THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 64: 203–214, 1975
Vol. 64, No. 3
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Printed In U.S.A.

ABSTRACTS

PROGRAM

THIRTY-SIXTH ANNUAL MEETING
THE SOCIETY FOR INVESTIGATIVE DERMATOLOGY, INC.

Chalfonte-Haddon Hall
Atlantic City, New Jersey
May 2 and 3, 1975

WORKSHOPS
(JOINTLY SPONSORED WITH THE NATIONAL PROGRAM FOR DERMATOLOGY)
Thursday, May 1, 1975, 7:30 P.M.

Workshop	Directors
Pathophysiology of Psoriasis	Irwin M. Braverman, New Haven, Connecticut; B. Allen Flaxman, Providence, Rhode Island
Biology of Sebaceous Glands	Alan R. Shalita, New York, New York
Genodermatoses	Howard P. Baden, Boston, Massachusetts; D. Martin Carter, New Haven, Connecticut
Photobiology	Madhu A. Pathak, Boston, Massachusetts
The Inflammatory Process	Kirk D. Wuepper, Portland, Oregon; Steven Katz, Bethesda, Maryland
Biology of Melanoma	James J. Nordlund, New Haven, Connecticut
Immunodermatology	William L. Weston, Denver, Colorado; Gerald G. Krueger, Salt Lake City, Utah

MORNING SESSION
THE SIXTH IRVIN H. BLANK RESIDENT-FELLOW FORUM
Friday, May 2, 1975, 7:30–9:45 A.M.
Complement and Cutaneous Disease
Moderator: Kirk D. Wuepper
Portland, Oregon

Fundamentals of Complement Action	Michael M. Frank, Bethesda, Maryland
Diseases Associated with Inherited Complement Deficiencies	Shaun Ruddy, Richmond, Virginia
Herpes Gestationis and Dermatitis Herpetiformis	Thomas T. Provost, Buffalo, New York
Complement Activation in Bullous Skin Diseases	Robert E. Jordon, Rochester, Minnesota

BUSINESS AND EXECUTIVE SESSION
8:30 A.M.
Clayton E. Wheeler, Jr., Chapel Hill, North Carolina, Presiding

SCIENTIFIC SESSION
10:00 A.M.
Farrington Daniels, New York, New York, Presiding

Presidential Address: Pathogenesis of Recurrent Herpes Simplex CLAYTON E. WHEELER, JR., Chapel Hill, North Carolina.

Complement Binding Factor in Herpes Gestationis. H. YAOITA, K. HERTZ, AND S. KATZ, Dermatology Branch, N.I.H., Bethesda, Maryland.

Herpes gestationis (HG) is a vesiculobullous disease of pregnancy and the puerperium. Prior studies of 3 patients demonstrated IgG, complement (Clq, C4, C3, C5), properdin, and C3 proactivator deposited at the basement membrane zone (BMZ) (Provost and Tomasi JCI 52: 1779, 1973, and Bushkell et al. Arch. Derm. 110: 65, 1974). The serum of one patient had a high titer of circulating IgG which bound to the BMZ of monkey esophagus. Those of two other patients contained no demonstrable anti-BMZ IgG but did contain a heat labile factor which bound C3 at the BMZ of normal human skin (NHS).

Extensive studies of a patient with HG are the subject of this report. Repeated direct immunofluorescence (IF) of lesional and normal skin demonstrated IgG, IgE, Clq, C4, and C3 at the BMZ, the site of blister formation. C3 activator and properdin could not be demonstrated, implicating the classical but not the alternate complement pathway. Serum C3, C4, and CH50 were normal. While circulating antibodies could not be found by conventional indirect IF techniques, a multi-step procedure (Jordon et al. J. Lab. Clin. Med. 74: 548, 1969) showed C3 binding at a 1:40 dilution of

the patient's serum, only with NHS as substrate. This binding factor was heat stable (60°C for 60 min.), lyophilizable, and characterized, by absorption studies, as an IgG.

Thus, for as yet unknown reasons, the IgG which was demonstrable by direct IF and by the multistep complement binding technique was not detected by the conventional indirect IF technique. The lesions in all of the patients studied to date may be due to similar immunopathologic mechanisms, but it would seem that the characteristics of the serum factor which binds C3 vary considerably from patient to patient.

Allergic Contact Dermatitis: A Fowl Deed. H. MAGUIRE, R. RANK, AND W. WEIDANZ, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pa. and Hahnemann Medical College, Philadelphia, Pa.

Studies of delayed-type hypersensitivity to low molecular weight allergens (allergic contact dermatitis - ACD) have been limited mainly to man, the guinea pig and, recently, the mouse. We now report the production of ACD in the chicken. In a typical experiment, different groups of white Leghorn chickens were sensitized to picryl chloride (PCL) or oxazolone (Ox.). A site on the comb was treated with 0.1 ml of 90% dimethylsulfoxide in water; 10 minutes later 0.1 ml of sensitizer in acetone (5% PCL or 2% Ox.) was given to the same site. This program was repeated on days 2 and 4. Challenge was made to one or the other wattle with each sensitizer on day 15. Swelling and severe exudative dermatitis developed at the sites challenged with the putative sensitizer but not with the other chemical. There were three birds per group; the average increase in wattle swellings (mm) at 24 hours are tabulated below:

Sensitized to	Challenged with	
	PCl	Ox.
PCl	1.10	0.27
Ox.	0.17	1.13

We have transferred Ox. hypersensitivity to naive B locus histocompatible chickens, in separate experiments, with spleen cells and with peripheral WBCs (Ca. 5 × 108); donors were chickens sensitized to oxazolone with Freund's complete adjuvant using the split adjuvant technique. This model extends to a new phyllum the phenomenon of ACD and provides the possibility of its study in animals selectively depleted of B or T cells.

Prostaglandins and cAMP in Retinoic Acid-Induced Epidermal Hyperplasia. K. ASO, I. RABINOWITZ, D. I. WILKINSON AND E. M. FARBER, Department of Dermatology, Stanford University, Stanford, California.

Epidermal cell proliferation in psoriasis is characterized by low levels of prostaglandins (PGs) and cyclic AMP. These substances were studied in retinoic acid (RA)-induced guinea pig ear hyperplasia which resembles that of psoriasis in many respects.

RA (acetone, 0.5%) was applied daily to an ear and acetone to a control ear for 6 days. Animals were sacrificed daily and ears taken for analysis and histologic study. cAMP was assayed using Cheung's bovine kidney cAMP binding protein and PGE by radioimmunoassay. Ear slices were incubated with ^{3}H-thymidine and uptake measured by scintillation counting.

At day 3, erythema, thymidine uptake (cpm/mgDNA) and PGE levels were maximum. PGE amounts at this time were 5–10 times control values (57pM/gm wet wt). Cyclic AMP increased control value of 1pM/mg wet wt. Hyperplasia was greatest on days 4 and 5.

PG release coincided with erythema increase, and possibly initiated cAMP accumulation which it preceded. There was no evidence of a fall in cAMP levels which might be associated with the wave of DNA synthesis or cell division. Highest cAMP was observed during the period of greatest hyperplasia. The results favor the idea that cAMP increases in response to cellular attempts to restore normal homeostasis.

FIRST WILLIAM MONTAGNA LECTURE

John S. Strauss, Boston, Massachusetts, Presiding

Cell Function, Cyclic Nucleotides and the Epidermis. KENNETH M. HALPRIN, Miami, Florida.

