. Small increases observed were not significant.

The increased levels of arachidonic acid found in inflamed skin can be metabolised by both the cyclo-oxygenase and lipoxygenase pathways. It is probable that the lipoxygenase product 12-HETE is involved in mediating these inflammatory reactions.

Altered Release of Prostaglandins from Human Epidermis and Dermis in the Early Phase of Inflammation. W. SCHALLA, C. HENSBY, A. CIVIER, J. CAMBROU, L. JUHLIN AND H. SCHAEFER, Centre International de Recherches Dermatologiques, Sophia Antipolis, Valbonne, France

We have studied the kinetics of PGD_2, and $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ (hydrolysis product of prostacyclin) release from human skin after stimulation with UV or IR irradiation and after inhibition with indomethacin using the superfusion method. Fractions (15 & 30 min) were collected and analysed for PG's after extraction with ethyl acetate and purification by t.l.c. and finally analysed by quantitative GC-MS.

No difference was detected between the epidermal and dermal levels of PGD_2 and $PGF_{2\alpha}$ (about 40 pg/cm-2/min-1) whereas the 6-oxo-$PGF_{1\alpha}$ release was only one third of that released from the dermis (25 pg/cm-2/min-1). This suggests that in the skin the 6-oxo-$PGF_{1\alpha}$ is mainly derived from blood vessels. After stimulation by UV a dose-dependent increase in $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ liberated from the epidermis was found. This increase started at a dose of ½ to 1 MED (minimal erythema dose), was more pronounced at 3 MED and could already be seen in the first 15 min fraction after irradiation. A similar increase could be induced by a high dose of IR. The production of prostaglandins was nearly completely inhibited immediately after adding indomethacin (10^{-5} M) to the superfusion system. The changes seen after irradiation appear not to be related to membrane damage since

the LDH activity was not influenced. The time course of the prostaglandin production and their close relationship to erythema formation indicates that they may be important in the very first steps of the inflammatory response of human skin.

Elevated Levels of Arachidonic Acid and Prostaglandins D_2, E_2, $F_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ in Tuberculine Delayed Hypersensitivity. J. M. PADILLA†, C. N. HENSBY*, B. SHROOT*, A. CIVIER* AND J. P. ORTONNE+, *Department of Biochemistry, Centre International de Recherches Dermatologiques, Sophia Antipolis, Valbonne, France and +Laboratoire de Recherches Dermatologiques, Hôpital Pasteur, Nice, France

Various inflammatory stimuli (UVB, Trafuril) increase the levels of prostaglandins in human skin. The purpose of our study was to determine their involvement in chronic inflammation of the skin due to tuberculine delayed hypersensitivity (TBDH), which can be considered one of the better models of delayed hypersensitivity reactions. Suction blisters were raised on TBDH induced lesions 24 and 48 h after intradermal injection of 10 units tuberculine in 100 μl 0.9% saline and also on normal control skin. After extraction and preparative TLC the exudates were subjected to quantitative GC-MS for the determination of arachidonic acid and prostaglandins D_2, E_2, $F_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$. The results (ng ml^{-1}) are given for AA and PGE_2 in the table. They support the involvement of arachidonic acid and its metabolites in the pathogenesis of tuberculine delayed hypersensitivity.

	Control	Tuberculine challenged	
		24 hr	48 hr
Arachidonic acid	2234 ± 115	4199 ± 420***	7110 ± 574***
n =	(27)	(5)	(14)
PGE_2	17.7 ± 0.9	36.4 ± 3.5***	52.9 ± 5.0***
n =	(28)	(6)	(16)

Values are mean ± S.E.M.
*** $p < 0.001$ relative to control.

Histamine and Bradykinin Induced Prostaglandin $F_{2\alpha}$ Release and Increased Vascular Permeability in Mini-Pig Skin. C. N. HENSBY*, A. CHATELUS*, T. J. WILLIAMS†, I. WILLIAMS‡, P. JOSE‡, F. VUILLE*, A. CIVIER* AND B. SHROOT*, *Centre International de Recherches Dermatologiques, Valbonne, France, †Dept. of Pharmacology, University of Bath, England, ++Dept. of Pharmacology, Royal College of Surgeons, London, England

In addition to the direct measurement of individual mediators of skin inflammatory responses, it is important to know what, if any, are the pharmacological interactions between different classes of mediators. We have examined two pharmacological interactions between histamine, bradykinin and prostaglandins, known mediators of various skin inflammatory reactions, in mini-pig skin, an animal with inflammatory responses very similar to man. The superfusion study clearly demonstrated a dose related stimulation in both histamine and bradykinin of mini-pig skin cyclooxygenase, as determined by the measurement of $PGF_{2\alpha}$ release from mini-pig skin superfused in the presence and absence of these agents. The permeability study demonstrated that PGE_2 (0.3 nmoles) could synergistically increase the dermal vascular permeability to ^{125}I labelled human serum albumin caused by both histamine and bradykinin. In addition, the vascular permeability due to histamine was blocked by mepyramine (10 nmol), a known H_1 antagonist, whereas cimetidine, a known H_2 antagonist, had little or no effect.

These results suggest that great care must be taken when interpreting the role of individual mediators in a skin inflammatory response.

Mediator Studies in Skin Blister Fluid (SBF) from Late Cutaneous Reactions (LCR) after Allergen Testing. WALTER DORSCH AND JOHANNES RING, Pediatric and Dermatology Dept., Ludwig-Maximilians-Universität, München, W-Germany

Late cutaneous reactions (LCR) occur not infrequently after allergen skin testing. They have been shown to be IgE-dependent. We reported earlier that LCR can be transferred by skin blister fluid (SBF). The mediators involved, however, are not yet clearly characterized. In this study SBF was drawn at different time intervals over LCR developing after allergen skin testing in 26 patients with atopic diseases and examined for various mediators. Results were compared to those of 10 non-atopic volunteers (untested skin). In the early phase (30 min) of LCR histamine concentrations in SBF (measured spectrofluorometrically) were markedly elevated (from normal 21 ± 5 to 206 ± 40 ng/ml) returning to normal after 6 hours. Measurable kallikrein activity (bioassay on rat uterus) was found in SBF from 10 out of 13 patients and in 2 out of 10 controls. SBF from both atopics and normals induced in vitro serotonin release from normal human platelets. With the development of LCR thromboxane B_2 content (radioimmunoassay) of SBF showed a significant rise up to 1770 pg/ml after 6 h. The oral application of a thromboxane biosynthesis inhibitor, dazoxiben, led to a reduction in LCR formation, while the immediate wheal and flare reactions in the same individuals were increased (randomized, double-blind study in 8 atopics and 9 normals after anti-IgE skin testing). It is concluded that among other possible mediators thromboxanes might play a role in LCR formation.

Effects of Low Oxygen Tension on Prostaglandin Biosynthesis by Neonatal Mouse Epidermal Cell Cultures. ALICE PENTLAND, MARY ANNE JORDAN AND CYNTHIA MARCELO, Dept. of Dermatology, Univ. of Michigan Medical School, Ann Arbor, MI

Prostaglandins (PG) are important modulators of inflammation. In their biosynthesis, 2 moles of molecular oxygen are incorporated for each mole of PG produced. Since oxygen tensions are known to change significantly in inflammation and wound healing, we studied the effects of lowered oxygen tension on PG biosynthesis *in vitro*. BALB/c neonatal mouse epidermal cell cultures were grown in 5%, 7.5%, 10%, 15% and 21% O_2. Cells grown in 21% O_2 were each exposed to medium equilibrated to either 21% O_2 or a lower O_2. The medium's O_2 tension was measured by polarographic electrode before adding it to the cultures. 2 μM Ca^{++} ionophore (to aid Ca++ influx for phospholipase A_2 activation), was also added to selected cultures. Cultures aged 1, 3, 5 and 8 days were exposed to fresh medium for 15, 30, 45, 75 minutes, 2 hours and 24 hours. PGE_2 and $PGF_{2\alpha}$ in the medium were then measured by radioimmunoassay and the μg DNA/culture measured. At all time points, the amount of PG per μg DNA in cultures exposed to a 7.5% oxygen atmosphere was only 50% of the control value. The amount of PG synthesized was primarily related to the O_2 tension of the medium, and not the gas phase O_2 tension during the growth period. Addition of Ca^{++} ionophore stimulated PG production at low oxygen tensions to the 21% control value. These results show that oxygen tension affects the quantity of PG produced/μg DNA by epidermal cells in culture. This relationship between oxygen tension and prostaglandin biosynthesis may be important in the early modulation of the inflammatory response after wounding. In inflammatory skin disease such as psoriasis, with abnormal PG synthesis, altered barrier function and increased blood flow, this relationship between oxygen tension and PG synthesis may play a role.

Localization of Human Neutrophil Histaminase to the Specific Granule. EILEEN W. RINGEL, NICHOLAS A. SOTER, AND K. FRANK AUSTEN. Harvard Medical School, Boston, MA

Human neutrophil histaminase is one of a group of diamine oxidase enzymes which catalyses the oxidative deamination of histamine. Viable human polymorphonuclear leukocytes containing over 90% neutrophils were isolated from citrated, whole blood on Ficoll-Hypaque cushions and stimulated with phorbol myristate acetate (PMA), formyl-methionyl-phenyalanine (fMLP) or ionophore A 23187, all of which have been shown to selectively release enzymes of the specific granule at appropriate concentrations. There was a dose-dependent release of histaminase, as measured by radioenzyme thin layer chromatography, which was accompanied by release of lysozyme and B_{12} binding protein, two components of the specific granule. The addition of Ca^{2+} (1.25 mM/L) to experiments with fMLP produced an enhancement of release of both specific granule markers and histaminase. Little of no B-glucuronidase, which resides in the azurophilic granule, or lactic dehydrogenase was released in any experiment. In order to confirm findings from the release experiments, azurophilic and specific granules were physically separated in a density gradient of 31% to 53% sucrose. Three major bands of granules were obtained. 58% of the total histaminase activity recovered in the gradient was found in band c (41.7% sucrose), whereas band a (47.7% sucrose) and band b (44.5% sucrose) contained only 1.5% and 3.3%, respectively. B_{12} binding protein showed a similar distribution to that of histaminase. These findings strongly suggest that human neutrophil histaminase is contained solely within the specific granule. Secretion of specific granule enzymes may have a significant regulatory function in allergic disorders, such as urticaria, where histamine is an important mediator of inflammation.

Early and Late Inflammatory Effects of PAF-Acether in the Skin of Experimental Animals and Man. C. B. ARCHER,* C. P.

PAGE, W. PAUL, J. MORLEY AND D. M. MACDONALD.* Departments of Dermatology,* Guy's Hospital, London, SE1 and Clinical Pharmacology, Cardiothoracic Institute, London, SW3, England.

The phospholipid, PAF-acether, is released in both allergic and nonallergic events and has properties appropriate to a mediator of inflammation. Intradermal injection of PAF-acether in experimental animals causes immediate extravasation of plasma protein accompanied by intense accumulation of platelets and neutrophils.

We have examined the immediate and late inflammatory responses in guinea-pig and human skin after intradermal injection of 0.1 to 250 ng doses per site, and have observed the effect on these responses of locally administered drugs including disodium cromoglycate (DSCG).

In guinea-pig skin there was an immediate response which persisted, being clinically detectable at 24 hours when there was, in addition to neutrophils, a mononuclear cell infiltrate of activated macrophages.

In man PAF-acether elicited an acute wheal and flare response which resolved within one hour, but which was succeeded, with larger doses, by an erythematous response approximately 3 hours after injection, at the site of the initial wheal. This late onset response was accompanied by a mononuclear cell infiltrate.

DSCG selectively inhibited the late response, an effect not attributable to antihistamine or mast cell stabilising activity.

We conclude that PAF-acether acts not only as a mediator for acute inflammation but may also mediate more presistent inflammatory responses which may be associated with influx of mononuclear cells. Clinical effects of DSCG may therefore be due to inhibition of such late onset inflammatory responses.

Gastrin Induces Histamine Release from Human Cutaneous Mast Cells. MICHAEL D. THARP AND TIMOTHY J. SULLIVAN. Depts. Dermatol., Int. Med., and Microbiol. University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Gastrin-induced histamine release from mast cells has been proposed as an important force in gastric acid secretion and some postprandial flushing syndromes, but little direct evidence of gastrin effects on mast cells has been presented. We investigated the ability of the human heptadecapeptide gastrin (G_{17}) and related peptides to stimulate histamine release from human cutaneous mast cells. When 200 μ thick sections of skin were incubated in the presence of 10^{-15} to 10^{-8} M G_{17}, a dose-related release of histamine was observed. A maximal release above controls of 15.6% (± 2.5 SEM) of the total skin histamine content was observed at 10^{-9} M gastrin. Histamine release was detectable 10 minutes after 10^{-9} M gastrin challenge and was complete within 30 minutes. Pentagastrin (PG), a synthetic analog of the carboxy terminal portion of G_{17} also provoked a dose-related release of histamine over a concentration range of 10^{-12} to 10^{-8} M with an optimal release of 18.6% (± 5.6 SEM) at 10^{-9} M. A synthetic analog of the first thirteen amino acid residues of gastrin (G_{1-13}) failed to stimulate release above controls at 10^{-12} to 10^{-8} M. Intradermal injections of PG (10^{-8} to 10^{-4} M) caused a wheal and flare reaction in 6 out of 7 human volunteers. These results demonstrate that physiologic concentrations of gastrin can induce histamine release from human skin mast cells, and suggest that gastrin binds to mast cell receptors that recognize the carboxy terminal region of the molecule. These observed stimulatory effects of gastrin on human cutaneous mast cells may explain the pathophysiology of some postprandial flushing and urticarial disorders.

Leukotriene and Histamine Induced Cutaneous Capillary Permeability, Elucidated by a Skinwindow Method. *Hans Bisgaard, Axel Lerche.* University Hospital of Copenhagen, Hvidovre, Denmark.

Evaluation of the capillary permeability increasing capacity of mediators in humans has so far been restricted to the estimation of wheal area and depth. A new method is introduced for qualitative and quantitative measurements of the permeability increase in the cutaneous microvascular circulation in humans.

Skinwindows were produced through suction and sealed off by skinwindow-chamber. ^{131}I-albumin and ^{99}Tc were given i.v. After a 2 hour resting period the skinwindows were exposed to histamine. The amount of labelled macro- and micromolecules in the exudate could thereafter be quantitated at selected intervals. A steep sigmoidal dose-response curve was found for histamine in the dose-range of $10^{-4} - 10^{-8}$ M.

Compared to the method of estimating the wheal volume in a double-blind design, this new principle seems to be more sensitive. Applying the method to the investigation of the effect of the SRS-A leukotrienes LTC_4 and LTD_4 as well as the LTB_4, we found these leukotrienes to increase the cutaneous capillary permeability in humans. In equimolar concentrations of 10^{-5} M, a comparative potency order was found as follows: histamine >LTB_4 >LTD_4 >LTC_4.

Comparisons of the Permeability of Human and Laboratory Animal Skin. P. H. DUGARD AND M. WALKER. ICI PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England

Results of studies involving applications of drugs and chemicals to animal skin are extrapolated to man despite possible differences in absorption, a process which often determines the nature of a biological response. The permeabilities of excised skin samples (human abdominal, laboratory animal flank skin) were determined in glass diffusion cells using tritiated water (T_2O), a commonly employed marker of permeability, and ^{14}C-paraquat (PQ), a slow penetrant of human skin known to display large rat versus human absorption differences. ^{14}C-paraquat dichloride was applied to skin as a 1 mg/ml aqueous solution. Rates of absorption are expressed as permeability constants (k_p, units: cm/hr × 10^5) in the Table. In all cases, higher mean rates were recorded for animal skins than for human skin but the greatest factor of difference was only 4.8 for T_2O (guinea pig). The factors of difference were much higher for PQ absorption (in parenthesis in the Table) ranging from 40 to over 1000.

	Human	Rat	H′ Less Rat	Nude Rat	Mouse	H′ Less Mouse	Rabbit	Guinea Pig
Water k_p:	93	103	130	152	144	351	253*	442*
PO k_p:	0.73	27.2* (40)	35.3* (50)	35.5* (50)	97.2* (135)	1065* (1460)	79.9* (130)	196* (270)

* significantly different from human

These (and our other) results indicate that species differences in skin permeability depend greatly upon the nature of the chemical; differences may be very large for some slow penetrants. Previously recommended animal 'models' for human skin absorption have not been tested with such slow penetrants.

Hydrophobic Compounds—A Potential Problem for *In Vitro* Percutaneous Absorption Studies. ROBERT L. BRONAUGH AND RAYMOND F. STEWART, Food and Drug Administration, Washington, D.C.

In vitro skin absorption studies using standard diffusion cell techniques may likely give erroneously low results for hydrophobic compounds because of the tendency of these compounds to remain in the skin and not partition into an aqueous receptor fluid. This effect has been observed for 2 fragrances of toxicological interest, cinnamyl anthranilate (CA) and acetyl ethyl tetramethyl tetralin (AETT) in a petrolatum vehicle and in rat skin. A 7.9-fold (CA) and 94.5-fold (AETT) increase in permeation was observed when the compounds were tested *in vivo* under similar conditions. The inability of the compounds to freely enter the receptor fluid was partially reversed by replacing normal saline with other fluids: rabbit serum, 3% bovine serum albumin, organic solvents and dilutions of 2 nonionic surfactants (Volpo 20 and Triton X). The effect of the receptor fluids on the integrity of the skin barrier was assessed by measuring the permeability of control compounds (cortisone, urea, and water). A 6% solution of Volpo 20 was the receptor fluid of choice, but only 61% of the *in vivo* absorption of CA was obtained. When the lipophilic petrolatum vehicle was replaced with acetone, the *in vitro* absorption of CA (using Volpo 20) increased to 73% of the *in vivo* absorption obtained with the same vehicle.

The *in vivo* and *in vitro* comparability of CA and AETT was improved by facilitating the *in vitro* partitioning into the diffusion cell receptor. Better comparability for CA was obtained by using a volatile vehicle (acetone) and thereby eliminating the lipophilic petrolatum layer on the surface of the skin.

Proportionality Between Horny Layer Reservoir and Percutaneous Absorption in Hairless Rats *In Vivo*. A ROUGIER,* D. DUPUIS,* C. LOTTE*, R. ROGUET* AND H. SCHAEFER†, *Département de Biologie, Laboratoire de Recherche Fondamentale de l'Oréal, 1 Avenue Saint-Germain, Aulnay-sous-Bois, France and †Centre International de Recherches Dermatologiques, Valbonne, France

In order to better understand the interrelationship between horny layer reservoir and percutaneous absorption, both parameteers were determined in hairless rats in vivo for ten structurally unrelated radiolabelled substances. The substances were applied in a lotion

(ethanol-water) and at equal concentrations (200 nmoles/cm^2) for 30 minutes. Stratum corneum reservoir was measured by sampling with adhesive tape strippings. Percutaneous absorption was determined via excretion in urine and feces during 96 hours and subsequent whole body analysis. Linear proportionality between the reservoir in the horny layer and the percutaneous absorption was found for all the compounds, when relating the two variables by the equation of $y = ax + b$ ($a = 1.644$, $b = -0.536$), x = stratum corneum reservoir in nmoles/cm^2 × total thickenss, y = percutaneous absorption in nmoles/cm^2.

The correlation coefficient r is 0.998, i.e. the correlation is highly significant ($p < 0.001$). A confirmation of this straightforward interconnection in human skin of reservoir and penetration would tremendously facilitate investigations of a toxicologic and pharmacokinetic nature in percutaneous absorption since the determination of the stratum corneum reservoir can be easily performed in humans via stripping using non radiolabeled substances. Corresponding investigations are underway.

Percutaneous Absorption: An Automated Technique for *In Vitro* Measurement Using Continuously Perfused Diffusion Cells. C. G. TOBY MATHIAS, Department of Dermatology, University of California Medical Center, San Francisco, CA

A methodology has been developed for *in vitro* measurement of percutaneous absorption, utilizing continuously perfused glass diffusion cells and an automated sample collection system. Cadaver skin is mounted between halves of the diffusion cell, and normal saline is perfused at a specified flow rate through the collecting chamber. Heated water pumped through a surrounding glass jacket maintains constant skin temperature, and a magnetic stirring bar ensures complete mixing of the absorbed compound within the collecting chamber. Perfusate is collected at specified time intervals in an automated fraction collector. Technical problems encountered include elevated hydrostatic pressure and accumulation of gas bubbles within the collecting chamber; both are overcome by keeping perfusing and collecting tubules at skin surface level, and prior degassing of the perfusate in a vacuum box. The elimination constant (K) is calculated from the relationship: K = flow rate/volume of diffusion cell. Using a midpoint approximation technique, hourly excretion rates may be plotted against the midpoint of the sampling time interval; total amount excreted from diffusion cell is calculated from area under the resulting curve (AUC). Residual amount (RA) in the diffusion cell is approximated from the concentration of the last collected sample again using a midpoint technique. Total amount absorbed at any point in time equals AUC + RA. By keeping the length of the sampling interval less than 1.5 times the elimination half life, less than 5% error is introduced. Variations of flow rate may permit analysis of effects of blood flow on absorption. Advantages of the automated system include ability to generate a large number of data points for accurate analysis of the resulting pharmacokinetic curve, and a substantial saving of technician time.

Small Substance of P-Containing Neurons in the Trigeminal Ganglia, As an Origin of the Cutaneous Unmyelinated (Type C) Afferent Fibers in Macaques. HIDEO UNO, HIROSHI HACHISUKA, AND BRADLEY POFF, Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI

Neuropeptide, substance P, is widely involved in the somatic sensory nerves. In the cutaneous sensory nerves, SP is found in terminal fibers in the subepidermal regions, but a presence of SP in sensory receptor-neurite complex is uncertain. Using immunocytochemical method, we examined SP-containing fibers in the maxillary skin region, maxillary nerves, trigeminal ganglia, and midbrain of adult rhesus monkeys. After exsanguination under Nembutal anesthesia, fresh skin and nerve tissues were taken and fixed with 0.4% benzoquinon PBS solution for 2 to 4 hours at 4°C. Following wash with 7% sucrose PBS overnight, sections were cut with cryostat. After drying, the sections were immersed in 0.5% triton-X PBS solution for 1 hour, then covered with monoclonal antibody against substance P (Sera-Lab) for 1 hour at 37°C. Next, they were treated with fluorescin conjugated IgG. Fine varicose fibers containing SP were found on the subepidermal layer and around the outer sheath of the sinus hair follicles. SP-containing fibers were also found in the cutaneous nerve bundles and maxillary nerve tracts; the fibers were mostly unmyelinated. In the trigeminal ganglia, many small sized neurons among large principal neurons contain SP in the soma and axonal or dendritic glomeruli. Centrally, SP-containing terminal plexuses were found in the trigeminal principal sensory nucleus and spinal trigeminal tract and nucleus in the midbrain. The results suggest that small SP-containing neurons in the trigeminal ganglia appear to be the origin of cutaneous type C afferent fibers in the subepidermal free nerve ending and sinus hair follicles. These primary sensory fibers centrally project to the cerebral sensory neurons.

Warts: Langerhans Cells and T Lymphocytes in Local Immune Response. YVETTE CHARDONNET, PAULE BEAUVE, JACQUELINE VIAC AND DANIEL SCHMITT. U. INSERM 209—ERA CNRS 788—Pav. R—Hôpital E. Herriot, Lyon, France

Local immune response was studied on frozen sections of 50 cutaneous and mucosal wart lesions from 27 patients and normal epidermis, with the use of indirect IF test and monoclonal antibodies OKT3, OKT4, OKT8 and BL6 respectively specific for circulation, helper/inducer, suppressor/cytotoxic T cells and Langerhans cells (LC). In 27 cases with or without a low grade infiltrate, LC were either absent or their density was reduced when compared with normal epidermis. In 12 of these, LC were also found in the upper dermis. In 23 other biopsies, with a moderate or a strong inflammatory reaction, unusually high numbers of LC were seen in the epidermis and in most cases also in the dermis. In 12 out of 50 cases, with an infiltrate, both OKT4 and OKT8 cell population were present; high densities of LC were seen both in the epidermis and in dermis. The reactions of the various specimens taken from the same patients differed. Keratin expression is also modified in wart epidermis. Monoclonal antibodies to purified keratin, K1, reacted with the basal cell layer of warts while it reacted only with suprabasal layer of normal epidermis, recognizing the 55-57000 keratin subunit. Viral antigen was detected with antiserum raised to SDS dissociated purified virus. The types of virus were identified histologically and by their DNA cleavage pattern using restriction enzymes. Most of the viruses were of types 1 and 2. These data demonstrate a low local cellular immune response by the wart lesions without correlation with the virus type or their location. Moreover the presence of LC or their absence suggests that these cells have an important role in the pathogenesis of the lesions.

SESSION C — South American Room

Hans Schaefer, M.D., Presiding

The *Asebia* Mouse: An Animal Model of Psoriasis and Possibly Ichthyosis. WILLIAM R. BROWN AND MARGARET H. HARDY, Department of Biomedical Sciences, University of Guelph, Guelph, Ont., Canada

Extensive efforts have been made to create animal models of psoriasis that could be used to test therapies. Most of these models have been based on the induction of temporary epidermal hyperplasia in laboratory animals. The mutant mouse *asebia* (*ab/ab*) is born with epidermal hyperplasia affecting the whole skin throughout life. We found, using tritiated thymidine autoradiography, that *asebia* mice had an epidermal proliferation rate 5 times normal. The mean labelling index (L.I.) of *asebia* mice was 7.5%; that of normal (+/+) mice, of the same strain (BALB/c), was 1.5%; and that of heterozygous (+/*ab*) mice was also 1.5%. Skin samples were taken at noon, the reported low point of the circadian rhythm in mouse epidermis. Heterozygous mice appeared phenotypically normal. *Asebia* epidermis was about twice the normal thickness, with extensive intercellular spaces. The dermis showed chronic inflammation, with twice the normal density of cells. The additional cells were mostly mononuclear. Mast cell density was increased about 20-fold. Numerous macrophages were identified ultrastructurally, and many of them, both intact and disintegrating, contained electron lucent cholesteryl ester crystals in lysosomes. Similar crystals are found in the liver in the human genetic disorder Wolman's disease, but none were found in *asebia* livers in the present study. Although the L.I. of psoriatic epidermis (about 20%) is greater than that of *asebia*, this mouse has an advantage over previous models that have only temporary hyperproliferation. Reports of abnormal cholesterol metabolism in ichthyoses suggest that the *asebia* mouse, with its thickened stratum corneum and cholesterol abnormalities, may provide insights into these diseases as well.

Free Radical Formation from Anthralin and Its "Dimer". M. WHITEFIELD, Dermal Laboratories Limited, Gosmore, Hitchin, Herts., U.K.

The mode of action of anthralin (AH) in the treatment of psoriasis remains to be elucidated. Its extreme sensitivity to oxidation, particularly in the presence of epidermal protein, strongly indicates that the drug is altered metabolically before reaching the site of action, and

recent evidence has suggested that, in the skin, it converts rapidly to a "dimer" (A_2) at the 10-position by oxidative coupling.

The properties of AH and A_2 were compared using electron spin resonance spectroscopy which demonstrated that

i) AH exchanged with D_2O resulting in the uptake of 4 D atoms at the 1-OH, 8-OH and 10-CH_2 groups, whereas with A_2 only the 1-OH and 8-OH groups are exchanged and not the protected 10-CH groups.

ii) after photolysis or thermolysis, the same C_{10} radical, A·, is obtained from both AH and A_2.

These results confirm that, if A· is the reactive species, the conversion of AH into A_2 in the horny layer provides a protective mechanism for the conveyance of the radical through the epidermis.

A Quantitative Comparison of the Irritancy of Pure Anthralin and Dithranol B.P. TIMOTHY KINGSTON, PETER DYKES, IHSAN HAMAMI, MARGARET CORBETT AND RONALD MARKS, Dept. of Medicine, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN

Topical anthralin remains one of the most effective treatments for psoriasis. Howevere, its irritancy remains a bar to its usage. In a previous study we established the minimal irritancy dose for dithranol B.P. using the rise in skin temperature as a measure of the skin's response. The purposes of the present study were 1) to compare the irritancies of pure anthralin and dithranol B.P. (a mixture of anthralin and its oxidation products, Anthralin Dimer and Danthron) 2) to compare temperature measurement and skin thickness measurements in the assessment of the skin's response to anthralin. 25 microlitres of freshly prepared acetone solutions of pure anthralin, prepared by thin layer chromatography, and dithranol B.P. in concentrations of 0.01% to 2.0% were evaporated onto paper discs. The discs were placed on the normal forearm skin of 20 subjects (10 normal controls, 10 psoriatics) with 'Finn chamber' occlusion for 24 hours. The irritant response was then assessed by measuring the increase in skin temperature (with a Cr-Al Thermocouple) and skin thickness (with Harpenden skin calipers and with pulsed ultrasound) at 24 and 48 hours. No significant differences were found in the dose-response curves of the increase in skin temperature or skin thickness for the two solutions (Fig 1). The following conclusions were drawn:— 1. Chromatographically pure anthralin and dithranol are of equal irritancy. 2. At all concentrations above 0.05% the irritancy of anthralin is easily detectable by the techniques chosen. 3. Pulsed ultrasound and skin calipers correlate well in the measurement of the thickness of acutely inflamed skin. 4. The responses of the skin of normal subjects and the uninvolved skin of psoriatics to anthralin are identical. 5. Dose-response curves for strong irritants can be accurately quantified.

A Comparison of Continuous and Short Contact Application of Anthralin in the Hairless Rat. D. CAVEY, R. DICKINSON AND B. SHROOT, Department of Biochemistry, Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, Valbonne, France

We have studied the in vivo fate of topically applied anthralin (A.) in intact and scotch-tape stripped skin of the hairless rat. To permit easy drug removal, A. was applied in a plastic disc in the presence of both ^3H- and ^{14}C-A. as tracers. The exposed skin was excised and extracted with isopropylether and free A., its dimer and quinone assayed by HPLC. In parallel, both ether soluble and residual fractions were assayed by liquid scintillation spectroscopy.

With continuous application to intact skin, A. rapidly concentrated in the horny layer (H.L.). The corresponding dimer was continuously formed in both normal and stripped skin, and ether insoluble material predominated over soluble material, especially when the H.L. was absent. In addition, about 40% of the penetrated drug could not be found in the skin, and the loss was not due to tritium exchange. After a short contact time (0.5–1 hr), A. quickly disappeared from the skin (normal and stripped). The level of the dimer was stable. In normal skin, the depletion of the horny layer of its radioactive A. content resulted in an equivalent incorporation of the ^{14}C label into the ether insoluble fraction. In the stripped skin, this insoluble fraction remained constant for the duration of the experiment (24 hr).

With a suitable formulation and washout procedure, short contact therapy should minimise the quantity of A. in the horny layer, whilst permitting action of bound drug in the diseased skin. A. predominantly decomposes in the living part of the skin.

Anthralin Short Contact Therapy—Clinical Response in Plaque Psoriasis. MICHÈLE VERSCHOORE*, JEAN-PAUL LAHMY*, HANS SCHAEFER*, H. KOUDSI**, NICHOLAS J. LOWE**, *Centre International de Recherches Dermatologiques, Sophia Antipolis, Valbonne, France; **Division of Dermatology, UCLA School of Medicine, Los Angeles, California, USA

Although anthralin has been used for more than 60 years in the topical treatment of psoriasis, it has suffered from severe side effects such as staining and irritation. To overcome these side effects, and maintain clinical efficacy, we have recently introduced Anthralin Short Contact Therapy (ASCT). After a daily 10 minute application of 0.3% Anthralin in vaselin, the patients wash with acid soap to remove excess drug and apply a local emollient. Comparison of the treatment with either Puva photochemotherapy, 0.5% fluorosone di acetate (Fluorone®) or TAR-UVB, classical anti-psoriatic treatments, demonstrated in a randomised study on 39 patients that after a 3 week period of therapy, the ASCT and steroids were significantly efficient ($p < 0.01$). Using a 1 to 4 grade for evaluation of clinical scores (CS) and side effects, no significant difference was observed either between the clinical efficacy of all treatments or in their reported side effects, such as local irritation and staining. The table presents the average values at 3 weeks:

Treatment (n=)	SCT (19)	PUVA (5)	TAR UVB (3)	Steroids (12)
CS treated lesions	4.63	4.60	2.35	4.00
CS differences untreated-treated lesions	2.53	1.40	2.00	2.00
Side effects	4.89	4.40	5.00	4.45

There is no statistical difference for the 3 criteria.

This supports the idea that the use of anthralin in ASCT is an advance in the topical treatment of psoriasis and may result in a more clinically acceptable treatment than corticosteroids, PUVA, TAR UVB.

Human Absorption of Crude Coal Tar Products. JAMES S. STORER, JOHN L. LASETER, ILDEFONSO DE LEON AND LARRY E. MILLIKAN, Department of Dermatology, Tulane University School of Medicine, Center for Bio-Organic Studies, University of New Orleans, New Orleans, Louisiana

To determine absorption of crude coal tar (2%) and its components as used in psoriatic patients during Goeckerman therapy, we examined urine and blood samples of five healthy volunteers. Samples were obtained before application and after two successive days, during which time 2% crude coal tar was applied for 8 hour periods each day.

Samples were analyzed by high resolution computerized gas chromatography and mass spectrometry.

Serum levels of naphthalene, biphenyl, acenaphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene were increased after treatment. Increases ranged from two to thirty times that found in pretreatment samples.

We conclude that there is absorption of polyaromatic hydrocarbons from crude coal tar as used in Goeckerman therapy, and that absorption is quantitatively and qualitatively variable. The potential carcinogenesis of some of these compounds is well known.

Alterations in Enzyme-Mediated Binding of Polyaromatic Hydrocarbon Carcinogens to Epidermal DNA by Coal Tar and Clotrimazole. BENJAMIN J. DEL TITO, JR., HASAN MUKHTAR AND DAVID R. BICKERS, Department of Dermatology, Veterans Administration Medical Center and Case Western Resereve University, Cleveland, Ohio

The polyaromatic hydrocarbons (PAH) are produced whenever fossil fuels are incompletely combusted and are among those chemicals in the environment known to be human carcinogens. The mechanism of PAH carcinogenesis is related to chemical transformation of inert precursors to highly reactive metabolites such as diol-epoxides by microsomal P-450-dependent monooxygenases. These diol-epoxides can bind to cellular macromolecules such as DNA thereby initiating tumor formation. This study assessed the effect of therapeutic coal tar and clotrimazole on enzyme-mediated binding of the PAH benzo(a)pyrene (BP) to epidermal DNA *in vivo* and *in vitro*. The *in vivo* binding of BP to epidermal DNA in coal tar-treated rats was 5–10 times that of untreated controls. *In vitro* binding of BP to calf thymus DNA in the presence of epidermal microsomes was 1–3 times that of controls. Clotrimazole, an imidazole antifungal agent significantly inhibited BP-binding to epidermal DNA *in vivo* and to calf thymus DNA *in vitro*. Epidermal aryl hydrocarbon hydroxylase (AHH) activity cor-

related with the degree of enzyme-mediated binding of BP to DNA. These studies indicate that topically applied therapeutic coal tar solution enhances the binding of BP to epidermal DNA *in vivo* and to calf thymus DNA *in vitro* and that clotrimazole, a widely using antifungal agent, is capable of largely inhibiting this reaction. The imidazole antifungals may prove useful in altering the risk of tumor development in the skin following exposure to polyaromatic hydrocarbon carcinogens.

Modifications of Mitochondria by Dithranol: A Morphological and Biochemical Study. PATRICE MORLIÈRE, LOUIS DUBERTRET, TERESA SAE MELO, CHRISTIAN SALET AND RENÉ SANTUS, Hôp. H. Mondor, Créteil, France; ERA CNRS 951 Paris, France; Lab. Biophysique INSERM U 201, Paris France

In order to look for ultrastructural modifications induced by Dithranol (Dit) in human skin, 0.5% Dit. in petrolatum was applied during 18 hours on the inner face of the thigh of normal volunteers. As control, petrolatum was applied on the other thigh. 4 mm punch biopsies of the treated and control skin were processed for electron microscopy, directly or after incubation in diaminobenzidine in order to visualize the cytochrome c oxidase (Cyt. Ox.) activity in mitochondria (Mit). Under these conditions no ultra-structural modification was observed in controls. In Dit. treated skin, epidermal Mit. were nearly completely destroyed. However some irregular empty vesicules were identified as Mit. by Cyt. Ox. cytochemistry. The effect of Dit. on rat liver isolated Mit. was monitored by measuring the ratio of oxygen consumptions in states III and IV (respiratory control ratio, RCR). The RCR is a measure of the Mit. capacity to use ADP to form ATP. The RCR decreases from 4.4 in control experiments to 3.0 and 1.4 at 10 μM and 100 μM Dit. respectively, while the state IV oxygen consumption is not inhibited. These results suggest that Dit. acts as an uncoupler of the oxidative phosphorylation. Parallel in vitro experiments were performed, showing that Dit. reduces ferricytochrome C which is involved in the electron transfer process in Mit. This very fast reduction, which does not require oxygen, only occurs when Dit. is bound to a protein (human albumin in the present experiments) stabilizing the Dit. mono anionic form. These morphological and biochemical results suggest that Dit. could inhibit the Mit. respiratory energy supply in epidermal cells. This could explain part of the therapeutic efficiency of this drug in psoriasis.

Effect of Medium Chain Length Dicarboxylic Acids on Mitochondrial Respiration. M. PICARDO, S. PASSI, M. NAZZARO-PORRO AND A. BREATHNACH*, St Gallicano Derm Inst, Rome, Italy; * St Mary's Hosp Med School, London, UK

C_8 to C_{12} dicarboxylic acids are effective in treatment of hyperpigmentary disorders and the possibility that they may act by inhibiting mitochondrial respiration is suggested by the fact that a) when administered to rats and humans, they are partially metabolized by β-oxidation; b) E.M. autoradiography of cultured normal and malignant melanocytes exposed to $^3HC_{12}$ diacid revealed concentration of radioactivity in mitochondria. We have tested this possibility by studying respiration on isolated rat liver mitochondria by O_2 electrode and spectrophotometric methods. ATP generation was determined by High Performance Liquid Chromatography on ion exchange column.

The results showed that the respiration was competitively inhibited by the diacids to different extents: the higher the number of carbon atoms up to C_{12}, the greater the inhibition. In particular, experiments on submitochondrial particles showed that the competitive inhibition concerned the following enzymes: NADH dehydrogenase, succinic dehydrogenase and ubiquinone-cytochrome-C reductase. The results clearly indicate that dicarboxylic acids do act by inhibiting mitochondrial respiratory enzymes, and this may explain their melanocytotoxic effect, in line with Wilkie's (J. Roy. Soc. Med. 72: 599, 1979) view that some antineoplastic drugs affect some steps of mitochondrial function.

The Proliferative and Stimulatory Effects of Para-Substituted Phenols (PSP). J. J. NORDLUND AND A. E. ACKLES, VA Medical Center, Dermatology Svc., West Haven, CT. and D. SAUDER, McMaster University, Hamilton, Ontario

PSP are known to be potent pigment cell (PC) toxins although the mechanisms by which they kill PC are unknown. We have studied in murine epidermis the effects of two PSP, monobenzyl ether of hydroquinone (MBEH), and 4-tert-butyl pyrocatechol (PTBC) on PC, keratinocytes (KC), and Langerhans cells (LC). 20% MBEH and 2% PTBC were applied for 1 up to 50 days to murine ears. Ears were surgically removed and the epidermis split from the dermis after incubation in 0.025M EDTA for 2 hours at 37°C. We observed the following effects: 1) the epidermis thickened by a factor of 2–4 fold, 2) basal KC were columnar in treated skin and cuboidal in control skin, 3) the number of epidermal cell layers increased from 2 to 4; the granular layer was very prominent, 4) by electron microscopy, the cells appeared normal. Keratohyalin granules were numerous. Some KC were in mitosis. 5) The number of PC visible after incubating the epidermal sheet in dopa (1 mg dopa/ml; pH 6.8) for 18 hours increased by a factor of 2. 6) The number of Ia positive LC increased from a mean of 430 cells/mm^2 (control) up to 1,200 cells/mm^2. The LC were greatly enlarged in size. These effects were partially blocked by concomitant topical treatment of the skin with indomethacin. In separate experiments, mice were treated with MBEH and PTBC for 7 days. The ears were surgically excised, the epidermis removed and incubated in tritiated thymidine for 2 hours, and exposed to nuclear track emulsion. We observed that the nuclei of many KC, some PC, and some suprabasilar cells were labelled. In another experiment, we observed that the thickened epidermis from MBEH and PTBC treated animals released very large amounts of epidermal thymocyte activating factor (ETAF).

