PROGRAM

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

Pick-Congress Hotel
Chicago, Illinois

Friday, Saturday and Sunday, June 21, 22, and 23, 1974

OFFICERS

RICHARD B. STOUGHTON, M.D., La Jolla, California, President
CLAYTON E. WHEELER, JR., M.D., Chapel Hill, North Carolina, President-Elect
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MORNING SESSION

FRIDAY, JUNE 21, 1974

7:30-9:45 A.M., THE FIFTH IRVIN H. BLANK RESIDENT-FELLOW FORUM (open only to residents and fellows)

TOPIC: CUTANEOUS VIROLOGY, HARVEY BLANK, M.D., Miami, Florida, moderator

FRANCIS E. PAYNE, M.D., Ann Arbor, Michigan, Macromolecular Aspects of Viruses

BERNARD ROIZMAN, Sc.D., Chicago, Illinois, Biology of Herpes Simplex

FRANKLIN PASS, M.D., Minneapolis, Minnesota, Wart Biology and Current Therapy

PHILLIP A. BRUNELL, M.D., New York, New York, Varicella-Zoster: A Latent Virus Infection

8:30 A.M. BUSINESS AND EXECUTIVE SESSION: RICHARD B. STOUGHTON, M.D., La Jolla, California, presiding.

10:00 A.M. SCIENTIFIC SESSION: ISADORE A. BERNSTEIN, PH.D., Ann Arbor, Michigan, presiding.

1. PRESIDENTIAL ADDRESS: PERCUTANEOUS ABSORPTION—A PERSONAL VIEW. R. B. STOUGHTON, M.D., Scripps Clinic and Research Foundation, La Jolla, California.


Cell cycle periods can be distinguished on the basis of cellular DNA content. Cytophotometry of Feulgen stained materials allows measurement of DNA contents on an individual cell basis and thus provides a tool by which cell proliferation can be studied.

* By invitation.
Specimens of normal skin from subjects without dermatologic disease as well as involved and uninvolved skin from psoriatic patients were obtained with a 4 mm punch. Paraffin sections were cut at 7 micra, Feulgen stained, and measured using a Vickers M85 scanning microdensitometer.

Involved psoriatic skin DNA profiles were bimodal as is characteristic of actively proliferating populations. This was due to the presence of cells with 2 C (G1) or 4C (G2) amounts of DNA separated by the intermediate values of cells undergoing scheduled DNA synthesis (S). DNA profiles of uninvolved psoriatic as well as normal epidermis were unimodal with the majority of cells containing a 2C amount of DNA. These profiles represent an instantaneous, random sample, thus the proportion of cells in a given period is an indication of the relative duration of that period if all cells are cycling. In this manner findings comparable with those by autoradiographic techniques can be obtained.

3. THE BRANCHED CHAIN FATTY ACIDS OF ADULT HUMAN SKIN SURFACE LIPID. N. NICOLAIDES, PH.D. AND J.M.B. APON, B.S.* Department of Dermatology, University of Southern California School of Medicine, Los Angeles, California 90033.

Identification of the lipid components of skin may suggest their biological function(s). Adult human skin surface lipid (AHSSL) contains 2 to 3% branched chain fatty acids other than iso or anteiso. Earlier we determined the structures of 37 monomethyl and 35 dimethyl fatty acids occurring in a comparable group of acids from vernix caseosa lipid (VCL). We now report that most of these acids also occur in AHSSL.

We concentrated the branched fatty acids by first removing the straight chain acids as urea adducts, then separated individual molecular species of branched acids on a high efficiency capillary gas liquid chromatographic column, feeding the effluent directly into a mass spectrometer. This provided the data from which the structures of the acids could be deduced.

As in VCL in methyl branches of AHSSL fatty acids appear only on the even numbered C-atoms counting the carboxyl carbon as 1. Replacement of a molecule of methylmalonyl-CoA for malonyl-CoA at various points in the fatty acid synthetase system would place methyl branches precisely at these positions. The fact that these acids occur in the wax ester fatty acids of VCL strongly indicates that they are endogenous products of skin and are made in the sebaceous gland.