AFTERNOON SESSION

Friday, May 2, 1975, 2:00 P.M.

POSTER SESSION

Radioimmunoassay for the Epidermolytic Toxin of Staphylococcus aureus. DIANE HAAS BAKER, ROBERT L. DIMOND, KIRK D. WUEPPER, AND JAMES E. RASMUSSEN, Department of Dermatology, University of Oregon Health Sciences Center, Portland, Oregon, and SUNY-Buffalo School of Medicine, Buffalo, New York.

The epidermolysis of toxic epidermal necrolysis associated with *S. aureus* infection is believed to be due to the protein exotoxin, epidermolysin. In order to measure epidermolysin, a radioimmunoassay was established using highly purified epidermolysin and monospecific rabbit antibody. Epidermolysin, from the supernatant fluid of *S. aureus* cultures, was purified by zone electrophoresis in Pevikon, followed by carboxymethyl Sephadex C-50 chromatography. Following radioiodination of epidermolysin by the chloramine T method, ^{125}I was shown to be associated with epidermolysin by disc gel electrophoresis and radial immunodiffusion.

Separation of free from antibody bound ^{125}I-epidermolysin was accomplished using half-saturated neutral ammonium sulfate (SAS/2). Free toxin was soluble in rabbit serum upon

addition of SAS/2; whereas, toxin in hyperimmune rabbit serum was precipitated by SAS/2. The antigen binding capacity of the rabbit antibody to epidermolysin (ABC-33) at .017 μg. antigen N was 22.7 μg. The minimum detectable concentration of unlabeled toxin added to human serum was 20 ng/ml.

Specific antibody to epidermolysin was detectable in convalescent, but not acute, sera from patients with toxic epidermal necrolysis associated with *S. aureus* infection.

Subcellular Localization of Cutaneous Aryl Hydrocarbon Hydroxylase Activity. D. R. BICKERS, Dept. of Dermatology, College of Physicians and Surgeons, Columbia Univ., New York, N. Y.

Skin exposure to environmental pollutant chemicals particularly chlorinated hydrocarbons such as the polychlorinated biphenyls (PCB) has increased in recent years. PCB are potent inducers of hepatic drug-metabolizing enzymes and cytochrome P-450. Following cutaneous application increased levels of the carcinogen-metabolizing enzyme aryl hydrocarbon hydroxylase (AHH) and cytochrome P-450 are found in the liver and skin. Maximum specific enzyme activity in the liver has been found in the 100,000 x g pellet (microsomal fraction). No such studies have been done in skin. This study was designed to determine the localization of maximum specific cutaneous AHH activity. Neonatal rats received one application (100 mg/kg) of PCB (Aroclor 1254) to the skin of the back. Controls received solvent alone. AHH activity was measured in skin and liver of control and treated animals. A non-PCB-exposed skin site of the treated animals was also assayed. Enzyme activity was determined in whole homogenate, 9000 x g pellet and supernatant, and the 100,000 x g pellet and supernatant fractions of cutaneous and hepatic tissue. PCB-treatment caused greater than 10-fold enhancement of cutaneous and hepatic AHH. Maximum specific activity was found in the 100,000 x g skin and liver pellet. Substantially less AHH activity was found in non-PCB-exposed skin sites of treated animals. These studies indicate that inducible AHH in skin and liver is found primarily in the microsomal fraction of each tissue.

Organization and Ultrastructure of the Microcirculation in the Papillary Dermis of Normal and Psoriatic Skin. IRWIN M. BRAVERMAN AND AGNES YEN. Department of Dermatology, Yale University, New Haven, Connecticut.

This study was undertaken to establish the normal pattern of the microcirculation in human skin. Serial sections (1 to 2μ) of plastic embedded tissue with frequent thin sections were used to reconstruct and determine the organization and nature of the papillary dermal vessels. Normal skin as well as psoriatic plaques before and after Goeckerman therapy were studied. Arterioles were followed through the formation of capillaries to the venule stage in the superficial dermal plexus. The elastin which initially had a spiral arrangement in the wall became peripheral in location as the vessel became smaller and formed a layer external to the wall at the level of the arterial capillary. The basement membrane of arterioles was homogenous, and undisrupted unlike that of venous capillaries and postcapillary venules in which the basement membrane was often layered. In the normal papilla the capillary loop had features of the transitional stage between the arterial and venous capillary. Most of the loop was venous capillary with fenestrations. In the psoriatic papillae the loops were entirely venous capillaries with the inflow and outflow vessels arising from a venous plexus-analogous to a portal system. After Goeckerman therapy, the capillary loops revert to the normal pattern noted above. These changes in ultrastructure and organization probably reflect the importance of the venous capillary and postcapillary venules in the exchange of nutrients and metabolites and water absorption, as well as the reversible responses of small vessels to the changes in local blood flow as shown by studies using tissue chambers implanted into the skin of rabbit ears.

Characterization of Lymphocyte Inhibitory Factor in Lupus Erythematosus Plasma. R. EDELSON, F. FINKELMAN, A. STEINBERG, M. STRONG, AND I. GREEN, NCI, NIAID and NIAMD, NIH and Naval Medical Research Institute, Bethesda, Maryland.

To identify and characterize circulating inhibitors of lymphocyte function in patients with lupus erythematosus (LE), plasmas from 11 untreated patients were tested in mixed leukocyte cultures (MLC) in which normal lymphocytes from 2 unrelated individuals interacted. The proliferative response of lymphocytes from each normal donor to histocompatibility antigens on the mitomycin C blocked lymphocytes from the other donor was measured by tritiated thymidine incorporation. Nine of the 11 plasmas inhibited normal MLC reactivity by more than 50%. The active factor (s) was nondialyzable and eluted with the IgG fraction from Biogel A 1.5M columns by gel filtration. All activity could be removed from the LE plasmas by absorption with an equal volume of leukemic T cells which were selected because of their failure to stimulate normal lymphocytes in MLC. The inhibitory activity was only partially removed by extensive absorption with B cell lymphoblasts which themselves stimulated allogenic lymphocytes in MLC. The active fractions from 3 patients' plasmas failed to significantly suppress mitogenic response to phytohemagglutinin, suggesting that the inhibition was specific for MLC.

These results indicate that plasma from a substantial percentage of LE patients contains inhibitory material, most likely IgG, with specificity against membrane antigens on T cells. This inhibi-

tor(s) may interfere with T cell function by combining with a membrane receptor important in MLC triggering.

Urea-Soluble Polypeptides in Cornified Cells of Man and Rat. N. Inoue, K. Fukuyama, and W. L. Epstein, Department of Dermatology, University of California, San Francisco, California.

This study compares the polypeptide patterns of insoluble proteins obtained from cornified cells of man and rat by biochemical and immunological methods. Proteins were extracted with 8 M urea containing 0.1 M 2 mercaptoethanol from cornified cells of normal human palm and newborn rat skin. They were dialyzed against 5 mM Tris-HCl, pH 8.5 and precipitated by reducing the pH. The maximum precipitation occurred at pH 6.3 for rat (rat F 6.3) and pH 5.5 for human (man F 5.5). The precipitates were dissolved in 6 M urea and subjected to SDS gel electrophoresis. The major polypeptides of rat F 6.3 were M.W. 68,000 and 61,000; those of man F 5.5 were M.W. 66,000, 63,000 and 59,000. Injection of rat F 6.3 in rabbits produced separate antibodies; one of which reacted with rat protein M.W. 61,000. The other reactive protein of rat was not characterized (most probably to a minor band), but appeared not to be rat protein M.W. 68,000. When these antisera were tested against crude man F 5.5, a preciptin line formed corresponding to the unidentified protein of rat, but not to rat protein M.W. 61,000.