Effect of Temperature on Selective Vascular Damage Made by Pulsed Laser Radiation (577 nm). R. W. GANGE, B. PAUL, R. R. ANDERSON AND J. A. PARRISH, Dept. of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, MA

Unfocussed pulsed 577 nm laser radiation of human skin causes selective damage to microvessels without damage to the surrounding tissue. The mechanisms have been explored in hamster cheek pouch and human skin. The cheek pouch was everted and exposed on a temperature-controlled microscope stage. Laser irradiation was administered in graduated doses through the 10x objective of the viewing microscope. Each vessel was exposed once only and observed immediately after exposure. A sequence of slowing of flow, brown discoloration of vascular contents or hemorrhage was seen with increasing doses. The effect of preirradiation target temperature alteration was studied in order to estimate the final target temperature resulting in vessel disruption. Hemorrhage dose thresholds were studied at 8°C and 34°C. At the higher temperature, the dose threshold was reduced by a mean of 0.41 J/cm^2. This is consistent with a target temperature rise of 63°C/joule of incident radiation and a post-irradiation temperature near 100°C. In an analagous experiment, the effect of preirradiation skin temperature upon energy thresholds for visible purpura induction was studied in human skin at 10°C and 42°C at the time of laser exposure. The energy threshold for induction of purpura was significantly less at the higher temperature (1.4 ± 0.08 J/cm^2, vs. 1.6 ± 0.11 J/cm^2 at 10°C; $n = 8$ $p < 0.001$). This is consistent with a target temperature rise of 71°C/joule of incident laser radiation, and a post-irradiation target temperature exceeding 100°C for purpura to occur. These observations are consistent with microvaporization as an event in the induction of vascular disruption in the cheekpouch and of purpura in human skin.

Mechanisms of Angiogenesis *In Vitro*. T. TSUJI AND M. KARASEK, Dept. of Dermatology, Stanford University, Stanford, Ca

Development of methods to isolate and maintain endothelial cells *in vitro* has provided a new tool for the analysis of the factors that promote endothelial cell growth and neovascularization. When the luminal surface of a monolayer of microvascular endothelial cells is exposed to a collagen matrix the cells immediately reorganize into vascular channels. We have studied and compared the early and late stages in this reaction sequence by phase contrast, scanning (SEM) and transmission electron microscopy (TEM).

Endothelial cells were cultured on a fibronectin-treated plastic surface, and vessel formation was induced by a collagen matrix formed over a 0.5 cm diameter annulus cut into the center of a 1.5 cm diameter filter. The collagen matrix containing the reorganized cells was fixed and processed for SEM and TEM.

Four stages in the reorganization patterns of endothelial cells were characterized. At Stage 1 (12 hours), migration and a change from a cobblestone pattern to a spindle-shaped pattern are involved. At Stage 2 (24 hours) the cells form a network of closely apposed spindle cells. At Stage 3 (48 hours) the network changes to a series of channels of three-dimensional architecture with a smooth surface of the walls and enwrapping a lumin. Cross-sections through these channels show cylinders of several cells with tight junctions surrounding a lumin in which cellular debris is often evident. At State 4 (96 hours) various sized holes in the vessel wall can be seen, and the structures subsequently lyse.

These studies define the time sequence and the three-dimensional architecture of the unusual response of microvascular endothelial cells to a collagen matrix which leads to vascular neogenesis *in vitro*.

Scanning Electron Microscope Study on Pericytes and Smooth Muscle Cells of the Dermal Blood Vessels. SHUHEI IMAYAMA, TAKAMICHI TOKUNAGA, TOMOKO IKAWA, TAMIKO SAKU, AND HARUKUNI URABE, Department of Dermatology, Faculty of Medicine, Kyushu Univ. Fukuoka, Japan

This study was carried out to observe the external surface of the dermal blood vessels following removal of the connective tissue elements. Under anesthesia, the foot skin of adult rats were perfused through the femoral artery with karnovsky's fixative. Methacrylate resin was, then, injected via the same artery to preserve the entire architecture of the vascular bed. After removal of the epidermis with sodium thiocyanate, materials were treated with HCl and collagenase to remove the extracellular material. The present procedure preserved the pericytes and the smooth muscle cells of the dermal blood vessels, enabling us to the arrangement and the surface features of the tells of various vascular segments. Small arteries were characterized by a layer of densely packed and circularly disposed smooth muscle cells. Small veins, on the other hand, showed a discontinuous smooth muscle layer. The muscle cells of both arteries and veins were interconnected together by a number of thin lateral projections. On capillaries, two types of pericytes; with two or more long branching processes and with a number of short lateral projections were observed. Most of the pericytes and their long processes were disposed not circularly but longitudinally on the capillary endothelium. In addition, the pericytes extending their processes to the neighbouring capillaries were frequently observed. These findings, coupled with the previous report that the capillary loops and networks show tortuous courses (S. Imayama, J. Invest. Dermatol. 76:151–157, 1981), lead us to a speculation that the contraction of the pericytes do not necessarily narrow the vascular lumen, but could cause tortuousness of the capillaries.

Human Platelet Regulation of Angiotensin II Formation. R. A. SNYDER, AND B. U. WINTROUB, Dermatology Service, VAMC and Department of Dermatology, University of California, San Francisco, California

Angiotensin II (AII) is a biologically active octapeptide formed by the sequential action of renin and endothelial cell-derived angiotensin converting enzyme (ACE) on angiotensinogen. The recent appreciation of a human neutrophil-dependent pathway for the generation of AII and the observation that inhibition of ACE reduces chronic inflammatory processes involving mononuclear cells suggest that AII mediates and/or modulates inflammation. Since platelets are mobile cells present at sites of inflammation we examined human platelets for the ability to regulate the availability of AII. Human platelet extracts (5×10^7 platelets/ml) were incubated with 10^{-5} M angiotensin I (AI) or AII for varying times at 37°C, pH 7.4 and the degradation of each peptide was measured by an HPLC assay. 10^7, 5×10^7, and 10^8 platelets metabolized 43, 290, and 350 pmoles of AI and 83, 281, and 307 pmoles of AII respectively after 60 minutes of incubation. The pH optimum of AI and AII metabolism was 6.0 and 7.0 respectively. The major detectable product of platelet dependent AI metabolism was a peptide that eluted with a retention time of ~4.4 minutes. A second product had a retention time of ~5.7 minutes which corresponded to the retention time of the AII standard and accounted for 9–10% of the AI input. A platelet extract (2×10^{10} platelets) was filtered on Sephacryl S-200 gel filtration in .01 M Tris, pH 7.2, 0.15 M NaCl buffer and the major region of AI and AII metabolizing activity was detected at 50–54% bed volume corresponding to a MW of ~100,000. These observations suggest a possible role for platelets in the regulation of intra- and/or extravascular levels of AII.

Relative Potency of Tissue-Derived Chymotryptic Enzymes That Generate Angiotensin II. B. U. WINTROUB, L. B. KLICKSTEIN, AND L. B. SCHWARTZ, Department of Dermatology, VA Medical Center and University of California, San Francisco, CA; Department of Immunology and Rheumatology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Conversion of angiotensin I (AI) to angiotensin II (AII) requires cleavage of the Phe^8-His^9 peptide bond of AI, an activity attributed to the action of angiotensin converting enzyme. Human leukocyte cathepsin G and rat salivary gland tonin are cell-derived chymotryptic enzymes known to rapidly convert AI to AII. Purified cathepsin G, tonin (supplied by J. Genet), and chymase (the predominant protein component of the rat peritoneal mast cell secretory granule) were examined for AI conversion by HPLC assay, benzoyl-tyrosin ethyl ester (BTEE) hydrolysis and immunologic identity. 350 and 500 fmol of each enzyme were incubated with 10 nmol AI at 37°C, pH 7.2 for 0, 5, 15 and 30 min. The rates of AII formation were 4000, 1000, and 400 mol AII/mol enzyme/hr for tonin, cathepsin G, and chymase, respectively. Cathepsin G and tonin generated only AII from AI while chymase generated Asp-Arg-Val-Tyr, the carboxy-terminal tetrapeptide of AII, at a rate 14-fold faster than formation of AII. The BTEE hydrolyzing activities of the three enzymes and chymotrypsin were compared; the relative activities were: tonin/cathepsin G/chymase/chymotrypsin = 1/51/844/1468. Monospecific goat antisera to cathepsin G formed a single arc when diffused against 4 μg cathepsin G but failed to precipitate 4 μg tonin or chymase. Similarly, monospecific rabbit antisera to chymas failed to precipitate 4 μg tonin or cathepsin G. Tonin, cathepsin G and chymase may modulate AII levels *in vivo* and regulate blood flow and/or vascular permeability during inflamation.

Regional Differences in Skin Blood Perfusion. ETHEL TUR, HOWARD I. MAIBACH AND RICHARD H. GUY, Dept. of Dermatology and School of Pharmacy, University of California, San Francisco, California

Non-invasive optical techniques of laser doppler velocimetry (LDV) and photoplethysmography (PPG) have been used to identify regional variations in skin blood flow. The procedures assess either velocity (LDV) or amount (PPG) of cutaneous blood vessel perfusion. Data, collected via small probes placed on the skin surface, were displayed as voltage differences from a control baseline. Both methods observe the microcirculation encountered to a depth of *ca.* 1.5 mm. 52 positions were studied in 10 subjects resting horizontally. Selecting, as an example, the upper back as a reference site, the LDV technique identifies positions which are statistically ($p < 0.05$, using the Hotelling t^2-test) (a) perfused more highly, e.g. face, ears, post-auricular region and finger tips, (b) perfused at an equivalent level, e.g., palm, toes, cheek, ventral and lateral surfaces of leg and dorsal surface of arm, and (c) perfused less, e.g. foot, sides of trunk and ventral surface of arm. Further, subtle variations within these groups are apparent; for example the cheek has significantly ($p < 0.05$) greater blood flow than the temple. PPG results concurred with LDV observations at many sites but the agreement was not general. For example, relative to the upper back (at $p < 0.05$), PPG classified the palm as highly perfused; LDV found the heel poorly perfused whereas PPG indicated no difference from the upper back. Other disparities were noted on face and ear. We conclude that real and substantial variations in regional skin blood perfusion exist. However because of the different modes of operation of LDV and PPG (i.e. measurement of blood flow *vs.* volume), it appears that the origin of different levels of regional skin perfusion is complex and remains incompletely understood.

The Enzyme-Histochemical Studies on the Blood and Lymphatic Capillary, Portwine Stain, Lymphangiectasia and Superficial Lymphangioma. MORIYA OHKUMA, SEITARO NISHIDA, TOMOMITSU NAKANO, AND TADASHI TEZUKA, Department of Dermatology, Kinki University, School of Medicine, Osaka, Japan

These investigations have been performed to find any good histological method to differentiate the blood and lymphatic capillary, to understand the metabolism of the vessels and to see any differences between the normal capillaries and the nevus- or tumor-vessels. Four specimens from the genital skin, 7 portwine nevus, 2 lymphangiectasia and a case of lymphangioma were investigated for the following enzyme histochemical stainings; alkaline phosphatase is useful to tell the weak positive lymphatic from the strong positive blood capillary and so is histochemical leucine aminopeptidase which is positive in the blood capillary. Lymphangioma and portwine can be differentiated after aminopeptidase and endogenous peroxidase reaction, which are positive in the latter. The isozyme of the alkaline phosphatase belongs to liver-bone type in all capillaries in the above specimens. Lymphangioma is not different from lymphangiectasia and from lymphatic capillary. However the portwine stain is not the same as blood capillary in alkaline phosphatase and in peroxidase. In conclusion, the blood and lymphatic capillary can be differentiated after alkaline phosphatase or aminopeptidase. Lymphangioma and portwine stain can be separated after aminopeptidase or peroxidase staining. The portwine stain is not merely dilated blood capillary as far as the enzyme metabolism is concerned. On the contrary the superficial lymphangioma, lymphan-

giectasia and lymphatic capillary are not different from each other which may suggest the nature of the lymphangioma is merely lymphangiectasia.

SESSION D Pan-American Room
Joseph S. McGuire, Jr., M.D., Presiding

Fate of Nuclear Proteins During Keratinization. J. MACEIRA, K. FUKUYAMA, D. L. TUFFANELLI AND W. L. EPSTEIN. Department of Dermatology, University of California, San Francisco, California

Both DNA and RNA disappear from the nuclei during differentiation of granular cells into cornified cells but the fate of nuclear proteins is unknown. We used anti-nuclear antibodies obtained from patients in the scleroderma spectrum to investigate localization of the antigenic nuclear protein in rat epidermis by light and electron microscopic immunoperoxidase techniques. Three sera, each of which reacted with nucleolus (Nu), karyoplasm (Kp) and nuclear membrane (Nm) of basal cells of 2-day-old rat epidermis were selected for this purpose. Anti-Nu serum also stained the cytoplasm of basal cells, but the other 2 sera did not. The staining patterns of the 3 sera were unchanged in spinous cells. Intensity of the nuclear staining increased in granular cells in anti-Nu serum and anti-Kp serum. In addition, both sera stained cytoplasm in granular cells. Immunoreaction was seen on ribosomes, filaments and edges of keratohyalin granules and the staining appeared diffusely in cornified cells. In contrast, anti-Nm serum stained only the nuclear outline in granular cells and it continued to stain nuclear contour in the cornified cells of the 4th or 5th proximal layers. The antigenic component surrounded amorphous, but not filamentous materials in cornified cells. These findings suggest that some nuclear proteins accumulate in the cytoplasm during keratinization and finally become indistinguishable as nuclear protein. However, the nuclear membrane protein maintains its localization even after nucleic acids are absent. The nuclear space is retained in cornified cells which metabolically are no longer active.

Lipids in Pathogenesis of Abnormal Cornification: Induction of Scaling with Topical Cholesterol Sulfate in Hairless Mice. MARY E. MALONEY, MARY L. WILLIAMS, MICHAEL Y. L. LAW, AND PETER M. ELIAS, Derm. Serv., VAMC, and Dept. of Derm., Univ. of Calif. School of Medicine, San Francisco, CA

Although several abnormalities of stratum corneum lipid composition have been associated with abnormal cornification in man, direct evidence for the role of these lipids in the provocation of abnormal scaling is lacking. One recently described example of a lipid abnormality in ichthyosis is elevated cholesterol sulfate in recessive x-linked ichthyosis (RXLI) (J. Clin. Invest. 68:1404, 1981). But patients with RXLI also have decreased free sterols in their stratum corneum and abnormal metabolism of circulating sulfated steroid hormones, such as dehydroepiandrosterone (DHEAS). To determine whether cholesterol sulfate itself is a specific cause of scaling, we applied cholesterol sulfate, DHEAS, cholesterol (0.5–5 mg/ml DMSO, 0.04 ml – total daily dose), or vehicle alone to the backs of hairless mice. In animals treated with cholesterol sulfate, but not with either of the other lipids or with vehicle alone, scaling without erythema appeared after one week, peaking at two weeks, after which it began to decrease. Cessation of treatment resulted in reversal of scaling in 3 days. By further doubling the dose of cholesterol sulfate, scaling reappeared, then disappeared again, suggesting that cholesterol sulfate may induce steroid sulfatase, leading to its own desulfation. Whereas cholesterol sulfate-treated mice demonstrated increased stratum corneum thickness, they displayed no acanthosis, dermal inflammation, or increased mitoses, a further indication of a direct effect on the stratum corneum. We conclude that cholesterol sulfate provokes the scaling in RXLI by a direct effect on the stratum corneum.

Retinoids Suppress Cholesterol Synthesis in Cultured Human Epidermal Keratinocytes. M. PONEC, J. KEMPENAAR, AND B. J. VERMEER, Department of Dermatology, University Hospital, Leiden, The Netherlands

Cholesterol is an important component of the plasma membrane and plays a role in the regulation of various membrane functions. Retinoids were reported to affect terminal differentiation of keratinocytes which is accompanied with their enhanced desquamation. In addition, the retinoids also increase the membrane fluidity in erythrocyte ghosts. Both these effects might be related to cellular cholesterol synthesis. Therefore we decided to study the effect of retinoids on cholesterol synthesis in human epidermal keratinocytes, cultured according to the method of Rheinwald and Green (Cell, 6, 133, 1975).

Confluent cultures were incubated in dark in the presence of various concentrations (10^{-8} to 10^{-5}M) of etretinate (Ro-10-9359), Ro-10-1670 and 13-cis-retinoic acid, where 2 μl of ethanolic stock solution was added per ml medium. Control dishes obtained 2 μl ethanol only. As a measure of cholesterol synthesis the incorporation of ^{14}C-acetate or ^{14}C-octanoate into ^{14}C-cholesterol was taken.

All retinoids studied have been found to suppress the cholesterol synthesis in a dose-dependent manner. The suppression is observed already after a 6 hr preincubation of cells with retinoids and remains unchanged even when the incubation time is extended up to 3 weeks. The same degree of the retinoid-induced suppression of cholesterol synthesis is similar in young (1 week) and old (3 weeks) cultures.

Suppression of cholesterol synthesis in keratinocytes by retinoids may influence the differentiation and cell-to-cell adherence of keratinocytes by inducing changes in plasma membrane functions.

Distribution of Cholesterol in Membrane Systems in Human Keratinocytes As Studied by Filipin-Sterol Complexation. YASUO KITAJIMA, TAKASHI SEKIYA*, YOSHINORI NOZAWA*, KAZUFUMI YONEDA, MAKOTO YANAGIHARA AND SHUNJI MORI, Department of Dermatology and Department of Biochemistry*, Gifu University School of Medicine, Gifu City, Japan

Filipin (a polyene antibiotic) interact specifically with cholesterol in membranes, producing characteristic 25 nm-diameter deformation (pit-like lesions) on the membrane plane detectable by freeze-fracture electron microscopy. Utilizing this reaction, the distribution of cholesterol molecules in the membrane systems and in lamellar structures between horny cells were investigated in human skin. Since basal cell epithelioma has been known to be rich in gap and tight junctions, basal cell epithelioma was also used in order to study the cholesterol distribution in these junction-rich membranes.

The plasma membranes of basal, spinous and granular cells were well labeled with filipin except for desmosomal membrane portions. The plasma membranes of horny cells were rarely labeled with filipin, while lamellar structures between horny cells were well labeled. This indicates the distinct difference in susceptibility to filipin between the horny cell membranes and the lipid lamellar structures. The low susceptibility of the horny cell membranes may be due to the membrane rigidity rather than the content of cholesterol, since the horny cell membranes were labeled in some areas, although very weakly. Odland bodies were well labeled in the limiting membranes but poorly in the internal lamellar structures. Gap junctions and desmosomes were absolutely unlabeled. As to tight junctions, "pit-like" filipin cholesterol lesions were produced very close to the junctional strands but did not appear to disrupt the junctional structure.

Is the Non-ionic Detergent Soluble (NIDS) Material from Human Stratum Corneum Intercellular in Origin? P. J. DYKES, D. L. WILLIAMS AND R. MARKS, Dept. of Medicine, Welsh National School of Medicine, Cardiff, CF4 4XN

The intercellular region of the stratum corneum is of particular importance because of its roles in the barrier function and desquamation of the stratum corneum. Knowledge of the biochemistry of this region is sparse for technical reasons. However, it is known that mechanical stimulation of the skin surface in the presence of buffered detergent leads to the release of large numbers of corneocytes. These are intact and indistinguishable electron microscopically from those seen in normal tissue sections (Barton et al (1980). In particular the corneocyte surface membrane is clearly seen suggesting that only cell surface or intercellular material is being released into the supernatant during the stimulated desquamation. Studies on the non ionic detergent soluble (NIDS) protein released during this procedure have provided further evidence that this is indeed the case (King, Dykes and Marks 1982). Here we wish to report the results of lipid analysis of the NIDS material from human stratum corneum. Quantitative analysis of the major lipid classes was performed by thin layer chromatography with flame ionisation detection using the Iatroscan TH-10 Analyser (Bradley, Rickards and Thomas 1979). Results indicate that in 5 normal individuals studied the composition of the major lipid classes (ceramides, triglycerides, cholesteryl esters, free fatty acids and squalene) in isolated corneocytes does not differ significantly from that in whole stratum corneum. On the other hand the NIDS material, which represents less than 8% of the total stratum corneum lipid does show differences in lipid composition. In particular, an increase in free fatty acids and a decrease in cholesteryl ester fractions was observed (see

Table). This finding supports the 'brick wall' hypothesis of stratum corneum lipid composition proposed by Elias and co-workers and provides further evidence that the NIDS material is intercellular in nature. REFERENCES: Barton S P et al (1980) Brit J Dermatol 102: 63. Bradley D M, Rickards C R, Thomas N S T (1979) Clin Chim Acta 92: 293. King C S, Dykes P J, Marks R (1982) J Invest Dermatol 79: 297.

Association of Absent Steroid Sulfatase Activity with Reduced Levels of Immunologically Detectable Enzyme Protein in Recessive X-Linked Ichthyosis. ERVIN H. EPSTEIN, JR., AND JEANNETTE M. BONIFAS, Medical Service, San Francisco General Hospital Medical Center, and Dermatology Department, University of California, San Francisco CA

To investigate the molecular defect underlying the absent steroid sulfatase (SS) activity in patients with recessive X-linked ichthyosis (RXLI), we prepared antibodies against normal enzyme and assayed extracts of placentae and leukocytes from patients with RXLI for immunologically cross-reactive material (CRM).

Rabbits were immunized with microsomes from normal human placentae, and sera were tested for anti-SS antibody by the presence of SS activity in immunoprecipitates. All 10 immunized rabbits produced anti-SS antibodies. To test for CRM, extracts of 10 RXLI patients' tissues were incubated with immune sera and detergent-solubilized SS. Patients' extracts did not inhibit immunoprecipitation, indicating they contained little or no immunologically detectable, catalytically inactive enzyme protein. In contrast, enzyme rendered catalytically inactive by maleyation did inhibit immunoprecipitation of active enzyme.

Rabbits immunized with microsomes from RXLI placentae produced minimal amounts of anti-SS antibody, confirming that the placentae contained little or no CRM. Thus, the genetic defect in RXLI reduces or eliminates not only SS catalytic activity but also SS enzyme protein.

Enzymatic Activities of Metal Binding Proteins (MBP) in Rat Epidermis. YOSHIMASA ITO, NOBORU HORIE, AND KIMIE FUKUYAMA, Department of Dermatology, University of California, San Francisco, California

Histochemical studies demonstrated that proteins which localized in keratohyalin granules and cornified cells bind *in vitro* with Zn^{++} and Cu^{++}. To examine their function in the epidermis we measured enzymatic activities of MBP. Granular and cornified cells were scraped off from the epidermis of 2-day-old rats and homogenized. Protein (TBS fr.) was extracted in 0.1 M Tris-HCl, pH 8.0, containing 0.14 M NaCl. Since MBP in keratohyalin granules were not extracted by this step, the precipitate was homogenized again and the protein (KSCN fr.) solubilized in 0.1 M Tris-HCl, pH 8.0, containing 2 M potassium thiocyanate. Each protein fraction was separately applied on a Zn^{++} or Cu^{++} chelate Sepharose 4B column and the protein was eluted with buffers of different pHs and finally with EDTA. Enzyme activities known to exist in epidermal cells were measured, by methods previously reported, in both bound (MBP) and nonbound proteins on an affinity column. In TBS fr. only histidase bound on the column with Cu^{++}, but not Zn^{++}, while soluble acid phosphatase, cysteine proteinase, dipeptidase, cathepsin D, β-galactosidase and superoxide dismutase passed through from Zn^{++} and Cu^{++} columns. In contrast, in KSCN fr. plasminogen activator, neutral proteinase and solubilized acid phosphtase bound to both Zn^{++} and Cu^{++} columns, but additional proteinase activity was detected in the passed through fraction. These findings suggest that MBP in the epidermis, particularly those in keratohyalin granules, may exhibit enzymatic activity during keratinization. Keratohyalin granules may be directly involved in regulating cell metabolism by metal chelating enzymes.

Electron Paramagnetic Resonance: A Technique to Study Free Radicals in Intact Skin at Ambient Temperatures. B. SHROOT* AND C. BROWN†, * Department of Biochemistry, Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, Valbonne, France and † Department of Chemistry, University of Kent at Canterbury, England

The presence of free radicals in skin following UV irradiation under vacuum at very low temperature (−196°C) has been reported[1]. We now present evidence for the existence of drug induced paramagnetic species in intact skin at ambient temperature by means of electron paramagnetic resonance (EPR) spectrometry.

Small sections of the epidermis from the inside surface of the ears of freshly sacrificed pigs were mounted in a quartz tissue cell and covered by a thin quartz plate. The cell was then inserted in the cavity of a JEOLPEIX EPR spectrometer operating at ca. 9.4 GHz. Spectra were recorded before and after topical application of chloroform solutions of the antipsoriatic drug anthralin. Addition of anthralin (which in the absence of skin gave no paramaagnetic signal) resulted in the appearance of a strong resonance ($g = 2.0020$) whose itensity increased steadily with time. This signal was highly persistent and could still be detected several days after application of the anthralin. From the spectral data we conclude that this species is formed from anthralin. The radical is extensively resonance stabilised and of a type that has been theoretically predicted to be highly persistent, and this is supported by studies carried out with derivatives of anthralin in which the radical forming stability is altered. These findings can be correlated with in vitro, clinical and other biological observations. 1. A. Meybeck, *Int. J. Cosm. Sci.*, *1*, 199 (1979).

Effect of Hydrocortisone on Skin Development in the Chick Embryo. PHILIPPE SENGEL, MICHEL DÉMARCHEZ, DANIEL HERBAGE, AND ANNICK MAUGER, ERA CNRS 621, Laboratoire de Zoologie et Biologie animale, Université de Grenoble I, France

The effect of hydrocortisone on the development of dorsal skin was analyzed in the chick embryo by 1) transmission electron microscopy, 2) indirect immunofluorescence histology of extracellular matrix components (collagen types I, III, and IV, fibronectin and laminin), and 3) quantitative determination of collagen content and synthesis, between administration of the drug at 6 or 6 1/2 days and final retrieval of skin pieces at 11 days of incubation. Treatment caused the formation of featherless skin areas which were characterized by an early maturation of the epidermis, an increase of collagen content and synthesis, uniform distribution of interstitial collagen and rarefaction of fibronectin in the dermal extracellular matrix. The distribution of type IV collagen and of laminin was unchanged. The absence of feather formation in hydrocortisone-induced apteria is interpreted as resulting primarily from an early extinction of epidermal morphogenetic competence, and secondarily from modifications in the amount and distribution of extracellular matrix components in the dermis.

Epidermal Growth Factor Excretion in Urine Is Dependent upon Age, Sex and Race in Man. DAVID M. STOLL, DAVID N. ORTH, AND LLOYD E. KING, JR. Department of Medicine, Divisions of Dermatology and Endocrinology, Vanderbilt University and V.A. Medical Centers, Nashville, TN

Human epidermal growth factor (hEGF) is a peptide hormone that is presumably important in regulating the growth, differentiation and function of a variety of tissues, including epidermis. Urinary hEGF is thought to derive from plasma hEGF, the source of which is not yet known. If syndromes of hEGF excess or deficiency are to be defined, then normal values for urinary hEGF excretion must first be established. Therefore, urinary hEGF excretion, expressed as a function of creatinine excretion, was measured by homologous radioimmunoassay in random urine specimens from 261 normal subjects from 18 to 90 years of age who had normal renal function. Based on preliminary results, subjects were divided into four subgroups according to sex and race. Data were subjected to linear regression analysis and to analysis with the Newman-Keuls multiple range test to determine if there were significant differences between subgroups. Urinary hEGF excretion varied inversely with respect to age ($p < 0.01$), decreasing at a constant rate of 0.43, 0.29, 0.45 and 0.28 μg/g creatinine per year for white females, black females, white males and black males, respectively; the differences in slope (i.e., rate of decline) were not significant ($p > 0.05$). In contrast, each subgroup had a significantly different intercept ($p < 0.01$), indicating that EGF excretion was significantly different. For example, the values at age 40 were 42.0, 32.6, 28.8 and 23.8 μg/g creatinine for white females, black females, white males and black males, respectively. Estrogen administration has previously been reported to increase urinary hEGF, but the female:male difference in this study appeared to extend even into the postmenopausal age range. The physiological significance of the differences in urinary hEGF excretion observed in this study is not yet clear, but it appears that age, sex and race all affect urine hEGF levels. Establishing normal values for urinary hEGF excretion with respect to age, sex and race will be useful in defining possible abnormal hEGF metabolism in disease states.

Investigation of Epidermal Growth Factor (EGF) Receptors in Skin Tumours. MANFRED HAGEDORN, NORBERT WALTER, GERD GROSS AND THOMAS BAUKNECHT, Department of Dermatology and Gynaecology, University of Freiburg, FRG

Several investigations have shown the mitogenic activity of EGF in different cell systems. There exist some evidence that the proliferation is controlled by growth factors, which may be influenced by tumour promotors or transforming viruses. All these findings are based on "in vitro" experiments which can not be transferred automatically "in vivo." In this paper we want to clarify if human skin tumours are sensitive against growth factors, by looking for specific binding sites. The specific binding of 125-J-labelled EGF was determined at a membrane preparation of different skin tumours. The membranes were purified and experiments done according to Hock and Hollenberg (1980). A total of 40 benign, semimalignant and malignant tumours have been investigated. The benign tumours (lipoma, dermatofibroma, trichoepithelioma, naevuscellnaevi) showed no difference compared to the normal skin. Condylomata acuminata however usually revealed 2–3 fold, one tumour a 10 fold, higher binding capacity. 6 of 10 basaliomas showed a 2–4 fold increase. The same was true in precancerous lesions. The highest binding capacity could be detected in 2 squamous cell carcinomas. No EGF binding was found in 3 melanomas. The se findings reveal a connection of increased EGF binding sites in squamous cell tumours, depending on the differentiation. Similar results have been described by us in epithelial tumours as ovarian-, endometrial- and squamous cell carcinomas of cervix, where the poorly differentiated types predominantly are positive. In ovarian carcinomas the EGF receptor status seems to have a prognostic value, showing in the positive group a better response to cytostatic agents.

The Immunobiology of Basal Cell Carcinoma: An In Situ Monoclonal Antibody Study. DANIEL R. SYNKOWSKI, JOSEPH C. ORLANDO, A. LEE DELLON, Departments of Dermatology and Surgery, Johns Hopkins, Baltimore, MD

Twenty-four patients (ages 20–82) had excision of primary (16) or recurrent (8), small (≤0.9 cm, 6) intermediate (1–1.9cm, 14) or large (≥2cm, 4) and ulcerated (10) or non-ulcerated (14) basal cell carcinomas (BCC). Portions of the tumors were snap frozen in O.C.T. and stored at >70°C. Cryostat sections (4–6μg) were placed on slides, air-dried, fixed in acetone and processed in a peroxidase-anti-peroxidase technique using the following primary antibodies: all T cells, T65 and Leu 1; T Helper/Inducer cells, T4 and Leu 3a; T Suppressor/Cytotoxic cells, T8 and Leu 2a; Monocyte/Macrophages, M1; B cells/Monocytes/Activated T cells, Ia; and B cells, Leu 10. A representative section was stained with hematoxylin and eosin to assess the predominantly round cell infiltrate. Using this slide as a guide the antibody slides were reviewed and scored for the percentage of cells stained: 0, no cells stained; 1+ up to 25%; 2+ 25–50%; 3+ 50–75%; 4+ 75–100%. Lymph node and negative controls were run. Results (total number of tumors studied in parenthesis):

All T Cells T65		T Helper/ Inducer-T4		T Suppressor/ Cytotoxic-T8		Macrophages-III	Activated T cells-Ia	B Cells Leu 10
3.9 (24) Leu 1 3.7 (23)	3.8	2.5 (24) Leu 3a 3.3 (23)	2.9	2.9 (24) Leu 2 2.5 (23)	2.7	1.6 (20)	3.7 (20)	3.3 (19)

Thus the immunity involved in BCC is cell-mediated with vigorous T cell response but also humoral with marked B cell response. Interestingly, the macrophage score is low (1.6) supporting the observation of defective macrophage mobilization in BCC. All T Helper and T Suppressor and T to B cell ratios approximate one, whether the tumors are taken as a whole or broken down according to recurrence, size or ulceration.

Is Defect in Low Density Lipoprotein Metabolism a Marker of Epithelial Differentiation? M. PONEC, J. KEMPENAAR, L. HAVEKES*, AND B. J. VERMEER, Department of Dermatology, University Hospital and Gaubius Institute TNO*, Leiden, The Netherlands.

In order to gain more information on differences in cellular behavior during differentiation process a number of epithelial cells with or without defect in terminal differentiation was compared as to their regulation of cholesterol synthesis. The following cultured cells were used: normal (K) and by SV 40 virus transformed (SV K_{14}) epidermal keratinocytes, human squamous carcinoma cells (SCC-12F2 cells with low degree and SCC-25 cells with high defectiveness in terminal differentiation) and normal skin fibroblasts (F). As a measure of cholesterol synthesis the incorporation of ^{14}C-acetate into ^{14}C-cholesterol was taken.

The low density lipoprotein (LDL) enter the cells by receptor mediated endocytosis and are degraded in lysosomes, liberate cholesterol which in its turn interferes with the intracellular cholesterol synthesis. The LDL-induced suppression of cholesterol synthesis is very strong in F, lower in SCC-25 cells and very low in SCC-12F2 cells. In K and SV K_{14} cells virtually no suppression of cholesterol synthesis is observed. This effect is independent of the cell density. The amount of ^{125}I-LDL specifically bound to the membrane, internalized and degraded by the cells is very high in F, lower in SCC-25 cells and very low in SCC-12F2, SV K_{14} and K cells. By morphological studies similar results are obtained.

These results show that the normal keratinocytes and keratinocytes transformed by SV 40 virus into cells with impaired differentiation both have the same, i.e. virtually zero, response to LDL. However, the picture is different when the normal keratinocytes are compared with the SCC cells. Here it appears that the response to LDL increases with decreasing ability of the cells to differentiate.

Markers and Immunological Function of Langerhans Cells in Human Epidermal Cell Culture J. CZERNIELEWSKI, M. REGNIER, M. GRANGERET, AND M. PRUNIERAS, Dept. of Cell Biology, CIRD, Sophia Antipolis, 06565 Valbonne, France

Recently two distinct types of human epidermal cells (EC) expressing HLA-DR antigen were demonstrated, namely the Langerhans cells (LC) and Indeterminate cells (IC), the former exclusively OKT-6 positive. Here we used IF technique with OKT-6 and anti-HLA-DR monoclonal antibodies to study the presence of these antigen expressing cells in human EC culture. We also examined their immunological function as measured by the capacity of EC to stimulate allogeneic peripheral blood lymphocytes (PBL) in MSLR (Mixed skin cell Lymphocyte culture Reaction). EC suspensions were obtained from surgical skin specimens through skin trypsinization. They were cultured (collagen coated dishes, Dulbecco's MEM supplemented with 15% FCS, EGF, and cholera toxin, antibiotics and without hydrocortisone) for varying time periods prior to harvest and used as stimulator cells in MSLR. Responder (R) PBL and stimulator (S) EC were cocultured (R:S ratio 1:1) for 5 days:PBL proliferation was measured by 3H-thymidine uptake.

	Duration of culture (days)							
	0	2	4	6	8	10	12	20
Stimulation of allogeneic PBL expressed as mean SI*	22, 7	18, 3	11, 3	3, 3	1, 1	1	1	1

* Mean SI—stimulation index:ratio cpm MSLR/cpm L cultured alone. Like fresh EC, EC cultured for up to 3 days were capable of eliciting a vigorous response in the MSLR, after that it progressively diminished. No stimulation was observed after 8 days of culture. IF study demonstration OKT-6 staining only for 9 first days of culture. However DR(+) cells were still present up to 23 days of primary EC culture. Furthermore, we noticed more DR(+) than T-6(+) in both suspensions and cultures. Conclusion:the capacity of cultured keratinocytes to stimulated lymphocytes is lost when OKT-6 (but not HLA-DR) positive cells disappear.

Monoclonal Antibodies (MCA) May Recognize Various Antigens of Unexpected Distribution in Skin. JEAN THIVOLET, DANIEL SCHMITT, AND JACQUELINE VIAC, U. INSERM 209—Pav. R—Hôpital E. Herriot, Lyon, France

Searching for unexpected specificities, the reactivity of 152 MCA has been controlled on skin sections by IF. We have tested 116 mouse MCA from various origins raised against human lymphoid cells reacting or not with those cells and 56 human sera with monoclonal IgG, M or A obtained from myelomas, macroglobulinemias or isolated dysglobulinemias patients. Indirect IF technique was carried out on frozen sections (4 μm) of human foreskin, rabbit lip and mouse skin which had been fixed in acetone, incubated at 37°C for 45 mn with MCA or sera at various dilutions, washed carefully and then incubated with fluorescein conjugated antimouse of human Ig. After screening, some crossreaction MCA have been studied by immunochemical methods. In one case, the antigenic protein has been characterized. Of 90 MCA reacting with human lymphocytes, 8 crossreacted with unexpected skin antigens (USAs): basal cells, epidermal cell surface, endothelial cells, smooth muscles, eccrin sweat glands, fibroblasts. Of 26 MCA showing no apparent reactivity with human lymphocytes, 2 reacted with USAs: basal cells, epidermal cells. of the 56 human monoclonal Ig, 3 out of 31 IgG reacted with USAs: stratum corneum, nuclei; 6 out of 19 IgM reacted with USAs: basal and endothelial cells, epidermal cell surface, collagen fibers, fibroblasts; and none of the 6 IgA showed a reactivity. Thus unforeseen crossreactivities may exist even in embryologically

unrelated tissues. MCA react with small antigens (epitopes or primary antigens) which may be shared by different large molecules or structures. The unpredictable existence of such epitopes necessitates the careful control of MCAs before using them as reagents. Mapping of tissue epitopes remains to be done as well as the evaluation of this new concept in terms of autoimmunity.

Neurofibromatosis: A Quantitative Analysis of the Epidermal Keratinocyte and Melanocyte Populations. E. FRENK, A. MARAZZI AND E. HALLER, Dept of dermatology and statistics, Faculty of medicine, University of Lausanne, Switzerland

The pigmentary disorder of neurofibromatosis being poorly understood, a study of the numerical relationship between epidermal keratinocytes (KC) and melanocytes (MC) was undertaken.

Skin biopsies were taken from café-au-lait spots and surrounding normally pigmented skin of 9 patients with neurofibromatosis (NF). The KC of the basal, spinous and granular layer were evaluated on 4 μm H&E sections, by counting the nuclei in 10 segments, each 0.15 mm wide. The MC were counted on NaBr-separated, Dopa incubated sheets of epidermis from the same biopsy (in 10 fields of 0.0225 mm^2). The values obtained were considered representative of the 2 epidermal cell populations and the ratio KC/MC was determined. The results in NF were compared to those obtained in normal skin of 15 healthy individuals, where the ratio KC/MC was found to be constant.

The ratio KC/MC was significantly decreased in café-au-lait spots and in normal skin of NF, if compared to skin of healthy subjects (Mann & Withney test); this decrease was significantly more pronounced in café-au-lait spots than in normally pigmented skin of NF (Wilcoxon matched pair test).

These results suggest an aberrant tissue organisation in the epidermis of NF with regard to KC—MC interrelation, the MC being relatively more numerous than the KC. Such a defect is compatible with a recent hypothesis postulating impaired cell-cell interaction as a major pathogenic factor in NF (Riccardi, NEJM, 305, 1717, 1981).

Immunopathological Investigation of Glycocalyx Material in Darier's and Hailey-Hailey Disease. EDWARD ABELL, Department of Dermatology, Univ. of Pittsburgh, Pittsburgh, PA

Epidermal Cell Glycocalyx (ECG) has been studied in Darier's and Hailey-Hailey disease by immunologic and nonimmunologic staining methods. Six pemphigus sera were used as a source of specific antibody to ECG and FITC labelled Concanavalin A was used as a non-immunologic stain of this cell surface glycoprotein. Unfixed 4μ cryostat sections of lesional and perilesional normal skin of 8 cases of Darier's and 2 cases of Hailey-Hailey disease were stained with these reagents by standard immunofluorescent methods. Pemphigus antibody binding was then shown by a second incubation with an FITC labelled antihuman IgG.