Quantitative differences in the amounts of these acids exist between VCL and AHSSL, and a repeating pattern of homologues was found.

4. CHANGES IN LIPID COMPOSITION OF SEBACEOUS GLAND HOMOGENATES AFTER INCUBATION WITH PROPIONIBACTERIUM ACNES AND PROPIONIBACTERIUM GRANULOSUM. S. M. PUHVEL, PH.D. AND R. M. REISNER, M.D., Division of Dermatology, University of California, Los Angeles, California 90024.

Propionibacterium acnes and Propionibacterium granulosum (C. acnes II) are believed to be significant factors in the pathogenesis of acne vulgaris, although the exact mechanism of their involvement remains unclear. The present study analyzes the effect of these intrafollicular bacteria on the lipid composition of sebaceous gland homogenates in vitro.

Isolated, dissected sebaceous glands from human scalp were pooled, homogenized and sterilized, then used as substrate for the growth of P. acnes and P. granulosum. After incubation, lipids were extracted from culture supernates and sterile controls, and analyzed by thin layer and gas liquid chromatography.

As expected, the most striking change in lipid composition effected by both species was the hydrolysis of sebaceous triglycerides to free fatty acids. Gas liquid chromatographic analysis suggested that although the spectrum of free fatty acids resulting from the triglyceride hydrolysis was similar with P. acnes and P. granulosum, there was considerable variation in the relative quantities of specific free fatty acids depending on the bacterial strains.

These studies using sebaceous gland homogenates as bacterial substrate give a much closer approximation of the effect of bacteria on freshly formed unmodified sebum than has been possible in previous studies.

5. MECHANISM OF STEROID-INDUCED VASOCONSTRICTION. J. E. WOLF, JR., M.D., W. R. HUBLER, JR., M.D. AND N. D. GUZICK, M.D., Department of Dermatology, Baylor College of Medicine, Houston, Texas 77025.
Human and animal models were utilized to investigate the mechanism of action of corticosteroid-induced vasoconstriction.

A modified hamster cheek pouch chamber was employed for direct, in vivo visualization of arterioles and venules. The topical application of hydrocortisone sodium hemisuccinate (Solucortef®) and dexamethasone sodium phosphate (Decadron®) produced minimal vasoconstriction. However, subsequent application of sub-threshold doses of epinephrine and norepinephrine evoked marked vasoconstriction (up to 80% of the luminal diameter of observed vessels measured with a calibrated oculair reticule). The alpha adrenergic blocking agent phenolamine (100 μg/ml) greatly reduced this enhancement of catecholamine-vasoconstriction; the beta-blocker propranolol (1000 μg/ml) had virtually no effect on post-steroid vascular reactivity.

Flurandrenolide-impregnated occlusive tape (Cordran®) was used to produce cutaneous pallor in twenty human volunteers. Each then received 0.1 ml intradermal injections of phenolamine (100 μg) and propranolol (2 μg). Propranolol had no significant effect on vasoconstricted skin, while phenolamine provoked a distinct erythema persisting up to four hours after injection.

Topically-applied corticosteroids potentiate catecholamine-induced vasoconstriction of integumental microvessels, possibly via increased sensitivity of alpha adrenergic receptors.

6. MAMMALIAN EPITHELIAL RIBOSOMES: A RE-EVALUATION. M. E. GILMARTIN,* B.A. and I. M. FREEDBERG, M.D., Department of Dermatology, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215.

Conflicting results in the literature have led us to a systematic re-examination of the parameters affecting the isolation of ribosomes from epidermis and hair roots. Hair root cells, harvested from guinea pigs by latex or wax epilation, yield ribosomal profiles containing "40s" and "60s" ribosomal subunits, a major peak of monomeric ribosomes and measurable amounts of small polyribosomes. Sedimentation velocities of isolated RNA from each class of particles were determined and accurate sedimentation values obtained. The profile from epidermal ribosomes differs in amount and distribution of ribosomal subunits and from both epidermis and hair roots larger concentrations of particles are found in young compared to adult tissue.