These findings indicate that distinct biochemical changes have occurred during evolution of insoluble proteins of cornified cells from rat to man, but immunologically extreme changes have not taken place. The sharing of antigenic determinants in man and rat may prove advantageous in immunochemical studies of human epidermal cells.

Histamine Inhibition of Human Lymphocyte Transformation. Henry E. Jones and William M. Artis, Department of Dermatology, University of Michigan, Ann Arbor, Michigan.

Histamine, injected along with the eliciting antigen, has been shown to be antagonistic to delayed-type cutaneous hypersensitivity (DH). Several mechanisms can be proposed to explain this observation. The most appealing is that histamine inhibits lymphocyte function via the second messenger adenosine-3', 5'-cyclic monophosphate (C-AMP). The purpose of this study was to investigate the effect of histamine on phytohemagglutinin (PHA) and antigen induced lymphocyte transformation measured by the incorporation of thymidine-^{3}H (T3H) into DNA. Histamine (10^{-2}-10^{-4} molar) inhibited the incorporation of T3H (97% at maximal and 30% at minimal concentrations) in PHA stimulated cultures. In like manner histamine inhibited PPD, trichophytin, and candidin induced T3H incorporation. There was no concomitant decrease in cell viability as measured by trypan blue dye exclusion. Metiamide (10^{-4}-10^{-6} molar), an antigonist of type 2 (H-2) histamine receptors, reversed the effect of histamine, thus suggesting that inhibition is dependent upon a membrane associated event. Incorporation of T3H was also inhibited by dibutryl C-AMP and isoproterenol. Theophylline (10^{-4}-10^{-6} molar) potentiated the inhibitory effect of histamine. This was interpreted to indicate the histamine-induced inhibition of lymphocyte transformation might be mediated through C-AMP. These data support the concept that histamine antagonized DH by direct inhibition of effector lymphocyte function.

In Vitro Metabolism of ^{3}H-Testosterone by Scalp and Back Skin. M. E. Karunakaran and P. E. Pochi, Department of Dermatology, Boston University School of Medicine, Boston, Massachusetts.

The in-vitro metabolism of ^{3}H-testosterone by human scalp and back skin was examined in a search for possible differences in enzyme activity in skin from these 2 areas, both of which contain large sebaceous glands, but only one of which, the back, is prone to develop acne.

Four-mm. punch biopsy specimens of skin were obtained from the scalp and back from each of 5 adult males. Each specimen was minced and incubated with ^{3}H-testosterone and cofactors at 37° for 90 min. In 3 cases the incubations were done in duplicate, using half of the biopsy specimen in each incubation. The metabolic products were diluted with carrier steroids and separated and measured by thin-layer chromatography and by gas chromatography performed on an instrument equipped with a splitter. Identification was based on retention times and, for 3β-androstanediol, on recrystallization to constant specific activity.

The results showed that of the 5α-reduced metabolites identified, the major ones in both scalp and back skin incubations were 3β-androstanediol and dihydrotestosterone. Scalp skin was more active in producing 5α-reduced metabolites, possibly reflecting a difference in the density of sebaceous glands in skin from the 2 sites. 3β-Androstanediol was the predominant 5α-reduced metabolite in 7 out of 8 of the scalp skin incubations and in 4 out of 8 of the back skin incubations. This finding that 3β-androstanediol is a major product of testosterone metabolism by human sebaceous gland-containing skin in vitro supports the possibility, previously suggested by studies which showed that the steroid can stimulate sebum secretion in the rat, that 3β-androstanediol is an active androgen in the sebaceous gland.

Thyroid Secretions and the Control of Periodic Epidermal Shedding in Snakes. Paul F. A. Maderson and K. W. Chiu, Department of Biology, Brooklyn College of C. U. N. Y., Brooklyn, New York.

An inhibitory role for thyroid gland secretions

on epidermal proliferation and differentiation is indicated by the fact that thyroidectomy will invariably eliminate the resting phase within approximately 14 days, and produce continual subsequent cycling through renewal phases. We have attempted to identify the active agent(s) by administering various dosages of a variety of iodo-amino acids (thyroxine-T_4, tri-iodothyronine-T_3, di-iodothyronine-T_2, di-iodotyrosine-DIT, mono-iodotyrosine-MIT) and L-thyronine to operated animals, have monitored their action by gross examination and biopsy of the skin, and compared these observations with appropriate intact and operated control animals. T_4, T_3 and T_2 restore the inhibition of epidermal activity, but L-thyronine, DIT and MIT have no effect. Histological examination suggests that patterns of epidermal cell activity in "accelerated" animals are quite normal except for the total elimination of the resting phase. Analyses of circulating iodo-compounds in snake blood indicate that MIT and DIT are invariably present in high quantities, but only a low level of T_4 is present, while T_3 and T_2 cannot be demonstrated. These apparently contradictory data emphasize the subtlety of the relationship between thyroid hormones and epidermal cell activity, and raise the possibility that normal epidermal cycling is controlled by differential patterns of thyroidal synthesis and/or release of the iodo-amino acids.

Octadecadienoic Acid in Human Skin Surface Lipid. ANN M. MORELLO, DONALD T. DOWNING, AND JOHN S. STRAUSS, Departments of Dermatology and Biochemistry, Boston University Medical Center, Boston, Massachusetts.

Previous workers claimed that the sebum of acne patients contained a *significantly higher proportion* of a constituent which we subsequently indentified as octadeca-5,8-dienoic acid (Δ5,8).

We have now developed a procedure for measurement of this compound and linoleic acid (Δ 9,12) in 2 mg. sebum samples. The fatty acid methyl esters formed by transesterification of a sebum sample are isolated by thin layer chromatography. The dienoic esters are then isolated on a silica gel-$AgNO_3$ plate and analyzed by quantitative gas chromatography. The overall recovery of the dienoic acids is measured by the recovery of [1-^{14}C] linolecic acid added to the original sebum sample. The analyses, carried out in triplicate, are summarized below.

	Acne	Non-Acne	Mature
Number of Subjects	5	3	3
Age range	18 – 27	19 – 24	38 – 48
%Δ5,8	0.61 ± 0.08	0.68 ± 0.21	0.73 ± 0.20
Range Δ5,8:	0.51 – 0.73	0.53 – 0.92	0.53 – 0.94
% Δ9,12	0.20 ± 0.05	0.52 ± 0.38	0.35 ± 0.20
Range Δ9,12:	0.14 – 0.26	0.27 – 0.96	0.16 – 0.56

The absolute amount of Δ9,12 was highly variable and because of the position of its emergence in gas chromatography of the total methyl esters this can influence the apparent amount of Δ5,8, the concentration of which was relatively constant in all of the sebum samples analyzed. For 10 normal subjects there was no correlation between the rate of sebum excretion and the ratio of Δ5,8 to Δ9,12.

Effects of the Spleen on the Immune Response (IMR) to Tumor Antigens. J. NORDLUND, A. ACKLES, AND R. K. GERSHON, Departments of Dermatology and Pathology, West Haven VA Hospital and Yale University, New Haven, Connecticut.