Binding of pemphigus antibody was uniformly and precisely absent within the acantholytic portion of all 10 lesions. Binding of FITC Concanavalin A was absent or markedly reduced in all 10 lesions also.

These findings demonstrate a major defect of the glycalyx material in both these diseases and represents evidence that acantholysis may be related to this defect rather than to a primary abnormality of desmosomes, as previously suggested.

An Animal Model for Altering the Irritability Threshold of Normal Skin. SUSAN S. ROPER AND HENRY E. JONES, Emory University School of Medicine, Atlanta, Georgia

Theory behind conditioned hyperirritability (autoeczematization) predicts the lowering of the irritation threshold in the presence of certain pre-existing dermatitides. We have attempted to develop an animal model that parallels the syndrome seen in man. Groups of ten guinea pigs were shaved and epilated, and irritation thresholds to benzylkonium chloride and trichloroacetic acid were determined using 1 cm diameter open patches. Reactions were scored 24 hours later on the basis of erythema and induration. Animals having as little as 1.56cm^2 of skin acutely inflammed with a known irritant had lowered irritation thresholds to this same irritant in normal skin at remote sites ($p < 0.01$). Mild irritation of a much larger surface area produced the same effect ($p < 0.01$). More extensive severe dermatitis had no additional effect on lowering the irritation threshold further. Acute dermatitis induced by a contact allergen (DNCB) lowered the irritation threshold of normal skin to the same level as that obtained with irritants ($p < 0.01$). Induction of chronic cutaneous ulcers 3–4 cm in diameter lowered the irritation threshold of normal skin to the same point defined by the acute studies ($p < 0.01$). We interpret these results to indicate that an acute irritant or contact dermatitis, as well as chronic skin ulceration, may alter the reactivity of unaffected normal skin to exhibit a heightened response to irritation. This model appears to differ from that seen in humans, in that a more extensive or chronic dermatitis did not further heighten the susceptibility to irritation.

Sunday, May 1, 1983

8:00 AM–12:00 PM CONCURRENT SCIENTIFIC SESSIONS

SESSION A — Presidential Ballroom

Peter Fritsch, M.D., Presiding

An Improved Assay for Vitamin A Cytoplasmic Binding Proteins. JAMES H. MILLER, JR., RONALD E. GATES, AND LLOYD E. KING, JR., Dept. of Medicine (Dermatology), Vanderbilt and VA Medical Centers, Nashville, TN

While the mechanism of action of vitamin A is unknown, specific cytoplasmic binding proteins for vitamin A and its analogs (retinoids) suggest similarities to steroid hormone action. These cytoplasmic receptors retinol binding protein (cRBP) and retinoic acid binding protein (cRABP) have binding affinities for retinoids which correlate well with biological activity. Preferred methods for measuring cRBP and cRABP are adaptation of methods used with steroid binding proteins in which labeled ligand is incubated with cytosol and specific binding quantitated after resolution of binding protein on a sucrose gradient. To reduce both time and equipment requirements of the preferred assay, we have substituted brief centrifugations of molecular sieving chromatography columns for the 20 hr sucrose gradient ultracentrifugation and subsequent tedious gradient fractionations. Brief centrifugation greatly reduces the time for ligand dissociation which occurs with the usual, much slower column separation methods. [^{3}H]-retinol or [^{3}H]-retinoic acid were purified by HPLC and then incubated with cytosol for 2 hr at 0°C. After removal of unbound ligand with dextran-coated activated-charcoal, treated cytosol (200 μl) was applied to a 3 ml column of hydrated polyacrylamide beads (Bio-Gel P-60). Binding protein was eluted from the column with a series of 200 μl buffer aliquots combined with 3 min centrifugations at 80×g. Three well-resolved peaks of radioactivity were eluted which correspond, in order of elution, to ligand non-specifically bound to serum albumin, to ligand bound specifically to cRBP or cRABP, and to unbound free ligand. While correlating well with results from sucrose gradient assays, cRBP and cRABP levels from our improved assay are obtained more rapidly with less effort and equipment.

Cellular Retinoic Acid Binding Proteins in Human Epidermis and Sebaceous Glands. S. M. PUHVEL AND M. SAKAMOTO, Division of Dermatology, UCLA School of Medicine, Los Angeles, California

13-cis-retinoic acid (13-cis RA) induces dramatic clinical improvement and remission from nodulo-cystic acne. A very specific effect of 13-cis RA in human skin is suppression of sebaceous gland lipogenesis. Some research suggests that effects of retinoids in tissues are mediated by specific cellular binding proteins. In this study we compared the cellular retinoic acid binding proteins (cRABP) in human epidermis versus sebaceous glands.

Using microdissection of human facial skin (after pre-treatment with collagenase), we collected large quantities of isolated sebaceous follicles and sebaceous gland-free epidermis. Tissues were homogenized, cytosol fractions isolated and cRABP activity assayed by standard procedures using incubation of cytosol with ^{3}H-RA and ^{3}H-13-cis RA, followed by charcoal absorption and sucrose density gradient centrifugation. Competitive inhibition assays and Scatchard plot analyses were performed to establish the specificity of binding, and to compare the number and affinity of the binding sites. At least 4 different pools of cytosol were run for each tissue. Each pool was obtained from skin of 3 to 4 face lift patients.

The approximate binding of RA per mg of cytosol protein was 10.1 ± 1.1 pmols in epidermal cytosol, and 8.0 ± 1.6 pmols in cytosol from sebaceous follicles. Approximate binding of 13-cis RA was 2.1 ± 0.3 pmols in cytosol from epidermis, compared to 3.12 ± 0.7 pmols in ducts and glands.

The slight difference in affinity of binding proteins for 13-cis RA in sebaceous follicles compared to epidermis suggests that the dramatic clinical effect of this retinoid in human skin cannot be attributed to differences in the cRABP in these tissues.

Qualitative and Quantitative Analysis of Cytosol Retinoid Binding Proteins in Human Skin. J. J. DiGiovanna, G. Chader, and R. T. Fletcher, Derm. Br., NCI and Lab Vision Research, NEI, NIH, Bethesda, MD

The distribution of the cytosol retinol (CRBP) and retinoic acid (CRABP) binding proteins varies widely within the different layers of the eye, a retinoid target organ. CRABP has been reported in chick embryo, human and rat skin, and dermal fibroblast cultures. CRBP has been reported in rat skin. We now have identified CRBP in 0.3 mm keratomed normal adult human skin and examined the relative contribution of the epidermis and dermis to the total retinoid binding. CRBP and CRABP were measured as the binding of ^{3}H-retinol (^{3}H-ol) and ^{3}H-retinoic acid (^{3}H-RA) to cytosol preparations using sucrose gradient centrifugation. Saturability and specificity were determined by demonstrating the presence or absence of competition with an excess of nonradiolabeled retinoid. The mean specific activity of ^{3}H-ol and ^{3}H-RA binding (0.57 ± 0.07, 3.43 ± 0.57 pmol/mg protein) to cytosol preparations from different specimens of adult human skin was determined. Skin obtained from one sample was assayed for cytosol ^{3}H-ol and ^{3}H-RA binding as full thickness skin (0.36 ± 0.09, 2.11 ± 0.38 pmol/mg protein), EDTA-separated epidermis (0.36 ± 0.03, 3.69 ± 0.13 pmol/mg protein) and subjacent dermis (neither detectable). The epidermis alone demonstrated binding for each retinoid at least as great as the full thickness skin. Adult human skin was keratomed at 0.1, 0.2 or 0.3 mm, and histology was obtained. Increasing the thickness from 0.1 to 0.2 mm (which added almost all of the lower epidermis) increased the specific activity for both retinoids. As thickness increased to 0.3 mm (adding significant dermal contamination), the specific activities decreased. These results suggest that CRBP and CRABP in adult human skin are predominantly located in the epidermis.

Effect of the Retinoid Ro13-6298 on Epidermal Differentiation in Mice. Roy Grekin, D. Tsambaos, E. Duell, and J. Voorhees, Dept. of Derm., Univ. of Mich. Med. School, Ann Arbor, MI and Freie Univ. Berlin, Berlin, W. Germany

Arotinoid (Ro13-6298), a tricyclic analog of vitamin A, is up to 500 times more potent than etratinate. Early trials with arotinoid show a stimulation of the granular cell layer of skin. As this layer is lost in some epidermal diseases, e.g. psoriasis, arotinoid is of great clinical interest. Skin samples were obtained from hairless mice: control or treated orally with arotinoid (0.1 mg/kg/day) for 1 week or (4 μg/kg/day) for 4 weeks. Serial extractions of the epidermis resulted in 6 fractions: keratohyalin granule proteins (R2), high salt soluble proteins (S2), SDS soluble nucleated cell content (S3), non-covalently (S4) and covalently (S5) crosslinked keratins and cornified cell envelope (R5). The proteins were studied using the Lowry protein assay, SDS polyacrylamide gel electrophoresis (PAGE) and isoelectrofocusing plus SDS-PAGE (2D-gels). Results from 1 week samples showed an increase (↑) in R2 (133%) and S5 (64%) and a decrease (↓) in S4 (27%) in percent of total protein isolated in treated vs control skins. 4 week samples showed similar changes but to a lesser degree with an ↑ in R2 (19%) and S5 (59%) and a ↓ in S4 (19%). The SDS-PAGE gels verified these quantitative changes and also showed qualitative changes in protein patterns. The 1-D gels showed a general ↓ in keratin proteins in the treated S4; in treated S5, a selective ↑ of keratins MW 46K to 53K occurred. 2-D gels augment these results and indicate a ↓ compared to controls in the treated S4 fractions of proteins MW 66K over a wide pH range and an ↑ in treated S5 fraction over controls in proteins at MW 68K and 60K at various pHs. S5 gels show an ↑ in acidic proteins at MW 52K. These data indicate that arotinoid may enhance epidermal differentiation by stimulating a shift in fibrous keratins from non-covalently to covalently crosslinked proteins.

Effect of a New Retinoid, Arotinoid, Ro 13-6298 on *In Vitro* Keratinocyte Proliferation, Differentiation and Cellular cAMP Content. R. Stadler, C. Marcelo, J. Voorhees, and C. Orfanos, Departments of Dermatology, Univ. Medical Center Steglitz, Free Univ. Berlin and Univ. of Michigan Medical School, Ann Arbor, MI

The new retinoid, Arotinoid, (Ar), is effective therapy in hyperproliferative and keratotic disorders. To understand the mode of action of Ar, we use primary keratinocyte cultures, and technics to study proliferation and differentiation. Ar-induced changes in cAMP content were determined using cholera toxin (CT) to stimulate $cAMP_3$. Neonatal mouse keratinocyte proliferation was determined using ^{3}H-tdr uptake into DNA and autoradiography. Differentiation was assessed using autoradiography of one dimensional polyacrylamide gel electrophoresis (PAGE). PAGE was done on proteins serially extracted from the cells using 6 buffers (keratins, cell envelope and keratohyalin granule-associated, KHG). cAMP content was determined using RIA of partially purified fractions. Ten doses of Ar ranging from 12 μg (32 μM) to 50 pg (1.3 fM) were added to the cells 1 day after plating (D = 1). Controls contained the 0.2% DMSO vehicle. After 7 and 10 d ≃ 50% inhibition of keratinocyte proliferation was induced by 12 μg to 0.05 μg of the Ar (9 doses, inclusive). The 12 μg dose was cytotoxic by D = 15. Cells treated with 0.5 μg Ar were pulse labeled with 2 μCi/ml ^{3}H-histidine and the proteins were studied by PAGE (D = 12). Ar significantly decreased the amount of fibrous (keratin) and cell envelope proteins produced while increasing the amount of KHG proteins. Ar (0.5 μg/ml) did not change keratinocyte cAMP content whereas a decrease of 50% in the cAMP content of CT stimulated cells was seen. In conclusion: 1) Ar decreased *in vitro* keratinocyte proliferation, and keratin and cell envelope protein production while stimulating KHG protein formation; 2) Ar did not directly change the cellular cAMP content of the cells, although Ar partially decreased the effect of cAMP stimulating agents.

Stability and Metabolism of Retinoids in Culture. Yukio Kitano, and Jun Adachi, Department of Dermatology, Osaka Univ. School of Medicine, Osaka, Japan

With the development of new retinoids which have strong therapeutic actions and less side effects, retinoids have become important drugs for the treatment of skin disease. In the study of the mechanism of action of retinoids, tissue culture system is widely used. Retinoids are unstable compounds. The metabolism by enzymes is important in the understandings of pharmacokinetics of retinoids. The stability in tissue culture medium and the metabolic activity of cells were investigated. Retinoic acid, 13-cis-retinoic acid and etretinate were added to the medium at a concentration of 10^{-6} M and the medium was taken after one to several days. Analysis and measurement were made by high performance liquid chromatography. Retinoic acid and 13-cis-retinoic acid were interchangeable in the medium. Four days after the addition of retinoic acid in Eagle's MEM supplemented with 5% fetal bovine serum, 30% of retinoic acid was 13-cis-retinoic acid. When 13-cis-retinoic acid was added to the medium consisted of TC199 with 5% fetal bovine serum, 54% was found in the form of retinoic acid after 4 days. Etretinate was stable in the culture medium. In the culture of B16 mouse melanoma cells in TC199 with 5% fetal bovine serum, the total amount of retinoic acid and 13-cis-retinoic acid was 57% to that in the medium without the cells after 4 days of incubation. B16 mouse melanoma and human melanoma cells had a strong capacity to hydrolyse etretinate to form Ro10-1670. HeLa cells did not hydrolyse etretinate. The stability of retinoids in the medium were variable and the cells had different capacities not only in the response to retinoids but also in the metabolism of retinoids.

Effect of Topical Application of 13-cis-Retinoic Acid on Skin of Hairless Rats. M. Sakamoto and S. M. Puhvel, Division of Dermatology, UCLA School of Medicine, Los Angeles, California

Oral administration of 13-cis-retinoic acid (13-cis RA) is effective in treatment of nodulo-cystic acne. In humans, suppression of sebaceous gland lipogenesis is among the most striking effects of this drug. There have been no reports on the effect of 13-cis RA following topical application. This study was aimed at evaluating this question, using hairless rats as experimental models.

Five concentrations (1.0, 0.2, 0.02, 0.01 and 0.005%) of 13-cis RA were prepared in a lipid base. Hairless Sprague-Dawley rats, housed individually and fitted with collars were treated for 2 to 4 weeks, once daily, by spreading 0.5 ml of 13-cis RA emulsions over the skin of the backs. Matched controls were treated with lipid base alone. In addition to monitoring the weight of animals and changes in cutaneous morphology, sebaceous gland activity was assessed by either: gravimetric determination of sebum production using a modification of the method of Ebling and Randall (J. Invest. Derm. 77: 458, 1981); *in vitro* lipid biosynthesis using 3 mm skin biopsies; or, histologic evaluations of sebaceous glands.

Results indicated that in rats topical application of 13-cis RA is more toxic than has been reported following oral administration. Four applications of 0.2% (total of 4 mg) induced severe emaciation and subcorneal cutaneous inflammation resulting in denudation of large areas following peeling of skin. Four applications of 1.0% were rapidly fatal. At non-toxic doses (.e. 0.01 and 0.005%) the effect of 13-cis RA was indistinguishable from the effects of the lipid base. The 0.02% emulsion induced mild brown scaliness of skin, and reduced normal weight gain by the animals, but had no suppressive effects on sebeceous glands.

Effects of Aromatic Retinoid (Ro 10-9359) on Epidermal Cell Production and Metabolism in Normals and Patients. A. D. PEARSE AND R. MARKS, Dept. of Med., Univ. Hosp. of Wales, Cardiff CF4 4XN, Wales

Although the retinoids produce clinical improvement in disorders of epidermal differentiation, their mode of action remains uncertain. In order to obtain data regarding the molecular basis of the retinoids' clinical effectiveness, a cytochemical and cell kinetic study has been completed on normal individuals and patients receiving etretinate. Etretinate (1 mg/kg/day) was given to 10 normal subjects and 8 patients with ichthyosis over 4 weeks. Biopsies taken before and at the end of treatment were studied by a novel cytochemical technique for glucose-6-phosphate dehydrogenase (G6PHD), succinic dehydrogenase (SDH), lactate dehydrogenase (LDH) and non-specific esterase (NSE) activities utilizing the densitometer module and image editor of an image analysing computer. Epidermal enzyme activities were characteristically altered after etretinate, the normals showing increases in all activities examined (Fig. 1) and the ichthyotics demonstrating decreases in (pre-existing elevated) G6PDH and SDH activities and increases in NSE activity. Biopsy fragments were also assessed for mean epidermal thickness (MET) using the image analysing computer, and thymidine (^{3}H) autoradiographic labelling indices (LI). In normal subjects before treatment the LI was 4.9% ± 1.2 and the MET was 60.23 μm ± 1.07, after treatment the LI was 6.8% ± 4.2 and the MET was 60.19 μm ± 1.94. The results indicate (1) the effect on epidermal metabolism persists after 4 weeks although the kinetic alterations have returned to normal (other studies indicate this trend), and (2) the changes recorded in patients reflect normalization of differentiation. REFERENCE: Fritson PO, Pohlin G, Langle U, Elias P. (1981). Response of Epidermal Cell Proliferation to Orally Administered Retinoid. J. Invest. Dermatol. 77: 287–291.

The Influence of Retinoic Acid on Collagen Synthesis by Skin Fibroblasts. DOUGLAS L. NELSON AND GARY BALIAN, Departments of Dermatology, Orthopedics and Biochemistry, University of Virginia, School of Medicine, Charlottesville, Virginia

The effect of all-trans retinoic acid (RA) on the incorporation of radioactive thymidine and amino acids by neonatal skin fibroblasts in culture was examined. Concentrations of RA varying from 10^{-6} to 10^{-11} M were used for a 24 hour preincubation prior to addition of radiolabel, and then throughout the labelling period. The incorporation of ^{3}H-methyl thymidine showed that cell proliferation decreased in response to increasing concentrations of RA. Analysis of ^{3}H-proline labelled proteins secreted into the culture medium before and after digestion with purified bacterial collagenase revealed a decrease in the synthesis of both collagen and non-collagenous proteins as a result of increasing RA concentrations. SDS-polyacrylamide gel electrophoresis (PAGE) of secreted proteins showed increased processing of procollagen. The appearance of intermediates in the enzymatic conversion of procollagen to collagen indicated that RA enhances the activity of procollagen amino protease. RA had no apparent effect on the migration of cellular or secreted fibronectin on SDS-PAGE. The morphology of cells was determined by phase-contrast microscopy and shown to be unchanged at all RA concentrations. Our results showing a decrease in thymidine incorporation, collagen and total protein synthesis are consistent with previous observations on the inhibitory effect of RA on cell proliferation. We have observed increased protease activity involving the processing of collagen precursors which may be an important event in the overall influence of RA on the elaboration of the connective tissue matrix.

The Induction of Epidermal Ornithine Decarboxylase by Retinoic Acid. MICHAEL J. CONNOR AND NICHOLAS J. LOWE, Division of Dermatology, UCLA School of Medicine, Los Angeles

The activity of the putrescine synthesizing enzyme ornithine decarboxylase (ODC) (EC 4.1.1.17) is raised during proliferation and its induction may be an obligatory component of tumor promotion. The induction of ODC activity is inhibited by retinoids, and this may explain their antiproliferative and antipsoriatic properties. Several retinoids despite the ability to inhibit the induction of ODC activity, can stimulate DNA synthesis and induce hyperproliferation of the epidermis. This dissociation of ODC activity and DNA synthesis suggests that the induction of ODC activity is not essential in proliferation. We now report that topical retinoids, particularly all-*trans*-retinoic acid (RA) can induce ODC activity in the epidermis of hairless mice. The induction of ODC activity by RA is dose dependent and is measurable in the epidermis 24 hours or more after application.

Treatment	ODC Activity at 24 Hr	Relative Activity
Vehicle (acetone)	.081 ± .048	1
3.4 nmol RA	.139 ± .080	1.7
10 nmol RA	.34 ± .15	4.4
100 nmol RA	2.07 ± 1.41	25

The induction of ODC activity by RA is itself suppressed by the retinoid analog arotinoid ethyl ester. The inhibition of the expression of ODC activity by retinoids must occur at a stage later than the induction process *per se*, since it is unlikely that retinoids induce and inhibit the expression of the enzyme by the same mechanism. These observations may explain recent conflicting and contradictory reports of retinoid effects on skin-carcinogenesis and support the hypothesis that ODC activity is a component of proliferation.

Sebum Suppression After Topical Application of Retinoids (Arotinoid an Isotretinoin). GERD PLEWIG, ANETTE RUHFUS, AND WINFRIED KLÖVEKORN†, Departments of Dermatology, University of Düsseldorf and Munich†, FRG

Sebum suppression after oral or parenteral use of isotretinoin is well documented in man and animals. This study describes sebum suppression after topical use in Syrian hamsters with a new retinoid (arotinoid) and isotretinoin, and in humans with isotretinoin.

Animals: Arotinoid (Ro13-6298) and isotretinoin (Ro4-3780), dissolved in aceton, was pipetted to the left ventral ear lobe and left flank organ (0.3; 0.05; 0.01; 0.005; and 0.001%) twice daily (5/7) for 3 weeks to groups of 10 12-h-light adapted mature male Syrian hamsters. The right ear and flank organ (aceton) and untreated animals served as controls. Sebaceous gland surface was planimetrically measured with a semi-automatic picture analyzer. The experiments were repeated 3 months later.

Humans: A 0.2% isotretinoin vanishing cream was used twice daily (7/7) for 16–20 weeks by 20 patients with seborrhea and acne. Casual sebum and 1-h-replacement levels were measured with a lipometre (μg/cm^2) and acne lesions from the face counted.

Both retinoids suppress sebum production significantly in the ear lobe and flank organ, dose-dependent, compared to controls. Arotinoid is almost 500-fold more effective, but similarly toxic, causing dermatitis, hair-loss, bone deformation and fractures. Isotretinoin was well tolerated. Non-toxic arotinoid concentrations are 0.0001% and less. Isotretinoin improved acne with a significant drop of lesion counts, caused no irritation, and lowered skin surface lipids up to 30% and more. Arotinoid may not be used in humans due to its high toxicity.

Follicular Distribution of Skin Bacteria and the Effect of Sebum on Abundance. J. N. KEARNEY, G. GOWLAND, D. HARNBY, K. T. HOLLAND AND W. J. CUNLIFFE, Departments of Immunology, Microbiology and Dermatology, University of Leeds, U.K.

Quantitative data on the distribution and depth of penetration of viable bacteria in human skin follicles is lacking but is pertinent to both disease processes and treatment. Our investigations used cryostat sectioning not adversely effecting microbial viability. Dependence of bacterial abundance on sebaceous gland products was investigated using split and full-thickness human skin grafts on a murine host.

6 mm punch biopsies of cadaver anterior thorax were sectioned to a depth of 2.4 mm. Quantitative microbial counts of successive 0.2 mm homogenised fractions were made. The major reservoir of *P. acnes* was always >0.2 mm below the surface, but the model fraction varied even amongst biopsies from the same donor. Staph. distribution varied from a primary upper location to an even distribution throughout the biopsy.

Split-thickness (0.6 mm) skin would normally contain high bacterial numbers but would exclude sebaceous glands. The fate of the major microbial groups over a 9 day period on both split- and full-thickness (1.5 – 2.0 mm) human skin grafts was observed using hairless mice as recipients. On full-thickness grafts the density of microorganisms remained unchanged. Exclusion of sebaceous glands significantly decreased *P. acnes* density (≏2.5 log cycles) but not staphylococci. This suggests that *P. acnes* depends on sebaceous gland products but staphylococci do not and corroborates circumstantial evidence available in the literature.

Long-Term Inhibition of Quantitative Sebum Production with Isotretinoin. E. G. GROSS, G. L. PECK, P. G. GANTT, AND M. N. WESLEY*, Dermatology Branch, Biometrics Branch*, NCI, NIH, Bethesda, MD

Dose-dependent inhibition of quantitative sebum production (QSP)

precedes the therapeutic benefits of oral isotretinoin in cystic acne. Forty patients with cystic acne were treated with a high dose (1.0 or 2.0 mg/kg/d) of isotretinoin for 2 or 4 weeks followed by a low dose (0.25 or 0.5 mg/kg/d) for 16 weeks. Three hour forehead QSP was measured gravimetrically (Strauss & Pochi 1961) during each follow-up visit. When compared during and after treatment there were no statistically significant therapeutic or QSP differences between these different treatment groups. There was a median decrease of 81% in QSP after 4 weeks of therapy which continued through the 16th week of treatment. Twelve partially responding patients (<75% improvement) were given a second course of therapy (2.0 mg/kg/d) for 6 months. One year after therapy there was an overall 97% clinical improvement. One month after stopping therapy, there was no statistically significant difference in reduction of QSP between responders (46%) and partial responders (56%). No correlation was seen between the percent inhibition of QSP and the therapeutic response to isotretinoin. Patients given the second course of isotretinoin showed a 10% further depression of sebum production while on therapy ($p = .01$, significant rank test). Furthermore, one year or more after stopping therapy this group showed a 60% decrease in QSP as compared to pretreatment values, while patients who received only a first course of therapy had a 39% decrease from their original value ($p = .03$, blocked Wilcoxon test). QSP in 5 patients approximated their pretreatment levels within one year. Dose-dependent prolonged partial suppression of QSP may be one mechanism by which prolonged remissions of cystic acne are induced by isotretinoin.

Effect of Varying Dosages of Isotretinoin on Nodulocystic Acne. RONALD R. RAPINI, ELIZABETH A. KONECKY, BRENT SCHILLINGER, HARRIET COMITE, JOHN H. EXNER, JOHN S. STRAUSS, ALAN R. SHALITA, AND PETER E. POCHI, Departments of Dermatology, University of Iowa College of Medicine, Iowa City, Iowa, State University of New York Downstate Medical Center, Brooklyn, New York, and Boston University Medical Center, Boston, Massachusetts.

While it is generally acknowledged that isotretinoin is very useful in the management of severe nodulocystic acne, the range of effective dosage of the drug in the treatment of severe acne has not yet been determined. Because a preliminary study demonstrated that small dosages of the drug could be effective, a double-blind tri-center study has been conducted using three different dosages, namely 0.1, 0.5 and 1.0 mg/kg/day for 16 to 20 weeks. A total of 150 patients were entered into the study. Of this number, 137 patients completed the study (0.1 mg/kg/day-47 subjects, 0.5 mg/kg/day-45 subjects, 1.0 mg/kg/day-45 subjects). All nodulocystic lesions, 4 mm or greater in size, were counted prior to treatment, at no less than 1-month intervals during treatment, and for 2 months post-treatment. The data on lesion counts are as follows:

	% reduction of nodulocystic lesions					
	2 wks	4 wks	8 wks	12 wks	16/20 wks	Post-treatment
1.0 mg/kg/day	13.1	24.6	41.6	42.0	68.5	88.2
0.5 mg/kg/day	20.1	39.2	56.5	73.3	81.1	88.9
0.1 mg/kg/day	11.8	31.1	31.7	52.9	69.5	82.4

The data show that the lowest dosage, 0.1 mg/kg/day, was virtually as effective as the higher dosages in reducing the number of lesions. The average reduction in the number of facial lesions as compared to trunk lesions was greater for each of the doses used, but the differences were not significant.

Evidence for the Sebaceous Origin of Human Skin Surface Cholesterol Esters. MARY ELLEN STEWART, ALLISON M. BENOIT, DONALD T. DOWNING, AND JOHN S. STRAUSS, Marshall Research Laboratories, Department of Dermatology, University of Iowa College of Medicine, Iowa City, Iowa.

The presence of cholesterol esters (CE) on the skin surface has been variously attributed to epidermal production, sebaceous secretion, skin surface esterase activity, or a combination of these sources. In order to identify the source of CE, we measured the sustainable rates of secretion of CE, and of other skin surface lipid components, on the foreheads of 20 acne patients who were undergoing oral treatment with 13-cis-retinoic acid, a drug which suppresses sebum production profoundly. Sebum which had been allowed to accumulate on the scalp for several days was also collected and the % CE measured. Our hypothesis was that if CE are of sebaceous origin, the amounts secreted should fall as sebum secretion is suppressed. If CE are entirely epidermal, the absolute rate of production should be unchanged by the drug. If CE are derived from both sources, the amount produced by the epidermis should be measurable by extrapolation to zero sebum production. If esterification occurs on the skin surface, the % CE should be higher in the scalp lipid than in the freshly secreted forehead lipid.

We found that the amounts of CE produced on the forehead fell proportionally to sebum secretion. The regression line of a plot of absolute amounts of CE vs. absolute amounts of squalene (a purely sebaceous lipid) passed through the origin, indicating that CE were derived entirely from the sebaceous glands. In the scalp lipid, the % CE rose somewhat as sebum secretion was suppressed, indicating that some esterification of cholesterol may have occurred on the scalp. It appears, therefore, that skin surface CE are derived predominantly from sebum and possibly also from skin surface esterification of free cholesterol.

Estrogen Stimulates Preputial Lipogenesis. ANN ALVES, ANTHONY THODY, LINDA SHEPHERD AND SAM SHUSTER, Dept. Dermatology, University of Newcastle-upon-Tyne, UK

Although estrogens are generally thought to inhibit sebaceous activity they stimulate secretion of sex attractant odors from the preputial sebaceous glands. We therefore examined the effect of estrogen on preputial lipogenesis. Isolated cell suspensions prepared from preputial glands of adult female rats (collagenase, 37°C, 40 min) were incubated with 2 mM [$U^{-14}C$] glucose in KRB (95% O_2:5% CO_2) for 2 h. Lipids were extracted and analysed by t.l.c. and scintillation counting. In isolated cells of intact rats the major labelled fractions were polar lipids (40%) and triglycerides (46%). Ovariectomy decreased rate of lipogenesis/cell and changed the pattern of labelling such that polar lipids incorporated 17% of the label and triglycerides 72%. Estradiol benzoate (2 or 10 μg/day) for 7 days increased lipogenesis. Lipogenesis was also increased 24 h after a single injection of estradiol benzoate (10 μg) and the pattern of lipid labelling was restored to normal. A similar pattern of labelling occurred at 2 h after OB, although at this time lipogenesis was decreased. Dose related increases in lipogenesis also occurred in isolated cells, prepared from preputial tissue of either intact or ovariectomised rats that had been pre-incubated with 10^{-6}, 10^{-8} and 10^{-10}M OB for 2 h and 18 h.

These results show that estrogen stimulates preputial lipogenesis. Whether this occurs in other sebaceous glands is not yet known but we suspect it will prove specific to the pheromonal odor producing sebaceous gland.

The Effect of the Intracutaneous Injection of Progesterone on the Flank Organ of Intact, Sexually Mature Male Syrian Golden Hamsters. JONATHAN R. MATIAS, NORMAN ORENTREICH, AND VIRGINIA MALLOY, Orentreich Foundation for the Advancement of Science, Inc., 910 Fifth Avenue, New York, New York 10021

In order to drastically inhibit the action of testosterone in the flank organ of intact male hamsters, progesterone must be constantly available at the tissue site and it must be present at a sufficient dose to compete effectively against testosterone for the binding site of the enzyme 5α-reductase. We have accomplished this objective by the intracutaneous injection of progesterone into the hamster flank organ which resulted in the production of a depot underneath the sebaceous glands. A unilateral decrease in the weight of the flank organ and the area of the pigmentation occurred when progesterone was injected at a dose of 5 mg per week for a period of 3 weeks. The weight of the testes and the seminal vesicles were not affected by the treatment. The injection of dihydroprogesterone and pregnenolone did not show any inhibitory activity, suggesting that the effect of progesterone was not due to mechanical factors. When the duration of the treatment was extended to 6 weeks, the flank organ of the male hamster declined to a size which closely approximated that of a normal female. The dose response curve indicated that the minimum effective dose was 1 mg of progesterone per week. Histological examinations showed that progesterone caused a marked reduction in the size of the flank organ sebaceous glands.

Elevated Androgens and Adrenal Hyperfunction in Two Adolescents with Acne and Hirsutism. ANNE W. LUCKY, JOSEPH MCGUIRE, AND ROBERT L. ROSENFIELD, Departments of Dermatology and Pediatrics, Yale Medical School, New Haven, CT and Department of Pediatrics, University of Chicago, Chicago, IL

We prospectively evaluated 15 females with acne and/or hirsutism known to have elevated androgens, to determine whether they might be responsive to hormonal therapy. All had high free testosterone (T > 9 pg/ml) and dehydroepiandrosterone sulfate (DS > 324 ng/dl) levels

and were tested with adrenocorticotropic hormone (ACTH) stimulation and dexamethasone suppression. An abnormal pattern of adrenal steroid response to ACTH was found in two adolescents, 14 and 17 years of age. Both had progressive hirsutism, comedonal acne, and irregular menses. The younger had pubic hair at age 7. Their levels of free T (14 and 18 pg/ml) and DS (438 and 548 μg/dl) were high. Thirty minute responses of plasma 17 α-hydroxypregnenolone (17 Preg) to ACTH (10 $\mu g/m^2$ IV) were greater than 2 S.D. above the mean of 8 normal controls (1636 and 1833 μg/dl, nl < 1272). The responses of other intermediates and cortisol were normal. Dexamethasone, 2 mg/day for 7–14 days, suppressed the high androgens in these two patients as well as in two-thirds of the other women in the study.

Adrenal hyperfunction manifested by hyperresponsiveness of 17 Preg to ACTH suggests deficiency of 3 β-hydroxysteroid dehydrogenase, the enzyme which converts 17 Preg to 17 α-hydroxyprogesterone. Dexamethasone responsiveness of elevated androgens in most of the patients tested suggests a possible therapeutic role for corticosteroids in selected cases of acne and hirsutism.

Suppression of Sebum Excretion Following Treatment of Acne and Hirsutism with Spironolactone. T. M. KEAHEY, N. MARTINEZ, L. BLASCO, K. J. MCGINLEY AND J. J. LEYDEN, Departments of Dermatology and Gynecology, Univ. of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Recent reports have demonstrated the efficacy of spironolactone (SL), an antiandrogen, in the treatment of hirsuit females. The dependence of sebaceous glands on androgens and the role of sebum in the pathogenesis of acne has prompted us to measure sebum excretion and hormone levels in women with acne or hirsuitism before and during treatment with SL.

Seven female patients, mean age = 24.4 ± 4.8 years, with either inflammatory acne (4) or hirsuitism (3) were treated with increasing doses of SL (100–300 mgs/day) over a three month period. Patients were evaluated monthly at which time serum and facial lipid samples were collected. Analyses of skin surface lipids were determined by thin layer chromatography and endocrine determinations were performed by radioimmunoassay. A significant reduction in sebum excretion was seen in all patients ranging from 25 to 63% of pre-treatment levels. An average reduction of 20% occurred after the first month increasing to 46% by the end of the third month (mean total lipid pre-treatment = 271.1 $\mu g/cm^2/3$ hours, post-treatment = 148.8, p = .001). All patients with acne demonstrated remarkable clinical improvement and most patients reported an increase in the amount and frequency of menstrual bleeding. Total testosterone (T) levels were initially elevated in all patients (mean T = 141.4 ± 30.0 ng/dl). After treatment with SL there was no definite pattern of reduced levels of either T or dehydroepiandrosterone sulfate, but a marked increase in unbound estrogen (E) was noted in all patients (mean % bound E pretreatment = 32.9, post-treatment = 10.4). The reduction in sebum excretion and clinical improvement in patients treated with SL may reflect a change in the ratio of estrogen to testosterone.

Antiandrogenic Effects of Topically Applied Spironolactone on the Hamster Flank Organ. A. WEISSMANN, J. BOWDEN, B. FRANK, S. HORWITZ, AND P. FROST, Dept. of Derm., Mt. Sinai Hosp., Miami Beach, Florida

The effects of topically applied spironolactone (SL) on the sebaceous glands of the flank organs (FO) of adult male golden hamsters were investigated. Groups of four animals received daily applications of .003 mg, .03 mg, .3 mg, or 3 mg of SL in 5% isopropyl myristate in isopropyl alcohol to the left FO. The right FO's were untreated. To the FO's of other animals the vehicle, or cyproterone acetate (CA), .3 mg, was applied. Castrated, untreated animals served as additional controls. Longitudinal diameters (D_L) and transverse diameters (D_T) of the palpable bulk of flank organs were measured every four days to determine gland size ($D_L \times D_T$). On day 28, flank organs were excised and sebaceous gland volume was determined by planimetric evaluation of serial sections using the Zeiss Videoplan image analyzer system.

Treatment with SL, .3 mg, and 3 mg, resulted in reduction of FO sizes averaging 39.3% and 29.5%, respectively ($p < 0.01$), while the size of untreated contralateral FO's remained unchanged. SL, .003 mg and .03 mg, and the vehicle, did not significantly alter FO size. CA, .3 mg produced bilateral reduction averaging 35.5% ($p < 0.02$), compared to pretreatment values. Volumes of untreated glands were significantly smaller than the contralateral (treated) glands in animals receiving SL, .3 mg (treated, 4.4 mm^3 ± 1.4; untreated, 6.5 mm^3 ± 1.9; $p < 0.03$) and SL, 3 mg (treated, 4.4 mm^3 ± .6; untreated, 6.7 mm^3 ± .8; $p < 0.03$). The effect was absent in the other groups.

In male hamsters, topically administered spironolactone appears to have local antiandrogenic effects only, as indicated by the lack of changes in the untreated contralateral flank organs.

SESSION B — Congressional Room

Otto Braun-Falco, M.D., Presiding

Metabolism of (1-^{14}C)-Arachidonic Acid by Peripheral Blood Cells from Psoriatic Patients. JOHN R. WALKER, SHEELAGH M. LITTLEWOOD, WILLIAM DAWSON, AND B. ROGER ALLEN. Lilly Research Centre, Erl Wood Manor and Department of Dermatology, University Hospital, Nottingham

Recent observations have indicated that the levels of lipoxygenase products may be increased in psoriatic lesions. Studies with benoxaprofen, an inhibitor of 5-lipoxygenase product formation, have shown beneficial effects in some psoriatic patients. We have taken peripheral blood cells from controls and patients with psoriasis and studied their metabolism of ^{14}C-arachidonic acid *in vitro*.

Dextran sedimented, mixed cell populations from psoriatic patients produced significantly elevated (100%) levels of 12-HETE compared to control cells. Purified polymorphonuclear leucocytes (PMNs) failed to produce significant levels of 12-HETE suggesting that another component of the mixed cells, possibly platelets, is responsible for this aspect of lipoxygenase mediated metabolism.

Neither mixed cells nor purified PMNs showed any significant differences in their ability to produce 5-lipoxygenase products (5-HETE and 5:12 di HETE). These observations were obtained with both resting cell populations and cells stimulated with the calcium ionophore, A23187. Limited experiments with patients receiving benoxaprofen indicated that purified PMNs had a reduced ability to produce 5-HETE (40% reduction).

The only significant change seen in cyclo oxygenase product formation from these cell preparations was a reduction (60%) in TxB_2 formation by the mixed cell populations.

These results suggest that platelets should not be excluded from studies on peripheral blood concerned with the pathogenesis of psoriasis.