Detergent treatment (desoxycholate) increases the yield and alters the pattern of epidermal ribosomes and causes a decrease in the yield of hair root ribosomes due to trapping of particles in the extracting medium. Sulphydryl reagents are not required in either type of tissue. Changes in ionic environment produce alterations in ribosomal patterns and yields. More particles are isolated in high potassium medium and K⁺ exhibits a synergistic effect in stabilizing the ribosomal profile in the presence of increased Mg²⁺ concentrations. Hair ribosomes are more resistant to changes in the ionic strength than are epidermal particles.

Our studies prove that the ribosomes of epidermis and hair root cells show distinct physicochemical characteristics, apparently due to changes occurring during differentiation.

AFTERNOON SESSION

FRIDAY, JUNE 21, 1974

2:00 P.M. SCIENTIFIC SESSION: RICHARD B. STOUGHTON, M.D., La Jolla, California, presiding.

1. METABOLISM IN EPIDERMAL DIFFERENTIATION. R. K. FREINKEL, M.D. AND K. A. WIER, M.D.,* Department of Dermatology, Northwestern University, Chicago, Illinois 60611.

Disruption of intact postnatal tissue has been employed previously to study metabolism at various stages of epidermal differentiation. We have investigated intact epidermis during fetal development when metabolic potential is limited by morphogenesis.

Electronmicroscopy of rat epidermis at day 18 and 21 antepartum (AP) and 5 postpartum (PP) was correlated with in vitro studies of 1) incorporation of H³ thymidine (T) as index of mitotic activity; 2) incorporation of H³ leucine (L) and C¹⁴ histidine (H) to assess protein synthesis; 3) hexose monophosphate shunt activity (HMP) (via fatty acids labelled with 1-C¹⁴ and 6-C¹⁴ glucose) to assess differential glucose disposition during glycolysis.

Uncornified epithelium with fetal keratohyaline (D18 AP) progressed to increased proportions of differentiated cells, mature keratohyaline, and cornification (D21 AP), and fully stratified epidermis (D5 PP). Concurrently T fell progressively to 10% of initial value at D5 PP, while the
percent of HMP remained constant. Protein synthesis from L declined less dramatically while that from H remained constant. The conclusions are: 1) deployment of glucose via HMP remains proportionately fixed independent of demands of replicative or biosynthetic functions for glycolytic products; 2) protein synthesis from L diminishes while that from H parallels keratinization; and 3) ratios of H/L may provide a useful index for distinguishing protein synthesis in early and late stages of epidermal differentiation in small tissue samples.

2. EFFECT OF DRUGS ON THE PILLEMER PATHWAY—DAPSONE. L. E. MILLIKAN, M.D. AND F. R. CONWAY,* Division of Dermatology, Department of Medicine, University of Missouri, Columbia, Missouri 65201.

Recent findings of Complement C₃ pro-activator (C₃PA) and the involvement of IgA in the skin in dermatitis herpetiformis (DH) suggest that the pathology is mediated by the Pillemer or alternate complement pathway. Patients with DH on sulfones, show changes in amounts of C₃PA and C₃ in the epidermis, correlating well with clinical improvement. This suggests that sulfones are affecting this pathway. This study was done to determine the action of sulfones in this disease.

Activity of the alternate pathway was studied through the technique of Insulin white cell degranulation, which is complement-mediated. The classical pathway was blocked by selective calcium chelation, using ethyleneglycol-bis (beta amino-ethyl ether) N,N'-tetra-acetic acid (EGTA) preserving normal magnesium levels. Degranulation was measured by P₃2 release from DFP incorporated in white cell membranes. Membranes P₃2 release was blocked by Dapsone in a typical dose response curve, supporting the thesis that they affect the Pillemer pathway. Steroids are less effective, correlating well with clinical and therapeutic findings of this disease. The role of other drugs in this pathway will be discussed.

This technique presents a useful approach to the screening of drugs that may be effective in diseases involving the alternate complement pathway.