The intensity of the immune response to antigens varies with the dose of antigen which the animal receives. We studied the role of the spleen in this phenomenon. The Cloudman melanoma grown in tissue culture elicits an IMR when injected into syngeneic DBA/2 mice. In one experiment, groups of normal and splenectomized mice were injected subcutaneously with 8 x 10^5 or 3 x 10^6 tumor cells. The appearance of the tumor is detected by palpation of the injection site. Results showed that low doses of tumor cells developed more rapidly into tumor nodules in splenectomized mice than in normals. In contrast, high doses of cells appeared sooner in normal mice than in the splenectomized animals. In another experiment groups of DBA/2 mice were: 1) thymectomized and injected with ALS, or 2) splenectomized or 3) subjected to sham procedure. Some mice from each of the three groups received weekly intraperitoneal injections of extracts containing tumor antigens in high concentration or low concentration. Antigens for these injections were prepared from melanomas grown in immunodeficient DBA/2 mice and diluted 1:2 or 1:10. Controls received no antigens. All animals were injected with 4 x 10^5 tumor cells (low dose). We observed that the high dose of antigen was immunosuppressive in animals with spleens and the low dose was immunosuppressive in animals without spleens. Both sets of experiments suggest that the spleen enhances or supresses the IMR to tumor antigens depending on the quantity of antigen given. Spleen seeking T-cells could be responsible for this bidirectional response.

Junctional and Compound Pigmented Nevi Induced in Albino Guinea Pigs. A. PAWLOWSKI, H. F. HABERMAN, AND I. A. MENON, Department of Medicine (Dermatology), University of Toronto, Toronto, Canada.

A model for the production of nevi in albino guinea pigs was established. 70 albino Hartley guinea pigs were used in the experiment to investigate the influence of 9,10 dimethyl-1,2 benzanthracene (DMBA) on amelanotic melanocytes. On a flank of each animal the field 5 sq. cm was marked. Hair from these fields were clipped twice a week during the whole experiment and painted for 20 consecutive weeks with 0.3ml of 1% DMBA in acetone.

Initial pigmented spots appeared 8 months after the first painting. These spots varying in number from 1 to over 200 appeared in 40 animals during the one-year observation period. Biopsies were taken under local anesthesia and sections were prepared for light microscopic studies (using staining with hematoxylin and eosin, DOPA, silver and PAS) and electron microscopic observations. Isolated melanocytes actively producing melanin as well as focal collections of melanocytes or nevus cells were found in the epidermis and in the dermis. The appearance of these melanotic melanocytes with varying number of functioning melanosomes was observed in serial sections. These sections also revealed various stages of nevus development (i.e. junctional, compound, etc). Therefore, a suitable animal model both for the study of the histogenesis of pigmented nevi including those with junctional activity along with a model showing the "transformation" of amelanotic melanocytes to melanotic melanocytes has been developed.

Marked Inhibition of Sebaceous Gland Secretion from Combined Glucocorticoid-Estrogen Treatment. PETER E. POCHI AND JOHN S. STRAUSS, Department of Dermatology, Boston University School of Medicine, Boston, Massachussetts.

Sebaceous gland secretion is acknowledged to be an important factor in the pathogenesis of acne vulgaris, and significant inhibition of glandular activity can prove to be clinically beneficial.

Sebaceous gland activity was studied in 5 women, four with hirsutism and one with acne. They were treated for these disorders initially with prednisone (P) 5-10 mg daily for varying periods, followed by the concomitant daily administration of 80-100 ug of ethinyl estradiol (E) given in 21-day cycles. Duration of estrogen treatment ranged from 2-15 months. Sebum production from the forehead was measured by the quantitative recovery of surface sebum delivered in a 3-hour period. Tests were performed in each individual 2 to 4 times before treatment and at various intervals thereafter during drug administration.

The results were as follows:

Patient	Sebum Production (mg/10 sq cm/3 hr)		
	Pre-treatment	P	P+E
1	2.97	2.05	0.87
2	3.17	2.71	0.64
3	2.21	1.23	0.46
4	3.62	2.47	0.37
5	3.52	0.96	0.35
MEAN	3.10	1.88	0.54

Combined estrogen and low-dose glucocorticoid administration led to marked suppression of sebaceous gland secretion—an average 83% reduction. These low sebum levels were attained 1-5 months after estrogen was begun.

Quantitation of Bacterial Flora Within Isolated Sebaceous Follicles from Normal Skin. S. M. PUHVEL, R. M. REISNER, AND D. AMIRIAN, Division of Dermatology, UCLA, Los Angeles, California.

Quantitation of the bacteria on human skin is complicated by the presence of sebaceous follicles. Previous studies have been limited to the quantitation of surface flora. The present study is a quantitative analysis of the flora whithin isolated sebaceous follicles. The techniques for such an analysis were made feasible by the discovery that 1 M $CaCl_2$ solution has no adverse effect on the survival of cutaneous staphylococci and propionibacteria *in vitro*. Therefore the method of pretreating skin with 1 M $CaCl_2$ solution (Kellum 1966) was used for the isolation of sebaceous follicles and hair follicles. Isolated sebaceous follicles were homogenized and quantitatively cultured. Surface flora of skin from areas adjacent to the biopsies was quantitatively cultured using Williamson's buffer scrub procedure.

Results from studies of 28 biopsies from the backs of 14 normal volunteers, 66 biopsies of scalp from 11 hair transplant patients, and 24 open comedones from 8 volunteers, indicated that: 1. The anaerobic flora of isolated follicles in upper back skin (average = 3.37×10^6 organisms/follicle) is similar to the anaerobic count in isolated comedones. The aerobic count in isolated follicles (average = 60 organisms/follicle) is in sharp contrast to the aerobic count in isolated comedones (average = 8.5×10^5/comedo). Thus in comedones, the aerobic, rather than the anaerobic flora, is markedly increased. 2. There is a correlation between the weight of the sebaceous glands and the density of anaerobic organisms within the corresponding follicles (coeff. of correlation = 0.6). 3. The follicular anaerobic flora is 10 to 46 times the adjacent anaerobic surface flora.

Nonspecific Impairment of Cell-Mediated Immunity (CMI) in Disseminated Coccidioidomycosis (DC). T.H. REA, H. EINSTEIN, AND N.E. LEVAN, Sections of Dermatology and Pulmonary Disease, Univeristy of Southern California School of Medicine, Los Angeles, California.

To further study the problem of whether the nonspecific deficiency of CMI observed in patients with disseminated granulomatous infections is a cause or a result of dissemination, 19 patients with active DC and 20 controls were subject to dinitrochlorobenzene (DNCB) sensitization.

A 2 mg sensitizing dose and 0.1, 0.05 and 0.025 mg challenges were used. Specific complement-fixing (CF) antibodies and skin test responses were measured in DC patients.

Response to DNCB in percent (p-value compared with control):

Challenge Dose	Controls	All DC (19) Patients	DC (7) with CF Titer 1/32 or less	DC (12) with CF Titer 1/64 or more
0.1	85	44	83 (1.0)	25 (0.002)
0.05	60	22	67 (1.0)	0 (0.002)
0.025	50	11	33 (0.6)	0 (0.002)

Since CF titer is probably a reflection of extent of dissemination and/or antigen load, the statically significant DNCB unresponsiveness seen only in high CF titer DC patients is probably a reflection of extent of dissemination and/or antigen load, and neither a cause of nor a necessary consequence of dissemination.