Enhancement of 5-Lipoxygenase Activity in Soluble Preparations of Human Psoriatic Plaque Preparation. V. A. ZIBOH, T. CASEBOLT, C. L. MARCELO AND J. J. VOORHEES. Dept. of Dermatology, Univ. of Calif. School of Medicine Davis, CA and Univ. of Michigan Medical School, Ann Arbor, MI

Although enhanced 12-lipoxygenation of arachidonic acid (AA) by homogenates from psoriatic plaques and corresponding uninvolved skin have been reported (Hammarstrom *et al.* PNAS, *72,* 5130 (1975), the possibility of AA transformation via the 5-lipoxygenase pathway by human epidermis has remained speculative. To determine the presence of this pathway in psoriatic skin, keratome sections of upper epidermis (mainly st. corneum and granulosum) or lower epidermis (mainly st. basale and spinosum) were taken from psoriatic plaques (3), or whole epidermis from uninvolved psoriatic skin (3) and normal volunteers (2). Portions of each specimen were kept for histological evaluation, or homogenized in 50 mM phosphate buffer, pH 7.4 containing 1 mM EDTA. The homogenate (H) was centrifuged at 900 g to remove debris and nuclei. The resulting supernatant was centrifuged at 105,000 g for 60 min. to give pellet (P) and supernatant (S) fractions. Each fraction was incubated with ^{14}C-AA (5.8×10^5 cpm) at 37°C for 15 min. Reaction was stopped and extracted with organic mixture. Radioactive metabolites were identified on silicic acid chromatography, TLC, and by HPLC. Data from these experiments demonstrate a greater biosynthesis of ^{14}C metabolite chromatographically similar to leukotriene B_4 (LTB_4) in the H and S fractions of psoriatic plaques, 6.7% and 6.2% respectively, than in their paired uninvolved skin H (3.4%) and S (2.6%). Negligible ^{14}C-metabolite was observed in the H and S fractions of normal skin. In lesional skin, more ^{14}C-metabolite was formed in the upper than in the lower keratome sections. Localization of a soluble 5-lipoxygenase activity in psoriatic skin offers a useful tool for further studies on the regulators of this pathway.

Monohydroxy Metabolites of Arachidonic and Linoleic Acids in Psoriatic Skin. R. CAMP, A. MALLET, P. WOOLLARD, S. BRAIN, A. KOBZA BLACK, AND M. GREAVES. Institute of Dermatology, London, U.K.

We have shown that extracts of lesional psoriatic skin contain chemokinetically active amounts of monohydroxy-eicosatetraenoic acid

(HETE) like material, and have now attempted to identify this material by gas chromatography-mass spectrometry(GC-MS).

Lipid extracts of lesional scale (50-150mg) and of chamber fluid (2 ml) from abraded lesional and uninvolved skin were purified by straight phase HPLC. This system was able to separate the following metabolites of arachidonic and linoleic acids: 15-HETE, 12-HETE, 11-HETE, 9-HETE, 8-HETE, 5-HETE, 13-hydroxy-octadecadienoic acid (13-HODD) and 9-HODD. GC-MS analysis after HPLC purification showed that all of the above compounds occur in psoriatic scale. 13-HODD was present in highest concentration (2.56 ± 1.82) followed by 12-HETE (1.31 ± 0.80), 9-HODD (0.27 ± 0.35) and 8-HETE (0.07 ± 0.02) (ug/100mg scale, mean ± S.D., n = 3). Except for 5-HETE, all of the above compounds were also detectable in purified chamber fluid extracts. In each case the concentrations in samples from lesional skin were higher than in samples from uninvolved skin, with the exception of 9-HODD. 12-HETE was present in highest concentration (104.3 ± 60.2 lesional, 47.5 ± 35.0 uninvolved) followed by 13-HODD (10.6 ± 4.1 lesional, 5.4 ± 3.8 uninvolved) (ng/ml chamber fluid, mean ± S.D., n = 4). We have found that the HODDs are chemokinetically inactive, but their other properties are under study. Apart from 15-HETE, all of the above HETEs are leukotactic and may be relevant to the psoriatic neutrophil infiltrate. With the exception of 12-HETE (Hammarstrom et al., 1975) the *in vivo* production of these compounds has not been conclusively reported. Further study should clarify their pathogenetic significance in psoriasis.

Biological Activity Due to Arachidonic Acid Lipoxygenase Products in Psoriasis. S. Brain, R. Camp, A. Kobza Black, P. Dowd, and M. Greaves. Institute of Dermatology, Homerton Grove, London, U.K.

Arachidonic acid is metabolized by lipoxygenases to produce hydroxy-eicosatetraenoic acids (HETEs). The di-HETE, leukotriene B_4 (LTB_4) is a potent leukotactic agent, whilst mono-HETE compounds have similar but weaker properties. We have examined psoriatic scale extracts for the presence of chemokinetically active lipoxygenase products.

Scale (approx. 150mg) from untreated plaques was vortexed with sodium acetate buffer, pH 3.5 and ethyl acetate; the resulting organic residue was partitioned between n-heptane and methanol to remove nonpolar lipids. The methanolic extract was applied to a straight phase HPLC column eluted with hexane/propan-2-ol/methanol/acetic acid (88:7:5:0.1) at 1ml/min. One ml fractions were collected and chemokinetic activity was assessed in each by using leukocytes (70 − 80% neutrophils) in an agarose microdroplet assay. Chemokinetically active material with the same retention time as LTB_4 was seen (180 − 800pg LTB_4 equivalents/100mg scale, wet weight, n = 4). Comparable amounts of activity caused by material with a similar retention time to mono-HETEs was also seen in each case. This suggests that considerable quantities are present in psoriatic scale, as mono-HETEs are chemokinetically much less potent than LTB_4.

We have recently found that LTB_4 and mono-HETE-like material are released from abraded psoriatic plaque, into skin chamber fluid. These results show that superficial psoriatic scale contains LTB_4-like material and also other lipid compounds which could induce and/or maintain the neutrophil infiltrate observed in psoriatic skin lesions.

A Comparison of Abundance and Labelling Pattern of Keratins Extracted from Involved and Uninvolved Skin of Psoriatic Patients. Michael P. Osber and Joseph McGuire. Yale University, School of Medicine, Department of Dermatology, New Haven, CT

Slices were taken with a keratome from the areas of interest. Each slice was extracted with low salt buffer and then the keratins were extracted with 2% SDS, 10 mM DTT, 0.1 mM PMSF.

The keratin patterns of involved and uninvolved skin were compared using SDS-PAGE electrophoresis and Coomassie blue staining. Uninvolved skin had bands at 70.8 K (I), 67.9 K (IA), 59.7 K (II), 58.5 K (III), and 52.6 K (IV) daltons. Involved skin had bands at 71.1 K (I), 60.0 K (II), 59.0 K (III), 58.2 K (IV), 52.5 K (V), and 50.9 K (VI) daltons. As Thaler et al. (J.I.D. 70:38–41) and Hunter and Skerrow (BBA 714:164–169) have previously shown, the highest molecular weight species in the involved skin were less abundant than in the uninvolved skin. When the skin slices were incubated with ^{35}S-methionine, a fluorogram of the SDS extract of uninvolved skin revealed an additional band undetected by Coomassie blue staining at 50.9 K (IVA) daltons. Similarly, the ^{35}S-methionine labelled SDS extract of involved skin revealed an additional band at 67.9 K (IA) daltons. The ^{35}S-methionine labelling also showed that band VI in psoriatic skin and band IVA in uninvolved skin had higher specific activities than the other keratins.

The keratin patterns of the skin slices can be divided into 3 groups: 71 K-67 K, 60 K-58-K, and 53 K-51 K daltons. When each band from both types of skin was analyzed according to the method of Elder et al. (JBC 252:6510–6515)—two dimensional thin layer chromatography of ^{125}I-labelled tryptic peptides—the keratins within each of the 3 groups were found to be very similar to each other with isomobile bands being nearly identical.

Keratin Hydrolyzing Enzyme in Psoriatic Scales. Toshihiko Hibino, Seiichi Izaki and Masakatsu Izaki. Department of Dermatology, Iwate Medical University School of Medicine, Morioka, Japan

Proteinases including trypsin-like enzymes and plasminogen activator (PA) have been demonstrated in psoriasis scales. In the present study, we investigated keratin hydrolytic activity in psoriasis scales. The detected enzyme was partially purified and characterized.

Scales from three patients with psoriasis vulgaris and three with pustular psoriasis were collected and extracted with 0.1 M Tris-HCl (pH 8.0) containing 0.14 M NaCl. Hydrolytic activities on Glu-Gly-Arg-p-nitroanilide (S-2444) and Ile-Pro-Arg-p-nitroanilide (S-2288) were measured. PA activity was measured with fibrin plates. For keratin hydrolysis, we prepared keratin-agarose plates. The enzyme activity was quantitated and expressed as KU. 1) Crude extracts from six patients showed similar activities. The extract hydrolyzed S-2444 and S-2288 (2.58 ± 1.37 and 5.96 ± 2.74 nmol/min/mg protein, respectively), and keratin (0.1 ± 0.06 KU/mg protein). PA activity (0.46 ± 0.31 CTA U/mg protein) was also detected. 2) Sephacryl S-200 gel chromatography revealed a peak with peptide hydrolysis at 25 K in molecular weight. Keratin hydrolytic activity was found in this fraction but not in other fractions. PA activity was seen at the 25 K fraction as well as 75 K and >150 K fractions. 3) Further separation of the 25 K fraction with DEAE Sepharose chromatography demonstrated a peak with peptide hydrolytic activity. This fraction clearly demonstrated degradation of keratin in SDS polyacrylamide gel electrophoretic pattern. This fraction did not contain PA, whereas three peaks with PA activity were separated. 4) The enzyme was found diisopropyl fluorophosphate (DFP) sensitive, relatively stable and alkaline (pH 8-9) proteinase.

Keratin hydrolyzing enzyme may be responsible for psoriatic condition.

Growth Profile of Cultured Keratinocytes from Patients with Psoriasis. Su-Chin C. Liu. Department of Dermatology, Stanford University School of Medicine, Stanford, California

We are comparing growth profiles of keratinocytes isolated from involved (PP), uninvolved (PN) skin of psoriatic patients and normal individuals (NN) to test the hypothesis that the defect in cell proliferation in psoriasis may exist in the keratinocyte itself.

Using a combination of the techniques of Rheinwald and Green, and Liu and Karasek, large quantities of proliferative keratinocytes from PP, PN, and NN skin can be obtained. Primary cultures from a 6 mm punch biopsy are grown on a 3T3 feeder layer seeded on a collagen surface in DMEM containing 20% fetal calf serum, hydrocortisone, epidermal growth factor, and cholera toxin. Two weeks later, when cells have divided 5–6 times, keratinocytes are passaged. These cells, plated on a collagen surface without feeder layer and maintained in unsupplemented DMEM plus serum, are used in comparing growth profiles.

No difference in morphology among these cells is observed. PP keratinocytes grow initially at a slower rate than do PN and NN keratinocytes in primary cultures, especially when isolated from active lesions. The growth rate, determined by cell number and DNA content, in passaged cells differs insignificantly among PP, PN, and NN cells. The cells have high metabolic and mitotic activities during the first week after subculturing. Waves in the mitotic index and metabolic activity suggest that the cells are synchronized during subculturing.

We have not found the enhanced proliferation characteristic of psoriatic keratinocytes *in situ* in the cultured cells; however, it is too early to reach the conclusion that the defect(s) responsible for psoriasis do not exist in the keratinocyte itself, since these three cell groups may respond differently to agents that enhance or inhibit growth.

Proliferative Defects in Psoriasis. G. Weinstein, P. Ross, J. McCullough and A. Colton. Department of Dermatology, University of California, Irvine, California College of Medicine, Irvine, California

A major problem in defining the proliferative defect(s) in psoriasis is whether the increased cell production is due to a rapid cell cycle time (T_c) in psoriatic (PS) cells compared to normal and/or to differences in

the respective growth fractions (G.F.). Using the double peaked fraction labeled mitosis method we determined that the PS T_c = 36 hrs. A 100% G.F. in psoriasis was demonstrated by a) autoradiographic analysis of 4 intralesional (IL) thymidine-^{3}H injections/36 hrs; b) mathematical calculations using kinetic data and exponential age distribution; and c) previous studies with IL MTX/T^{3}H injections. The T_c in normal epidermal cells (N.ep) was calculated to be 311 hrs based on T_c = 14 hrs, L.I. = 2.7% (Cl. Res. 28:136, 1980), and *in vivo* human epidermal G.F. = 60% (Gelfant, 1982). In the current study a higher G.F. of 75–100% was found in human epidermis xenografts in nude mice.

We have developed mathematical models of PS and normal epidermis based on quantitation of proliferative and differentiated compartment cell populations/mm^2 surface area. The psoriatic proliferative compartment contains 52,000 cells/mm^2 and produces 35,000 new cells/day (d), while the comparable N.ep values are 26,000 cells and 1,218 new cells/day. The viable differentiated cell populations contain 47,000 and 17,000 cells/mm^2 respectively. Based on the above values, estimated differentiated compartment transit (turnover) times (T.T.) are almost identical with previously experimentally observed T.T. of 1.75d in PS and 12d in N.ep. These experimental data and mathematical interpretations indicate that the major proliferative defect in psoriasis is the 8 fold differences in T_c (36 vs 311 hrs) while G.F. differences are of relatively minor significance.

Thermal Dosimetry Studies on Human Skin: Association of Depth of Heating with Clinical Efficacy in the Treatment of Isolated Psoriatic Plaques. F. Richard Noodleman, Douglas Pounds, Eugene M. Farber and Elaine K. Orenberg. Department of Dermatology, Stanford University School of Medicine and the International Psoriasis Research Foundation, Stanford, California 94305

Localized heating (42–44°C, 30 min, t.i.w.) induced by ultrasound has been used successfully to clear psoriatic plaques. For each degree increase in temperature, the treatment time needed is decreased. Thermal dosimetry studies on psoriatic and uninvolved skin were performed to determine the heating profiles achieved within skin heated by direct contact methods, ultrasound (5 MHz, 2 MHz), and infrared CO_2 laser (10 μ wavelength). We analyzed the relationship between depth, temperature and time of heating required to produce therapeutic results.

Thermocouple microprobes (specially constructed with rt. angle bends to allow precise depth location) were placed at the skin surface and at 0.5, 1, 2 and 3 mm depths. Dosimetry curves were generated over 1 hr as a function of power output. With contact heating of the surface (43.5°C) skin loses 1.5–2°C within the first mm; between 1 and 3 mm, up to a 1°C further drop in temperature occurs. More power was required to heat a psoriatic plaque to 44°C than uninvolved tissue, probably as a consequence of the increased vascular perfusion of the lesion. However, after 25 min, heating and power requirements decreased as did psoriatic skin cooling capabilities suggesting vascular occlusion. This same phenomenon has been observed with hyperthermia treatment of tumors. Localized heat deposition within 3 mm of the surface (44°C) is sufficient to produce plaque clearing. Further development of heat delivery instrumentation suitable for larger body areas can be optimized through consideration of the time, temperature and depth of heating requirements.

Regulation by Different Stimuli of the Increased Production of Oxygen Radicals by Monocytes and Polymorphonuclear Leukocytes in Psoriasis. Rudolf E. Schopf, Elke Straussfeld, and Bernd Morsches. Department of Dermatology, Johannes Gutenberg University, Mainz, Fed. Rep. Germany

Based on recent findings indicating an increased respiratory burst activity of zymosan-stimulated monocytes (Mø) and polymorphonuclear leukocytes (PMN) in psoriasis we asked if stimulation by different receptors always results in augmented oxidative metabolism compared to healthy controls. We used five different stimulants to activate Mø (2×10^5) and PMN (5×10^5): 1. aggregated immunoglobulin (aggIg), 2. serum-treated zymosan (C3b), 3. phorbol myristate acetate (PMA), 4. concanavalin A (ConA), and 5. zymosan (Z). Activation of Mø and PMN isolated from the peripheral blood of 12 patients with psoriasis vulgaris and 12 controls was measured by luminol-enhanced chemiluminescence.–AggIg and Z equally well yielded increased stimulation of Mø and PMN of patients compared to controls ($p < 0.05$). C3b led to higher stimulation of Mø only from patients ($p < 0.025$). By contrast, incubation with PMA and ConA resulted in significantly augmented activation ($p < 0.05$ and <0.001, resp.) with PMN but not Mø from patients. The activity of the respiratory burst could be inhibited by superoxide dismutase, colchicine, hydrocortisone, protease- and transglutaminase-inhibitors as well as β-adrenergic stimulation.–We conclude that the increased respiratory burst activities found depend both on the stimulus and on the cell type. These differences may be important to control inflammation and immunoregulation in psoriasis.

Demonstration of Chemotactic Lipoxygenase Products in Psoriatic Scales. Beate M. Czarnetzki, Juergen Grabbe, and §Mithat Mardin. Universitaets-hautklinik, Muenster, and §Bayer, Wuppertal, W.-Germany

In order to examine whether products of the lipoxygenase pathway are involved in the emigration of leukocytes into psoriatic epidermis, scales from patients with psoriasis were weighed and extracted at pH 3 into ether or hexane. Neutrophil and eosinophil chemotactic activity, measured in modified Boyden chambers, was found in the ether, but not the aqueous extracts, and corresponded to a biological activity of 10^{-9} M leukotriene B_4/10 mg scales. After heating (100°C, 10 min) of the samples, the activity in the ether extract was not diminished, while chemotactic activity appeared in the aqueous phase. By separate analysis, a potent, heat-labile inhibitor of leukotriene B_4-induced chemotaxis could be demonstrated in the non-heated aqueous extracts of scales. The presence of lipoxygenase products in the ether extracts, as suggested by the above experiments, could be confirmed by high pressure liquid chromatography, with leukotriene B_4, and at times 5-HETE, being the main products. These findings show therefore that lipid chemotactic factors as well as inhibitors of chemotaxis are present in psoriatic scales and that they potentially modulate the influx of leukocytes into the psoriatic epidermis.

Receptor Specific Differentiation Between Chemotaxis and Superoxide Anion Release in Psoriatic Polymorphonuclear Leukocytes. Heinz-E. Schlaak Jens-M. Schröder, Christoph Schubert and Enno Christophers. Dept. of Dermatology, Univ. of Kiel, W.-Germany

In psoriasis increased chemotactic activity of polymorphonuclear leukocytes (PMN) exposed to common chemotaxins has been shown by various authors. The mechanisms leading to such alterations in PMN function are poorly understood. We established a model system which enabled us to study the binding mechanisms of chemotaxins and their modulating effects upon chemotaxis (Boyden chambers) as well as upon the release of oxygen radicals (O_2^-, cytochrome c reduction). PMN were incubated with wheat germ agglutinin (WGA, 0.1 – 6 μg/ml) and subsequently stimulated by the chemotaxins C5a or formyl-methionyl-leucyl-phenylalanine (FMLP). WGA caused a dose-dependent inhibition of chemotaxis against both chemotaxins whereas chemokinesis was present. Similarly the release of O_2^- was not affected. The inhibitory effects of WGA could be blocked by N-acetyl-glucosamine (GlcNAc) whereas N-acetyl-neuraminic-acid-pretreatment showed no blocking effects. On the other hand preincubation of PMN with neuraminidase reduces the chemotactic responsiveness while pretreatment with glucosaminidase failed to affect PMN chemotaxis. At higher concentrations of WGA (6-60 μg/ml) the cells remained chemotactically non-responsive towards both chemotaxins. However the release of O_2^- could still be elicited by C5a and FMLP. Treatment with glucosaminidase further increased this effect whereas it was abolished by neuraminidase. Phorbol-12-myristat-13-acetat (PMA) was not able to enhance O_2^- release under these conditions. These data demonstrate that at low lectin concentrations arrest of cellular motility does not necessarily block the release of oxygen radicals. Further, the blocking experiments suggest binding specificity of C5a to NANA.

Reduction of Chemotactic Activities in Psoriatic Lesions by Hyperthermia Treatments. M. Oosaki, M. Isoda, S. Yasumoto, and H. Urabe. Department of Dermatology, Faculty of Medicine, Kyushu Univ. Fukuoka, Japan

Accumulation of polymorphonuclear leukocytes (PMNs) in the stratum corneum as Munro's abscess is a characteristic histological feature of psoriasis. Psoriatic scale extracts have been shown to contain leukotactic substances (Br J Dermatol 95:1, 1976). Recently local hyperthermia has been found to be effective in the treatment of psoriasis (Arch Dermatol 116:893, 1980). In this study we investigated the effect of hyperthermia on the psoriatic scale leukotactic factor. Four psoriatic patients were treated by patching the "Erhitzen" (chemical heat-pad) of 42°C or steeping the site of the eruptions in a water-bath (42–43°C). Scales from the psoriatic lesions before and 2 weeks after the treatment were suspended respectively in 50 times their weight of 6M urea and the supernatant fluids were dialysed against phosphate-buffered saline of pH 7.4. After sterilizing filtration with Millipore filter, the extracts

were tested for their neutrophil chemotactic activity by using Boyden chamber method. The results of migration of PMNs are shown below.

Case No.	1	2	3	4	Average
Pre-treatment	208	94	107.6	322	182.9 ± 91.6
After treatment	38.4	28.8	40.2	63	42.6 ± 2.6

Two weeks after treatment, there was a prominent decrease in the chemotactic activities ($p < 0.05$) with clinical and histological remissions. This finding suggests that the effect of local hyperthermia on psoriasis may be due to inhibition of chemotactic activity.

Neutral Proteases and Other Granule Enzymes of Neutrophils As Related to Activity, Extent and Duration of Psoriasis. W. GLINSKI, D. BARSZCZ, Z. ZAREBSKA, E. JANCZURA, AND S. JABLONSKA. Dept. of Dermatology, Acad. of Medicine, 02008 Warsaw, Poland

The activities of elastase/E/cathepsin G/C/, lysozyme/L/, and peroxidase/P/ of peripheral blood polymorphonuclear leukocytes /PMNs/ were determined by spectrophotometry in 33 patients with psoriasis and 15 healthy volunteers. Two fractions of the enzymes were studied, which eluted at 4°C from PMNs with 0.05 M citrate buffer, pH 3.5, alone for 24 hr /I/, and next with buffer + 1%Triton ×-100 for 24 hr / II/.

In guttate psoriasis of short duration, the C activity was only about 20% higher in both enzymatic pools, whereas the easy releasible E and L/I/ were reduced as compared to controls/$p < 0.05$/, but their stable pools/II/ were normal. This argued for *in vivo* partial degranulation of PMNs and against the increase in E and C to be a cause of relapse.

In active extensive plaque psoriasis of 2–8 mo duration, an 55–75% increase of the E, C and L activities was found in both eluates as compared to normals/$p < 0.01$/, which may be responsible for the persistence of psoriatic lesions.

There were no relations between: 1. the P activity and disease activity, and 2. the activities of all enzymes studied and extent as well as duration of recent skin lesions.

In patients with stationary lesions despite their longlasting character/ > 4 mo duration/, both neutral proteases, E and C, were about 40% less active/I and II/ than in controls/$p < 0.05$/, which was presumably due to an excess in inhibitors of these enzymes accumulated during relapse.

Effect of Various Pharmacological Agents on Mitogen Responsiveness of Psoriatic Lymphocytes. J. J. GUILHOU, M. ANDARY, AND J. CLOT. Clinique de Dermatologie, Hôpital Saint-Charles and Département d'Immunologie, Hôpital Saint-Eloi, 34059 Montpellier R Cedex, France

Pharmacological disturbances have repeatedly been demonstrated in psoriatic (Pso) epidermis. Such imbalances, if present at the lymphocyte (Ly) level, could possibly be of importance in the impairment of immune responses.

We have studied the effect of various pharmacological agents on PHA and ConA responsiveness of peripheral blood mononuclear cells (PBMC). In each experiment normal and Pso. PBMC were tested simultaneously using previously determined optimal concentrations of isoproterenol (10^{-5} and 10^{-7} M) histamine (10^{-5} and 10^{-7} M), L-phenylephrine (10^{-4} and 10^{-6} M) and theophylline (10^{-5} and 10^{-7} M).

In controls, an inhibition of mitogen responsiveness was observed with isoproterenol (50% inhibition) histamine (40% inhibition) and theophylline (30% inhibition) whereas L-phenylephrine induced no significant modification.

In pso, the proliferative response of PBMC in the presence of histamine and L-phenylephrine was the same as in controls. In contrast, isoproterenol and theophylline-induced inhibition was reduced or absent in certain patients. Moreover in some of them a paradoxal increase of the proliferative response was observed in the presence of these two agents.

Such results could indicate interactions between immunological and pharmacological responsiveness of Pso PBMC.

Topical Indomethacin Exacerbates Lesions of Psoriasis. CHARLES N. ELLIS, JEROME D. FALLON, JAN L. HEEZEN, AND JOHN J. VOORHEES, Department of Dermatology, University of Michigan Medical School, Ann Arbor. MI

Free arachidonic acid (AA) and its derivatives 12-HETE, leukotriene (LT) B4 and prostaglandins (PG) are elevated in lesional psoriasis. Since 12-HETE, LTB4, and free AA are chemotactic for polymorphonuclear leukocytes, these substances may be involved in the pathogenesis of psoriasis. In support of this concept, the oral 5-lipoxygenase inhibitor, benoxaprofen, has been found to markedly improve psoriasis. In epidermis in vitro, indomethacin (IM) is known to inhibit the formation of PG resulting in an accumulation of free AA, 12-HETE, and presumably LTB4. If free AA, 12-HETE, and LTB4 are factors in psoriasis, we theorized that topical IM would cause local exacerbation of psoriatic lesions. To investigate this hypothesis, bilaterally symmetrical lesions of psoriasis and adjacent normal appearing skin in 20 patients were treated for 4 weeks in a double-blind fashion with twice daily applications of either 1% IM in hydrophilic cream or placebo cream (P) alone. Patients were seen weekly and evaluated for erythema, thickness, scale, symptoms, and overall response. After 4 weeks, P therapy caused significant improvement in scale and thickness of lesions ($p < .05$); with IM, however, none of the signs was statistically improved. At the end of treatment, the IM side was significantly worse than P in overall response ($p < .05$). Combining signs and symptoms, the IM side was rated worse in 14 patients, better in 5, and there was no difference in 1 ($p < .05$). New psoriatic lesions were not induced by IM. Thus, psoriasis demonstrates a significantly poorer clinical response with topical IM compared to P alone. In summary, the exacerbation of psoriasis by IM strengthens the hypothesis that free AA, 12-HETE, and LTB4 are important participants in the pathophysiology of psoriasis.

Koebner Phenomenon Induced by Topical Cantharide in Psoriatics: A Consequence of Epidermal Protease Activation. LOUIS DUBERTRET, BRIGITTE BERTAUX, MICHÈLE FOSSE, RENÉ TOURAINE. Hôp. H. Mondor, Créteil, France

A filtered alcoholic solution of Cantharide (0.1 mg/ml) was applied during 1 hour, under occlusion, on the uninvolved skin of psoriatics leading to an intraepidermal blister. In normal controls the blister disappeared in a few days without scar. In 29 among 81 psoriatics (36%), taken in random fashion a Koebner phenomenon (K.Ph.) was present two weeks after. An abnormal proteolysis was recently described in the upper stratum spinosum of psoriatic plaques and in 7 out of 17 biopsies (41%) of uninvolved psoriatic skin (1). Using the same technique on sequential biopsies of Cantharide blisters (CB) we have looked for a pathogenic relation between C.B. healing, activation of epidermal proteinases and the appearance of a K.Ph. In 24 hours of C.B. no granular layer and no proteolysis in the three controls and the three psoriatics studied. In 48 hours C.B. a granular layer reappeared; no proteolysis in the three controls; in contrast a proteolysis was observed in the upper stratum spinosum in the six psoriatics studied. Subsequent biopsies were performed in four psoriatics. In three patients the proteolysis disappeared: at day 6 for one, without histological abnormalities; at day 10 for two; in these two last cases a typical psoriatic hyperacanthosis was observed in the last biopsy. However, a clinical K.Ph. did not occur in any of these three psoriatics. In one patient the proteolysis did not disappear and a clinical and histological K.Ph. was observed. These kinetic studies strongly suggest that the protease-anti protease equilibrium is deficient in uninvolved psoriatic skin and that the Cantharide K.Ph. is a direct consequence of an increased proteolysis occurring in the upper stratum spinosum during the intra epidermal blister healing process. (1) L. Dubertret et al. Br. J. Dermat., 1982, 107, 499–504.

Psoriasis: An Immunocytochemical Study. I. M. LEIGH*, E. B. LANE† AND K. PULFORD◊. *St. John's Hospital for Diseases of the Skin, London; †Imperial Cancer Research Fund, London; ◊Department of Research Pathology, University of Oxford

Monoclonal antibodies to epithelial cells have been used to determine the immunocytochemical profile of psoriasis as part of a search for psoriatic markers. Direct immunofluorescence of uninvolved and involved psoriatic skin and normal controls was performed using monoclonal antibodies LP34 (immunogen = cultured psoriatic keratinocytes); AJ4 (immunogen = leukaemic cells) and K92 (immunogen = human hair).

LP34 a cytokeratin antibody showed no difference between psoriatic tissue and normal, nor between cultured psoriatic keratinocytes and normal keratinocytes: simpler epithelial cytokeratins were not detectable using other monoclonal antibodies (including LE61) even in involved psoriatic plaques. K92 stains suprabasal cells in normal skin and there is an increased negative basal zone in involved plaque psoriasis. Staining is also weaker in the first suprabasal layers. AJ4 stains basal cells only in normal skin but staining was absent in psoriatic plaques. Uninvolved psoriatic skin showed erratic staining. This provides immunological evidence to support recent biochemical studies that there are detectable differences between uninvolved psoriatic skin and normal skin. These changes are more exaggerated in psoriatic plaques.

Lectin Studies on Keratinocyte Maturation in Psoriasis. J. N. MANSBRIDGE AND A. M. KNAPP. IPRF, Box V, Stanford, CA 94305

This study is designed to distinguish between the possibilities that the altered keratinocyte maturation in psoriasis results from premature termination or an alternative differentiation pathway.

Ulex europeus lectin (UEA) binds to a site in the granular layer of normal skin which is absent in psoriasis. Studies with isolated cells show that the reaction has a dissociation constant of 3 nM, an association rate of 7.7×10^5/M/sec and a dissociation rate of 4.6% per minute. By flow cytometry, about 15% of cells possess about 8.2×10^5 sites. The site is a surface component of the cytoskeleton. Loss of such a site in psoriasis is consistent with either possibility. Cytoplasmic staining seen in normal skin is considerably enhanced in psoriasis, however, which supports the alternative pathway hypothesis.

The binding of Helix pomatia lectin (HPA) to the stratum spinosum in normal skin is triton-sensitive. In psoriasis, a triton-resistant HPA site appears early in the maturation process. This component is not found in normal skin and represents an early departure from normal maturation. The result is difficult to explain on the basis of premature termination of maturation and supports the view that psoriatic skin is executing an inappropriate program, perhaps related to one of the alternative differentiation pathways open to the multipotent basal cell. Experiments to confirm this conclusion by monoclonal antibody and recombinant DNA methods are in progress.

Presence of Typical Chromosomal DNA-Binding Proteins in Psoriatic Scales and Reaction with Lymphocyte Bound Antibodies of Psoriasis Patients. G. REIMER, E. F. ALI-BRIGGS*, R. H. CORMANE* AND P. K. DAS*. Dept. of Derm., University Frankfurt, FRG; *Dept. of Derm., University Amsterdam, NL

Accelerated epidermopoesis and incomplete keratinisation indicate a changed gene regulation in psoriatic epidermis cells. As the pattern of chromosomal DNA-binding non-histone proteins (DBP) is shown to be changed after different gene regulation stages, its composition in healthy and pathologic skin was analysed by SDS-electrophoresis and compared by densitometric readings.

Related tissues correspond in their DBP spectra as shown by human dermis, epidermis and cell cultures. The key fraction of psoriatic scales are two DBP with mw of 90 kdal and 94 kdal, respectively. Most likely, they derived from the condensed nuclei of the parakeratotic horny layer of psoriatic scales. These DBP are neither present in healthy epidermis nor in scales of other dermatoses. During therapeutic treatment of psoriasis using different regiments the amount of DBP decreases to normal. DBP preparation showed no activity of DNases, DNA-polymerases or RNA-polymerases. Furthermore, no binding activity for cAMP, cGMP or steroid hormones was measured. However, in literature and own studies, DBP with mw of 90 kdal are found to be present in cultured cells growing without contact inhibition.

Additionally, in some cases of psoriasis, auto-antibody extracted from peripheral blood lymphocytes has been found to be directed against basal cell nuclei by indirect immunofluorescent technique using non-involved skin as substrate. Preliminary results show that this activity could be absorbed by the above mentioned DBP preparation. We postulate that these DBP play a role in the pathogenesis of psoriatic epidermis cells.

SESSION C — Federal Room

Thomas J. Lawley, M.D., Presiding

Evidence of Chronic T-Cell Activation in Patients with Dermatitis Herpetiformis and Gluten Sensitive Enteropathy. DANIEL O. CLEGG, SETH H. PINCUS, AND JOHN J. ZONE, University of Utah School of Medicine, Salt Lake City, Utah

Dermatitis herpetiformis (DH) and gluten sensitive enteropathy (GSE) are immune-mediated diseases in which gluten is the suspected antigen. T-cells expressing HLA DR antigen are thought to be immunologically activated, presumably a reflection of antigenic stimulation. These activated T-cells are seen in increased numbers in patients with sepsis, rheumatoid arthritis, Type I diabetes mellitus, and after tetanus toxoid booster.

The purpose of this study was to determine whether DH and GSE patients had elevated numbers of activated T-cells and whether such an increase was related to the ingestion of gluten. We studied 12 DH patients and 6 GSE patients. Half were adhering to a strict gluten-free diet; half were not. T-cells were isolated by double rosette technique using sheep red blood cells. A mean of 11% (range 6–21%) of DH and 13% (11–17%) of GSE circulating T-cells expressed HLA DR antigen. Our normal controls express HLA DR on a mean of 3% (0–7%) of their peripheral T-cells. In addition, we administered tetanus toxoid booster to 3 individuals. On day 0 a mean of 1.7% (1–2%) of circulating T-lymphocytes were HLA DR positive and on day 4, 24.3% (21–29) of T-cells expressed HLA DR, indicating that the level of T-cell stimulation in some of our patients approximated that stimulated by tetanus toxoid booster. There was no correlation of HLA DR positivity with age or sex of the patient, disease severity, duration of disease, ingestion of gluten or daily Dapsone dose necessary to control the skin lesions of DH.

These data support the concept that patients with DH and GSE are undergoing chronic antigenic challenge which continues after attempted removal of gluten from the diet.

An Increased Prevalence of Thyroid Abnormalities in Dermatitis Herpetiformis Patients. JOHN J. ZONE, AND MICHAEL C. CUNNINGHAM, Division of Dermatology, Univ. of Utah School of Medicine, Salt Lake City, Utah

Reports of exacerbation of Dermatitis Herpetiformis (DH) by iodide ingestion and by thyroid abnormalities prompted us to evaluate thyroid abnormalities in DH patients in search of factors which may be important in the pathogenesis of DH. We studied fifty patients with clinical DH and granular IgA on skin biopsy for clinical and serological evidence of thyroid disease. Two patients had a previous history of hyperthyroidism, while five had a history of hypothyroidism and were on thyroid hormone replacement therapy. In addition, there were 3 patients who had had thyroidectomies for nodules. Five patients had asymptomatic goiter, and two were felt to be pre-hypothyroid on the basis of thyroid function tests. Thyroid microsomal antibodies were found in 38% of our DH patients compared to 12% of 50 age matched sex matched controls. Thyroglobulin antibodies were seen in 12% of patients, but in none of the controls. Thyroid hormone therapy appeared to aggravate skin disease in three of ten patients.

We identified some type of clinical or serological thyroid abnormality in eighteen of twenty-three female DH patients, and in eight of twenty-seven male DH patients. The association between DH and thyroid disease is clinically significant. The dramatic effect of iodine on exacerbating DH together with the intricate role of iodine in thyroid metabolism suggests an interaction between thyroid abnormalities and DH which transcends simple grouping of autoimmune disorders.

Immuno Electron Microscopical Studies in Patients with Linear IgA Deposits. G. P. HAFFENDEN, NICOLA P. RING, J. N. LEONARD, AND LIONEL FRY, Departments of Histopathology and Dermatology, St. Mary's Hospital, London, W.2

Immuno electron microscopic studies have been carried out on 22 patients with linear IgA deposits, 15 of whom had homogenous linear (HL) and 7 had granular linear deposits (GL). In the GL group the deposits were similar to those found in papillary IgA dermatitis herpetiformis.

In patients with HL IgA deposits were found either in the lamina lucida or just below the basal lamina, often associated with anchoring fibres. Ultrastructural localisation of IgA cannot therefore be predicted by immunofluorescent microscopy. Furthermore there must be 2 antigenic determinants, one in the lamina lucida which may be a glycoprotein and one below the basal lamina which may be a collagen subtype.

When IgG or IgM are found in association with IgA they always have the same localisation. Complement (C3) was found to be homogeneous linear in 2 cases which had sub basal lamina IgA, and granular linear in one case which had IgA confined to the lamina lucida. No C1q was found even when IgG was present.

None of the patients with lamina lucida deposits of IgA investigated so far has had an associated enteropathy, 50% of them had HLA B8. 33% of patients with sub basal lamina deposits had an associated enteropathy and all but one had HLA B8.

Evidence That the IgA in Linear IgA Disease Is Qualitatively Different to That in Dermatitis Herpetiformis. JONATHAN LEONARD, GERALD HAFFENDEN, NICOLA RING AND LIONEL FRY, Dermatology and Histopathology Departments, St. Mary's Hospital, London W2 1NY

A previous study, using immunofluorescent techniques, has shown that in vivo bound J-chain is present in the uninvolved skin of patients with papillary IgA dermatitis herpetiformis (DH) in a distribution that is co-extensive with the IgA. This implied that the IgA was dimeric and was consistent with origin from the gut mucosa. In this study, only 1 of 15 adult patients with homogeneous-linear (HL) deposits of IgA had in

vivo bound J-chain. However, J-chain was present in all 5 patients tested with granular-linear (GL) deposits of IgA.

These findings show that the IgA in the HL patients is qualitatively different from that in papillary IgA DH suggesting that these two diseases have a different pathogenesis. However, the IgA in the GL group does appear to be dimeric indicating that these patients have a disease of similar pathogenesis to DH. These results make distinction of the two patterns of linear IgA deposition particularly important.

Identification of IgA Subclasses in Skin of Patients with Dermatitis Herpetiformis. R. P. HALL, D. L. NELSON, AND T. J. LAWLEY, Dermatology and Metabolism Branches, NCI, NIH, Bethesda, MD

Although cutaneous deposits of IgA are a constant finding in patients with dermatitis herpetiformis (DH), the origin of the IgA is not known. Two subclasses of human IgA have been identified. IgA_1 is predominately monomeric, and comprises 80–90% of the normal serum IgA. IgA_2 is mainly polymeric, comprises 10–20% of the human serum IgA, 40–50% of secretory IgA, and is the predominant subclass present in the IgA producing cells found in gut associated lymphoid tissue (GALT). Since patients with granular deposits of IgA have an associated gluten sensitive enteropathy (GSE), it has been suggested that the IgA in their skin may be of GALT origin. In contrast, patients with linear IgA deposition have no associated GSE, and their IgA may be of a different origin. We have used monoclonal antibodies against human IgA_1 and IgA_2 to examine the normal skin of 28 patients with DH. 22 had granular IgA deposits while 6 had a linear pattern of IgA. Mouse anti-human IgA_1 and IgA_2 were determined by ELISA assay to be monospecific against human IgA_1 and IgA_2 myeloma proteins, of similar sensitivity, and have no light chain reactivity. 22 of 22 patients with granular IgA deposits at the dermal-epidermal junction (DEJ) had IgA_1 present whereas 0 of 22 had IgA_2 deposits. Deposition of IgA around the papillary blood vessels was seen in 5 patients (4 IgA_1, 1 IgA_2). 6 of 6 patients with linear IgA deposits had IgA_1 at the DEJ and 1 of 6 had additional deposits of IgA_2. There was no correlation found between the ultrastructural location of the linear IgA deposits and the IgA subclass detected. These findings indicate that the IgA deposited in the skin of patients with granular and linear DH is IgA_1, and suggests that it is most likely monomeric, and may not arise from GALT.