3. SPECIAL LECTURE. MUCOPOLYSACCHARIDES, ALBERT DORFMAN, M.D., Professor, Department of Pediatrics, University of Chicago, Chicago, Illinois 60637.

4. AN ABNORMALITY OF CELL BOUND IgE IN URTICARIA. M. W. GREAVES, V. M. PLUMMER, P. MCLAUGHLAN, D. R. STANWORTH* (intr. by J. VOORHEES), Department of Dermatology, University of Newcastle upon Tyne, England.

We report qualitative and quantitative studies of cell-bound and serum IgE which reveal a previously unrecognized abnormality of basophil leukocyte IgE in urticaria. Basophil-bound IgE was investigated by the Ishizaka method of reversed anaphylaxis, using a specific anti-human IgE serum. Washed basophil suspensions were incubated with concentrations of anti-IgE ranging from 0.002-2.0 μg AbN/ml and evoked histamine release, measured by bioassay. Serum IgE was measured by radioimmunoassay. Results were obtained in 11 patients with urticaria and 16 healthy controls. Histamine release (γ) evoked by 0.02-0.2 μg AbN/ml anti-IgE in the urticaria group was reduced by 38-70% compared with corresponding values in the control group. This difference is highly significant (analysis of variance, 0.05 > p > 0.001). There was no quantitative difference in basophil-bound IgE since maximum release was produced by the same concentration in the two groups. The mean and individual serum IgE concentrations were all within normal limits in both groups. There was no significant difference in chemical (compound 48/80) histamine release between the two groups, and basophil counts and basophil histamine content did not differ. Our results indicate a qualitative abnormality of basophil IgE which could be explained by a conformational change in the IgE molecule, or an abnormal distribution of IgE on the plasma membrane.

5. EFFECT OF PEMPHIGUS OR BULLOUS PEMPHIGOID SERA & LEUKOCYTES ON NORMAL HUMAN SKIN IN ORGAN CULTURE. AN IN VITRO MODEL FOR THE STUDY OF BULLOUS DISEASES. B. MICHEL, M.D. AND C. S. KO, M.D., Department of Dermatology, Case Western Reserve University, Cleveland, Ohio 44106.

Since no satisfactory animal models for the study of pemphigus (P) or bullous pemphigoid (BP) exist the effect of P or BP sera on normal human skin in organ cultures was studied as a possible model. Skin specimens 2 mm³ and 0.5 mm thick were grown in 1 ml normal (N) serum for up to two
weeks with no pathologic changes on H&E sections. (Sarkany et al. Brit. J. Derm. 77, 65, 1965). Similar specimens grown in P sera alone show no changes on H&E sections at 6 hours incubation. At 12 hours intercellular edema is noted in the epidermis. At 24-72 hours suprabasilar or diffuse acantholysis is seen and extensive acantholysis is present at 120 hours. The changes were similar in 3 experiments with pemphigus foliaceus and 6 with pemphigus vulgaris sera. Heating sera at $56^\circ C \times 30$ min did not prevent the changes. P antibody titers ranged from 80-640. Direct immunofluorescent studies showed questionable or weak intercellular staining at 6, 12, 24 hours and generally negative staining at 72 and 120 hours.

Heated BP sera, however, with or without added fresh serum as a source of complement did not produce histologic changes. The addition of homologous WBC 5-8 x $10^9/mm^3$ to culture caused epidermal-dermal (E-D) separation in the presence of N or BP serum in 6-24 hours, but no acantholysis. BP blister fluid produced E-D separation similar to that seen with WBC suggesting probable presence of proteolytic enzymes.

Thus, skin organ cultures can serve as an excellent model for future studies of the pathophysiology of acantholysis due to P sera alone and of E-D separation caused by WBC or BP blister fluid.

6. VITAMIN A ANTAGONISM OF CORTICOSTEROID-INDUCED COLLAGEN SYNTHESIS INHIBITION. R. F. WEHR, B.S.,* J. G. SMITH, JR., M.D. AND K. CUTRONEO, Ph.D. Departments of Dermatology and Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30902.