By implication the nonspecific impairment of CMI observed in other disseminated granulomatous infections is neither a cause of nor a necessary consequence of dissemination, but perhaps a result of antigen excess.

The Reliability of the Indirect Immunofluorescent Test For Pemphigus and Bullous Pemphigoid. JOSE RODRIGUEZ AND JEAN-CLAUDE BYSTRYN, Department of Dermatology, New York University School of Medicine, New York, New York.

This study was conducted to determine the reliability of the indirect immunofluorescent (IF) test in the diagnosis of pemphigus and bullous pemphigoid.

Clinical diagnoses were obtained in 517 consecutive sera submitted for routine determination of anti-skin antibodies by indirect immunofluorescence. Patients with bullous disease were included in the analysis only if their diagnosis was unequivocal by clinical, histological and therapeutic criteria. Diagnoses were available and satisfied these criteria in 363 (70%) sera obtained from 209 patients. Intercellular (IC) antibodies were found on at least one occasion in 51 of 64 (80%) patients with pemphigus and in 6% of 145 patients with unrelated dermatoses. The incidence of false positive reactions, determined from the incidence of IC antibodies in sera of patients without pemphigus (11 of 185 sera) was 5.9%. Basement zone (BMZ) antibodies were found on at least one occasion in 27 of 30 (90%) patients with bullous pemphigoid and in 3% of 179 patients with unrelated dermatoses. The incidence of false positive reactions for BMZ antibodies in patients without pemphigoid (10 of 305 sera) was 3.3%. Titers of IC and BMZ antibodies in patients without pemphigus or bullous pemphigoid were usually low. There was a correlation between IC antibody titer and activity of pemphigus but not between the titer of BMZ antibodies and severity of bullous pemphigoid.

Thus, the detection of IC and BMZ antibodies is a sensitive but not completely specific test for pemphigus and bullous pemphigoid.

Solubility of Organic Molecules in the Lipids of the Stratum Corneum. ROBERT J. SCHEUPLEIN AND PAUL N. KING, Department of Dermatology, Harvard Medical School, Boston, Massachusetts.

The relatively larger skin permeability of lipid soluble molecules originates from their great affinity for the lipids of the stratum corneum. It was the purpose of this study to directly measure the extent and the strength of this affinity by using isolated stratum corneum (s.c.) lipids per se as the substrate for sorption.

Measured extracts (in 2:1 chloroform:methanol) of s.c. lipids were spread onto shallow sorption trays and permitted to evaporate into firm, coherent lipid-films approximately 10^{-4} cm thick. Known volumes of dilute aqueous solutions of tagged organic molecules were applied and the amount of uptake by the film was measured by analysis of both the solution and the lipid-film. Heats of reaction were computed from the temperature dependence of the measured solubilities.

These lipid-films were found to be stable to both acidic and neutral aqueous solutions and to moderately concentrated alcohol-water solutions. Aqueous solutions of pH 8 or greater dispersed the film presumably because of the diminished lateral cohesion resulting from ionization of the film fatty acids. Lipid-film-solubilities of homologous series of alcohols, fatty acids and steroids were uniformly many times greater than the corresponding values measured in whole stratum corneum. Heats of reaction in the two cases were identical as was the relative solubility ranking within a given series. These data provide evidence that s.c. lipids, whether in a condensed film or within the tissue, act in much the same way. The technique offers promise as an accessible, inexpensive and rapid method of predicting the relative rates of percutaneous absorption of lipid soluble molecules.

Lymphomatoid Papulosis: Immunologic and Ultrastructural Studies. P. SCHNEIDERMAN, R. EDELSON, M. LUTZNER, M. GUILLINO, AND I. GREEN, NCI and NIAID, NIH, Bethesda, Maryland.

To determine the relationship of lymphomatoid papulosis to other cutaneous lymphocytic infiltrative processes, ultrastructural and immunoreceptor studies were performed on involved skin from 5 patients with lymphomatoid papulosis. In addition to electron microscopic (EM) examination, tissue was studied using a method (J. Invest. Derm. 61:82 1973) by which B (bone-marrow derived) lymphocytes can be identified in tissue section by their receptors for C3 (third component of complement) and histiocytes by their receptors for IgG. T (thymus-derived) lymphocytes can be identified after extraction from tissue biopsies by their receptors for sheep erythrocytes (E). The infiltrating cells in the lesions from each patient with lymphomatoid papulosis lacked receptors characteristic of B cells or histiocytes. The infiltrating lymphocytes

obtained from biopsies from 2 of 2 patients studied bound E, thus establishing their T cell identity. In EM sections of lesions from all 5 patients, large numbers of infiltrating cells with convoluted nuclei were observed. These infiltrating cells morphologically resembled those seen in lesions of mycosis fungoides and the Sezary syndrome. In one patient who simultaneously had lesions of lymphomatoid papulosis and mycosis fungoides, the morphologic characteristics of the infiltrating cells in the 2 separate types of lesions were indistinguishable.

These data suggest that there are morphologic and immunologic similarities between the infiltrating cells of lymphomatoid papulosis and those of the cutaneous T cell lymphomas. Thus, lymphomatoid papulosis may be a part of the spectrum of the cutaneous T cell lymphomas.

The Heterogeneity of Bovine Epidermal α-Keratin. PETER M. STEINERT, Dermatology Branch, National Cancer Institute, N.I.H., Bethesda, Maryland.

The α-fibrous protein of bovine epidermis has been shown to consist of at least two different polypeptide chains by polyacrylamide gel electrophoresis (PAGE) using sodium dodecylsulfate (SDS) with a continuous buffer system. In order to explore the subunit structure of epidermal α-keratin further, protein extracted from bovine snout and hoof epidermis with urea buffers, and prekeratin extracted with sodium citrate buffer from the same tissues, were examined on gels with discontinuous buffers containing SDS. The same band pattern of seven different polypeptide chains was found in all cases. The increased number of bands was not due to electrophoretic artefacts or random proteolytic degradation, but was due to improved resolution effected by the isotachophoretic property of the discontinuous buffer systems.

Each of the polypeptide chains was isolated by a combination of DEAE-cellulose and preparative PAGE and individually characterized. All of the polypeptide chains have generally similar characteristics, but significant differences were evident. However, on the basis of close similarities in such properties as amino acid compositions, α-helix contents and amino- and carboxyl-terminal amino acid residues, it is possible to classify the polypeptide chains into three distinct groups of molecular weights of about 58,000, 52-54,000 and 48,000 daltons.

The evidence favors the view that bovine epidermal α-keratin consists of a heterogeneous group of polypeptide chains, as has been found for the fibrous keratin proteins of wool, hair and feather.

In Vivo Effects of Lymphokines: An Ultrastructural Study. E.G. THORNE, I. KOLSCH, N. SCHWARTZMAN, AND W.L. WESTON, Division of Dermatology, Veterans Administration Hospital and University of Colorado Medical Center, Denver, Colorado.