Connective Tissue Degrading Enzymes in the Blister Fluids from Bullous Skin Diseases: Demonstration of Two Separate Elastolytic Enzymes in Bullous Pemphigoid and Dermatitis Herpetiformis. AARNE I. OIKARINEN, JOHN J. ZONE, A. RAZZAQUE AHMED, URPO KIISTALA and JOUNI UITTO, Harbor-UCLA Med. Ctr., UCLA Sch. of Med., Univ. of Utah, and Univ. of Helsinki

Blister fluids were obtained from patients with dermatitis herpetiformis (DH), bullous pemphigoid (BP), chronic bullous disease of childhood (CBDC), and pemphigus vulgaris (PV). The cells were recovered by centrifugation, and the supernatants as well as the cell pellets were assayed for elastase activity using a synthetic Suc-$(Ala)_3$-PNA or soluble tropoelastin as substrate. For comparison, samples from burn blisters or vacuum-induced suction blisters were also assayed. High levels of elastase activity were present in all DH patients, while lower, but clearly detectable, levels were also found in BP, CBDC and PV, when the activity was determined with the synthetic substrate. The enzyme activity in BP was inhibited by EDTA, but not by PMSF, and Ca^{2+} stimulated the activity, suggesting that the enzyme in BP was a metalloproteinase. In DH, the elastase activity was markedly decreased by PMSF, but not by EDTA, indicating that the enzyme was a serine protease. The cells recovered from DH blister fluids also contained high levels of elastase activity which could be inhibited by PMSF. Thus, in DH, the elastase activity may be derived from PMNs abundantly present in the lesions. Further studies indicated that BP blister fluids, and the supernatants and the cell pellets derived from DH blisters contained enzyme activity which degraded soluble tropoelastin. The results indicate that active proteases are present in the blister fluids of bullous skin diseases, and they may play a mechanistic role in the blister formation by degrading connective tissue components of the dermis and the dermo-epidermal junction.

Isolation of Pemphigoid Antigen from Normal Human Esophagus. RICHARD J. PYE, G. D. HARKISS AND T. E. CAWSTON, Department of Dermatology, Addenbrooke's Hospital, Cambridge, England

The study was undertaken to develop a simple technique for the extraction of purified pemphigoid antigen with a view to its characterization.

Post mortem human esophagus was obtained within 24 hours of death. Mucosa was separated from submucosa by immersion in cold 2M sodium thiocyanate (NaSCN), homogenised in PBS and then centrifuged. The supernatant was dialysed, lyophilized and when resuspended blocked indirect immunofluorescence (IIF) produced by pemphigoid antibody on normal human esophagus. This activity was not retarded by DEAE cellulose. The exclusion peak was applied to a sepharose-IgG affinity column. The IgG for this column was purified by DEAE cellulose chromatography from a patient with high titre pemphigoid antibody and coupled to sepharose 4B using the cyanogan bromide method. The retarded protein was eluted off with 2M NaSCN, dialysed, lyophilized and shown to block IIF of pemphigoid antibody. This preparation was radiolabelled with 125I and run on 17.5% SDS-PAGE and shown on autoradiography to have two consistent bands with molecular weights of 68,000 and 33,000 daltons. Radiolabelled material was also present close to origin and the bottom of the gel. The radiolabelled preparation was subjected to enzyme digestion and run on SDS-PAGE. It was resistant to DNase, RNase, trypsin, collagenase and hyaluronidase, partially degraded by pepsin and completely degraded by protease.

A simple two step purification method has been established. The data suggest that pemphigoid antigen is a protein and is present as a polymer. Further characterization work is in progress.

Heterogeneous Nature of Bullous Pemphigoid-like, IgG Associated Basement Membrane Zone Disorders. ROBERT A. BRIGGAMAN, W. RAY GAMMON, ALFRED O. INMAN, III, BETH A. J. LAMB AND LAURINDA L. QUEEN, Department of Dermatology, The University of North Carolina School of Medicine, Chapel Hill, North Carolina

Bullous pemphigoid (BP) is generally defined by the following criteria: generalized bullous disease, sub-epidermal blister separation with variable inflammation, linear IgG deposition at the basement membrane zone (BMZ) on direct immunofluorescence and circulating IgG antibody with BMZ specificity. Evidence will be presented that these criteria do not define a homogeneous disease entity. Using as substrate 1 M NaCl separated skin which is cleaved in the lamina lucida (LL), 44 sera from patients fulfilling the above criteria were examined by immunofluorescence. Five sera were identified in which IgG localized to the dermal side of the separation instead of the epidermal side as in "classic BP". Indirect immunoelectron microscopy (immEM) using these sera on a normal human skin substrate found the IgG deposit in the deeper (dermal) portion of the lamina densa (LD) and beneath the LD, but not in the LL. Direct immEM was performed on skin from three of these patients and showed the same IgG localization. Other clinical features distinguished these patients from "classic BP" including: mild atrophic scarring with milia, mechanobullous component and poor response to systemic corticosteroids. Distinction from acquired epidermolysis bullosa (EBA) is more difficult but includes different clinical features (BP like), absence of amorphous sub-LD deposits on electron microscopy and different distribution of the immune deposits on immEM. These patients may constitute a sub-group of the bullous disorders associated with IgG deposition in the sub-LD area.

Suppressor T Cell Function in Bullous Pemphigoid Patients. A. RAZZAQUE AHMED, Division of Dermatology, Department of Medicine, University of California, Los Angeles, California

T cells were separated from the peripheral blood leukocytes of eight patients with active bullous pemphigoid by SRBC rosetting technique. Eight patients with inactive disease and 15 controls were similarly studied. The percentage of total T cells, helper/inducer and cytotoxic suppressor cells were determined using Leu 1, Leu 2, and Leu 3 monoclonal antibodies by an immunofluorescent assay.

Peripheral blood from 11 patients and controls were separated into T and B cells by buoyant density sedimentation of spontaneous rosettes formed by T cells and SRBC pretreated with 2-aminoethylisothiouronium. T cells were irradiated with 3000 rads to remove suppressor activity. Cells were cultured in various combinations and concentrations of patient and normal cells to determine helper and suppressor effects. Cells were cultured in 1640 RPMI, fetal calf serum, glutamine and gentamicin for five days in humidified air with 5% Co_2. Pokeweed mitogen was added to the cultures. The total IgG synthesized by the cells was measured by a sensitive solid phase radioimmunoassay for IgG using 125 I labelled goat anti-human IgG antiserum. Comparisons were made of the IgG synthesized by cells in various combinations and after irradiation. No statistically significant differences were observed in the total IgG synthesized by cells from the patients and controls.

This study indicates that using non-specific assays there is no loss of helper cell or increased suppressor cell function in the peripheral blood leucocytes of patients with bullous pemphigoid.

Regional Variations in the Expression of Bullous Pemphigoid Antigen in Human Skin. D. J. GOLDBERG AND J-C. BYSTRYN, Dept. of Dermatology, New York University School of Medicine, New York, NY

To study how uniformly bullous pemphigoid antigen (BP Ag) is distributed we estimated its concentration at different sites on the body from the highest dilution of BP sera capable of giving a positive reaction at a site by indirect immunofluorescence. Three sera with high titers (640–1280) of BP antibodies against monkey esophagus were reacted with 87 specimens of human skin obtained from normal individuals. All skin specimens were immediately frozen in liquid N_2 and used within 48 h.

There was a marked variation in the maximum dilution of BP sera giving positive reactions against skin from different sites. The highest concentration of BP Ag was in skin obtained from the flexor surface of the arm, leg or thigh. Eighteen specimens were tested from these sites. All were positive at high dilutions (1/640–1/2560, average 1/1280) of BP sera. The lowest concentration of BP Ags was in the scalp, face and extensor surface of the arm. Thirty-nine specimens were tested from these sites. No BP Ag could be demonstrated in 10 (25%) specimens. In the remainder, low dilutions (1/40–1/160, average 1/80) of BP sera were required to demonstrate BP Ag. Skin from trunk, knee and forearm had intermediate amounts of BP Ag. Similar results were obtained with all three BP sera. Of note is that the highest concentration of BP Ag was in areas commonly involved in the disease (flexural surfaces), whereas the lowest was in areas (head and extensor arm) which are rarely involved.

Thus, there is marked heterogeneity in the concentration of BP Ags at different sites in skin of normal individuals. The apparent correlation between the concentration of this antigen and the location of lesions in BP, suggests that BP Ag concentration may play a role in the distribution of skin lesions in this disease.

Proteolytic Disruption of the Lamina Lucida and Bullous Pemphigoid Antigen by Activated Leukocytes Adherent to the Basement Membrane Zone. CAROLYN C. MERRITT, LAURINDA L. QUEEN, ALFRED O. INMAN, III and W. RAY GAMMON, Department of Dermatology, The University of North Carolina School of Medicine, Chapel Hill, North Carolina

Leukocyte proteases may be responsible for injury to the lamina lucida and dermal-epidermal separation (DES) in bullous pemphigoid (BP). In a previous study we described a model of DES in cryostat sections of normal human skin treated with BP antibodies (BPAb), leukocytes and complement (C) and showed DES was due to attachment of activated leukocytes to the basement membrane zone (BMZ). In this study, we examined DES in the model using immunofluorescence and antibodies to BMZ proteins to determine if a leukocyte protease-sensitive site could be identified. Sections of skin were treated with BPAb, C and leukocytes with and without 1–2 mg/ml soybean trypsin inhibitor (TI) or BPAb, buffer and leukocytes as control, incubated for 30–120 minutes at 37°C, cleared of leukocytes by hypotonic lysis and examined by immunofluorescence for laminin, type IV collagen and BP antigen (BPA). In skin treated with BPAb, C and leukocytes, foci of DES developed above laminin and either below or through BPA. There was no change in type IV collagen or laminin immunofluorescence but a reduction in BPA immunofluorescence and fragmentation of BPA were observed. Neither DES nor changes in BPA immunofluorescence were observed in controls or skin treated with TI. These results show that the site of DES in the model corresponds to BP lesions and that there is a leukocyte protease-sensitive site in the lamina lucida associated with BPA. These results support a role for leukocyte proteases in BP.

Effector Proteases and Pemphigus Antibody Action. T. Y. WOO, P. A. BAROUSKI-MILLER, T. D. GELEHRTER, G. J. ANHALT, V. HOGAN, H. PATEL, AND L. A. DIAZ, Departments of Dermatology and Human Genetics, U. of Michigan, Ann Arbor, MI and Department of Dermatology, Johns Hopkins U., Baltimore, MD

Pemphigus IgG (PV IgG) induces epidermal cell detachment *in vivo* and *in vitro*. Using Balb/c primary epidermal cell cultures (PECC), we have investigated the role of enzyme inhibitors and plasminogen activator (PA) in the detachment process induced by PV IgG. Duplicate PECC grown with or without human IgG (normal donors (ND) or PV) were treated with pharmacological doses of protease inhibitors and dexamethasone. Also, PECC were grown in plasminogen (P)-free media obtained by lysine-agarose affinity chromatography. The % cell detachment was determined at 96 hours (Patel, et al JID, 76:474, 1981) and supernatants of these cultures were tested for fibrinolytic (FA) and (PA) activities by the ^{125}I-fibrin plate assay. FA and PA activities were expressed as the % of total cpm released by trypsin. We found: 1—Cell detachment induced by PV IgG was inhibited by α-2 macroglobulin by 70%, trasylol (66%), soybean trypsin inhibitor (62%) and pepstatin (49%). Chymostatin, antipain and leupeptin were ineffective. 2—Cell detachment induced by PV IgG was not altered in PECC maintained in P-free growth media. 3—There was no difference in FA or PA in media from PECC treated with either PV IgG or control ND IgG. 4—Dexamethasone inhibited FA and PA by 98% in both, without affecting cell detachment. 5—FA and PA did not differ between control ND and PV IgG treated cultures grown in P-free serum.

In conclusion the increased cell detachment induced by PV IgG in PECC is inhibited by certain protease inhibitors and appears to be independent of PA or FA activities in the growth media.

Modulation of the Synthesis of Pemphigoid and Pemphigus Antigens by Extracellular Calcium Concentrations Which Regulate Differentiation of Epidermal Cells. J. R. STANLEY AND S. H. YUSPA, Uniformed Services, Univ. of the Health Sciences and N.I.H., Bethesda, MD

The calcium concentration in the medium of cultured epidermal cells has been shown to modulate the transition between proliferating monolayer cells in low (.07 mM) Ca^{2+} medium (LCM) and nonproliferating stratifying cells in high (1.2 mM) Ca^{2+} medium (HCM). Specific changes in protein synthesis occurring with this process of maturation induced by Ca^{2+} have been difficult to identify. In this study we sought to determine if the synthesis of bullous pemphigoid antigen (BPA) a basal cell-associated protein, and pemphigus antigen (PA), a cell surface glycoprotein of stratifying cells, was modulated by Ca^{2+} concentrations which regulate this process of differentiation. In order to detect newly synthesized antigen, cultures maintained in LCM or switched to HCM for 24 hr before labeling were radiolabeled with ^{14}C-amino acids or ^{14}C-glucosamine. The results of immunoprecipitations of radiolabeled cell extracts and medium from these cells indicated that BPA, a 220 kd protein, is synthesized mainly by proliferating cells cultured in LCM and synthesis is decreased over 90% in cells switched to HCM. In contrast to BPA, the synthesis of PA, a 130 kd glycoprotein, was detected only in stratifying cells in high Ca^{2+} medium. Immunofluorescence performed to detect BPA and PA was consistent with these results. Thus these studies indicate that extracellular Ca^{2+} concentrations which modulate the transition between proliferating and stratifying epidermal cells also modulate, in parallel, the synthesis of BPA and PA. These antigens may prove important in understanding epidermal differentiation.

Interleukin 2 Deficiency in Pemphigus. VALI KERMANI-ARAB, SUNILKUMAR A. PATEL, JOHN F. FAHEY and A. RAZZAQUE AHMED, UCLA School of Medicine, Los Angeles, California

Altered immune regulation with production of autoantibodies is a feature of pemphigus. A key factor in lymphocyte response are the lymphokine, particularly interleukin-2 (IL-2). Thus, investigations of IL-2 production and persistence were undertaken in patients with pemphigus vulgaris, to determine if altered immune regulation of IL-2 was characteristic of this autoimmune disorder.

Kinetics of IL-2 production was studied on peripheral blood mononuclear cells (PBMC) of 16 patients with active pemphigus. 1×10^6 PBMC/ml from each patient or corresponding sex and age matched normal controls were cultured with 10 μg phytohemagglutinin P (PHAP) for 24 to 120 hours. The supernatants were assayed for IL-2, using IL-2 dependent CTLL-2 murine T lymphocytes proliferative response. To evaluate the IL-2 activity from each specimen, the logarithmic dilution of the sample were plotted and resulted in a sigmoid curve. A standard curve was similarly prepared. IL-2 activity was transformed into units/ml by linear regression analysis, using 30% of maximal CPM of the standard. In normal controls IL-2 production is evident at 24 hours. Maximum peak production occurs at 48 hours and then decreases by 72–120 hours. In contrast the pemphigus patients IL-2 production reached peak levels later, e.g., at 72–120 hours, and decreased more slowly. It is interesting to speculate that the abnormalities in the production and/or response of IL-2 may have a significant role in auto-antibody production.

Dexamethasone Effect on Experimental Pemphigus in Mice. G. J. Anhalt, R. S. Labib, H. P. Patel and L. A. Diaz, Department of Dermatology, Johns Hopkins University, Baltimore, Maryland

Our laboratories have reported that pemphigus (PV) may be induced in neonatal mice by intraperitoneal (IP) injections of human PV IgG. Using this animal model, we have investigated the role of dexsamethasone (Dex) in the induction of disease by a highly pathogenic PV IgG obtained from a single donor. BALB/c neonates received an initial IP injection of Dex, and controls a similar volume of saline. After 6 hours, 7 test animals received twice daily IP injections of PV IgG [14.4 (n = 5) and 20.0 (n = 2) mg IgG/g body weight/day] and Dex in doses of 0.01 to 0.02 mg/g body weight/day. Control littermates (n = 7) received identical doses of PV IgG without Dex. At 48 hours, mice were graded for disease extent (# of lesions) on a scale of 1^+ to 3^+. The extent of disease was similar in mice treated or untreated with Dex. Histologic exam of lesions from both groups of animals showed intraepidermal vesiculation with acantholysis, and PV IgG bound to the mouse epidermal cell surfaces (by direct immunofluorescence).

PV IgG Dose	Dexamethasone Dose	# Subjects	Disease Extent
14.4 mg/g/day	0.010 mg/g/day	n = 2	1^+, 1^+
14.4 mg/g/day	0	n = 2	0, 1^+
14.4 mg/g/day	0.020 mg/g/day	n = 3	1^+, 1^+, 3^+
14.4 mg/g/day	0	n = 2	1^+, 0^+
20.0 mg/g/day	0.020 mg/g/day	n = 2	3^+, 3^+
20.0 mg/g/day	0	n = 3	3^+, 3^+, 3^+

This study demonstrates that very high doses of Dex (up to 20 mg/kg/day) do not prevent the development of cutaneous lesions in mice receiving IP injections of PV IgG. This suggests that corticosteroids may be of therapeutic value in PV patients by their immunosuppressive effect rather than by modifying the antibody mediated target tissue responses.

In Vitro Production of Anti-intercellular Cement Substance Antibodies and Anti-basement Membrane Zone Antibodies by Bone Marrow Cells from Patients with Pemphigus Vulgaris and Bullous Pemphigoid. A. Razzaque Ahmed, Joe Aiello, David Chia, and Martin J. Cline, Division of Dermatology and Hematology Oncology, Department of Medicine, University of California, Los Angeles, California

Four patients with clinical and immunopathologically proven pemphigus vulgaris and bullous pemphigoid were studied. 5 ml of bone marrow was obtained from the patients. Leucocytes were separated on ficoll-hypaque. 1×10^6 and 1×10^7 cells per ml were cultured with and without pokeweed mitogen (PWM) in McCoy 5A medium with fetal calf serum, glutamine, thioglycerol, penicillin and streptomicin in a humidified incubator with 5% Co_2. The supernatants were assayed for anti-Basement Membrane Zone (BMZ) by an indirect immunofluorescence assay using monkey esophagus as a substrate. Anti-Intercellular Cement Substance (ICS) antibody was assayed by an ELISA using COLO-16 cell lines as substrate. Supernatants were assayed at 0, 2, 4, 5, and 7 days of cultures. Anti-BMZ and anti-ICS antibody could not be detected in cultures at day 0. Anti-ICS and anti-BMZ antibody were found in supernatants at day 2; were maximum at day 5 and decreased by day 7. There were no statistically significant differences in the levels of antibody produced by the cultures with or without PWM. This study provides an assay to study the immunoregulatory mechanisms that control autoantibody production and pathogenetic mechanisms. The availability of such an assay permits the study of the effect of pharmacological agents on the bone marrow production of autoantibodies and its relevance to clinical disease activity.

The Effect of IgG Class Pemphigus Antibodies on Keratinocyte Plasma Membrane. Hideo Yaoita, Yasuo Kitajima*, Kiyomi Eguchi, Kazuo Ohki*, and Yosinori Nozawa*, Department of Dermatol. Jichi Medical School, Tochigi, *Department of Biochem. School of Medicine, Univ. of Gifu, Gifu, Japan

To know the role of keratinocyte plasma membrane in the acantholysis, we studied the effects of pemphigus antibodies on the keratinocyte plasma membrane, using electron spin resonance (ESR) and freeze fracture. Pemphigus antibodies were obtained from the sera of three patients with pemphigus vulgaris who were diagnosed clinically, histopathologically and by immunofluorescence. (age: 38–68, male, pemphigus antibody titer; 1:320–1280) Control sera were obtained from two healthy volunteers. IgG class antibodies were purified using DEAE column and protein A column. Keratinocytes used in these experiments were obtained from donor skin for transplantation and were cultured for two weeks. Some were used for freeze fracture study and the others were suspended just before ESR. In freeze fracture study the cultured keratinocytes were incubated with the purified pemphigus antibodies (5 mg/ml) for 15 min at room temperature, and treated for observation. In the ESR study the suspended keratinocytes were labeled with stearate spin probe and incubated with the purified antibodies. The membrane fluidity was then measured by ESR. Freeze fracture study showed that desmosomes decreased in size and in number and tight junctions were broken after the keratinocytes were incubated with the pemphigus antibodies. ESR study revealed that the membrane fluidity of keratinocytes increased when the keratinocytes were incubated with the pemphigus antibodies. However, one of pemphigus antibodies had no effect on the plasma membrane. These results suggest that some pemphigus antibodies directly affect the plasma membrane of keratinocytes which may result in acantholysis.

The Cellular Response of Human and Guinea-Pig Keratinocytes to Pemphigus IgG-Cell Death Related to IgG-Receptor Surface Migration and Internalization. Vitaly Citovsky, Shulamit Naishtat, Rina Timberg, Yoram Milner, and Beno Michel*, Department of Biological Chemistry, The Hebrew University of Jerusalem, Israel; *Department of Dermatology, Case Western Reserve University, Cleveland, Ohio

When keratinocyte suspensions are treated with purified fluorescent Pemphigus-IgG (P-IgG) or fluorescent lectins, a series of migratory steps occur at the cell surface, as seen microscopically. These migration steps, from diffuse patterns to microclustered and partially internalized IgG receptors, precede the cell death induced by the bound P-IgG. Thus, maximal microclustering occurs at 24–28 hs of incubation, whereupon P-IgG induced cell death increases dramatically. D-Galactose specific lectins, on the other hand, induce much faster microclustering with very little internalization and little correlation to the induction of cell death. Internalization of P-IgG into the cell was shown by following the fate of [^{125}I]P-IgG and ferritin conjugated IgG, using autoradiographic and electron microscopic techniques. Some degradation of [^{125}I]P-IgG was detected in cells incubated for 32 hours with labeled IgG, showing possible contact of internalized P-IgG with lysosomes. Further observations which strongly implicate surface mobility of P-IgG receptor complex on the keratynocyte surface with the mechanism of P-IgG induced cell damage are: (a) cells responsive to P-IgG (20–30 μM cells were separated from the rest by Ficol density gradients) were also active in surface mobility of bound IgG; (b) monovalent Fab fragments of P-IgG did not elicit surface migration or Pemphigus induced cell death; (c) microtubuli disruptive drugs, *e.g.*, colchicine, theophylline and theobromine, inhibited cell surface migration of bound P-IgG and also P-IgG induced cell death in similar dose-response relationships.

Effects of Prednisolone and Cyclophosphamide on the Synthesis of Pemphigus Autoantibodies. J. C. Guillaume, J. Revuz, and R. Touraine, Department of Dermatology, Univ. Paris XII, Créteil. France

It has been shown previously in animals that the removal of a circulating antibody induces a rebound in antibody synthesis. We observed such a rebound (i.e. an increase of serum antibody titers after an initial decline) in some of our patients undergoing plasma exchange (PE) for the treatment of pemphigus and studied the suppressive effect of various drug regimens on the de novo synthesis of pemphigus autoantibody.

Sixteen patients were treated by repetitive (PE) together with various doses of cyclophosphamide (CY) and/or prednisolone. Serum antibody titers were sequentially determined by indirect immunofluorescence on rat oesophagus.

The magnitude of the decrease in antibody titer after 3 PE was related to the daily dose of prednisolone ($r = 0.77$ $p < 0.001$) and not to the dose of CY. Nine of 16 patients experienced a rebound in antibody synthesis despite further PE. The magnitude of this rebound was inversely related to the daily doses of prednisolone ($r = -0.76$ $p < 0.001$). On the contrary there was no relationship between the rebound and the daily dose of CY.

Both effects—on the decrease of antibody titers and on the magnitude of the antibody rebound—suggest that prednisolone has a suppressive effect on pemphigus and antibody synthesis. This was not observed with CY. These results challenge the current concepts about the effects of these drugs on antibody synthesis in man.

An Organ Culture Model of Autoantibody Mediated Subepidermal Bullous Disease. W. RAY GAMMON, ALFRED O. INMAN, III, ROBERT A. BRIGGAMAN, AND CLAYTON E. WHEELER, JR, Department of Dermatology, The University of North Carolina School of Medicine, Chapel Hill, North Carolina

Complement (C) activating autoantibodies to the cutaneous basement membrane zone (BMZ) may be responsible for skin injury in several subepidermal bullous diseases. However, neither dermal inflammation nor dermal-epidermal separation have been successfully produced by passive transfer of anti-BMZ antibodies in animals or organ culture. In this study, an organ culture model of dermal inflammation and dermal-epidermal separation caused by C activating anti-BMZ antibodies from 4 patients with epidermolysis bullous acquisita (EBA) is described. 0.4 mm thick sections of keratomed normal human skin were precultured with EBA serum or EBA IgG, or normal human serum (NHS) or NHS IgG. Skin was then incubated for 4 hrs at 37°C with: 1) normal human peripheral blood leukocytes (PBL) and fresh NHS; 2) PBL and heat-inactivated NHS or 3) fresh NHS alone and subsequently processed for direct immunofluorescence and routine histology. In skin precultured in EBA serum or EBA IgG, IgG and C3 were deposited at the BMZ. In skin with IgG and C3 deposits subsequently incubated with PBL and fresh NHS, leukocytes were present in the dermis and densely concentrated beneath the BMZ, and focal to confluent sites of dermal-epidermal separation were observed. Leukocyte infiltration and dermal-epidermal separation were not observed in skin incubated with PBL and inactivated NHS or fresh NHS alone. These results show that C-activating anti-BMZ antibodies can produce cellular infiltration and BMZ separation in organ culture, suggest the antibodies may be pathogenic in vivo and provide a model for further study of the interaction between anti-BMZ autoantibodies, C and leukocytes in the pathogenesis of subepidermal bullous diseases.

SESSION D — South American Room

Rona MacKie, M.D., Presiding

Photochemotherapy of Vitiligo with Oral Phenylalanine. RUDY H. CORMANE, ANWAR H. SIDDIQUI, AND ILONKA M. NENGERMAN, Department of Dermatology, Academic Medical Center, University of Amsterdam, The Netherlands

Since the administration of tyrosine and incubation of ultraviolet light-irradiated phenylketonuria skin have led to normal melanin synthesis we tried the same to the vitiliginous skin but with no clinical results. We then tried another aminoacid, L-phenylalanine in combination with UVA light and to our surprise vitiliginous lesions showed a remarkable improvement in repigmentation.

After a careful preliminary pharmacokinetic study we worked out a protocol whereby L-phenylalanine in a dose of 200 mg/kg body weight was orally administered about an hour (time to reach peak phenylalanine serum level) before the UVA exposure on three times a week schedule. Dramatic repigmentation of hypopigmented macules occurred especially in the adipose area of the skin in 5 and reasonably good in 10 out of 20 patients in about 6 months time. Initial duration of the therapy needed to start repigmentation was 3 months (36 phenylalanine + UVA) exposure. The UVA dose ranging from 1.5 to 5.0 J/cm^2 (according to skin type) was gradually increased. To discard the possibility that phenylalanine, UVA, UVA + UVB or UVB alone are responsible for causing the repigmentation of the hypopigmented macules in vitiligo patients we treated them separately for three months on a thrice weekly schedule but without any success.

A big advantage in this modality is that L-phenylalanine is a natural essential amino acid and constitutes a part of daily dietary protein. As such there is no danger of getting serious side effects due to the administration of this kind of photochemotherapy. Moreover vitiliginous skin becomes less sensitive to sunlight as a result of this therapy.

Vitiligo: Racial Variations in the Response to Topical Photochemotherapy. PEARL E. GRIMES, HAROLD R. MINUS, REBAT M. HALDER, JOHN ENTERLINE AND JOHN A. KENNY, Department of Dermatology, Howard University College of Medicine, Washington, D.C.

We have previously demonstrated that optimal topical photochemotherapy of vitiligo can be achieved in black and East Indian patients utilizing 0.1% 8-MOP and UVA (J Am Acad Dermatol 7: 771–778, 1982). In the present investigation, racial variations in the response to topical photochemotherapy were assessed in 3 groups of patients: 32 blacks and East Indians, 21 whites and 12 comprising other racial groups (Hispanics and Orientals). Patients with 1–20% cutaneous surface involvement were treated weekly. 0.1% 8-MOP was applied, followed by exposure to UVA (365nm) radiation sources after an interval of 30 minutes. Repigmentation was defined as 50% or greater return of pigment to vitiliginous areas. There were no statistically significant differences in mean age, duration of disease or duration of treatment between the three groups. 18/32 (56%) of blacks and East Indians, 2/21 (10%) Caucasians and 3/12 (25%) of others repigmented ($p = 0.0017$). One or more episodes of blistering occurred in 6/32 (19%) blacks, 13/21 (65%) Caucasians and 8/12 (67%) others ($p = 0.0074$). Thus, maximal repigmentation with minimal phototoxicity occurred in blacks and East Indians; maximal phototoxicity and minimal repigmentation occurred in the other racial groups studied. These data suggest that in contrast to blacks and East Indians, 0.1% 8-MOP is not the optimal concentration for topical photochemotherapy in non-black vitiligo patients (Caucasians and other racial groups). Lower concentrations may be required to achieve optimal responses in these patients.

Antibodies to Melanocytes in Vitiligo. G. K. NAUGHTON, M. EISINGER, AND J-C. BYSTRYN, New York University School of Medicine and Memorial Sloan-Kettering Institute, New York, NY

We have used melanocytes grown in tissue culture to develop a sensitive immunoprecipitation with Protein A-Sepharose antibodies binding detergent soluble, radioiodinated surface macromolecules of normal human melanocytes grown in culture. This assay was used to measure antibodies to melanocytes in sera of 120 patients: 61 with active vitiligo, 35 with non-pigmentary dermatoses, and 24 with melanoma. Antibodies to melanocytes were found in 82% of 61 patients with vitiligo. Antibodies were as frequent in patients with common vitiligo (100% of 14 pts) as in patients with vitiligo associated with other immune disorders (75% of 42 pts), or with chronic mucocutaneous candidiasis (100% of 5 pts). The antibodies were directed to antigens selectively expressed on melanocytes since they reacted to several different lines of melanocytes but not to three lines of human melanoma cells or to normal allogeneic keratinocytes or fibroblasts radioiodinated in a similar manner. No antibodies to melanocytes were found in 35 patients with non-pigmentary diseases. Low levels of antibodies were present in 12% of 24 melanoma patients. A linear relationship existed between specifically bound radioactivity and the volume of melanocyte antibody positive serum used in the assay, indicating that the assay provides a quantitative estimate of melanocyte antibody level.

This study indicates that most patients with active vitiligo have circulating antibodies to surface antigens on normal human melanocytes. This finding suggests, but does not prove, that vitiligo is an autoimmune disease mediated by antibodies to melanocytes.

Calcium Has Several Points of Action on MSH-Stimulated Melanosome Dispersion. ANGELA LUCAS, SAM SHUSTER, AND ANTHONY THODY, Department of Dermatology, University of Newcastle-upon-Tyne, UK

Calcium is required for MSH stimulation of melanosome dispersion but its mode of action is unknown. In this study we have examined the role of calcium on the rate of melanosome dispersion in the lizard, *Anolis carolinensis*.

We found a parallel and linear shift in α-MSH dose response curves (50pM–70nM) by changing the Ca^{2+} concentration between 10^{-5}–10^{-1}M, indicating that the effect of Ca^{2+} is at a point common to that of MSH action. To test this further we used a long acting dispersion in the presence of Ca^{2+}, and to a lesser extent in the absence of Ca^{2+} ($+10^{-4}$M EGTA). When a half-maximal dose of the peptide was added in the presence of Ca^{2+}, the subsequent response to α-MSH was reduced by 50%, but when added in the absence of Ca^{2+} the subsequent response to MSH was 100% indicating unoccupied receptors. This suggests that Ca^{2+} is required for initial receptor-peptide binding. Once the peptide was bound however, removal of Ca^{2+} abolished darkening but this could be restored with the addition of Ca^{2+}, suggesting that Ca^{2+} is also required for mediating the effect of the peptide after binding. To test the possibility that this may involve a Ca^{2+} influx experiments were done with the Ca^{2+} ionophore A23187 ($>1.6 \times 10^{-5}$M) which showed that Ca^{2+} influx can stimulate melanosome dispersion.

We conclude that (1) Ca^{2+} is required for MSH-receptor binding, (2) Ca^{2+} may also mediate the effect of MSH by acting as an additional second messenger. The possibility that it also acts by modulating transduction of the MSH signal cannot yet be ruled out.

Immediate Pigment Darkening. A Reevaluation of Proposed Mechanisms. H. HÖNIGSMANN, G. SCHULER[+], W. ABERER, AND K.

WOLFF, Depts. of Dermatology, Universities of Vienna & Innsbruck+, Vienna & Innsbruck, Austria

Proposed mechanisms of IPD are controversial. They include photooxidation of "pre-melanin," changes in the distribution pattern of microfilaments and microtubules, movement of melanosomes to melanocyte dendrites, increased transfer of melanosomes to keratinocytes and changes in the melanosome distribution pattern in keratinocytes. We have investigated the following aspects of IPD: 1) Production of IPD by UVA under physiological and non-physiological conditions in full thickness skin and epidermal sheets. 2) Reversibility of IPD in vitro after in vivo and in vitro production. 3) Blocking of IPD by disruption of the microfibrillar or microtubular system in vitro. 4) Alterations of the cytoskeleton of melanocytes. 5) The melanosome distribution pattern in melanocytes and keratinocytes.

The results are as follows: IPD can be elicited in vitro in full thickness skin and in epidermal sheets. Its production is temperature independent (0°–37°) and is not inhibited by repeated freezing and thawing, or by formalin fixation. IPD is reversible in vitro under tissue culture conditions but only in viable skin. IPD cannot be blocked by substances which disrupt the microfibrillar or microtubular system (Cytochalasin B, Colcemid, Vincristine). As seen with a monoclonal antivimentin-ab, IPD-producing UVA doses do not induce changes in the cytoskeleton of melanocytes. No changes in number and distribution patterns of melaonosomes can be observed electromicroscopically. IPD production does not depend on the structural and functional integrity of the melanocyte cytoskeletal apparatus and is not confined to viable skin, whereas its reversibility is. The fact that no increased melanosome transfer occurs may explain the lack of an UV protective action.

Abnormal Cutaneous Melanocytes Within Pigmented Regenerating Feathers of Delayed Amelanotic (DAM) Line Chickens. RAYMOND E. BOISSY AND J. ROBERT SMYTH, JR.*, Dept. of Dermatology, Yale Univ. School of Medicine, New Haven, Ct. and *Dept. of Veterinary & Animal Sciences, Univ. of Massachusetts, Amherst, Ma

Feather amelanosis in the delayed amelanotic (DAM) line of chickens resembles cutaneous depigmentation in patients with vitiligo. We have reported previously that melanocytes are absent in regenerating feathers of amelanotic adult chickens. We have now evaluated by light and electron microscopy the melanocytes in pigmented regenerating feathers from 1) DAM chicks at various times prior to the onset of amelanosis and 2) DAM adults displayng erratic amelanosis. Melanocytes in pigmented DAM chicks contained melanosomes with curved pigmented extensions of different lengths and continuous with the margin of the melanosome. Melanosomal abnormalities increased as the bird aged and approached amelanosis. Immediately preceding amelanosis, melanosomes became compartmentalized within the melanocytes, and mononuclear leukocytes appeared in the central pulp. In erratic DMA adults, melanocytes of identical appearance occurred randomly throughout the pigmented areas of regenerating feathers. However, pigmented extensions frequently formed an electron lucent chamber, partially or completely surrounding the melanosome. Occasionally, extensions from adjacent granules appeared to coalesce. The degree of melanocyte dysfunction varied considerably between and within erratic DAMs and the amount of the amelanotic area per feather appeared to correlate with the amount of mononuclear leukocytes in the pulp. We conclude that the DAM mutation affects the function of the melanocyte and interferes with melanoblast repopulation into regenerating feathers.

Characterization of Melanosome Morphogenesis by Type of Melanogenesis and Physico-Chemical Properties of Melanins in Malignant Melanoma. K. JIMBOW, Y. MIYAKE*, K. HONMA, Y. IZUMI*, AND M. KIYOTA, Dept. of Dermatol., Sapporo Med. Col., and *Dept. of Polymer Sci., Fac. of Sci., Hokkaido Univ., Sapporo, Japan

Two major types of melanin synthesis, eu- and pheomelanin, occur in mammals. In normal mouse hair, the type of melanogenesis strictly reflects melanosome (MS) morphogenesis. However, in malignant melanoma (MM) it is not known to what extent the type of melanogenesis and MS morphogenesis correspond each other. This study characterized the type of melanogenesis and physico-chemical properties of melanin(s) to elucidate the MS morphogenesis in MM, using B16 and Harding Passey (HP) mouse MMs because they produce 2 forms of MSs commonly seen in human MM, and B16 MSs are identical in morphology and color to those of eu-MSs while HP MSs are similar to those of pheo-MSs. By acid hydrolysis B16 and HP MSs were found to contain a markedly different amount of melanins, the % compositions of which were, however, basically similar. Permanganate oxidation of B16 and HP melanins resulted a high amount of pyrrole-tricarboxylic acid, eumelanin indicator, and a low content of amino-hydroxyphenylalanine, pheomelanin indicator. The infrared spectra of B16 and HP MSs revealed a similar pattern except for one unique peak in HP which was also detected by nuclear magnetic resonance. By x-ray small angle scattering B16 and HP MSs revealed 3 major peaks, the intensity and stacking profiles of which were quite different between 2 MSs. Our results indicate that B16 and HP MSs are mainly eumelanic, though both contain a trace amount of pheomelanin, and that the major differences for MS morphogenesis in 2 MMs are related to the content of melanin, not the type of melanin, and the mode of the chemical binding of melanin with structural protein.

Are Melanoma Antigens Modified Expressions of Normal Gene Products? DOUGLAS M. GERSTEN, VINCENT J. HEARING, AND JOHN M. MARCHALONIS, Dept. Path., Georgetown Univ. Med. Cen., Wash. D.C.; Derm. Br., NCI, NIH, Bethesda, MD; and Dept. Biochem., Med. Univ. So. Carol, Charleston, SC

We have previously described a cross-reacting, melanoma-specific antigen with a molecular weight of approximately 70,000 daltons (Proc. Natl. Acad. Sci. 78:5109, 1981); this antigen has now been purified by immune-affinity chromatography, gel filtration, and preparative gel electrophoresis, and has been partially characterized. Analysis of its amino acid composition and N-terminal sequence have confirmed our previous serologic evidence that this 70,000 dalton antigen is not related to histocompatibility antigens, immune response gene antigens, known viral antigens, or species-related glycoproteins. Computer analysis of the data suggests that there is some homology between this antigen and serum albumin with respect to their amino acid composition and primary sequence, although many significant differences have also been detected. In light of recent data published by two other groups on different melanoma antigens, which indicate that they are at least partially homologous to transferrin/lactoferrin (Nature 296:171, 1982) and HLA-DR (IA) antigens (Amer. J. Pathol. 107:357, 1982), it has begun to appear that the production of modified normal proteins by transformed cells might be a common phenomenon to malignant melanomas. The degree of variability between these antigens from different tumor sources has not yet been established. It has already been proposed that tumor antigens might diversify by a genetic process mediated by genes comparable to the immunoglobulin variable regions, and the recently emerging data on the characterization of different melanoma antigens serves to promote this hypothesis.

Spontaneous and Inducible Natural Cytotoxicity in Lymph Nodes Draining Primary Melanoma. EVA-MARIA KOKOSCHKA,* ATUSHI UCHIDA,** EMURA YANAGAWA,** MICHAEL MICKSCHE,** AND ROMAN KOKOSCHKA,*** *II. Dept. Dermatol., Univ. Vienna, **Inst. for Cancer Research, Univ. Vienna, ***Surgical Dept. Kaiserin Elisabeth Spital Vienna, Austria, Europe

There exists little information about local immunological defence mechanisms in tumor or draining lymph nodes of primary melanomas especially, such as natural cell mediated cytotoxicity. In the following study we could demonstrate, that lymphocytes isolated from the first 3 draining lymph node stations adjacent to a primary tumor process variable levels of natural killing (NK) activity against K 562 target cells as determined in a 4-hour ^{51}Cr release assay. Augmentation of lymph node cell (LNC) cytotoxicity by interferon (IFN) α, β and the IFN γ inducer protein A showed considerable variations when LNC were taken from different node levels of the same patient. Furthermore adherent cells isolated from these lymph nodes showed appreciable levels of cytotoxicity against tumor cells in vitro. In patients receiving preoperative peritumoral injections with a streptococcal preparation OK 432 (Picibanil) lymph node NK activity was augmented, whereas in peripheral blood natural cytotoxicity was found to be not significantly influenced by this therapy. Thus the lymph node can be regarded as an NK deficient tissue. Two populations of cells i.e. NK cells and macrophages showing natural cell mediated cytotoxicity are present in primary melanoma draining lymph nodes and their activity can be modulated in vitro as well as to same extend in vivo. The biological significance of this phenomenon, i.e. variable pattern of local tumor defence mechanisms in different regional draining lymph node stations should be taken inconsiderable when elective lymph node dissection is performed for treatment of primary melanoma of extremities.