The effect of systemic Vitamin A on the collagen synthesis inhibition which occurs with corticosteroid therapy in sponge implanted granulomas in Sprague-Dawley rats was studied. Sponges were implanted by making a small cut on the dorsal posterior skin, inserting a rumen-trocar under the skin, and advancing it until the tip was about shoulder high. The plunger was removed and a sterile saline impregnated sponge was inserted into the space left by the withdrawing trocar. The wound was closed with autoclips. Saline, triaminocinolone acetonide (2 mg), Vitamin A (15,000 international units), and Vitamin A plus triaminolone were injected daily. The animals were sacrificed on the fourth day. The tissue covered sponges were dissected away from the skin, granuloma tissue stripped off, and weighed, and tissue homogenates prepared for prolyl hydroxylase activity assay. Dose response levels were determined separately for triaminolone and Vitamin A. Collagen synthesis inhibition was demonstrated by a decrease in weight of sponge-induced granuloma tissue and a decrease of prolyl hydroxylase activity. Vitamin A administered simultaneously with triaminolone reversed the reduction in weight of granuloma tissue as compared to animals treated with triaminolone alone. Prolyl hydroxylase levels in Vitamin A treated animals, however, did not return to control levels nor did the body weight.

7. ISOLATION AND GROWTH OF SKIN ENDOTHELIAL CELLS IN CELL CULTURE. M. KARASEK, Ph.D. AND M. CHARLTON, B.S.,* Department of Dermatology, Stanford University, Stanford, California 94305.

Skin endothelial cells from blood vessels of postembryonic rabbit ear may be isolated simply and rapidly by slow perfusion of trypsin through the marginal ear vein. Phase microscopy of both living and stained endothelial cells reveals multiple fine branching processes at the apical ends. Such cells may be easily distinguished from mesenchymal cells and epithelial cells by noting their dense, refractive cytoplasm and typical circular growth pattern. Lumen formation occurs in vitro and a mechanism of vacuolization and degeneration is postulated to explain this process. Addition of fibrin to endothelial cells markedly stimulates proliferation. Other conditions for optimal growth (pH, type of medium, surface, serum) have been established.

Isolated endothelial cells inoculated into a collagen gel reorganize and multiply in close apposition to form columnar structures resembling native blood vessels. They may be cultured in collagen gels in the presence of both trypsin-release epithelial cells and fibroblasts.

These studies represent the final stage in the development of a method to isolate and maintain in cell culture three of the major cell types of normal skin (epithelial, fibroblast, and endothelial). The unique behavior of endothelial cells in culture suggests that they may be used to assay those factors that regulate endothelial cell proliferation in vivo.

MORNING SESSION

SATURDAY, JUNE 22, 1974

1. EXTRACELLULAR LIPASE(S) IN CORYNEBACTERIUM ACNES. I. VARIATION OF TRIGLYCERIDE HYDROLYSIS WITH CULTURE AGE AND ACYL CHAIN-LENGTH. S. A. GILBERT, M.S.*, M. S. CHRISTENSEN, PH.D.*, E. H. GANS, PH.D., AND M. S. RHEINS, PH.D.*, Vick Divisions Research and Development, Mount Vernon, New York 10553, and the Department of Microbiology, Ohio State University, Columbus, Ohio 43210.

Previously reported studies of extracellular lipase activity of *C. acnes* have not taken into account possible changes in lipolytic activity in relation to culture age. We investigated the time-related changes using 5 strains of *C. acnes*, grown in broth culture. The rate of cell growth was determined by the measurement of insoluble protein. Cell-free aliquots of the culture media were assayed for trioctanoin hydrolytic rate. There were distinct curves for the development of maximal activity to trioctanoin for each organism, which did not necessarily parallel cell growth. In some strains, the peak activity was observed early in the growth after which the activity fell rapidly while other strains demonstrated relatively stable lipolysis rates.

Culture fluids were harvested at the optimal time for trioctanoin activity and aliquots were tested against triglycerides of different acyl chain-length (from C-4 to C-18). The pattern of lipolysis varied in each strain.