An intradermal injection of lymphokines (LK) (soluble mediators of delayed hypersenitivity liberated by lymphocytes after culturing with antigen or mitogen) causes a mononuclear cell infiltrate, which morphologically simulates delayed hypersensitivity. In order to further characterize the mononuclear infiltrate, an ultrastructural study was undertaken. We obtained LK from guinea pig peritoneal exudate cells (PEC), by culturing the PEC with either Con A or PPD. The mediator-rich supernatants were then injected into female Hartley strain guinea pigs. Biopsies obtained from the injection sites were processed for light and electron microscopy. Phosphate-buffered saline, negative supernatants, and Con A were injected in a similar manner and served as controls. A total of 32 biopsies were examined. The ultrastructural findings indicate that LK injected intradermally produce activation of monocytes, as judged by their numerous microvilli and the formation of aggregates. A similar aggregation of mononuclear cells was also seen in biopsies from positive mumps and Candida skin tests. Additionally, aggregation has been seen after incubation of guinea pig PEC with LK. Similarities between the in vitro and in vivo assays suggest a functional role for LK in the production of delayed hypersensitivity.

Lymphocyte Abnormalities in the Small Cell Variant of Sezary Syndrome. CORWIN VANCE, ANDREJ SABAD, JOHN KERSEY, ROBERT GOLTZ, WILLIAM GENTRY, AND JAROSLAV CERVENKA, Departments of Dermatology, Laboratory Medicine & Pathology, and Oral Pathology, University of Minnesota, Minneapolis, Minnesota.

A case of the small cell variant of Sezary Syndrome, with the characteristic lymphocytes identified in the skin and peripheral blood was studied before treatment. His WBC was normal, and the Sezary cells comprised about 50% of the lymphocytes. The clinical course of ten years has been relatively benign. Peripheral blood was cultured for 24 hours without stimulation as well as for 72 hours with phytohemagglutinin (PHA). Many mitoses were identified in the unstimulated 24 hour cultures. 32 to 38 metaphases recovered from the 24 hour cultures had 46 chromosomes which morphologically appeared normal using nonbanded preparations. Banding studies are in progress. In stimulated 72 hour cultures, mitoses were 68% hypodiploid (40 to 45 chromosomes). Banding studies revealed abnormal marker chromosomes in most of the PHA stimulated metaphases. The patient was anergic to multiple skin test antigens in vivo, and demonstrated marked depression of his in vitro lymphocyte response to PHA and Concanavalin A; response to Pokeweed mitogen and allogeneic lymphocytes was normal. The percentage of circulating immunoglobulin staining and third component of complement (C'3) receptor carrying (B) lymphocytes was normal. Spontaneous sheep RBC binding and Human

Thymic Lymphocyte Antigen (HTLA) positive (T) lymphocytes were low normal to depressed. A spontaneously dividing diploid lymphocyte population in unstimulated 24 hour cultures appears to be a new finding in Sezary Syndrome. The benign clinical course may possibly be related to partial maintenance of his cell-mediated immunity.

A New Rapid and Easy Technique of Extracting DNA From the Epidermis and Its Application To An Animal Model For Screening Drugs For Potential Use In The Treatment of Psoriasis. A. du Vivier and R. B. Stoughton, Division of Dermatology, Scripps Clinic and Research Foundation, La Jolla, California.

A new hairless mouse model of psoriasis using UVL to stimulate increased epidermal cell turnover has been designed so that drugs may be tested both topically and systemically for possible therapeutic value in this disease. Two methods of determining drug activity have been used, the mitotic index and a new procedure for determining the incorporation of radioactive thymidine into DNA. The latter involves hydroxyapatite, a compound which has an unique property of high affinity for DNA but not for RNA and proteins. Thus DNA may be extracted from the epidermis by loading crude epidermal cell lysate onto a hydroxyapatite column, washing the RNA and proteins through with a low salt buffer whilst the DNA remains bound until eluted with a high salt buffer. The DNA is measured quantitatively in a spectrophotometer and then the radioactivity of the sample is determined in a liquid scintillation counter. Results show that 2% 5-fluorouracil, 2% nitrogen mustard and Lidex cream topically and cyclophosphamide systemically significantly reduce the mitotic index. The effect of the topical agents is shown to be local and not systemic. DNA synthesis for the latter 3 drugs is similarly decreased. The DNA extraction procedure is very quick, accurate and simple and results may be obtained from 20 samples of skin within 5 hours. The technique is being used to screen a large number of compounds for topical and systemic antiproliferative properties in the epidermis.

MORNING SESSION
Saturday, May 3, 1975, 9 A.M.

BUSINESS AND EXECUTIVE SESSION
Clayton E. Wheeler, Jr., M.D., Chapel Hill, North Carolina, Presiding

SCIENTIFIC SESSION
John A. Kenney, Jr., Washington, D.C., Presiding

Biochemical Aspects of a Protein Which Restores Contact Inhibition of Growth to Malignant Melanocytes. Margarete E. Knecht and George Lipkin, Dept. of Dermatology, New York University Medical Center, New York, N. Y.

A distinctive protein was identified in conditioned medium of contact-inhibited hamster melanocytes by polyacrylamide gel electrophoresis, and isolated on a Sephadex G-200 column. This protein restores one aspect of in vitro growth control to malignant melanocytes, i.e., contact inhibition of growth. It is a glycoprotein of molecular weight over 180,000 and is destroyed by pronase treatment. Partial purification of the protein has been accomplished on ion exchange columns and on slab gels. Cell fractionation on discontinuous sucrose gradients revealed it to be present both in plasma and ER-membranes. To examine the outer surface of contact- and non-contact-inhibited hamster melnocytes for the presence of this melanocyte contact inhibitory factor (MCIF), the cell surface was labeled with ^{125}iodine. Autoradiographs showed distinct differences between the surface proteins of the cell lines. MCIF is present on the outer surface of contact-inhibited melanocytes, but in non-contact-inhibited cells is replaced by several bands in this region. Examination of proteins from various culture media and sera on polyacrylamide gels discloses a protein with identical migration properties to MCIF in media of contact-inhibited human and 3T3 mouse fibroblasts, but not in SV40-transformed 3T3 fibroblasts. This band is also found in sera of hamster and porcine origin. MCIF, a component of the melanocyte surface required for in vitro growth control, may be biochemically and/or functionally related to analogous surface proteins of other normal cell types.

Contact Inhibition of Growth: Restoration In Malignant Melanocytes of Man, Mouse, and Hamster. George Lipkin and Margarete E. Knecht, Department of Dermatology, New York University Medical Center, New York, New York.

A previous report described the isolation, from cultures of contact-inhibited hamster melanocytes, of a protein which restores contact inhibition of growth to hamster malignant melanocytes [PNAS(USA) *71*:849–853,1974]. The present study examined the species specificity of this effect. Hamster melanocyte contact-inhibitory factor (MCIF) from conditioned media of contact-inhibited cultures was identified on analytic polyacrylamide gels, and separated on Sephadex G-200. MCIF (60 μg/ml) was added to freshly plated subconfluent cultures of established lines of hamster malignant melanoma (RPMI 1846), mouse B16 melanoma, and 4 human melanomas. Whereas control cultures, lacking MCIF, exhibited at confluence the usual disorientation, overgrowth and polymorphism characteristic of neoplastic cells in vitro, all cultures treated with MCIF were oriented, flat, and fibroblast-like in appearance. Saturation densities of treated cultures were 41–84% lower than in controls, with no loss of viability. These effects were reversible less than 24 hours after removal of MCIF, and could be prevented by pre-treatment of

MCIF with Pronase, but not DNase. The estimated molecular weight (>180,000) and kinetics of mitotic inhibition distinguish MCIF from cAMP and chalone. Its effects transcend species barriers. It appears to stabilize cell contacts, facilitating feedback inhibition of growth. MCIF is the first mammalian cell protein identified which restores contact inhibition of growth to malignant melanocytes, and may be the prototype for a class of closely related, surface-associated proteins concerned with regulation of normal cell-cell interactions.