Cytolysis of Melanoma Cells Mediated by Human Monocytes Activated with Liposome-Encapsulated Muramyl Dipeptide. KENT L. ERICKSON, ALAN J SCHROIT, EUGENIE S. KLEINERMAN,

AND ISAIAH J. FIDLER, Univ. of California School of Medicine, Davis, CA and NCI-Frederick Cancer Research Facility, Frederick, MD

The purpose of these studies was to assess whether phagocytosis of liposomes containing muramyl dipeptide (MDP) by human peripheral blood monocytes would render them tumoricidal toward human melanoma cells. Monocytes were obtained by leukapheresis and countercurrent centrifugation elutriation. Tumoricidal activity of monocytes could be generated by incubation with lipopolysaccharide or MDP. Multilamellar lipid vesicles, liposomes, composed of phosphatidylcholine and phosphatidylserine were maximally phagocytosed by the monocytes. Two types of MDP, hydrophilic and lipophilic, entrapped in liposomes generated optimal monocyte-mediated cytotoxicity in vitro against allogeneic target cells. Two populations of monocytes separated by the fluorescent activated cell sorter by the presence of phagocytosed liposomes were both tumoricidal toward the melanoma cells. This activation was not due to the release of MDP from the liposomes into culture because the calculated total amount of MDP entrapped into liposomes would not render normal monocytes tumoricidal. This activation by liposome-entrapped MDP required about 3000-times lower MDP concentration than unencapsulated MDP. This ability to activate human monocytes for immunotherapy is attractive since liposomes injected into circulation are cleared by phagocytic cells which have been shown to play a role in host defenses against invading melanomas.

Inhibition of Mitogen-Induced Lymphocyte Blastogenesis by B-16 Melanoma. TED BREZEL AND NEIL I. BRODY, Department of Dermatology, Downstate Medical Center, Brooklyn, New York

Considerable evidence exists that tumor cells and tumor cell products can interfere with host immune mechanisms. Previous work in our lab has shown that frozen-thawed B16 melanoma cells and a tumor facilitating factor (TFF) derived from the supernatant of B16 cells increase the incidence of melanomas in C57B1/6J mice inoculated with a threshold dose of B16 cells. In order to further elucidate a mechanism in which neoplastic cells evade host surveillance we chose to study the effects of irradiated B16 cells and a TFF derived from B16 cell supernatants on mitogen induced blastogenesis of lymphocytes from normal C57B1/6J mice.

B16 melanoma cells irradiated with 4000 rads were shown to depress tritiated thymidine incorporation by normal C57B1/6J splenocytes in response to PHA, Con-A, and LPS. This effect could be reproduced by substituting TFF. TFF was determined not to be toxic to C57B1/6J splenocytes by trypan blue dye exclusion. Pretreating with indomethacin does not alter the effects of TFF on mitogen induced blastogenesis suggesting that prostaglandin producing suppressor macrophages are not involved. Preincubation of C57B1/6J splenocytes for 18 hours with TFF followed by washing resulted in reversal of mitogen stimulated blastogenesis. We suggest that one mode by which TFF facilitates tumor growth is by reversibly suppressing the immune response of C57B1/6J mice in a non-prostaglandin mediated fashion.

Effects of Polyamine Antimetabolites and Retinoids on Proliferation and Tyrosinase Activity in Murine Melanoma Cells. J. LAUHARANTA*, K. KÄPYAHO**, L. KANERVA*, AND A. RANKI*, *Dept. of Dermatology, University Central Hospital, Helsinki; **Dept. of Biochemistry, University of Helsinki, Finland

Murine melanoma (S91) cell cultures were exposed to polyamine antimetabolites: difluoromethyl orinithine (DFMO) and methylglyoxal bis (guanylhydrazone) (MGBG), and to retinoids: etretinate (E) and its main metabolite, all-trans retinoic acid (RA), 13-cis retinoic acid (13-cis) and the new arotinoid (A). Cell number, DNA amount, polyamine levels (dansylation of perchloric acid extracts), and tyrosinase activity (^3H_2O released from ^3H-tyrosine) were determined after 4–6 days.

DFMO (3mM) inhibited cell growth by about 30%, 2μM MGBG by about 80% and 5 μM MGBG completely inhibited cell growth. Tyrosinase activity was inhibited by these compounds especially in α-MSH-treated cultures.

The inhibitory effect of retinoids on cell growth was dose-dependent at concentrations of 10^{-5}–10^{-6}M. Comparable effects were achieved with A at concentrations of only 1/10–1/50 of that with other retinoids. From the latter RA and 13-cis showed the strongest inhibition. At 10^{-6}M concentration they inhibited the growth by about 80%. Polyamine levels remained the same or were slightly elevated by retinoids. All retinoids caused a manyfold increase in tyrosinase activity.

The antiproliferative action of polyamine antimetabolites on murine melanoma cells is associated with decreased tyrosinase activity while that of retinoids is associated with increased tyrosinase activity. Furthermore, retinoids do not reduce polyamine levels in melanoma cells. Thus, different mechanisms seem to be involved in the antiproliferative action of these compounds.

Enhancement of 5-S-Cysteinyldopa Genesis in Cultured B-16 Melanoma Cells by Monochromatic UV-C Irradiation. MASAMITSU ICHIASHI, MANOJ MOJAMDAR, MASAYUKI TSUJI, AND YUTAKA MISHIMA, Department of Dermatology, Kobe University School of Medicine, Kobe, Japan

In contrast to UV-A and -B, the skin pigmentation induced by UV-C (254nm) is of less intensity and of shorter duration (Parrish et al. Arch. Surg. 104:276–283, 1972). Using B-16 melanoma cells cultured away from intricate biological influences of the dermal-epidermal environment, the possibility that UV-C selectively induces light colored pheomelanin formation rather than dark eumelanin has been explored.

20–40 J/m^2 UV-C radiation caused a dose dependent 1 to 3 days delay in the onset of growth phase of the melanoma cells. However, during the exponential phase there were no substantial differences in the growth rate. Tyrosinase activity in irradiated (10 J/m^2) cells has been found to be decreased as compared to non-irradiated controls. In contrast, γ-glutamyl transpeptidase activity has been found to manifest a 50% increase, 48 and 72 hours after irradiation. The levels of 5-S-cysteinyldopa secreted into the medium 48 hours after 0 and 10 J/m^2 UV-C irradiation has been found to be 5.2 and 10.1 μg/total medium respectively despite the number of cells being less in the irradiated group. Further, it has also been found that B-16 melanoma pellet to be lighter 2 to 5 days after irradiation. These findings indicate that UV-C can stimulate 5-S-cysteinyldopa genesis and possibly pheomelanogenesis in cultured B-16 melanoma cells, and may explain the low intensity of pigmentation induced by 254nm irradiation. The reasons for the shorter duration of UV-C induced pigmentation however, requires further studies.

Enzyme Dynamics in Pheomelanogenesis Caused by 4-Tertiary Butyl Catechol (TBC) in Melanoma Cells. K. YONEMOTO, T. KAWASHIMA, G. A. GELLIN, AND W. L. EPSTEIN, Dept. of Dermatology, University of CA, San Francisco

It has been suggested that the reduced form of glutathione (GSH), liberated by glutathione reductase (GR), forms cysteinyldopa by adding dopaquinone through γ-glutamyltransferase (GGT). However, glutathionedopa may first be formed and then be converted to cysteinyldopa by GGT in pheomelanogenesis, although the specific metabolic pathway remains to be clarified. Since glutathione S-transferase (GST) catalyzes conjugation of GSH, we measured this enzyme, in addition to GR and GGT, in tissue cultured melanoma cells in which pheomelanogenesis was chemically induced. B16 (HFH-18) melanoma cells cultured in MEM with 10% fetal calf serum were incubated in BME containing 10^{-4} M TBC dissolved in DMSO for stimulation of pheomelanogenesis. After 2 hrs of treatment the cells were allowed to grow in MEM with serum. Control cells were treated for 2 hrs in BME containing only DMSO. For enzyme assay, cells were scraped off from flasks and homogenized with a polytron. GGT was measured in the homogenate but GR and GST were assayed in the supernatant prepared by centrifugation at 105,000 g for 30 min. No change was seen in enzyme activities immediately after TBC treatment. Elevation of GR and GST activity occurred by 12 and 24 hrs, respectively, after TBC treatment. Activities of both enzymes continued to rise but GGT activity stayed at the control level until 48 hrs after treatment when all 3 enzymes showed more than a 20% increase over control levels. This finding indicated that TBC stimulated both pheomelanogenesis initiated by GR activation and production of excess GSH. Subsequent activation of GST is involved in the formation of glutathionedopa, a substrate for GGT to catalyze cysteinyldopa formation.

Dopachrome Oxidoreductase: A New Enzyme in the Pigment Pathway. JOAN I. BARBER AND RICHARD A. KING, Department of Medicine, University of Minnesota, Minneapolis, Minnesota

Recent studies by Korner and Pawelek have suggested the existence of conversion and blocking factors active in the pigment pathway distal to dopaquinone. We have characterized the dopachrome (DC) conversion factor and have found it to be an enzyme, dopachrome oxidoreductase (DCOR), that has the properties of accelerating the pathway at DC and blocking the pathway at 5,6-dihydroxyindole (DHI). DCOR was prepared from amelanotic B-16 melanoma through homogenization and centrifugation in 0.05M Na phosphate with 1% Triton X-100, pH 6.8 DC was prepared by the Ag_2O method. DCOR activity was determined spectrophotometrically by monitoring the decrease of DC at 475nm. DCOR is a protein as shown by loss of activity with trichloracetic acid precipitation, by boiling or by digestion with poteases. At 45°C the half life of DCOR was 42.5 minutes (first-order rate constant of 0.0163 min^{-1}). Loss of activity at −70°C and stable activity at 6°C

suggested a polymeric structure. Molecular weight was between 50,000–100,000 by Amicon filtration. Kinetic analysis revealed mixed enzyme inhibition with apparent Km of 0.02–0.04 mM DC and apparent Ki of 16 mM dopa. On cell fractionation of melanotic B-16 melanoma DCOR activity had a similar distribution to tyrosinase with 44 ± 7% melanosomal, 40 ± 6% microsomal, and 14 ± 0.5% soluble. DCOR in the absence of tyrosinase blocked the pathway at colorless DHI, most likely by reducing indole-5,6-quinone to DHI. With DCOR from amelanotic tumor, DC was converted to DHI but no melanin formed until tyrosinase was subsequently added. DCOR activity was also found in mouse hairbulbs, human hairbulbs, and Duroc Swine melanoma. In summary, DCOR catalyzes the conversion of DC to DHI, is capable of blocking the pathway at DHI, and may be an important regulatory enzyme in the pigment pathway.

Induction of Hepatic Monooxygenases After Chronic UV-Light Exposure. H. MERCK (1), G. GOERZ (1), K. BOLSEN (1), D. TSAMBAOS (2), AND H. BERGER (3); (1) Dep. of Dermatology, Uni. of Düsseldorf; (2) Dep. of Dermatology, Uni. of Berlin; (3) Dep. of Dermatology, Uni. of Göttingen; FRG

The activity and inducibility of cytochrome-P-450(P-450) dependent enzyme activities are influenced by genetic factors and environment agents. We investigated the hepatic and the cutaneous P-450 dependent enzyme activities after chronic UV-light exposure (24 weeks; 16h/d; UVA=106J/cm^2; UVB=0,62J/cm^2; total UVB-dose:104J/cm^2; TL40/W09 Phillips) of Ng/-mice (H. Berger et al., Z. Hautkr. 55:1510, 1981). Liver and skin microsomes were prepared and P-450 content, 7-ethoxycoumarindeethylase (7-EOD), aryl-hydrocarbon-hydroxylase (AHH), aminopyrine-N-demethylase (ADM) were studied as described previously (W. Vizethum et al., Chem. Biol. Inter. 28:291, 1979; 31:215, 1980). Cutaneous AHH were studied by radiometric assay according to VAN CANTFORT et al. (Biochem. Biophys. Res. Comm. 79:505, 1977). There was no significant difference of the AHH in the skin microsomes of the irradiated mice as compared to controls. However in the liver of the treated animals a significant rise in the weight, protein and P-450 content of the microsomes (0,78±0,01, controls:0,37±0,02nMol P-450 protein^{-1}) and P-450 dependent enzyme activities 7-EOD (270%), ADM (314%), AHH (349%) were observed. These results indicate that nearly pure UVA-light exposure itself is a further environmental factor which influences hepatic monooxygenases under in vivo conditions.

Analytical Ultrastructural Autoradiographic Localisation of a Dicarboxylic Acid in Murine Melanoma. BARBARA J. WARD, A. S. BREATHNACH, E. J. ROBINS, S. PASSI,* AND M. NAZZARO-PORRO*, St Mary's Hosp Med Sch, London, UK; *St Gallicano Derm Inst, Rome, Italy

As hyperpigmentary disorders such as lentigo maligna and malignant melanoma are effectively treated by dicarboxylic acids, which are competitive inhibitors of tyrosinase, we decided to study the intracellular location and possible sites of action of dodecanedioic acid in murine melanoma cells using EM autoradiography. 9 day cultures of Cloudman S91 melanoma and 12 day cultures of Harding-Passey melanoma were incubated with 33μCi/ml ^{3}H dodecanedioic acid (0.33×10^{-6}M) for 2, 5, 20 or 60 minutes. Autoradiographs of ultrathin sections were prepared and at least 500 grains were photographed for each incubation time. The micrographs were analyzed using a modified version of the computer-based hypothetical grain analysis (Ward & Harris, Tissue and Cell, 11: 793, 1979).

After only 2 minutes, significant levels of radioactivity were found in the mitochondria and in the nuclei, but not in association with membranes of ER, GERL, or Golgi apparatus, and not in melanosomes. These results suggest that the toxicity of dicarboxylic acids in melanoma cells is not related to anti-tyrosinase activity but may be due to interference with oxido-reductase enzymes in the mitochrondria and nucleus and to inhibition of DNA synthesis in the nucleus.

Increased Susceptibility to B16 Melanoma Tumor Takes Induced by Ultraviolet Light. RICHARD G. BOWEN AND NEIL I. BRODY, Departments of Microbiology and Immunology (RB) and Dermatology (NB), State University of New York, Downstate Medical Center, Brooklyn, New York

This study was designed to examine the effect of ultraviolet (UV) light on the modification of the host response to B16 melanoma. Unlike prior UV tumor immunology studies in which the tumors are directly UV induced, a causal association between melanoma and UV exposure has not been established. In addition, unlike the UV induced tumors (eg. UV-regressor tumors) B16 melanoma cells do not require an immunocompromised host for growth.

The shaven backs of C57Bl/6J mice were irradiated for 30 minutes at 0.274 mW/cm^2 of UVB, 3 times each week for six weeks. Controls were shaven but not irradiated. Mice were then challenged in dose response fashion with viable B16 cells with or without a simultaneous injection of tumor facilitating factor of B16 (TFF), a cell surface glycoprotein produced by B16 cells that enhances their growth in vivo. Irradiated mice grew tumors more frequently than non-irradiated mice (0% vs 67% for challenge with 5×10^3 B16 cells). Latency was also reduced (44 to 23 days). The addition of TFF to irradiation did not further increase susceptibility to tumor growth. The effects of irradiation were transient, stopping irradiation returned mice to control values. Peritoneal macrophage spreading previously shown to be increased in mice receiving TFF was also increased in UV irradiated mice.

These studies indicate that UV decreases host resistance to B16 melanoma growth. This may be a good animal model to help understand the increased frequency of melanoma in "sunworshiping" individuals.

Binding of Anti-Cell Surface Antibody to Human Melanoma Cells Stimulates Plasminogen Activator. K. SINGER, A. ROBERTSON, AND G. STUHLMILLER, Duke University Medical Center, Durham, NC 27710

Binding of antibody to normal epithelial cells can result in a loss of cellular adhesion induced via proteolytic mechanisms involving plasminogen activator (PA) (J. Exp. Med. v. 157, 1983). In a similar fashion, antibodies to antigens on tumor cell surfaces may influence adhesion of tumor cells and perhaps alter their metastatic potential. We have reported loss of cellular adhesion following incubation of human melanoma cells with protein A-affinity purified IgG prepared from a multispecific monkey anti-human melanoma serum. Loss of adhesion was blocked by the serine class proteinase inhibitors, diisopropylfluorophosphate and lima bean trypsin inhibitor. We report here that the serine class proteinase stimulated by antibody binding to melanoma is plasminogen activator. Conditioned medium and detergent lysates were prepared from 2 different human melanoma cell lines incubated with and without anti-melanoma IgG, and examined for secreted and cell associated PA activity, respectively. After incubation with anti-melanoma IgG, secreted PA activity increased in a time and IgG dose dependent manner >3 fold (0.5 mg IgG/ml for 24 hr). Conditioned medium was collected from antibody treated cells and electrophoresed in SDS-PAGE; gels were sliced and the slices examined for PA activity. Two peaks were observed in a MW range of 55-68K. In order to define the specificity of antibodies capable of stimulating PA activity in melanoma cells a panel of monoclonal anti-melanoma antibodies is currently being screened for PA stimulation.

Modulation of Plasminogen Activator in Mouse Keratinocytes by Extracellular Calcium. R. ISSEROFF, M. AGLEHAM, AND D. CARTER-PEOPLES, Dept. of Dermatology, Univ. of Calif., Davis, School of Medicine, Davis, CA

We have previously demonstrated that morphologic differentiation of mouse keratinocytes is accompanied by increased levels of the serine protease, plasminogen activator (PA). Since murine keratinocytes grown in medium containing decreased concentrations of calcium (0.07 mM) have been shown to differentiate less than those grown in normal calcium (1.4 mM), we measured PA in these cells to determine whether this marker of keratinocyte differentiation, increased levels of PA, was also modulated by extracellular calcium concentration. Neonatal mouse keratinocyte cultures were initiated in low calcium medium, LCM (calcium-free M199 + 10% Chelex-treated FCS) and maintained in either LCM or switched to normal calcium medium, NCM (standard M199 + 10% FCS) (Hennings, et al. 1980). At specific times after the medium switch, levels of cell-associated and secreted PA were measured using the 125-I fibrin plate method (Ossowski, et al., 1973). Levels of cell-associated PA rose 3–5 fold in the NCM cultures within 24 hours of the switch from LCM to NCM. In the medium, a two-fold increase in levels of secreted PA could be detected as early as one hour after switching the cultures from LCM to NCM, and PA levels remained approximately 4-fold higher in the NCM cultures over a 76 hour incubation period. PA activity was not associated with either increased cell number or protein in the NCM cultures.

These findings indicate that extracellular calcium concentrations regulate both cell-associated and secreted levels of keratinocyte PA. The shift to terminal differentiation induced by the increased calcium concentration is associated with increased keratinocyte PA levels, which may, in turn, effect morphologic changes such as squame detachment characteristic of terminal differentiation.

2:00 PM–4:00 PM CONCURRENT POSTER DISCUSSIONS

DISCUSSION I Presidential Ballroom
John S. Strauss, M.D. and Enno Christophers, M.D., Presiding

Effect of Short-Term Starvation and Refeeding on Ornithine Decarboxylase in Rat Skin. JEANNE LESIEWICZ, JEFFREY CHAIN,* AND LOWELL GOLDSMITH,** Hoffman-La Roche, Inc., Nutley, N.J. *Jefferson Medical College, Philadelphia, PA and **University of Rochester Medical Center, Rochester, NY

The effects of dietary growth restriction on skin enzymes have not been studied extensively. The correlation of increased ornithine decarboxylase (ODC) activity with growth processes and its short half-life suggested that this skin enzyme might be a sensitive indicator of altered nutritional status. Juvenile male rats were fed ad libitum, starved for up to 48 hours or starved for 24 hours and refed for up to 24 hours. Soluble protein concentration and ODC activity were measured in unstimulated skin and in skin stimulated by hair plucking. In unstimulated skin, neither soluble protein content nor ODC enzyme activity showed significant changes even with up to 48 hours of starvation. In stimulated skin, soluble protein content was decreased through 48 hours of starvation. Activity was restored to control levels by 12 hours of refeeding. Although ODC activity was stable, the half-life of skin ODC was decreased to 13.3 minutes by 24 hours of starvation. These data suggests that that in the absence of a secondary stress factor (hair-plucking), skin is able to compensate for the effects of short-term starvation.

A Comparison of Methods for Quantifying the Atrophogenicity of Topical Steroids. G. L. GROVE, P. M. LEHMANN, J. J. LEYDEN, AND A. M. KLIGMAN, Skin Study Center and University of Penna., Philadelphia, Pennsylvania

The purpose of this study was to evaluate three noninvasive assays for measuring the atrophogenicity of topical steroids. We applied 5 different commercially available glucocorticoid creams as well as white petroleum to the forearms 3 times weekly (M-W-F) for 3 weeks. On the final day, 6 hours after the chambers were removed a variety of measurements were taken. These included grading the test sites clinically for atrophy and telangiectasia on a 0–4+ scale, measuring transepidermal water loss with a Servomed Evaporimeter and skin thickness with a Sonometrics B-mode pulsed ultrasound unit. In order to check the validity of these measurements, we also took 3mm punch biopsies for each test site and measured the viable epidermal thickness histometrically with a Magiscan Image Analyser.

Although pulsed ultrasound did reveal changes in total skin thickness, this approach did not provide enough sensitivity to resolve the less potent steroid. However, all the other measurements did allow for distinctions to be made and showed excellent correlations in the rank ordering with 0.05% Clobetasol propionate as the most potent followed by 0.1% Halocinonide, 0.1% Betamethasone valerate and 1% Hydrocortisone. On the other hand, 0.1% Triamcinolone acetonide induced only a moderate increase in TWL but caused the second greatest reduction in viable epidermal thickness.

Lamellar Ichthyosis: Biochemical and Clinical Evidence of Heterogeneity. M. L. WILLIAMS AND P. M. ELIAS, Dermatology Service, VAMC, and Dept. of Dermatology, Univ. of California School of Medicine, San Francisco, CA

Lamellar ichthyosis, a severe autosomal recessive disorder of cornification, is characterized by two clinical groups: 1) classical lamellar ichthyosis (CLI) with large, dark, plate-like scales and marked ectropion, and 2) non-bullous congenital ichthyosiform erythroderma (NB-CIE) with finer, whiter scales and a more pronounced erythroderma. We report here that lipid extracts of untreated scales, analyzed by quantitative, sequential thin-layer chromatography (TLC), indicate biochemical correlates of this clinical heterogeneity:

Disorder	Neutral Lipid Fractions* (% Total Lipid ± SEM)				
	Lipid Wt%	Sphingo.	Sterols	Alkanes	FFA
Normal (n = 6)	10.5 ± 1.4	25.5 ± 1.0	15.4 ± 1.0	5.5 ± 0.2	15.0 ± 1.2
NB-CIE (n = 10)	10.7 ± 0.6	22.4 ± 0.8	16.0 ± 0.7	24.9 ± 0.7*	7.7 ± 1.9
CLI (n = 5)	10.7 ± 1.0	34.1 ± 0.6* *$p < 0.01$	23.2 ± 1.8* *$p < 0.01$	6.1 ± 1.5 *$p < 0.001$	20.8 ± 2.8

In CLI the sphingolipid and free sterol fractions are increased, producing a lipid profile very similar to normal sole stratum corneum (Lampe, et al. J. Lipid Res. In Press, 1983). In contrast, in NB-CIE a marked increase in n-alkanes occurs that is absent in both CLI and normals. Although the metabolic basis for these abnormalities is not known, these results: a) provide reliable criteria for separating these disorders; and b) indicate that lamellar ichthyosis comprises at least two distinct diseases. Studies are underway to determine how these abnormalities produce pathological scaling.

The Localization, Subcellular Distribution of Skin Sulfhydryl Oxidase, and Some Properties. H. YAMADA, K. TAKAMORI, AND H. OGAWA, Department of Dermatology, School of Medicine, Juntendo University, Tokyo, Japan

Disulfide bone formation is essential to the normal keratinization process in skin. Previously, we identified the disulfide forming enzyme (skin sulfhydryl oxidase) in rat skin which catalyzes the formation of disulfide bonds in proteins, and then purified it to a single band. (Biochem. Biophys. Acta: 615, 309, 1980). In this study, the localization of this enzyme in skin, and some properties, were examined. Cow snout skin was separated into four sections st. corneum, st. granulosum and st. spinosum-basal and dermis, and the enzyme activity was then measured. This enzyme activity was highest in the st. granulosum, and then decreased through the st. spinosum-basal and dermis, with the lowest activity being recorded in the st. corneum. These findings indicate that the conversion of sulfhydryl groups in protein to disulfide is carried out by this enzyme in the st. granulosum. The heat stability of this enzyme was most stable in the st. granulosum, while the enzyme in the st. corneum was the most labile. The substrate specificity for low molecular weight thiol compounds was highest in DTT followed by 2ME, GSH and Cys. The enzyme activity was inhibited, concentration dependently, by diethyldithiocarbamate, but not affected by either EDTA, EGTA, O-phenanthroline or α,α-dipyridyl. The enzyme activity increased two fold as a result of the addition of Cu^{2+}, but was not increased by another divalent cation. These findings suggest that skin sulfhydryl oxidase requires Cu^{2+} as a co-factor for the activity, and catalysed S-S crosslinking in the marginal band of st. cerneum.

The sub-cellular distribution of this enzyme will also be presented.

Electrophoretic and Immunoelectrophoretic Analysis of Epidermal Plasma Membrane Glycoproteins. IAN A. KING, ANNE TABIOWO, AND F. M. POPE, Dermatology Research Group, M. R. C. Clinical Research Centre, Harrow, Middlesex, U.K.

The glycoprotein components of plasma-membrane enriched vesicles from pig epidermis have been isolated using immobilized concanavalin A.

One dimension SDS polyacrylamide gel electrophoresis indicated the presence of at least 12 glycoproteins. The major PAS stained components had apparent mol. wts of 180000, 153000, 126000, 95000 and 80000. Treatment with neuraminidase removed at least 90% of the total sialic acid and caused an increase in the electrophoretic mobility of most components. Two dimension electrophoresis indicated that most components had a pI in the range 4.5 to 6. Some of the major glycoproteins showed charge heterogeneity in that they migrated as diffuse spots and also existed in a very acidic form. Much of this heterogeneity was due to varying sialic acid content since neuraminidase-treated glycoproteins migrated as symmetrical spots and their acidic forms were abolished.

An antiserum against the membrane glycoproteins was raised in rabbits. Antibodies were isolated and were used in cross immunoelectrophoretic analysis in the presence of 0.1% SDS/1% NP40. At least 9 immunoprecipitation bands were detected. The glycoprotein nature of these immunoprecipitates was demonstrated by their susceptibility to neuraminidase. Crossed immuno-electrophoresis was also used to examine the lectin binding specifity of the glycoproteins. Ricinus communis agglutinin retarded all glycoproteins to some extent. Wheat germ agglutinin only bound two components while soybean agglutinin did not affect any of the immunoprecipitates.

The Epidermal Response to Injection of Purified Cysteine Proteinase in Mice. C. MARINO, K. FUKUYAMA, Y. ITO, AND W. EPSTEIN, Department of Dermatology, University of California, San Francisco, California.

The inflammatory response is known to invoke epidermal hyperproliferation. Recently intradermal (ID), injection of cysteine proteinase, cathepsin B, isolated from rat liver, was shown to cause leukocyte (PMN) accumulation in the skin. We have investigated *in vivo* effects of cathepsin B on mitosis and DNA synthesis in epidermis. Ten ng of purified cathepsin B was ID injected into 1-day-old Balb/c mice 6, 12,

16 or 24 hrs prior to biopsy. Saline injected litter mates were used as controls. Biopsies taken from injected sites and from peripheral sites (ventral skin) were prepared for 4 μ paraffin sections or minced and placed in a medium supplemented with either 1 μC/ml ^{3}H-TdR or 1 μC/ml ^{14}C-orotic acid for 1½ hrs incubation. DNA and RNA were extracted from the organ culture specimens using 5% PCA and 0.5N KOH and the labeling of nucleic acids measured. Hematoxylin and eosin stained histological sections showed marked PMN accumulation at 6 hrs at the injected site, but also a minimal PMN response at the peripheral sites. The inflammatory change was observed even by 12 hrs but subsided by 16 hrs after injection. An increase in mitoses was seen as early as 6 hrs post injection in both injected (22%) and peripheral (15%) sites. It was most marked at the peripheral sites (30%) at 12 hrs post injection although the injected sites continued to show 18% increase. ^{3}H-TdR incorporation into DNA was increased at 16 hrs (18%) and 24 hrs (25%) at the peripheral site only. No uptake of ^{14}C-orotic acid occurred in the DNA at all time periods studied although RNA was labeled. The results show that the inflammatory response resulted in an early increase in mitoses. Subsequently, DNA synthesis increases at the site where the maximum increase of mitosis appeared. DNA synthesis utilized the salvage pathway exclusively.

Detection and Partial Characterization of Neutral Proteinase in Cornified Cells. N HORIE, Y ITO, AND K FUKUYAMA, Department of Dermatology, University of California, San Francisco, California

Proteinases regulate intracellular metabolism and are involved in a variety of cell functions. In order to better characterize proteinases in epidermal cells we used the methodology developed originally for the detection of tissue plasminogen activator. Cornified cells obtained from newborn rats were homogenized in Tris-HCl buffer saline to first extract proteinase inhibitors. The residue was homogenized in 0.1 M Tris-HCl buffered 2 M KSCN, pH 8.0, and protein extracted for 2 hr at 4°C. Hydrolytic activity for gelatin, α-casein and fibrin was detected by spectrophotometry and plate assay in the KSCN extract. For molecular weight determination of proteinases, gelatin of α-casein, with and without the addition of plasminogen, was incorporated into polyacrylamide gels at the time of casting plates and electrophoresis of the KSCN extract was performed. The gels were negatively stained and localization of proteinases was measured using urokinase (MW 53K and 33K) and trypsin (MW 23.3K) as references. Three enzymes with MW of approximately >100K, 68K and 41K were detected. The >100K enzyme digested gelatin without plasminogen while the 68K enzyme degraded gelatin and α-casein only with plasminogen. The 41K enzyme was not active on gelatin, but digested α-casein. All 3 proteinases were inhibited by the serine proteinase inhibitor, diisopropyl fluorophosphate. Co^{++}, Ni^{++}, Cu^{++}, Zn^{++} and Cda^{++} also inhibited the enzyme activity, but Mg^{++}, Ca^{++}, Mn^{++}, Fe^{++} and Hg^{++} did not. This study confirmed previous findings that epidermal cells contain plasminogen activator. In addition it demonstrates that there are at least 2 other serine proteinases similarly active, but having different MW and substrate specificity.

The Composition of Prekeratin and Keratin Polypeptides from Human Epidermis. PHILIP T. BLADON, NIGEL F. COOPER, EDWARD J. WOOD, AND WILLIAM J. CUNLIFFE, Departments of Biochemistry and Dermatology, The University and The General Infirmary, Leeds, U.K.

Living epidermal cells (basal, spinous and granular) produce prekeratin, the precursor of keratin of the dead cells of the horny layer. We have investigated the conversion of human epidermal prekeratin to keratin by amino acid analysis and peptide mapping. Amino acid analysis showed that in both callus and non-callus areas the conversion of prekeratin to keratin was accompanied by an increase in glycine residues implying the removal of glycine-poor peptide(s). Individual polypeptides of human prekeratin were recovered from SDS/polyacrylamide gels and analysed. Those of high mol. wt. (e.g. 70000 and 66000) were found to contain significantly more glycine and serine residues than those of lower mol. wt. Cleavage of prekeratin polypeptides with proteases, e.g. *Staph. aureus* V8, subtilisin or chymotrypsin, always gave rise to two groups of peptides; one group of mol. wt. 15000–20000 and another of mol. wt. 30000–40000. The kinetics of digestion suggested that the latter were intermediates in the cleavage and were susceptible to further digestion to yield peptides of mol. wt. 15000–20000. Cleavage of keratin polypeptides also gave rise to two groups of peptides with mol. wts. in the ranges described. The peptide patterns of prekeratin and keratin were distinct. Peptide mapping of human prekeratin polypeptides radio-labelled by *in vitro* incubation in the presence of [^{35}S] methionine, [^{14}C] glycine or [^{14}C] serine indicated that these amino acid residues were evenly distributed throughout prekeratin polypeptides. However [^{32}P] orthophosphate radio-labelled prekeratin polypeptides gave rise to peptides some containing little radio-label and others which were strongly radio-labelled.

Immunohistochemical Study of the Localization of Deoxyribonuclease in Epidermal Keratinocytes. TADASHI NOHARA*, HIROYUKI SUZUKI*, SADAO MORIOKA*, AND AKIRA KAWAOI**, Department of Dermatology, Nihon University*, Tokyo, and 2nd Department of Pathology, Yamanashi Medical College**, Yamanashi, Japan.

Cytochemical techniques have demonstrated that the disappearance of DNA from the nuclei of epidermal cells is a process that occurs gradually during the differentiation of basal cells into granular cells. This immunohistochemical study was undertaken to detect the localization of enzymes that hydrolyze DNA in the nuclei at different cell layers. Deoxyribonuclease I (DNase I), purified from bovine pancreas and purchased from Worthington Co, was injected into rabbits. The antibody produced was confirmed by Ouchterlony's method and the absorption test. Frozen sections of normal human skin were fixed in Zamboni's solution and stained with antisera by enzyme immunohistochemistry. In human skin, the reactivities against the enzyme were minimal in the basal cells, but showed a distinct enhancement in the nuclei located in more superficial layers, and the maximum reactions were seen in the granular cells. The reactions were also detected in the cornified cells. The reaction products were located in a mass in the center portion of the cells and were flattened in shape by vertical sections and made oval by horizontal sections. The results indicated that the nuclei of epidermal cells at different levels contain DNase, but the enzyme accumulated more distinctly in the superficial cells where most of the DNA seems to disappear. Furthermore, after the nuclei disappeared, DNase was present in the cornified cells as disk-like masses.

Ultrastructural Contrast in Human Skin Post-fixed with Karnovsky's OsO_4-Ferrocyanide Mixture. GISELA MOELLMANN, ELIZABETH KUKLINSKA, AND SIGRID KLEIN-ANGERER, Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut.

We have used ferrocyanide-reduced OsO_4 (MJ Karnovsky, 1971, Abs. #284, XIth Ann Meet Am Soc Cell Biol) to investigate glycogen deposits in human epidermis and have found that the OsO_4-ferrocyanide mixture, in 0.1M cacodylate buffer, pH 7.2, is superior to OsO_4 alone as a general secondary fixative for skin, following primary mixed aldehyde fixation.

Our observations to date have shown that, with light alkaline lead staining, there is a stunning increase in the contrast of all cytoplasmic and plasma membranes, of Langerhans cell granules and melanosomes. In particular, the lamellar contents of membrane coating granules is outlined in exquisite detail. The basement membranes of capillaries, Schwann cells and melanocytes appear to lack a lamina lucida.

The most striking improvement of contrast occurs in the epidermal anchoring complex. Anchoring filaments are discerned with ease. The intensified basal lamina appears to be widened at the expense of the lamina lucida. Owing to their sharply delineated transverse striations, anchoring fibrils stand out prominently, forming a reticulated border that is interrupted only where there is a melanocyte. Collagen fibers are barely visible in negative relief by virtue of a weakly electron dense investing layer of presumed carbohydrate.

Although the chemistry of the staining by OsO_4-ferrocyanide-lead is not known, it is tempting to speculate that this reaction is an ultrastructural counterpart to the histochemical PAS reaction for carbohydrates. Counterstaining with uranyl acetate does not abolish the fine details described above, but the stunning contrasts are lost because of the density imparted by UA to collagen fibers, tonofilaments, and ribonucleoproteins.

Topical Steroids Alter Dermal and Epidermal Healing. K. D. LEVENDORF, S. P. ALSTADT, P. M. MERTZ, O. M. ALVAREZ, AND W. H. EAGLSTEIN, Dept. of Dermatology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA

We studied the effects of topically applied glucocorticoids (triamcinolone acetonide, TA, and hydrocortisone, HC), an anabolic steroid (nandrolone decanoate, ND), and combination TA+ND on dermal and epidermal wound healing. Keratome induced wounds 0.3 mm deep on the skin of young domestic pigs were treated daily with either 0.1% TA; 1% HC; 1% ND + 0.1% TA; 1% ND; vehicle (USP petrolatum); or

control (untreated). Wounds were excised on days 2–7 after wounding and the epidermis was separated from the dermis. The dermis was assayed for collagen synthesis by measuring incorporation of ^{14}C proline into collagenase digestible protein and for collagen polymorphism by SDS-PAGE. The epidermis was evaluated macroscopically for re-epithelialization and keratin production was analyzed by SDS-PAGE densitometry. A significant decrease ($p < .01$) in relative collagen synthesis was observed in the wounded dermis in both HC and TA treated groups on day 3 after wounding, but there were no significant differences on day 4–7. Depressed collagen and non-collagen protein production was also noted in vehicle treated wounds on day 3. Topical application of ND and TA+ND did not influence collagen synthesis. The proportions of types I and III collagen were similar in all treatment groups. Topical ND accelerated wound re-epithelialization by 4.8% compared to vehicle and by 26% compared to untreated. TA delayed resurfacing by 22% and ND+TA delayed resurfacing by 17% compared to vehicle. HC enhanced resurfacing when compared to untreated wounds but did not differ markedly from its vehicle. No treatment group altered the proportions of epidermal keratin components. These results suggest that topically applied steroids influence dermis and epidermis in an independent fashion.

The Effect of Platelet-Derived Growth Factor on Outgrowth of Epidermal Explants. PATRICIA A. HEBDA AND WILLIAM H. EAGLSTEIN, Department of Dermatology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA

Platelet-Derived Growth Factor (PDGF) is a potent mitogen for connective tissue and glial cells, and stimulates collagen synthesis, lipid synthesis, certain enzyme activities, endocytosis and chemotaxis in these cells. Its effect (direct or indirect) on epidermal cells is not yet known. Skin explants (0.2 mm thick) from young Yorkshire pigs were grown for 5 days in RPMI 1640 supplemented with varying concentrations of fetal calf serum (FCS) and PDGF (80 explants/group). The average relative outgrowth for each group is shown in the table:

PDGF[b]	RELATIVE EPIDERMAL OUTGROWTH[a]				
	0% FCS	5% FCS		15% FCS	
(units/ml)			*(S.E.)*[c]		*(S.E.)*
0	0	0.85	(0.09)	1.00*	(0.07)
0.2	0	0.98	(0.06)	0.81	(0.08)
1.0	0	1.02	(0.03)	1.43	(0.04)
5.0	0	1.05	(0.02)	1.02	(0.06)

[a] All results are expressed relative to outgrowth in 15% FCS.
[b] 1 unit PDGF = the amount which evokes a response in fibroblast equal to that of 5% FCS.
[c] S.E.—Standard error

Our results seem to indicate that PDGF enhances epidermal outgrowth in low and high concentrations of serum, but not in the absence of serum. Data obtained with 15% FCS in the medium showed 1.0 unit PDGF/ml to be optimal for maximum outgrowth. These findings suggest that PDGF may be important (directly or indirectly) in epidermal cell outgrowth.

Lymphokine/Monokine (LK/MK) Regulation of Fibroblast Growth and Collagen Synthesis. MATTHEW R. DUNCAN, JEROME S. PERLISH, AND RAUL FLEISCHMAJER, Dept. of Dermatology, Mt. Sinai School of Medicine, New York, N.Y.