SPECIAL LECTURE

Molecular Features of Glycoproteins in Cell Membranes. VINCENT T. MARCHESI, New Haven, Connecticut.

Dermal Collagen Grafts. R. F. OLIVER, R. A. GRANT, M. J. HULME, AND A. MUDIE, Department of Biological Sciences, University of Dundee, Dundee, Scotland.

Since mature collagen has apparently low antigenicity there exists the possibility that dermal collagen allografts would not elicit an immune response. Accordingly whole rat skin was treated with a solution of crystalline trypsin at 15°C to remove all but the collagen (and elastin) components. Subcutaneous implants of such purified dermal collagen allografts became recellularised and revascularised without lymphocytic infiltration. The original collagen bundle architecture was also preserved, especially when further treated before grafting with low concentrations of glutaraldehyde. This procedure has been shown to render dermal collagen resistant to bacterial collagenase. Dermal collagen transplanted into full thickness loss skin wounds with conventional dressings became desiccated and was lost. However when the grafted collagen was covered with a primary dressing of dermal collagen, on removal of the dressings at 4 weeks the grafts were not only recellularised but were also reepithelialised with recipient epidermis. At this time wound contraction was also virtually suppressed. Even grafts of human collagen, although in this case associated with lymphocytic infiltration, became reepithelialised with rat epidermis.

It might prove possible, therefore, that purified dermal collagen, with an appropriate dressing, could be used to replace lost or damaged skin in humans as well as animals.

^{3}H-Thymidine Incorporation by Endothelial Cells From Scleroderma Capillaries. R. FLEISCHMAJER, J. S. PERLISH, R. E. STEPHENS, AND R. I. BASHEY, Division of Dermatology, Hahnemann Medical College, Philadelphia, Pennsylvania.

The purpose of this investigation was to ascertain whether endothelial cell proliferation occurs during the active stage of systemic scleroderma. Skin biopsies, usually from forearms, were performed in 5 active scleroderma patients and 8 normal controls. The specimens, about 1mm thick, were incubated in MEM with 2 × BME vitamins at 37°C in an atmosphere of 5% CO_2 and 95% O_2. Following a 2 hr. pulse with ^{3}H-thymidine (10μCi/ml), the specimens were fixed in formalin, serially sectioned, dipped in Kodak NTB-2 Nuclear Track Emulsion and processed in Kodak D-19. Endothelial cells around capillary lumens were counted in the papillary and subpapillary capillary plexus. Epidermal germinative cells were also counted and the labeling index determined.

	Epidermis		Endothelial Cells	
	Germinative cells	Labeling index	Total count	Labeling index
Scleroderma (5)	8,973	10.90 ± 1.26*	1,083	8.18 ± 0.71*
Control (8)	15,235	6.55 ± 0.30*	1,219	0.18 ± 0.13*

* Standard error of the mean.

During the active stage of scleroderma there is (a) an increase in epidermal ^{3}H-thymidine labeling, suggesting increased epidermal cell-turnover and (b) a proliferative process involving endothelial cells from capillaries, which may represent the early event of the disease, thus, preceding the stage of fibrosis.

Promotion of Ultraviolet (UV) Carcinogenesis by Topical Vitamin A Acid Applications. JOHN H. EPSTEIN, Department of Dermatology, University of California, San Francisco, California.

The present study examined the effects of topical vitamin A acid on UV induced cancer formation. Sixty-nine, 2-month-old, albino hairless mice (UC strain) were divided into 2 groups. Group I (38 mice) received topical applications of vitamin A acid (0.3%) cream, 0.5 grams to the right and left flanks, 3 times a week for 4½ months. Group II (31 mice) received identical applications of the cream base for 4½ months. The right flanks received 1.38 × 10^{7} ergs/cm^{2} of UV energy between 280 and 320 nm 3 times a week from a hot quartz contact source for 10 months. Tumors greater than 4 cu.mm., 50 cu.mm. and 100 cu.mm. were tabulated weekly. Autopsies were performed when feasible. No specific difference in tumor onset was noted between Group I and II. The tumor incidence and growth was significantly more notable in Group I than Group II. By 31 to 32 weeks the 4 cu.mm. tumor difference was significant (P value of 0.015), the 50 cu.mm. tumor difference had a P value of less than 0.01 and the 100 cu.mm. tumor difference had a P value of less than 0.05. No tumors occurred on the skin receiving the vitamin A acid or placebo alone. The tumors greater than 50 cu.mm. that we examined were squamous cell carcinomas. Thus

under the circumstances of this study, vitamin A acid was not carcinogenic but did promote UV induced cancer formation. This effect may have been related to irritant effects. However, preliminary autoradiographic studies indicate that vitamin A acid can inhibit post-UV repair replication. It is possible that vitamin A acid may influence UV induced tumor formation at a more basic cellular level.

AFTERNOON SESSION
Saturday, May 3, 1975, 2:00 P.M.

FIFTEENTH ANNUAL HERMAN BEERMAN LECTURE
Clayton E. Wheeler, Jr., Chapel Hill, North Carolina, Presiding

Interactions Between T and B Lymphocytes in Immune Responses. DAVID H. KATZ, Dept. of Pathology, Harvard Medical School, Boston, Massachusetts.

SCIENTIFIC SESSION
Ruth K. Freinkel, Chicago, Illinois, Presiding

Epidermal Ribosome Increases in the Early Phases of Two-Stage Mouse Epidermal Tumorigenesis. LAWRENCE M. DE YOUNG, THOMAS S. ARGYRIS AND GERALD B. GORDON, Department of Biology, Syracuse University, Syracuse, New York, and Department of Pathology, Upstate Medical Center, Syracuse, New York.

Ribosomes were isolated from the post-nuclear supernatants of normal, initiated (7,12-dimethylbenz(a)anthracene-DMBA), and initiated and promoted (12-0-tetradecanoyl phorbol-13-acetate-TPA) trypsinized dorsal epidermis of female CD-1 mice. Normal epidermis contains 0.541 mg rRNA/Gm epidermis or 0.125 mg rRNA/mg DNA. One week after initiation with DMBA when the epidermis appears normal, rRNA increases 42%/Gm epidermis and 64%/mg DNA. Forty-eight hours after one application of the promotor TPA to the backs of initiated mice the epidermis is hyperplastic and rRNA increases 121%/Gm epidermis and 157%/mg DNA over normal levels.

To estimate the total amount of ribosomes on the back of a mouse and to indicate the role of hyperplasia and hypertrophy, a technique was developed which measures the rRNA and epidermal mass overlying a standard area of 100 cm^2 dermis. In normal animals the rRNA overlying this standard area is 0.88 mg and the mass of epidermis is 0.165 Gm. In initiated skin total rRNA increases 78% and epidermal mass 27%. In epidermis initiated and promoted total rRNA increases 390% while epidermal mass increases 119%. These findings indicate that at each stage of epidermal tumorigenesis, i.e., initiation and promotion, there is an increase in epidermal ribosomes with respect to tissue concentration, cellular content, and total amount on the back of a mouse.

Bovine Epidermal and Hair Follicle Transglutaminases: Isoenzymes. MELODIE M. BUXMAN AND KIRK D. WUEPPER, Dept. of Dermatology, Univ. of Oregon Health Sciences Ctr., Portland, Oregon.

Epidermal (ET) and hair follicle (HFT) transglutaminases were compared biochemically and immunochemically, and localized in keratinizing tissues. HFT, isolated from hair follicle homogenates, was similar in charge, molecular weight (55,000) and calcium and sulfhydryl dependence to epidermal transglutaminase. The K_m for the substrate analog dansyl cadaverine (DC) was 1.5×10^{-4} (ET 1.2×10^{-4}). In contrast to its behavior with ET, antiserum to ET did not precipitate with HFT in agar nor did it inhibit crosslinking of fibrin or incorporation of DC into casein by HFT. Upon incubation of 5μ frozen epidermal sections with 2 mM DC-10 mM calcium, the fluorescent amine was bound in the cytoplasm of spinous and granular cells of the epidermis and in Huxley and Henle layers of hair follicles. The reaction was obrogated by EDTA, EGTA, and p-chloromercuribenzoate.

Fluorescein-conjugated antiserum to ET, applied to freeze-dried, paraformaldehyde vapor-fixed thin tissue sections was also used to localize the antigen. Fluorescent label was seen in upper spinous and granular cells but did not bind to inner root sheaths. Controls, prepared with nonimmune sera or antibody absorbed with ET were negative.

HFT and ET are biochemically similar but antigenically different isoenzymes.

Molecular Basis of Tyrosine-Induced Epithelial Damage. L. A. GOLDSMITH, Department of Medicine, Duke University, Durham, North Carolina.

Human tyrosinemia is a genetic disorder resulting from a deficiency of soluble tyrosine aminotransferase. Tyrosine levels greater than 40 mg/dl result. A metabolic state similar to the human disease results from feeding rats a 20% tyrosine diet. The dominant feature of the human disease and the animal model are inflammatory lesions limited to volar epidermis and corneal epithelium. These lesions histologically are characterized by very dense infiltrates of polymorphonuclear cells. It is proposed that the initial pathological event producing the lesions is lysosomal lysis induced by tyrosine.

Membrane lysis induced by tyrosine was assayed with human red cells in isotonic buffers. In this system tyrosine crystals caused hemolysis in a dose-response fashion. Kinetics of hemolysis was similar at 25° and 37°. Hemolysis was inhibited 70% by .25M sucrose, 66% by .25M glucose, 62% by 1.34 mM prednisolone, and completely with 5% serum. In preliminary experiments, incubation of mouse liver lysosomes suspended in isotonic buffered saline with tyrosine crystals resulted in a 47% increase in β-glucuronidase release which was inhibited in .25M sucrose. The behavior of tyrosine on red cell and lysosomal membranes is similar to

other membraneolytic agents which possess donor phenolic hydrogens.

It is proposed that in corneal and volar epithelium tyrosine, free of serum and other protective agents, lyses lysosomes releasing proteases and chemotactic factors which then attract polymorphonuclear cells initiating clinical inflammation.

Formation of a Melanin–Tyrosinase Complex in Vitro and Its Possible Significance as a Model for Melanosomes. I. A. MENON AND H. F. HABERMAN, Section of Dermatology, Clinical Science Division, Medical Sciences Building, University of Toronto, Toronto, Canada.

Melanosomes isolated from normal melanocytes and melanomas are known to contain melanin and tyrosinase as well as a few other enzymes. The tyrosinase is present in the melanosomes in a partially latent state and can be activated by a variety of physical and chemical procedures, such as treatment with sodium deoxycholate (DOC), p-chloromercuribenzoate (PCMB), Triton X-100, sonication, etc. The possibility of obtaining a particulate complex consisting of melanin and tyrosinase resembling melanosomes was investigated. Melanin was synthesized by the action of purified mushroom tyrosinase on L-3, 4-dihydroxyphenylalanine (dopa). Tyrosinase activity was determined by measuring the formation of tritiated water from tyrosine-3,5-^{3}H. When the melanin was incubated at 37° for 2 hours the tyrosinase activity of the suspension was decreased. The melanin sedimenting from this mixture has detectable tyrosinase activity. Treatment with DOC or Triton X-100 or sonication of this sediment enhanced the tyrosinase activity. Treatment with PCMB had no significant effect on the tyrosinase activity. These results indicate that tyrosinase forms a complex with melanin. As in melanosomes, the tyrosinase in this complex is present in a partially inactive state and can be activated by some of the procedures which increase the tyrosinase activity of melanosomes.

Response of Human Sebaceous Glands to Testosterone. GAIL SANSONE-BAZZANO, RONALD M. REISNER, KEITH SEELER, AND GAETANO BAZZANO, Department of Medicine, Division of Dermatology, U.C.L.A., Los Angeles, California, and St. Louis City Hospital, St. Louis, Missouri.

In order to study the specific response of human sebaceous glands to androgen stimulation an in vivo technique was utilized. Two baseline biopsies were taken from the upper lateral scapular region of volunteer adult females. Biopsies were subsequently taken from matching contralateral sites which had been intradermally injected with testosterone 2 mg/day for 3 to 5 days. The biopsies were incubated with testosterone-1,2-^{3}H and glucose-6-^{14}C. Following incubation the sebaceous glands were isolated and separately analysed for testosterone uptake and triglyceride synthesis. Testosterone administration caused a 50 to 200 per cent increase in the uptake of radioactive testosterone in sebaceous glands and dermis. Synthesis of triglyceride in the sebaceous glands and dermis was stimulated up to 5 fold. The label from glucose-6-^{14}C was found mainly in the glycerol portion of the triglycerides. Administration of exogenous testosterone resulted in an increase in the uptake of testosterone-^{3}H by sebaceous glands and dermis and this was positively correlated with an increase in triglyceride synthesis.

Characterization of Heart Valve Collagen. D. COLLINS, B. MCLEES, AND S. R. PINNELL, Department of Medicine, Duke University, Durham, North Carolina.

Heart valves are involved in a wide variety of unique pathologic processes including inflammation and dysfunction secondary to connective tissue abnormalities. The heart valve is a leaflet containing predominantly collagen, with considerably lesser amounts of elastin and mucopolysaccharide. We report the first isolation and biochemical characterization of heart valve collagen.

Pig atrioventricular valves were found to contain 71% collagen per unit dry weight based on hydroxyproline assay. Pig heart valve collagen was insoluble to extraction with salt, acid and denaturing solvents but could be extracted following pepsin digestion at 11°. Pepsin solubilized collagen was chromatographed on carboxymethyl cellulose in 8M urea at 23° and on 6% agarose in 2M guanidine into α_1 and α_2 chains in a ratio of 2:1. The patterns of chains and their corresponding cyanogen bromide peptides on SDS polyacrylamide disc gel electrophoresis were similar to authentic Type I collagen chains. Amino acid analyses of α_1 and α_2 chains revealed a twice elevated hydroxylysine content of 8.4 and 13.6 Residues/1000 Amino Acid Residues respectively. In addition, the carbohydrate content of each chain was 5-10 times greater than previously reported Type I collagens: α_1 (4.9%) and α_2 (3.9%). Heart valves are uniquely subject to continuous, repetitive sheer and impact forces. Consequently, a Type I collagen molecule with specialized modifications may have evolved to meet these unique environmental stresses. These new techniques may be useful in the further definition of collagen in normal and pathologic heart valves.

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