In this study we have optimized the culture conditions necessary for the production and assay of LK/MK that regulate fibroblast proliferation and collagen synthesis. LK/MK supernatants were produced by stimulating human peripheral blood mononuclear cells in endotoxin-free DMEM + 0.5% HSA with 20 μg/ml lipopolysaccharide (LPS) or 12.5 μg/ml Concanavalin A (Con A) for 24 to 72 hours. Exhaustively dialyzed LK/MK supernatants were then assayed for activity that 1) stimulates proliferation of subconfluent quiescent normal human dermal fibroblasts in serum-free microcultures containing 0.5% HSA, 2) inhibits proliferation of subconfluent fibroblasts growing in 10% FCS, and 3) inhibits collagen synthesis of quiescent confluent fibroblasts in 0.5% HSA. Proliferation was assessed by ^{3}H-thymidine incorporation into DNA and collagen synthesis by ^{3}H-proline incorporation into salt precipitated collagen. Log-linear dilution analysis showed that LPS-induced supernatants harvested at 24 hours contained maximum proliferative activity (10 fold stimulation, EC_{50}: 5% supernatant) and minimal inhibitory activities, while 72 hours supernatants had maximum levels of both assayed inhibitory activities (90% inhibition, IC_{50}: 10% supernatant) and little proliferative action. In contrast, Con A induced no proliferative activity at any time and maximum amounts of both inhibitors (90% inhibition, IC_{50}: 5% supernatant) at 72 hours. Under assay conditions used, no supernatants were found to stimulate collagen synthesis. These results indicate that LK/MK are both positive and negative regulators of fibroblast proliferation, but under quiescent conditions used they only inhibit collagen synthesis.

Characteristics of a Human Epidermal Squamous Cell Carcinoma Line Grown in Media of Different Ca^{2+} Concentrations. ADELAIDE A. HEBERT, JOANNE MILLER, AND MIRIAM M. BRYSK, Department of Dermatology, Univ. of Texas Medical Branch, Galveston, Texas

A human epidermal squamous cell carcinoma, initially established in culture on 3T3 feeder layers, is now growing as a monolayer in culture. The growth and cell surface properties of this line were studied in media of different Ca^{2+} concentrations. The growth rate was the same at 0.07, 1.0 and 10.0 mM Ca^{2+} whereas the morphology was distinct at each level of Ca^{2+} with stratification occurring in high Ca^{2+}. Lactoperoxidase iodination profiles of cell surface proteins also differed with the level of Ca^{2+}. At 0.07 mM Ca^{2+}, most of the radioactivity was localized in a band near 165 Kd. At 1.0 mM Ca^{2+}, the 165 Kd band persisted but a band of comparable intensity appeared near 145 Kd. At 10 mM Ca^{2+}, the new 145 Kd band persisted while the 165 Kd band became attenuated and two new bands of more intense radioactivity appeared between 55 and 65 Kd. When cells grown at different Ca^{2+} concentrations were run on SDS-PAGE and then overlaid with ^{125}I-Con A, the radioactivity profiles obtained did not for the most part match those from lactoperoxidase iodination. The same glycoproteins were labeled at the three levels of Ca^{2+}, with the most intense bands near 110 Kd and 130 Kd; the 110 Kd band was attenuated with increasing Ca^{2+} concentration.

The Lack or Reduced Capacity of Contracting a Collagen Lattice by Dermatosparactic Calf and Sheep Skin Fibroblasts Is Not a Common Character of Fibroblasts from Ehlers-Danlos Syndrome. PIERRE DELVOYE*, THOMAS KRIEG,** AND CHARLES M. LAPIÈRE*, Department of Dermatology, University of Liège* and Munich**

Both dermatosparaxis (D-) in animals and Ehlers-Danlos (ED) type VII in the human are characterized by the persistence of precursor sequences in collagen type I of skin. In the D-animals the main symptom is skin fragility while in human ED VII it is bone and joint deformation. When cultured within a matrix made of reconstituted collagen fibers normal (N-) human, calf and sheep skin fibroblasts progressively contract the lattice at a rate proportional to the density of the cells. This capacity is lost by fibroblasts derived from the skin of two D-calves. Skin fibroblasts from homozygous D-sheep contract the lattice at a rate and to an extent lower than fibroblasts from age matched normal sheep. Three different strains of fibroblasts from patients presenting ED type VII (one from Munich, two from the American Type Culture Collection: CRL 1148 and 1183) contract normally the lattice as do several strains of fibroblasts from various other types of Ed patients (type I, 2; II, 2; III, 1; IV, 3). Our data support a similarity in the molecular defect responsible for D- in the two animal species and a difference with ED VII and other types of ED in the human. Addition of up to 25% N-calf skin fibroblasts at a density too low to contract the gel to D-calf skin fibroblasts does not correct their defective capacity of contracting the lattice. Furthermore N-fibroblasts are elongated along the collagen fibers in the lattice while D-fibroblasts remain round with little cell processes. The distributed relationship of the D-cells with the matrix is probably not related only to a lack of procollagen peptidase activity.

Characterization of Cell Populations Derived From Dermatofibrosarcoma Protuberans by Immunohistological, Ultrastructural and Biochemical Investigations. ADRIANA ALBINI[1,4], CHRISTIAN SCHMOECKEL[2], LUTZ WEBER[3], PETER K. MÜLLER[1], AND THOMAS KRIEG[2], [1]Max-Planck-Institut für Biochemie, Martinsried (FRG), [2]Dermatologische Klinik der Universität, München (FRG), [3]Abteilung für Dermatologie der Universität, Ulm (FRG), [4]Instituto Scientifico per lo Studio e la Cura dei Tumori, Genova (I)

Dermatofibrosarcoma protuberans (DFSP) is an invasively growing tumor, which rarely forms metastases. The nature of its cells is still controversary and it is believed to be of histiocytic, fibroblastic or neural origin. In order to characterize these cells we investigated synthesis of connective tissue components in vivo and under monolayer conditions. In addition, indirect immunofluorescence microscopy using antibodies against connective tissue proteins and electron microscopical

studies were carried out in both systems. Collagen production was measured after pulse labelling and the newly synthesized chains were characterized by slab gel electrophoresis and ion exchange chromatography. The ultrastructure of DFSP tumor cells was similar *in vivo* and *in vitro* and showed characteristic cytoplasmic extensions, a dilated endoplasmic reticulum and intracytoplasmic filaments. No basement membrane proteins could be identified in the tumors, only a faint staining with laminin antibodies could be detected. All cell strains synthesized type I collagen, whereas production of type III collagen was in most of the cases strongly reduced. No indication was found for the production of considerable amounts of type IV and V collagen. Most of the cells accumulated the newly synthesized macromolecules in the cell layer. These findings suggest that DFSP cells reveal some morphological and biochemical characteristics of fibroblasts but clearly differ from normal fibroblasts in other aspects.

Cholesteryl Ester Accumulation in Cultured Monocytes. J. G. VAN DER SCHROEFF, L. HAVEKES*, J. J. EMEIS*, AND B. J. VERMEER, Department of Dermatology, University Hospital Leiden, and Gaubius Institute TNO*, Leiden, The Netherlands

Xanthomas and early atherosclerotic lesions are characterized by the presence of lipid-laden macrophages (foam cells), which contain high amounts of cholesteryl esters (CE). The mechanisms which lead to the accumulation of CE *in vivo* are not yet fully understood.

In our study, incubation of cultured porcine monocyte-macrophages with acetylated low density lipoproteins (acetyl-LDL) produced intracellular storage of CE, whereas native LDL did not induce CE accumulation. Binding sites for LDL were demonstrated on the cell surface by immunoelectron microscopy and immunofluorescence microscopy. In contrast, binding of acetyl-LDL to the monocyte plasma membrane could not be visualized. High affinity and specific degradation of ^{125}I-LDL and ^{125}I-acetyl-LDL confirmed that monocytes metabolize these different lipoproteins by distinctive pathways. The accumulation of CE in the cells could be prevented by adding compactin to the medium. Other antifungal agents such as nystatin and amphotericine-B also inhibited CE accumulation.

This model for generation of foam cells *in vitro* provides a method for further investigations on factors which may promote or reduce xanthomatosis and atherosclerosis.

***In Vitro* Complement Fixation by Pemphigus Antibodies Using Organ and Tissue Cultured Skin.** S. KAWANA*, M. JANSON*, AND R. E. JORDON, VA Medical Center, Wood, WI, The Medical College of Wisconsin, Milwaukee, WI, and The Univ. of Texas Health Science Center, Houston, TX

Although complement is often detected in the intercellular substance (ICS) of pemphigus skin lesions, the ability of pemphigus antibodies to fix complement *in vitro* is controversial. The purpose of this study was to test *in vitro* complement fixation abilities of pemphigus antibodies further using organ and tissue culture methods. Epidermal cell monolayers from mouse tail were incubated with the purified IgG fraction of pemphigus serum followed by purified C1q. Binding of C1q, as well as IgG was demonstrated by immunofluorescence (IF) methods. When purified C1q was replaced with fresh normal human serum (NHS), positive C3 IF staining was also evident. When purified IgG of NHS was used in place of pemphigus IgG, similar IF staining was not observed. Further evidence for complement fixation *in vitro* by pemphigus antibodies was obtained using organ cultures. Only organ cultures of normal skin cultured in purified pemphigus IgG showed ICS binding of IgG. Additional organ culture sections were then treated with complement (fresh NHS) and tested by *in vitro* C3 staining. Fixation of C3 was noted in ICS areas of organ culture skin incubated with pemphigus IgG but not those incubated with normal IgG. Prior treatment of pemphigus IgG organ culture skin sections with unlabeled anti-C3, blocked positive ICS C3 staining. These results suggest that some pemphigus antibodies are capable of activating complement *in vitro*.

Post Receptor Defects Causing Insulin Resistance in Acanthosis Nigricans. FRANÇOIS GARCIER, ALAIN L. CLAUDY, ALINE KOWALSKI, AND FRANÇOIS BERTHEZÈNE, Department of Dermatology, Hôpital Bellevue, 42000 St Etienne, France

Although the pathogenesis of acanthosis nigricans (AN) is unknown, the association of insulin resistance with AN is well documented. Two patients, apparent monozygotic twins, presented with a 6-year history of typical AN of the neck, axillae and groin, without acral hypertrophy or signs of virilization. Histological and ultrastructural features of the skin were confirmative of AN. Fasting blood glucose was normal. Insulin resistance was clinically silent. Insulin receptor auto antibodies were not detectable. After simultaneous infusion of porcine insulin (0.77 mU/kg/mn), somatostatin (500 μg/hr) and glucose 10% (6 mg/kg/mn), blood glucose reached a peak at 19.8 μmole/ml (N: 6.2 μmole/ml). On 4.10^9 red blood cells at 15°C, the maximum binding obtained was 18.2% (N: 11.8 ± 3.4) and on fibroblasts the maximum binding was 8.9×10^{-3}/mg protein at 37°C (N: 7.9) and 27.5×10^{-3} at 15°C (N: 18.2). Thus, the number of insulin binding receptors was sharply elevated on red blood cells and fibroblasts, and the antireceptor antibodies were absent. B LPH and plasma growth hormone levels were within normal limits. These results support the concept 1) that insulin resistance may be associated with AN and may depend on a genetic basis, 2) that insulin resistance may not be due to type A (decreased insulin binding to receptor or to type B (circulating antibodies to the insulin receptor) but to post receptor defect, 3) that high plasma levels of insulin may promote papillomatosis and acanthosis characteristic of AN in the absence of elevated growth promoting peptides.

Langerhans Cells in Xeroderma Pigmentosum. LEENA M. KOULU AND CHRISTER T. JANSÉN, Department of Dermatology, University of Turku, Turku, Finland

It has been suggested that the occurrence of cutaneous neoplasms on sun exposed skin may be linked to a UV-induced reduction of the epidermal Langerhans cell (LC) density, which could lead to a deficient recognition of antigens, including neoantigens of transformed cells. This theory prompted us to compare the density and UV sensitivity of LC in normal controls and in patients with xeroderma pigmentosum (XP), a disease characterized by an increased skin UV sensitivity and a high risk of developing cutaneous neoplasms. Small skin areas on the buttocks of 5 XP patients and 10 healthy controls were exposed to a standard, erythematogenic dose of UV irradiation from a medium pressure mercury lamp. Skin biopsies were taken 4, 7, 14 and 21 days later, epidermal sheets separated by EDTA, and LC stained by the ATPase method. In non-irradiated skin, the LC density was 741 ± 89 cells/mm^2 in XP patients and 723 ± 80 in controls. After irradiation of the control skin, a nadir of 325 ± 159 cells/mm^2 was recorded at day 4, with a restoration to the original density by day 14. In contrast, XP epidermis showed a steady decrease in the LC density on days 4 through 14, to a nadir of 90 ± 7 cells/mm^2, and at day 21 the LC count was still only 408 ± 88. It is concluded that a standard UV dose causes a stronger and longer lasting LC depletion in the epidermis of XP patients, as compared to healthy controls. This increased Langerhans cell UV sensitivity may play a contributory role in the induction of UV dependent neoplasms in XP skin.

Increase of a DNA-Binding Protein ("C_3DP") in Sera of Patients Suffering from Malignant Melanoma. CH. SCHRÖDER, AND G. REIMER*, Inst. for Physiol. Chem. University of Mainz, FRG, * Dept. of Dermatology University Frankfurt, FRG

Human serum contains a variety of DNA-binding proteins (DBP). Among them a DNA-binding derivative of the complement protein C_3—called "C_3DP"—was shown to be increased in sera of patients with solid malignancies. In our study, the presence of certain DBP were determined in sera. DBP with mw of 22 kdal, presumably corresponding to the smallest subunit of "C_3DP", showed remarkable correlations to the presence of a malignant melanoma (MM): a) sera of 23 healthy volunteers contain low amount of 22 kdal DBP, b) sera of 26 patients suffering from MM with histological levels of III to V contain significantly higher amounts of these DBP, c) within 12 months after tumor excision, the amount of 22 kdal DBP decreased in the sera of approximately 55% of the patients. The remaining 45% of the patients with continuously high levels is presently being observed with regard to a possible increased risk of developing metastases, and d) the sera of 19 patients suffering from MM metastases contain high amounts of the analysed DBP. Within 12 months after excision of the metastases, however, no decrease in these serum proteins could be noted.

For clinical use of this serum indicator we have tried to elaborate an immunologic assay system. However, the purified DBP was not stable in buffer media and corresponding antibodies crossreacted with the complement factor C_3. Therefore, we intend to yield antibodies against the subunits of the "C_3DP" which should react in a more specific manner.

Soluble Tyrosinase in Melanoma Results from Cell Degradation. Y. TOMITA, A. HARIU, C. KATO, AND M. SEIJI, Department of Dermatology, Tohoku University School of Medicine, Sendai 980, Japan

Tyrosinase is localized in the specialized cytoplasmic particle called

the melanosome in the melanocyte. However, tyrosinase activity has been reported to be detected not only in the melanosomes, but also in smooth and rough endoplasmic reticulum and in the soluble fraction. The soluble fraction has been known to contain about 10 to 30% of total tyrosinase in mouse melanoma cells and the biological significance or role of this has been unclear. We tried to clarify whether tyrosinase in the soluble fraction was the precursor enzyme to be transferred into melanosomes, or was the result of leakage of tyrosinase from the endoplasmic reticulum and/or the melanosome during homogenization. The precursor-product relationship between tyrosinase in the microsome and in the melanosome was confirmed in Harding-Passey mouse melanoma by incorporation of ^{3}H-amino acids into tyrosinase and the immunoprecipitation of the labeled enzyme at various times by tyrosinase-specific antibody. However, little or no incorporation of ^{3}H-amino acids into tyrosinase in the soluble fraction could be detected, showing that soluble tyrosinase is not a precursor of the melanosomal enzyme and does not result from leakage from melanosomes or microsomes. Considering the data on the recovery of tyrosinase from the soluble fraction, it is suggested that most soluble tyrosinase comes from necrotic tumor cells. Furthermore, the enzyme activity in the soluble fraction could not be increased, even when the post-nuclear fraction of the tumor was further homogenized radically. As a result, tyrosinase in the soluble fraction appears to be released from the membranes of melanosomes and microsomes of necrotic tumors cells which are modified by various degradation enzymes.

Level of Free and Bound Dopa and 5-S-Cysteinyldopa in Melanogenic and Non-melanogenic Tissues and Its Relation to Tyrosinase Activity and Type of Melanogenesis. S. Ito, K. Jimbow*, H. Takahashi*, and K. Maeda*, Inst. for Comprehensive Med. Sci., School of Med., Fujita-Gakuen Univ., Toyoake, and * Dept. of Dermatol., Sapporo Med. Col., Sapporo, Japan

5-S-Cysteinyldopa (5-S-CD), a major intermediate of pheomelanin synthesis formed through dopa, is excreted in urine of melanoma (MM) patients and normal subjects, though melanin synthesis in their hair and skin is eumelanic. To elucidate the nature of 5-S-CD synthesis in terms of tyrosinase (TY) activity and type of melanogenesis, this study analyzed the free and bound forms of dopa and 5-S-CD in melanogenic and non-melanogenic tissues by high performance liquid chromatography. In normal mouse hair, the level of free dopa and 5-S-CD correlated well with type of melanogenesis and TY activity. In contrast, bound dopa and 5-S-CD were detected in all hairs and in all non-melanogenic organs examined. The in vitro experiment, using isolated TY and BSA, indicated that TY catalyzes the hydroxidation of tyrosine in any type of protein and subsequently oxidizes to dopaquinone which results in formation of bound 5-S-CD. Furthermore, the studies of 2 forms of mouse MM showed that the free and bound dopa and 5-S-CD are present in both soluble and melanosomal fractions, though these 2 were primarily eumelanic by analysis of % composition, infrared spectra and chemical degradation of melanin. Our studies show that (a) bound dopa and 5-S-CD are formed even in non-melanogenic tissues by oxidation mechanism other than that of TY and (b) a considerable amount of free and bound dopa and 5-S-CD is produced, irrespective of melanogenesis type, without being incorporated into melanosomes. Turnover of free and bound 5-S-CD may lead to the release of this catechol into blood, eventually excreted in urine of normal subjects at a low level and MM patients at a high level.

Postnatal Development of Hair Discs. Hiroyuki Suzuki, Yoko Ishida, Fukiko Takahashi, Hideaki Kamata, and Shunichi Baba, Department of Dermatology, Nihon University School of Medicine, Tokyo, Japan

Hair discs (HD) have been regarded as one of the cutaneous sensory receptors of mammals. The purpose of this study was to clarify the qualitative and quantitative changes of HD after birth. Two species of newborn mice and rats were used. More than five animals of each species were sacrificed at each of the five developmental stages (5, 10, 15, 20 and 25 days after birth). Specimens obtained from the abdominal skin were examined by LM, SEM, TEM, ARD(^{3}H-TdR) and morphometry. The results were basically same in both species. It was confirmed by morphometrical analyses that the HD increased gradually in size after birth. However, the size of HD of 25-day-old animals did not differ significantly from that of adults ($p > 0.06$). The HD were observed on the acute angle side of the thick hair. About 7–9% of the basal cells of the HD were labeled with ^{3}H-TdR during the examination period. Merkel cells (MC) seen at the basal portion of the HD did not change in number after birth. ^{3}H-TdR incorporation into the MC was not observed during postnatal development. At 25 days, the MC showed a typical fine appearance similar to that of adults. They contained the cytoplasmic granules and were associated with the nerve endings. A specific capillary system just below the HD was obviously seen at 20 days. These results suggest that HD develop postnatally and are structurally built up by 25 days after birth.

The Holocrine Secretion of Sebaceous Glands: A Histochemical and Ultrastructural Study. Masaaki Ito, Katsuhiro Motoyoshi, Megumi Tanizawa, Tomohiro Maruyama, and Yoshio Sato, Department of Dermatology, Niigata Univ. School of Medicine, Niigata, Japan

To know the mobility and concentration of the proteins with -SH groups of S-S linkages in sebaceous glands during holocrine secretion, a histochemical and ultrastructural study was performed on the skin specimens from the ear of male New Zealand white rabbits.

DACM (N-(7-dimethylamino-4-methyl-3-coumarinyl)-maleimide) staining method showed that the proteins with -SH groups were present in the cytoplasm and nuclei of the cells in the all layers from the peripheral through dead cells of sebaceous glands and that the proteins with S-S linkages were distributed in the dead cells and the picnotic nuclei of the differentiated cells of sebaceous glands. Ultrastructurally, these dead cells were electron dense and seemed to be suddenly formed from the differentiated cells.

These findings indicate that the conversion of -SH groups to S-S linkages of proteins in the sebaceous cells may play an important role in holocrine secretion.

It was also confirmed that deparaffinized tissue was able to be used for DACM staining and displayed very fine fluorescence findings by DACM.

A Comparison of Floating and Rocking Organ Culture Systems for Anagen Hair Follicles in Hamster Skin Explants. Karla L. Stoner, Don W. Coble, and Vera R. Usdin, Gillette Research Institute, Rockville, Md.

Floating and rocking organ culture systems were compared for their ability to maintain viability of hair follicles in Syrian hamster skin explants over periods of one to twelve days *in vitro*. Assessments were made using light microscopy, transmission electron microscopy and autoradiography of tissue labeled with ^{3}H-thymidine and ^{3}H-L-leucine. The effects of the deletion of serum in the rocking culture system were also investigated.

Results indicated that the rocking culture system maintained improved viability of hair follicles for longer periods *in vitro* than the floating culture system. Histologically, floated explants showed marked deterioration by 48 hours *in vitro* while rocked explants did not begin to show deterioration until 72 hours. ^{3}H-L-leucine uptake in hair shafts continued 24–48 hours longer in the rocking system than in the floated system, whereas ^{3}H-thymidine uptake was increased in the rocked explants at 24 and 48 hours when compared to the floated system, but not at later culture periods. The deletion of serum in the rocking culture system was found to have no effect on the viability of the hair follicles over four days of culture. The rocking culture system had the added advantage of being a cleaner, better defined system.

A New Antibiotic for Topical Use in Acne Vulgaris? E. A. Eady, K. T. Holland, W. J. Cunliffe*, and G. W. Taylor**, Microbiology Department, University of Leeds, * Dermatology Department, Leeds General Infirmary, and ** Biochemistry Department, Imperial College of Science and Technology, London, U.K.

Staphylococcus epidermidis NCIB11536, isolated from human skin, produces a peptide antibiotic active against propionibacteria and staphylococci. The purpose of this study was to purify and characterise this antibiotic in order to assess its possible therapeutic usefulness in the management of acne vulgaris and infected pyodermas. Up to 6 mg/L of antibiotic was produced by the organism in Brain Heart Infusion broth after 48 h incubation at 37°C on an orbital shaker at 160 rpm. Ammonium sulphate precipitation, ion-exchange chromatography on Sephadex C-25 and HPLC using μBondapak C_{18} were used to purify the antibiotic. Structural elucidation was by amino acid analysis and fast atom bombardment mass spectrometry.

The purified antibiotic, molecular weight 2,163 d, contains 17 known amino acids and 1–2 residues of an unknown amino acid. It is cationic and stable at acidic pHs. It is resistant to most proteases, only trypsin at 50 units/μg for 3 h and 25°C reduces biological activity.

Semi-purified antibiotic is bactericidal for staphylococci and propionibacteria. Minimum inhibitory concentrations are within 0.5–10 μg/ml for most organisms tested. Incorporated into solid media at 1.6 μg/ml

the antibiotic reduced the number of viable Micrococcaceae recovered from skin washings by ≥98%.

These data indicate that the antibiotic may be helpful in the topical management of acne vulgaris, without promoting resistance to oral antibiotics such as tetracycline and erythromycin, to which it is structurally unrelated.

Variation in Sebum Composition Among the Equidae. W. SABIN, VI. COLTON, AND DONALD T. DOWNING, Marshall Dermatology Research Labs, Department of Dermatology, University of Iowa College of Medicine, Iowa City, Iowa

Previous studies showed that the major component of horse sebum is a series of giant-ring lactones formed from branched chain ω-hydroxy-acids having 32 to 36 carbon atoms. Resemblance of these lactones to macrocyclic pheromones of other animals suggested a similar role in horses. We have now examined in detail the skin surface lipids from 5 equid species. Lactones were the major surface lipid of each species. They were isolated by TLC and separated into saturated (s) and unsaturated (u) fractions by TLC on $AgNO_3$. The structures of these lactone fractions, determined by nuclear magnetic resonance and GLC, showed distinct differences between species:

Structure	Horse (*E. caballus*)		Siberian horse (*E. przewalskii*)		Asian ass (*E. onager*)		Donkey (*E. assinus*)		Zebra (*E. grevyi*)	
	s	u	s	u	s	u	s	u	s	u
% Straight	—	—	—	40	19	45	3	97	33	67
% Branched	16	78	2	58	35	—	—	—	—	—

There were also distinctive differences in the lactone ring sizes between species. In addition, we found that of the lactones from the mule, half had the branched chains characteristic of the horse and half were straight chained as in the male parent, the donkey. This suggests that synthesis of the respective chain structures is under genetic control. Furthermore, the gradation in chain structures of the lactones between the equid species is in harmony with evolutionary relationships in this genus deduced from the composition of esterases and other serum proteins. These findings indicate that the sebaceous lactones in the genus *Equus* could function as species specific pheromones.

Oral Treatment of Conglobate Acne with Isotretinoin. Cooperative Multicenter Study Group from 19 Departments of Dermatology. HARALD GOLLNICK, WILHELM MEIGEL, GERD PLEWIG, AND HEINRICH WOKALEK, Departments of Dermatology, Universities of Berlin, Hamburg, Düsseldorf and Freiburg (FRG)

It was the purpose of this open study to find out the optimal dose and duration of treatment for a monotherapy with isotretinoin/Accutane (R) in 198 patients with conglobate acne. Three dose schedules (0.2, 0.5, and 1.0 mg/kg BW) were available for the first 12 weeks (phase I). Depending on the clinical response (improvement >66%), the initial dose was continued, increased or lowered from the 13th to the 24th week of treatment (phase II). At short intervals all acne lesions were counted and the intensity of seborrhea was estimated. At the same time all clinical side effects were registered, and various blood counts were obtained. All of the dose schedules tested showed clinical efficacy and nearly direct dose-dependence. However, a more than 75% improvement in the acne lesions could only be detected in patients with the combined dose schedule of 1 mg/kg in the first phase and 0.2 mg/kg in the following second. These dosages were optimal, too, because no pathological elevation of serum lipid levels could be measured in this group. Mucocutaneous and systemic side effects were dose related with the exception of the intensity of myalgias. The follow-up shows that some of the patients who had achieved a complete clearing of acne lesions after 24 weeks of treatment have now been in remission for over 18 months. In addition the number of patients in remission seems to be related to the initial dose.

Difficulties in Producing Antibodies to Purified *P. acnes* Exocellular Enzymes. EILEEN INGHAM, K. T. HOLLAND, G. GOWLAND, AND W. J. CUNLIFFE, University Departments of Immunology, Microbiology and Dermatology, Leeds, U.K.

P. acnes exocellular enzymes may play a role in the initiation and persistence of inflammation in acne. Fluorescein labelled antibodies to the enzymes are potentially of use to determine whether the enzymes are present in the follicles of both normal and acne skin. Therefore, we have attempted to produce antisera to purified preparations of *P. acnes* lipase, hyaluronate lyase (HL) and acid phosphatase (AP) in rabbits.

Antiserum to lipase (neutralising titre 1:32) was raised using conventional methods: ~30 μg enzyme in Freund's complete adjuvant (FCA) was injected into multiple sites thrice at weekly intervals. Antibody levels were boosted by i.v. injections of 30 μg in saline at 2 weekly intervals for 2 months. Such regimes failed to raise antibodies to HL or AP.

A novel technique was developed to raise antibodies to HL. Rabbit mononuclear cells were incubated with 45 μg HL *in vitro* (1h 37°C) prior to injection subcutaneously above the rabbit ankle joint. Simultaneous injections of HL in FCA (45 μg) were administered into the footpad and thigh. Three treatments at weekly intervals produced an antiserum with a neutralising titre of 1:256. A similar regime failed to raise antibodies to A.P.

Antibodies to *P. acnes* lipase and H.L. also neutralise the enzymes produced by *P. granulosum*. The enzymes produced by both species thus appear to exhibit antigenic similarities.

DISCUSSION II — Congressional Room

Georg Stingl, M.D. and Thomas T. Provost, M.D., Presiding

Changes of Activities of Hypoxanthine-Guanine Phosphoribosyl Transferase and Amidophosphoribosyl Transferase in Epidermis After UVB Irradiation. TOSHIHIRO IIDA*, HIROYUKI SUZUKI*, SADAO MORIOKA* AND SIGEKI NAKAGAWA**, *Department of Dermatology, **Department of Biochemistry, Nihon University School of Medicine, Itabashi, Tokyo, Japan

In purine metabolism, hypoxanthine-guanine phosphoribosyl transferase (HGPRTase) has been established to be a salvage enzyme and amidophosphoribosyl transferase (amidoPRTase) has been well known as one of the de novo enzymes. The purpose of this study was to examine the activities of two metabolic enzymes, HGPRTase and amidoPRTase, in order to clarify the changes of RNA synthesis in the epidermis post exposure. Newborn mice (ddY) were used. Skin samples were obtained from the back at 1, 18, 24, 36, 48 and 72 hours after UVB irradiation (total UV energy 0.6 J/cm^2). Epidermis was separated from the dermis by Kitano's method using trypsin. HGPRTase was assayed using the method by Gutensohn and Guroff. AmidoPRTase was measured by the method of Lewis and Hartman. HGPRTase activity started to increase at 18 hours after UVB irradiation, reached the peak at 24 hours and recovered to control level after 48 hours. This increase was not observed with the treatment of Actinomycin D. On the other hand, amidoPRTase did not change in activity during the experimental period. These results suggest that the increase of RNA synthesis after UVB irradiation mostly depends on the salvage enzyme (HGPRTase) which could be synthesized in ribosomal RNA, but de novo enzyme (amidoPRTase) does not relate to the RNA synthesis post exposure.

Extent and Fidelity of Reversible Bihelicity in Psoralen Crosslinked DNA. P. M. ROSS, C. A. AJELLO, AND D. M. CARTER, Laboratory for Investigative Dermatology, The Rockefeller University, New York, NY

To understand the molecular effects of photomediated psoralen DNA interstrand crosslinks, we have studied the reversible bihelicity of DNA molecules in solution. The bacterial nuclease S1 digests single-strand nucleic acids, sparing twin-helical DNA; thus S1 can be used to distinguish between crosslinked and uncrosslinked DNA in solution. If a mixed population of DNA molecules is denatured and then treated with S1, the reversibly bihelical, crosslinked DNA is spared, while uncrosslinked DNA is degraded.

How faithful is the renaturation process? How many nucleotide pairs can be renatured in a DNA molecule that contains a single interstrand crosslink? To answer these questions we used the 49,000 base pair DNA molecule from bacteriophage lambda. Lambda DNA was treated with 4,5′,8-trimethylpsoralen (10^{-3} dilution sat'd sol'n in EtOH) and irradiated with UVA (360 nM, 0-2 kJ/m^2), denatured in alkali (pH 12, 22°C, 30 min), digested with S1 nuclease (100-fold excess), then either fractionated by agarose gel electrophoresis or by precipitation in 7% perchloric acid.

Our results show that a single interstrand crosslink enables the lambda DNA molecule to renature and become S1-resistant when measured by acid precipitation. S1 treatment did not produce double-strand breaks or single-strand nicks in the renatured, crosslinked DNA. Apparently a single interstrand crosslink can cause at least 49,000 base pairs of DNA to renature with sufficient fidelity so that a single-strand endonuclease capable of recognizing very short regions of nonhomology (as little as a single base mismatch) cannot act on the DNA.

Predictive Testing of Chemicals with Photoallergenic Potentials in Mice. YOSHIKI MIYACHI AND MASAHIRO TAKIGAWA, Department of Dermatology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

The induction and elicitation method of contact photosensitivity in mice has been reported recently (J. Invest. Dermatol. 79:108, 147, 1982). Using this technique we studied the photoallergenic potential of both well known and newly discovered photosensitizers in mice. Animals were sensitized with paintings of the test agent on the shaved abdomen and irradiated with black light on days 0 and 1. On day 5, mice were challenged with the application of the chemicals on the ear lobes and were irradiated with black light. The degree of the sensitivity was assessed by measuring the ear thickness 24 hours after challenge. Tribromosalicylanilide, bithionol and chlorpromazine as well as tetrachlorosalicylanilide were readily demonstrated to be photosensitizers in mice. Hexachlorophene and sulfanilamide induced contact photosensitivity after pretreatment of mice with cyclophosphamide (20, 100 and 200 mg/kg) on day −2. The recently discovered photosensitizers such as musk ambrette, 6-methylcoumarin, 7-methylcoumarin and benzocaine showed photoallergic reactions, which were enhanced by cyclophosphamide pretreatment. Salicylanilide, salicylic acid and 3,4-dichloroaniline which were known not to be photosensitizers failed to demonstrate photosensitization potentials. We suggest that this mouse model and cyclophosphamide pretreatment is a useful strategy to determine photosensitization potentials for a variety of new chemicals.

Photoreactivity of Isolated Furocoumarins from *Heracleum laciniatum*. G. KAVLI[1], G. VOLDEN[1], H. KROKAN[2] AND J. RAA[3], Dept. Dermatology[1], Inst. Med. Biol.[2], Inst. Fisheries[3], University of Tromsø, Norway

The *Umbelliferous* plant *Heracleum laciniatum* evoke very strong phototoxic reactions.

Furocoumarins were extracted and purified. The purity was controlled by high-pressure liquid chromatography. The phototoxic potencies of plant materials from different parts of the plant or purified furocoumarins were assessed. Action spectrum studies were performed with crushed leaves and individual furocoumarins on normal volunteers and in a patient with subtotal vitiligo.

Roots, leaves and fruits contained approximately the same amount of furocoumarins while the stems contained less than 10% compared to the roots. The following order of phototoxic potency of the furocoumarins were found: Bergapten, pimpinellin, angelicin, sphondin, icobergapten and isopimpinellin. Action spectrum studies demonstrated photosensitivity in the range 315–375 nm with peak sensitivity at 330–335 nm for all of the following: Crushed leaves, bergapten, pimpinellin, angelicin and sphondin. Both the minimal erythema dose and the minimal phototoxic erythema dose in vitiliginous skin were approximately ⅓ of that of persons with skin type II.

Accidental photocontact allergy was induced in 2 volunteers after 5 respectively 6 provocations to the angular furocoumarins sphondin, isobergapten and pimpinellin.

Psoralen-Induced Depletion of Glutathione and Cutaneous Inflammation: A Dose Response Study. L. A. WHEELER, M. CONNOR, N. LOWE, AND M. V. W. BERGAMINI, Depts. of Pharmacology and Dermatology, UC, Los Angeles and Herbert Laboratories, Irvine, CA

Glutathione (GSH) is an intracellular thiol important in regulatory and synthetic functions of the cell and as a protective agent against chemical and light damage. Photochemical activation of 8-methoxypsoralen (8MOP) by ultraviolet light-A (UVA; 320-400 nm) can be phototoxic to the skin. The purpose of this study was to examine the dose response relationship between PUVA (psoralen + UVA) and both depletion of skin GSH and inflammation. Double-fold skin thickness (DFST), an index of cutaneous edema, was the measure of inflammation. Female hairless mice were given 8MOP (dissolved in corn oil) by gavage at doses of 0.1, 0.5, 5, 25 and 50 mg/kg. Two hours later the mice were irradiated with 5 J/cm^2 UVA light. Time-dependent changes in both GSH and DFST peaked at 24 hrs. At 24 hrs, DFST measurements were taken, the mice were killed, and GSH, oxidized glutathione (GSSG) and glutathione-S-transferase were measured in the epidermis and dermis. Epidermal GSH was depleted 0, 11, 45, 87 and 98% from vehicle and/or UVA light-treated levels (0.5 mM) after 0.1, 0.5, 5, 25 and 50 mg/kg respectively. In the dermis (0.3 mM) decreases of 47, 87 and 91% were observed, with 5, 25 and 50 mg/kg 8MOP. Increases in normal DFST (0.074 cm) were not observed until doses of 8MOP reached 5, 25 and 50 mg/kg. DFST increased 20, 241 and 337% respectively. GSSG accounted for a small portion of total GSH in the skin after PUVA, suggesting the GSH decrease was not due to oxidation. These dose response data suggest a relationship between depletion of GSH and cutaneous edema. The inflammatory mediator(s) released by PUVA are unknown; however, reduction of cellular GSH may lead to membrane damage and may influence synthesis and metabolism of released mediators.

Alterations in Epidermal Cell Surface Glycoconjugates by Psoralen Plus Long-wave Ultraviolet (UVA) Light Irradiation (PUVA). KIICHIRO DANNO, MASAHIRO TAKIGAWA, AND TAKESHI HORIO, Department of Dermatology, Faculty of Medicine, Kyoto University, Kyoto, Japan

PUVA-induced alterations in membrane glycoconjugates were visualized by fluorescence (FL) microscopy using: pemphigus autoantibodies, experimentally-produced anti-guinea pig epidermal cell membrane antibodies, and several fluorescein-conjugated lectins including Ulex europaeus I agglutinin (AGL) (Ulex), wheat germ AGL (WGA), peanut AGL (PNA), Bandeirea simplicifolia AGL (BSA), soybean AGL (SBA), and concanavalin A (Con A). A 1% 8-methoxypsoralen (8-MOP) solution was painted on the backs of total 32 guinea pigs (20 μL/sq cm) and 1 hr later UVA light (1.8–7.2 J/sq cm) was exposed. Serial biopsies were taken up to 14 days after a single PUVA treatment and processed for FL microscopy and routine histology. Following PUVA FL for the antibodies and all lectin stainings localized on cell membranes of whole (WGA, BSA, Con A) or upper (PNA, SBA) epidermal layers, except for Ulex, were decreased most remarkably in intensity after day 2 and recovered by day 7–8. These changes were dose dependent and did not occur following UVA light or 8-MOP alone. While Ulex staining was invisible in normal guinea pig skin, it depicted upper epidermal and horny layers after day 2; the Ulex FL disappeared by day 14. Controls using competitive sugars confirmed specificity of the lectin stainings. Dermal FL with WGA, BSA, and Con A was slightly affected. Histologically, there were no degenerative changes in PUVA-treated skin. These results suggest that PUVA induces deletion and/or changes in composition of epidermal cell surface glycoconjugates. In addition to a photobinding to cellular DNA, such membrane alterations by PUVA may provide another possible basis for its diverse clinical efficacy.

Effect of PUVA on Chemotactic Activity of Zymosan Activated Serum on Polymorphonuclear Leukocytes—Quantitative Comparison of Effects Among 8-Methoxypsoralen, 4,5,6-Trimethylpsoralen, and 3-Carbethoxypsoralen. KENJI ESAKI AND NOBUYUKI MIZUNO, Depart. of Dermatol., Nagoya City Univ. Medical School, Nagoya, Japan

PUVA is effective on neutrophilic dermatoses, but its action mechanism is not known. There is a possibility that PUVA may inhibit the chemotactic activity of anaphylatoxin C5a. This study was performed to explore this hypothesis more fully with zymosan activated serum (ZAS) as the anaphylatoxin.

Four groups of test samples were prepared for each psoralen. Namely (1) Z + A (absolute control), (2) Z + P, (3) Z + A + U, and (4) Z + P + U (PUVA). Where Z, A, P, and U represent 1 ml of ZAS, 5 μl of acetone, psoralen in 5 μl of acetone, and UVA respectively. The final psoralen concentration in the solutions was adjusted to 0, 7×10^{-7}, . . . , 7×10^{-4} M, and UVA dose ranged from 0 to 9 J/ml. ZAS was prepared by Vallota & Müller-Eberhard's method, and polymorphonuclear leukocytes (PMNs) were obtained by Bøyum and Chenowith's method. Chemotaxis to samples were tested by Boyden's method.

There were no significant differences in the number of migrated PMNs among control groups (1), (2), and (3), and in the effect of PUVA on chemotactic activity of ZAS among the 3 psoralens. There was dose-dependency in the PUVA effect on psoralen and UVA, but it was limited under the critical points of (7×10^{-6} M, 7 J/ml, Migration inhibition = 55%), (7×10^{-5} M, 7 J/ml, 65%), and (7×10^{-6} M, 5 J/ml, 65%) for 8MOP, TMP and 3CPs respectively.

It is very possible from these results that one of the therapeutic action mechanisms of PUVA on neutrophilic dermatoses is the inhibition of C5a anaphylatoxin activity.

Comparison of Erythemogenic Effectiveness of UVB and UVA Radiation by Skin Reflectance. SAN WAN, KURT JAENICKE AND JOHN PARRISH, Dept. of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, MA

We have previously reported a method of estimating the amount of cutaneous blood in the superficial plexus by skin reflectance. The present study applies this method to compare the erythemogenic effectiveness of ultraviolet-B (UVB) and ultraviolet-A (UVA) radiation.

The blood determination utilizes the optical model d = 1/63.6 [ln(R(544)-r) + ln(R(610)-r) − 2 ln (R(577)-r)], where d (in ml/cm^2) is the amount of cutaneous blood in the superficial plexus per unit skin area, R is reflectance of the skin surface and r is the minimum of R in 250–300 nm. The backs of 4 fair-skinned subjects were irradiated with 8 doses of broadband UVB and UVA each. The UVB source consisted of a bank of Elder FS36 sunlamps with a fluence rate of 0.8 mW/cm^2. The UVA radiation was obtained from a 2.5 kW xenon arc after passing through 6 cm of a 7% $CuSO_4$ and 7% $CoSO_4$ aqueous solution and a Schott WG335 filter. The fluence rate was 30 mW/cm^2. The estimated quantity of cutaneous blood at each site was determined from *in vivo* diffuse reflectance 8, 24 and 72 hours after irradiation. The doses of UVB and UVA that caused this quantity to increase to 1.5, 2 and 2.5 times the pre-irradiation value were used for comparison of their erythemogenic effectiveness. Our results show that on an equal dose basis, UVB is 2.28 to 3.12 × 10^3 times as effective as UVA in inducing erythema, whether erythema is evaluated 8, 24 or 72 hours after irradiation. This result agrees with the comparison of erythemogenic effectiveness obtained in terms of MED's (minimum erythema dose) by concurrent visual inspection. The potential applications of this objective method are discussed.

Melanosomal Alterations in PUVA Lentigines. HIDEMI NAKAGAWA, ARTHUR R. RHODES, KHOSROW MONTAZ-T, AND THOMAS B. FITZPATRICK, Department of Dermatology, Harvard Medical School, Boston, MA

Comparative ultrastructural studies were conducted to determine morphologic differences between melanocytes in PUVA lentigines (PL) and solar lentigines (SL) in white adults. Specimens included lesional and perilesional skin of 6 PL from the buttocks of as many psoriatics receiving PUVA for 4–6 yr, and 7 SL from the upper backs of as many individuals not on ultraviolet radiation therapy, and light-protected buttock skin (LPS) from 5 additional control subjects. Melanocytes were increased in PL and SL. Compared to SL, melanocytes in PL had longer, more numerous dendrites and more active melanogenesis. Melanocytes in some PL showed aberrant melanosomal changes, including giant pigment granules. Melanosomal pattern was determined from observations of 1000–1500 melanosomes (single plus complex) in basal keratinocytes of PL and SL, and 100–200 melanosomes of perilesional skin and LPS. Melanosome size was determined from measurements of the largest single melanosome and the largest melanosome in complex melanosomes for 50 single and 60 complex melanosomes, respectively. The average percent (mean ± SD) of single melanosomes was greater in PL than in SL and LPS (71 ± 15% vs 21 ± 20% and 16 ± 2%, respectively) ($p < 0.005$, each comparison), but not significantly different from perilesional skin of PL (69 ± 14%). The average mean greatest diameter of single melanosomes was larger in PL than in SL and LPS (0.61 ± 0.04 μm vs 0.42 ± 0.02 μm and 0.32 ± 0.02 μm, respectively) ($p < 0.005$). The average mean greatest diameter of the largest melanosome in complex melanosomes was also greater in Pl than in SL and LPS (0.43 ± 0.01 μm vs 0.32 ± 0.02 μm and 0.28 ± 0.04 μm) ($p < 0.005$). It is unknown whether abnormal melanosomal alterations in PL represent a reversible effect of PUVA or evidence of a somatic mutation.

Contact Sensitivity to Chrysanthemum and Photosensitivity in Guinea Pigs. ANDREW HETHERINGTON AND BRIAN E. JOHNSON, Department of Dermatology, University of Dundee, Ninewells Hospital, Dundee, Scotland, U.K.

Photosensitivity Dermatitis/Actinic Reticuloid (PD/AR) subjects have chronic photosensitivity associated with contact allergic reactions to substances such as oleoresin extracts from Compositae plants which also have a phototoxic potential. To investigate this association, we have studied the reactions of guinea pig skin to Chrysanthemum extracts and simulated sunlight. Guinea pigs were sensitized by topical, occluded applications of extract to nuchal skin accompanied by intradermal Freund's complete adjuvant, with and without erythemal or sub-erythemal exposures to xenon arc solar simulator (SS) radiation, with or without UV-B. Challenge tests were by topical applications of appropriate dilution series to flank skin. Phototesting, before and after sensitization, was done using graded exposures to SS radiation. 24 hour open photopatch tests with SS radiation minus UV-B were used to assess phototoxicity and photoallergy. Action spectrum studies were done using a xenon arch powered grating monochromator, ½ maximum bandwidth: 7.8 nm. Guinea pigs sensitized to Chrysanthemum showed cross reactions to related common weeds, Tansy and Yarrow but not to Alantolactone, a typical Compositae allergen. There was no change in inherent photosensitivity due to contact sensitization and photopatch tests to demonstrate photoallergy were negative. Phototoxicity was demonstrated as erythema, developing to a maximum at 4 hours and fading by 24 hours after exposure with an action spectrum peak around 350–360 nm.

Some features of PD/AR may be demonstrated in guniea pigs in that they may be sensitized to Chrysanthemum and show cross sensitivity to related plants. Phototoxicity is unequivocally demonstrable in vivo, as in vitro but there is no photoallergic potential in these extracts.

Inhibition of UV-Induced Epidermal DNA and Protein Synthesis by Polyamine Antimetabolites. KIRSTI KÄPYAHO, JORMA LAUHARANTA, AND LASSE KANERVA, Department of Biochemistry, University of Helsinki, and Department of Dermatology, University Central Hospital, Helsinki, Finland.

The study was aimed to investigate the feasibility of using a combination of two inhibitors of polyamine biosynthesis, 2-difluoromethylornithine (DFMO) and methylglyoxal bis(guanyl-hydrazone) (MGBG), to prevent epidermal hyperproliferation. Hyperproliferation was induced in mouse skin by UVB-irradiation, and DFMO and MGBG were administered topically in a cream base (Ambilan); before UVB DFMO was also given perorally. DNA and protein synthesis were followed by incorporation of radioactive precursors.

Polyamine accumulation appears to be essential for macromolecule synthesis and cell proliferation, and inhibition of polyamine synthesis is shown to prevent proliferation in cell cultures and animal tissues. As observed in other cells and tissues, DFMO and MGBG acted synergistically in UVB-irradiated mouse epidermis. DFMO depleted the epidermal cells of polyamines and, consequently, increased greatly the uptake of MGBG, an analog of the polyamine spermidine. MGBG is a strong inhibitor of polyamine synthesis, but has also other antiproliferative effects. Topical administration of either DFMO or MGBG inhibited UVB-induced mouse epidermal DNA-synthesis, but pretreatment of the mice with DFMO, followed by a combined treatment with DFMO and MGBG, produced a profound inhibition of both macromolecular syntheses. The inhibition could be partly reversed by a concomitant application of spermidine. The results show that a combination of the two polyamine antimetabolites may provide an effective tool for the treatment of epidermal hyperproliferation, e.g. psoriasis.

Topical Indomethacin—An Effective Sunscreen Agent. H. W. LIM, R. L. BAER, AND I. GIGLI, Division of Dermatology, Univ. of California Medical Center, San Diego, CA and Dept. of Dermatology, NYU Medical Center, New York, NY

Indomethacin (IND), a prostaglandin-synthetase inhibitor, markedly reduced ultraviolet-B (UVB)-induced erythema when applied topically after UVB exposure (J Invest Dermatol 64:322, 1975). We investigated whether topically applied IND could also act as a sunscreen agent. In vitro, IND, 3 × 10^{-2} mM, dissolved either in ethanol or in propylene glycol: ethanol:dimethylacetamide (PED), 19:19:2, showed similar absorption spectrum between 250 to 380 nm, with maximal absorption at 270 nm, and also at the UVA spectrum (325 nm). This absorption spectrum was identical to that of para-aminobenzoic acid (PABA) for the wavelength range of 250 to 320 nm. PABA, however, had no absorbance at the UVA range. The sunscreen property of IND solution in vivo was studied in guinea pigs (GPs). IND, 2.5% in PED, was applied topically to depilated back of albino GPs, followed by exposure to UVB (1800 mJ/cm^2) 20 minutes later. Two parameters of the effect of UVB radiation on the skin were assessed, namely, cutaneous erythema and the density of ATPase-positive epidermal Langerhans cells (LC). Irradiation of untreated GPs, or GPs treated with PED vehicle alone, resulted in marked cutaneous erythema, accompanied by a 60–70% decrease of the density of ATPase-positive LCs 2–4 days later. In contrast, IND-treatment prior to irradiation prevented both the development of erythema, and the decrease in LC density. This protective effect of IND is similar to that observed in GPs treated with 5% PABA solution, and is independent of its prostaglandin-synthetase inhibitor activity. These observations demonstrate that IND is a potentially clinically useful sunscreen agent, with a much broader absorption spectrum than PABA.

Detection of Early Stage Mycosis Fungoides Using a Combined Approach of Monoclonal Antibody Cell Surface Markers, Chromosomal Analysis, and Histopathology. ROLLIN H. HEINZERLING, MARK L. NELSON, MARGARET C. DOUGLASS, THOMAS K. BURNHAM, AND EDWARD A. KRULL, Department of Dermatology, Henry Ford Hospital, Detroit, Michigan

A combined approach of monoclonal lymphocyte typing antibodies, chromosomal analysis, and histopathology of peripheral blood lympho-

cytes (PBL), lymph nodes, and skin cellular infiltrates were used to determine the presence of mycosis fungoides (MF) in 11 clinically suspected MF patients (PT). OK-T(3,4,6,8,9,10,11), OK-M1, and NEN-IA monoclonal antibodies (ab) were incubated with PBL and frozen sections of suspected MF skin lesions and lymph nodes, followed by FITC-conjugated $F(ab')_2$ goat anti-mouse IgG. The PBL were enumerated by cytofluorographic analysis and the sections observed by fluorescence microscopy. Lymphocytes from peripheral blood, lymph nodes, and skin were cultured with and without T-cell growth factor (TCGF) for chromosome analysis. Tissues were incubated for 48–96 hours, the cells were then mitotically poisoned with colchicine and subsequent spreads were trypsinized for G-banding. Patients' karyotypes were compared to their corresponding monoclonal antibody phenotypes and tissue histopathology in attempting to identify significant diagnostic patterns for predicting MF.

The dermal lesions of 8 PT that were histopathologically only suggestive of MF contained 6 with 10–20% OKT-10 positive cells and 5 with chromosomal abnormalities. All the PBL specimens were completely normal. We believe that the combined criteria of lymphocytes with increased OKT-10 markers, chromosomal abnormalities, and histopathologic atypicality provide a more sensitive means to detect early MF than any single modality.

Typing of Immunoglobulin Producing Cutaneous B Cell Lymphomas Using an Immunoenzymatic Double Labeling Technique for Paraffin Sections. GÜNTER BURG†, ULF DETMAR†, DAVID Y. MASON*, PETER KAUDEWITZ†, PETER KIND†, AND OTTO BRAUN-FALCO†, Dermatologische Klinik, LMU München†, John Radcliffe Hospital, Oxford*

Monoclonal immunoglobulin (Ig) producing lymphomas (immunocytomas and immunoblastomas) constitute 15% to 20% of all cutaneous lymphomas. Their differentiation from polymorphous lymphoplasmacytoid inflammatory or pseudolymphomatous infiltrates may be difficult if sections are stained for one single light chain only.

It was the *aim of the study* to elucidate the ratio of kappa (k) vs lambda (λ) positive cells in 10 lymphoproliferative, 5 pseudolymphomatous, and in 13 inflammatory lymphoplasmacytoid cutaneous infiltrates and to characterize them by the type of Ig (γ, α, μ, k, λ) synthesized intracellularly.

An indirect immunoenzymatic double labeling *method* (alkaline phosphatase and peroxidase) was used for the simultaneous demonstration of k and λ light chains and γ, α, and μ heavy chains in paraffin sections.

Results. Ig producing lymphomas of the skin show patchy monoclonal proliferations of cells synthesizing IgM/k in 50% of the cases (5/10); the ratio of λ to k positive cells is at least 1:10. In polyclonal inflammatory and pseudolymphomatous infiltrates the λ:k ratio never exceeds 1:5. In high grade malignant lymphomas, the tumor cells may loose their capacity for Ig production (2/3).

Conclusions. The indirect immunoenzymatic double labeling technique for the simultaneous detection of k and λ light chains on paraffin sections is most useful for the differentiation between polyclonal pseudolymphomatous and monoclonal lymphomatous lymphoplasmacytoid infiltrates in the skin, which most commonly produce IgM/k.

The Distribution of Neoplastic and Non-neoplastic Cells in Cutaneous B Cell Lymphomas. R. WILLEMZE, C. B. DE GRAAF-REITSMA*, W. A. VAN VLOTEN, AND C. J. L. M. MEIJER*, Departments of Dermatology and Pathology*, University Hospital, Leiden, The Netherlands.

In the present study the distribution of the neoplastic B cells and non-neoplastic cells (modulatory T-cells, dendritic reticulum cells (DRC) and macrophages) in the dermal infiltrates of 12 patients with a cutaneous B-cell lymphomas (CBCL) was investigated by means of immunohistochemical, cytochemical, and ultrastructral techniques. This group comprised 5 centroblastic lymphomas (CB), 2 centroblastic/centrocytic lymphomas (CB/CC) and 5 immunocytomas (Kiel classification). Cutaneous involvement was the presenting symptom in 11 patients. In 9 of them the malignant lymphoma apparently remained confined to the skin for at least 6 months suggesting a primary cutaneous origin.

In all but one case a monoclonal staining pattern for S1g and/or C1g was observed. With respect to the non-neoplastic cells the following distribution was found. CB:occasional Leu-1^+, OKT3^+ T-cells, few DRC and relatively many macrophages; CB/CC:numerous DRC and macrophages, 30–50% Leu-1^+, OKT3^+ T-cells. The majority of these T-cells expressed the phenotype of activated T-helper cells (Leu-3^+, OKT4^+, HLA-DR$^+$); immunocytomas:few DRC, 20–50% OKT3^+, Leu-1^+ T cells. The majority of these T-cells were also reactive with Leu-3a and OKT4 antisera, but not with HLA-DR antiserum. No differences were found between B-cell lymphomas with primary and those with secondary skin involvement.

These results show that not only the neoplastic cells but also the distribution of the non-neoplastic cells in CBCL is very similar to that observed in B-cell lymphomas of the same type in lymph nodes. These findings might serve as an additional diagnostic criterium in the classification of CBCL.

Sequential Ultrastructural Analysis of Murine Graft-Versus-Host Skin Disease (GVHSD). JANE H. DEES, MICHAEL R. CHARLEY, RICHARD D. SONTHEIMER, AND JAMES N. GILLIAM, Dept Dermatology, UTHSC, Dallas, Tx

This study was undertaken to extend our morphologic description of the GVHSD that occurs in C57B1/6J → LP/J chimeras (JID 78:347, 1982). Abdominal biopsies at 5, 7, 9, 12, and 15 days after transplantation were processed for light and electron microscopy. At 5 and 7 days there was focal keratinocyte and fibroblast injury. By 7 days a few lymphocytes were seen in the epidermis, and dermal mast cells were prominent. By 9 days intercellular spaces were widened; desmosomes were isolated; and lymphocyte exocytosis was seen. Langerhans cells (L.C.'s) contained large residual bodies of phagocytosed debris but otherwise appeared normal. Apoptotic cells were present in the dermis and residual bodies were seen in fibroblasts. At 12 days there was widespread injury to keratinocytes and in some areas the epidermal injury had progressed to necrosis. Injured fibroblasts were present subepidermally, and mast cells were not seen. At 15 days, the epidermal injury was resolving while the dermis contained relatively acellular collagen and cell debris. Endothelial cells remained normal throughout the study period.

In summary, focal keratinocyte and fibroblast injury was detected earlier by E.M. (5 days) than by light microscopy (7 days). Endothelial cells and L.C.'s remained surprisingly normal in appearance throughout. Mast cells were prominent during the early time intervals but were absent after day 9. It has been suggested that vascular damage may be a prerequisite for the sclerosis seen in human scleroderma. This study has shown that dermal sclerosis can occur in murine GVHD without vascular damage and therefore raises the possibility that the vascular injury and sclerosis of scleroderma may not be causally related.

The Impaired Histamine-N-methyltransferase Activity in Arthus Reaction. SADAO IMAMURA AND SHINKICHI TANIGUCHI, Department of Dermatology, Faculty of Medicine, Kyoto University, Kyoto, Japan

In order to clarify the role of chemical mediators in the development of skin inflammations, the activities of histamine-N-methyltransferase which is a degrading enzyme of histamine, were measured in the lesions of Arthus reaction evoked by ovalbumine, croton oil dermatitis and allergic DNCB dermatitis in guinea pigs. Biopsied skin specimens were homogenized with 40 volumes of Tris-HCl buffer and were subsequently centrifuged at 105,000 × g for 60 min. The resultant supernatants were used as enzyme sources. The enzyme preparations were reacted with S adenosyl-L-[^{3}H-methyl] methionine (2 μM, 1 μCi) and histamine (0.2 mM) at 37°C for 30 min. Produced ^{3}H-methylhistamine was extracted by the method of Axelrod, J. and the radioactivities were measured. The specific activities of the enzyme were expressed as pmol/min/mg protein. The enzyme activities in the skin lesions of Arthus reaction were reduced to about ⅓ of the normal skin, while those in the lesions of croton oil dermatitis and DNCB dermatitis, and in uninvolved skin of Arthus animals were within normal limits. The decrease in the enzyme activities in Arthus lesions was time-dependently from the onset of the reaction. The apparent Km values of histamine-N-methyltransferase in normal and Arthus reaction skin for 2 substrates were; histamine:116 μM (normal), 66 μM (Arthus) and S adenosyl-L-methionine: 1.85 μM (normal), 1.16 μM (Arthus). These results indicate that the decreased enzyme activities due to the change of enzyme kinetics may play an important role for the development of Arthus reaction.

Antigens of Canine Stratified Squamous Epithelium Reactive with Human Pemphigus Antibodies. ROBERT J. SEILER AND JAY HUANG, Dept. of Veterinary Pathol., N.Y.S. College of Veterinary Med., Ithaca, N.Y. and Dept. of Microbiol., School of Med., S.U.N.Y. Buffalo, Buffalo, N.Y.

Pemphigus occurs spontaneously in dogs, so we studied some features of pemphigus antigens in canine esophageal epithelium. First we showed by conventional indirect immunofluorescence, and by a horse-

radish peroxidase labelled protein-A method, that human pemphigus sera (n = 4) bound, as expected, with canine esophageal epithelium (n = 12). Titers were similar to those on monkey esophagus and 4 normal sera did not bind. The pemphigus antigens were insoluble in phosphate buffered saline (PBS), while sediment from saline extraction retained antigenic activity. No activity was present in similar preparations of pooled canine liver, kidney and spleen. Prolonged exposure (12–24 hr at 5°C) to PBS or Tris-acetate buffered saline (TAS) inactivated antigens reactive with a pemphigus foliaceus (PF) serum, but did not affect reactivity with a pemphigus vulgaris (PV) serum. The PF reactivity was also removed in solutions containing phenylmethylsulfonylfluoride but was preserved, and could be restored, by the addition of dithiothreitol (DTT) and $CaCl_2$. The histochemical localization of the PF and PV reactive antigens also differed in that the PF serum did not bind in the basal epithelium. The antigens were not solubilized by the addition of non-ionic detergents (0.5% Nonidet P-40 or 0.2% Triton X-100) to TAS containing DTT and $CaCl_2$. Similarly, short exposure (up to 10 mn at 5°C) to ethyl alcohol, ether, acetone, dimethylsulfoxide and formalin had no effect on the reactivity. The results confirm the existence of canine pemphigus antigens, which were unexpectedly stable. Also there was an apparent difference in the requirement for free-SH groups and Ca^{2+} on the antigens reactive with PF or PF sera.

Antibodies to the Dermatan Sulfate Core Protein and Their Use for Localization of Proteodermatan Sulfate in Skin by Immunoelectron Microscopy. MARIA O. LONGAS AND RAUL FLEISCHMAJER, Department of Dermatology, The Mount Sinai School of Medicine, New York, New York

The purpose of this investigation was to develop polyclonal antibodies against the protein moiety of dermatan sulfate in order to study its interaction with collagen. Proteodermatan sulfate was isolated from human skin in 6 M urea at 70°C, containing proteolytic inhibitors, and purified by ion and gel exclusion chromatography. The final product displayed one band on agarose polyacrylamine gels, and was composed of 55% protein and 45% dermatan sulfate. This material was used to immunize rabbits. Double diffusion of antisera against proteodermatan sulfate or the protein obtained by exhaustive digestion with chondroitinase-ABC resulted in one precipitin line. Antisera, however, did not react with bovine collagen type I, bovine fibronectin, human or porcine skin dermatan sulfate, hyaluronic acid, heparin, heparan sulfate or chondroitin sulfate from various sources. Radioimmunoassay of the purified IgG fraction with [^{3}H]-proteodermatan sulfate indicated that a 40,000th dilution of antibody bound 50% of the total radioactivity of the antigen-antibody complex precipitated with goat anti-rabbit IgG. Anti-proteodermatan sulfate IgG was detected along collagen in human dermis by indirect immunofluorescence microscopy and by immunoelectron microscopy using ferritin-labelled goat anti-rabbit IgG. No labelling took place with preimmune sera or antisera depleted of antiproteodermatan sulfate. The results indicate that anti-proteodermatan sulfate IgG is highly specific for the core protein, and support the hypothesis that skin proteodermatan sulfate is associated with collagen.

A New Component of the Dermo-Epidermal Junction (DEJ) Is Revealed by an Anti-amnion Antiserum. JEAN-PAUL ORTONNE, BAE-LI HSI, CHANG-JING G. YEH, AND W. PAGE FAULK, Department of Dermatology and INSERM U 210, University of Nice, France

In view of common ectodermal ancestry of amniotic epithelium and certain major components of skin, we investigated whether an anti-amnion antiserum was able to identify cross-reacting sites in mammalian skin.

Deoxycholate solubilized human amnion was chromatographed on biogel P-200, and two peaks were obtained. A rabbit was immunized with an emulsion of the first peak in Freund's complexe adjuvant. Following absorption with human erythrocytes and placenta, the antiserum (AABM) was found to react with amniotic epithelial basement membrane (BM) in a pattern which was quite different from that of collagen IV and fibronectin.

AABM reacted brilliantly with skin epidermal BM and with BM surrounding epidermal adnexae. This was not blocked with bullous Pemphigoïd sera, and pattern of reactivity at the DEJ was independent of the type of epidermal differentiation. AABM also reacted with the following substrates : oesophagus, lip, vagina and breast duct epithelial BM. The following substrates did not react with AABM : liver, spleen and kidney. AABM reacted with tissues from human beings, pigs, rabbits, mice and rats. AABM reacted with cryostat sections of human thymus in an extracellular pattern, but did not react with Hassall's corpuscles.

Results of our present research on human skin following dermo-epidermal dissociation suggest that the antigen of AABM is localized in the lamina lucida. It appears to markedly differ from other described antigens of the DEJ.

A Comparison of Two Short-term Predictive Tests for Contact Sensitising Chemicals and the Effect of Cyclophosphamide on the Sensitivity of Each Test. G. J. A. OLIVER, P. A. BOTHAM, AND P. H. DUGARD, ICI PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

This study was designed to compare the reliability and sensitivity of two short-term predictive tests for contact sensitising chemicals, the Stevens Test (Stevens, 1967, Brit J of Ind Med 24 189) and the Lymph Node Test. The effect of cyclophosphamide on the sensitivity of each test was also determined.

Six known sensitisers were chosen: oxazolone, dinitrochlorobenzene, penicillin G, nickel chloride, sodium chromate, trichlorophenol. The Stevens Test involved the sensitisation of outbred albino guinea-pigs by three topical applications of each chemical to the ears, followed four days later by a topical challenge to the flank. Results were expressed both as the number of animals responding and as the average intensity of erythema. The Lymph Node Test involved a single topical application of each chemical to the ears of guinea-pigs. Six days later the weight of the draining (auricular) lymph nodes were determined and compared with those from control animals. Cyclophosphamide was injected on Day-3.

All six sensitising chemicals were positive in the Stevens Test; cyclophosphamide increased the intensity of response to sodium chromate and to dinitrochlorobenzene. Four of the chemicals were positive in the Lymph Node Test (nickel chloride and trichlorphenol were not detected); cyclophosphamide increased the response to sodium chromate and dinitrochlorobenzene and resulted in a positive response to trichlorophenol.

The qualitative Stevens Test thus remains as a first stage screening test for extreme and strong sensitisers but the quantitative Lymph Node Test is at Present not a viable alternative. The sensitivity of both models was not increased by cyclophosphamide.

Subacute Cutaneous Lupus Erythematosus (SCLE): Association of Anti-Ro (SSA) Antibodies with a Speckle-like Thread Nuclear Staining Pattern. D. J. WERMUTH, W. D. GEOGHEGAN, AND R. E. JORDAN, VA Medical Center, Wood, WI, The Medical College of Wisconsin, Milwaukee, WI, and the University of Texas Health Science Center, Houston, TX

SCLE, a recently defined LE subset, is characterized by a photo sensitive rash, mild systemic complaints and a high incidence of anti-Ro (SSA) antibodies. Controversy still exists as to whether the Ro antigen is of cytoplasmic or nuclear origin. Fourteen patients with clinical, histopathologic, and serologic evidence of SCLE had anti-Ro antibodies as demonstrated by double immunodiffusion using an ion exchange (DEAE Sephacel) chromatographic saline extract of human spleen as the source of antigen. All 14 serum samples were negative when tested for antibodies to the nuclear antigens Sm and RNP and native DNA. When tested for antinuclear antibodies (ANA) using immunofluorescence on mouse liver substrate, 3 of these 14 sera were ANA negative. When imprints of human spleen were utilized as substrate, 13 of the 14 serum samples yielded a nuclear IF pattern conforming to the speckle-like thread pattern of Burnham (Arch. Dermatol. 114:1343, 1978). After 2 of the Ro positive sera, which were ANA negative on mouse liver substrate, were incubated with human spleen extract containing Ro antigen, the speckle-like thread nuclear IF pattern on human spleen imprints, as well as the Ro-anti-Ro precipitins in double immunodiffusion, was inhibited. Our studies suggest that the presence of anti-Ro antibodies is associated with a nuclear reactive antibody yielding a speckle-like thread pattern on human spleen cells. Studies are currently underway to determine if this nuclear reactive antibody and the anti-Ro antibody are the same entity.

Phospholipase A2 Activity in Blister Fluid and Epidermis of Symptomless Skin of Psoriasis. HAROLD J. YARDLEY, ROY SUMMERLY, SUSAN FORSTER AND ELIZABETH ILDERTON, Departments of Biological Sciences and Postgraduate Medicine, University of Keele, Staffordshire, England

Suction blister fluid from uninvolved (symptomless) psoriatic skin has been reported (1) to contain a higher concentration of free arachidonic acid than blister fluid from normal skin: since arachidonic acid is released from phospholipids by the action of phospholipase A2 we measured the activity of this enzyme in blister fluid.

Suction blisters were raised, the fluid was aspirated and the epidermis forming the roof of the blisters was removed with scissors and homogenised in an all glass tissue grinder. Phospholipase A2 activity was measured in the high speed supernates from the blister fluid and the epidermal homogenates (2).

The specific activity of phospholipase A2 in blister fluid was very low, although higher than in serum. In contrast, the specific activity of the enzyme in the epidermal supernate was about 100 times greater.

We have previously shown (3) that the enzyme activity derived from symptomless psoriatic epidermis is significantly greater than that derived from normal epidermis. In vivo, the products of this presumed intraepidermal reaction are released into the intercellular spaces; this would explain the higher concentration of free arachidonic acid in suction blister fluid from symptomless psoriatic skin reported by others (1). We conclude that suction blister fluid is not a suitable source of epidermal phospholipase A2.

1) Plummer, N. A., Hensby, C. N., Warin, A. P., Camp, R. D. & Greaves, M. W. Clin. Exp. Derm. *3*(1978)367. 2) Freinkel, R. K., & Traczyck, T. N., J. Invest. Derm. *74*(1980)169. 3) Forster, S., Ilderton, E., Summerly, R. & Yardley, H. J. Brit. J. Derm. *107*(1982) in press.

Long-term Effect of PUVA on Psoriatic Dermis from Exposed Areas. J. S. Perlish, M. O. Longas, V. Carter, J. Petrozzi, and R. Fleischmajer, Dept. of Dermatology, Mt. Sinai School of Medicine, New York, N.Y. and V.A. Hospital, Philadelphia, Pa

The purpose of this study was to determine the effect of prolonged PUVA on fibroblasts derived from psoriatic dermis. Biopsies were taken from the forearms of 8 patients in continuous PUVA treatment (av. 3000 Joules/cm^2, 5–6 yr) and 5 normal controls. Doubling times were determined by serially sampling cultures over a 15-day period. Metabolic studies following the incorporation of ^{3}H-tryptophan (protein), ^{14}C-proline (collagen as ^{14}C-OH pro) and ^{3}H-glucosamine (glycosaminoglycan (GAG)) for 24 hrs were performed using standard radioassay procedures. Labelled GAG were isolated by ethanol precipitation and analyzed by two dimensional cellulose acetate electrophoresis.

No difference in doubling time could be found between PUVA and normal controls isolated from the papillary dermis.

Synthesis of Protein and GAG (Mean SEM)

	Fibroblasts (cpm/γ protein)		
	^{14}C-pro	^{3}H-tryptophan	^{3}H-glucosamine
Papillary layer			
Psoriasis (8)	265 ± 35	2142 ± 471	857 ± 147
Normal (5)	478 ± 124	2717 ± 417	818 ± 140

About 40% of the total ^{3}H-glucosamine was incorporated as GAG. The predominant GAG was hyaluronic acid. Eighty percent less GAG was synthesized by PUVA as compared to normal controls. There was no difference in collagen (^{14}C-OH pro) synthesis.

Growth and metabolic studies revealed no major difference between PUVA and normal controls except for a decrease in GAG synthesis in the PUVA group.

Urocanic Acid: A Marker for Psoriasis? J. C. Caron*, B. Martin*, B. Shroot*, P. Brun†, L. Juhlin† and S. Shroot†, *Department of Biochemistry, Centre International de Recherches Dermatologiques (CIRD), Valbonne, France and †Centre International de Traitement du Psoriasis, Antibes, France

There is evidence in the literature that in psoriasis the metabolism of histidine is disturbed. Urocanic acid, histidine and histamine levels in biological samples were measured using high performance liquid chromatography.

Significantly lower levels of histidine, histamine and urocanic acid have been observed in suction blisters raised on uninvolved psoriatic skin as compared to normal skin of healthy subjects (Table 1). In the suction blister fluid we detected only the E isomer of urocanic acid. In contrast, we found on the surface of the skin (psoriatic scales, plantar callus, washings) larger quantities of the Z isomer of urocanic acid. In the serum, no urocanic acid was found, and the histidine levels were identical in psoriatics and in healthy subjects. The reason for the decrease in histidine and its metabolites in psoriatics is still unknown.

Table 1: Levels (μg/ml) in blister fluid from normal appearing skin.

	1-Histidine	Histamine	Urocanic acid
Healthy subjects	22.8 ± 6.3 (n = 83)	2.5 ± 0.6 (n = 11)	10.9 ± 6.6 (n = 75)
Psoriatics	10.2 ± 4.3 (n = 18)	1.5 ± 0.2 (n = 6)	2.4 ± 0.8 (n = 8)

Superoxide Radicals and Ischemic Injury in Skin. Michael J. Im, Chun J. Pak, and John E. Hoopes, Div. of Plastic Surgery, Johns Hopkins Univ. School of Medicine, Baltimore, Maryland

The distal portions of skin flaps are destined to undergo necrosis due to a combination of diminished vascular inflow by transection of supplying vessels and the initial vasoconstriction induced by hyperadrenergic environment in the microcirculation. The initial hyperadrenergic state lasts approximately 24 hrs after flap elevation with relaxation after that time. Ischemia and reperfusion are thought to enhance the production of cytotoxic oxygen free radicals in tissues. In this report, the role of oxygen free radicals in the ischemia-evoked tissue damage in skin flaps was investigated by using the enzyme superoxide dismutase (SOD), a scavenger or superoxide radicals. Island skin flaps, 8 × 8 cm, were created on the abdominal wall of female Sprague-Dawley rats. The blood supply and innervation to the flap was provided by the right epigastric neurovascular pedicle. In experimental group, SOD (1,000 u in 1 ml of saline) was infused slowly to the flap through femoral artery. In the control group, the flaps received saline solution. Skin flap survival in the random flap on the left side of animal was evaluated after 7 days of daily observation. The tissue viability was 18% ± 6.0 s.d. (n = 6) of skin flap area in the control and 33% ± 7.9 (n = 5) in SOD-treated animals ($p < 0.01$). Xanthine oxidase activity was measured in optimal assay condition for skin: the enzyme activity was 57 pmoles/hr/mg dry weight in normal skin and increased 4.9 times normal in skin flaps. The beneficial effect of SOD on flap tissue survival suggests that superoxide radicals contribute to ischemic tissue damage in skin and that local ischemia and reperfusion injury occurs at the junction of the surviving area and irreversibly damaged portion of the flap.

Dose-Response Behavior of Local Nicotinate Pharmacodynamics Following Topical Application. Richard H. Guy, Ethel Tur, and Howard I. Maibach, School of Pharmacy and Dept. of Dermatology, University of California, San Francisco, California

To understand more clearly the time course of percutaneous absorption, the local pharmacodynamics of methyl nicotinate (MN), a topical vasodilator, have been followed non-invasively in man using laser Doppler velocimetry (LDV) and photoplethysmography (PPG). LDV and PPG are sensitive to blood flow changes through the skin microcirculation and are therefore responsive to the pharmacologic stimulus of MN. Drug was applied in aqueous solution for 15 sec and removed. The time course of the local effect of MN was then recorded by PPG and LDV via small probes on the skin surface directly over the application site. LDV data from 4 subjects is summarized below:

[MN]/mM	Peak response/mins	Response decay/mins	Max. response/mV
5	17 ± 2	33 ± 3	250 ± 85
10	13 ± 3	41 ± 3	495 ± 125
25	10 ± 3	54 ± 3	565 ± 50
100	7 ± 2	68 ± 6	625 ± 50

PPG results were in agreement. Analysis of the biexponential log (response) *vs* time behavior suggests half-lives for absorption to and elimination from the site of action of ~ 5 and 12 minutes, respectively. The response pattern following intradermal injection of MN confirms the elimination t½ and hence the very rapid absorption of this drug across the stratum corneum. Dose-response behavior obtained by variation of application area and skin contact time has also been investigated and results consistent with predictions have been observed. We suggest that pharmacodynamic measurements of this type have potential for elucidating aspects of dermal pharmacokinetics *in vivo*.

The Effect of Anti-inflammatory Drugs on Prostaglandin $F_{2\alpha}$ Release from Superfused Mini-pig Skin. A. Chatelus, B. Shroot, C. N. Hensby, F. Vuille, A. Civier, H. Schaefer, and K. Williams*, Centre International de Recherches Dermatologiques, Sophia Antipolis, Valbonne, France and *Department of Pharmacology, University of Bath, Great Britain.

The abdominal skin of mini-pigs, maintained under halothane anaesthesia, was stripped of the horny layer at 6 separate sites using cellotape. Flat glass flow chambers (diameter 2.5 cm, height 0.5 cm) were placed over the areas of exposed epidermis and held in position by vacuum. Tyrode's solution, 1 ml/min+ at 37°C was superfused over the sites via the chambers and consecutive 15 minute fractions were collected. The prostaglandin content of the samples after acidification, ethyl acetate extraction and preparative TLC was quantitated by deuterium stable isotope GC-MS.

Initial experiments revealed that the $PGF_{2\alpha}$ release over the first 15

minutes was 300–500 pg min^{-1} cm^{-2}, but within 45 minutes this had fallen to 150–250 pg min^{-1} cm^{-2} and remained within these limits for the next 6–8 h. Because of the initial high release, the fluid collected in the first 60 minutes was discarded, and only those in the next hour were taken for basal release. A variety of anti-inflammatory drugs have been tested (5×10^{-5} M to 5×10^{-7} M) for their ability to inhibit the basal release of $PGF_{2\alpha}$. All drugs showed a dose related inhibition of $PGF_{2\alpha}$ release with a rank order of potency of Indomethacin = Naproxen > Benoxaprofen > Aspirin ≤ BW755C.

Cutaneous Blood Flow Measured by ^{133}Xenon Washout and a Laser-Doppler Flowmeter. JOHANNES K. KRISTENSEN AND MERETE ENGELHART, Department of Dermatology, Rigshospital, Copenhagen, Denmark

Quantitative measurement of cutaneous blood flow is important in the study of temperature regulation. Noninvasive measurement can be performed by ^{133}Xenon washout and the laser-Doppler flowmeter, the latter technique being based on the fact that laser light is backscattered from moving red blood cells with Doppler shifted frequencies. Blood flow was measured in hand and fingertip skin of four normal persons during resting conditions and during reactive hyperemia after 6 min. occlusion of arterial inflow. Repayment during reactive hyperemia was calculated. Using the ^{133}Xenon method, no differences between hand and fingers were observed in the parameters measured, and repayment was 71.9 ± 9.8% in the hand and 61.4 ± 4.7% in the finger. Using the laser-Doppler flowmeter resting blood flow in the finger was more than 400% higher than the hand skin blood flow and repayment was 7.7 ± 1.8% in the fingers compared to 71.5 ± 13% in the hand. This difference was significant.

The phenomenon of reactive hyperemia is dependent on metabolic factors, and where repayment is low most of the blood flow probably serves temperature regulation. While the ^{133}Xenon method probably measures mainly nutritional blood flow, the laser-Doppler method measures at least part of the non-nutritional blood flow and may be of value in the study of temperature regulation.

The Effects of Uniaxial Tension on the Superficial Dermal Microvasculature. R. L. BARNHILL*, D. BADER**, AND T. J. RYAN*, Department of Dermatology, Slade Hospital and University of Oxford,* and Oxford Orthopedic Engineering Center, Nuffield Orthopedic Center,** Oxford, United Kingdom.

The role of pressure in the compromise of cutaneous blood supply has been recognized for many years. Moreover, there has been an appreciation that shearing forces may also influence the cutaneous vasculature, but almost no work has been done on this subject. In the present study, we used a spring loaded apparatus to apply uniaxial tension to forearm skin in seventeen human subjects-10 normals, 6 psoriatics, and one patient with scleroderma. Simultaneously, the effects of stretching on the upper dermal vasculature were observed stereo-microscopically. Progressive changes (collapse) in the superficial microvasculature-vertical capillary loops and horizontal subpapillary plexus-with increasing tension were photographed. Force and strains were recorded at the points of disappearance of virtually all vessels visualized. An average force of 11.9 newtons, accompanied by a mean strain of 10.3%, resulted in occlusion of all vessels. Although almost all subjects were relatively young, there appeared to be a trend toward greater force needed for vascular occlusion with increasing age. In addition, a much higher force (18.5 newtons) was needed to occlude blood flow in the one patient with scleroderma.

In conclusion, we have developed a new technique for the study of mechanical forces on the blood supply of the epidermis. The data have shown that uniaxial tension has important effects on the superficial dermal microvasculature, resulting in impedance and obliteration of blood flow at relatively low magnitudes.

4:00 PM–4:45 PM SID SPECIAL SPEAKER — Presidential Ballroom

WILLIAM MONTAGNA AWARD
D. Martin Carter, M.D., Presiding

Douglas R. Lowy, M.D.
"Viral and Cellular Transforming Genes"

4:45 PM–5:00 PM Closing Remarks — Presidential Ballroom
Gerd Plweig, ESDR President
Leonard C. Harber, SID President

5:00 PM–6:00 PM Board of Directors (SID) Meeting — Ohio Room