These studies have shown that the determination of lipolytic capabilities of strains of *C. acnes* must be performed at the time of optimal enzyme expression, and the hydrolysis of a wide range of substrates should be assessed, as there may exist a multicomponent enzyme system characteristic for each individual strain. Failure to observe these constraints may lead to spurious results.

2. EPIDERMAL ANTIBODIES OF UNIQUE SPECIFICITY IN PEMPHIGUS FOLIACEUS. J.-C. BYSTRYN, M.D., E. A. ABEL, M.D.*, and C. DE FEUO, M.D., Department of Dermatology, New York University School of Medicine, New York, New York 10016.

Distinctive antibodies reacting specifically with antigens present only in subcorneal intercellular substance of epidermis were found in a patient with pemphigus foliaceus. These antibodies were different from those found in pemphigus vulgaris which react with intercellular antigens present throughout the epidermis. Thus, antibodies in 2 sera of this patient bound only to subcorneal intercellular antigens in 2 guinea pig esophagi, and 7 different specimens of human skin. By contrast, antibodies in sera of 4 patients with pemphigus vulgaris bound to intercellular antigens throughout the width of the squamous cell layer in these specimens. There was no intercellular binding with 4 normal sera. These distinctive antibodies were found in 2 of 4 patients with pemphigus foliaceus, in none of 178 pemphigus vulgaris sera, and 1 of 339 sera of patients with various dermatoses. The concentration of antibodies binding pemphigus foliaceus antibodies appeared to be greater in guinea pig esophagus where they were present in all specimens tested, were located in the uppermost 2-4 intercellular spaces, and bound antibodies to a titer of over 1/1280. By contrast, these antibodies were not present in some specimens of normal human skin, were located only in the uppermost 1-2 intercellular spaces, and bound antibodies in the same sera to a titer of 1/80. These findings suggest that pemphigus foliaceus may be associated with distinct antibodies to antigens whose anatomical location corresponds to that of the earliest lesion seen in this form of pemphigus.

3. SPECIAL LECTURE. GENETIC CONTROL OF THE IMMUNE RESPONSE, ALFRED NISONOFF, PH.D., Professor, Department of Biochemistry, University of Illinois, Chicago, Illinois 60680.

4. INFLUENCE OF HUMIDITY ON ACUTE ULTRAVIOLET INJURY. D. W. OWENS, M.D., J. M. KNOX, M.D., H. T. HUDSON, PH.D.*, AND D. TROLL, M.D.*, Department of Dermatology, Baylor College of Medicine, Houston, Texas 77025.

Humidity may influence the response of skin to acute ultraviolet injury. This was tested using an environmental chamber in which the humidity was varied while hairless mice were irradiated with UV from Westinghouse FS-40 T-12 sunlamps. One group (12 animals in each group) received 10 MED of UV while maintained at 5% humidity and the other group received the same UV exposure while maintained at 80% humidity. Damage was recorded daily until the animals
recovered. The animals irradiated while kept at 80% humidity had much more severe damage than animals kept at 5% humidity. Mortality rate for the high humidity group was greater than 50% one week after irradiation, while there was no deaths in the low humidity group.

Additionally, MED determinations were made in 7 albino rabbits before and after hydration of skin with wet compresses. The mean MED before hydration was 8.0 mj/cm² and after hydration 5.4 mj/cm², (P < 0.0005).

5. EFFECT OF KERATINOCYTE-MELANOCYTE INTERACTION ON THE SIZE OF MELANOSOMES WITHIN KERATINOCYTES IN VITRO. K. TODA, M.D., PH.D. AND F. MORIKAWA, M.D.* Department of Dermatology, Tokyo Teishin Hospital, Tokyo, and Shisedo Laboratory, Yokohama, Japan.

Our earlier studies demonstrated that the exposure of Caucasian skin to longwave ultraviolet radiation after the application of psoralens resulted in an increase in the average size of the melanosomes and changes in the distribution pattern of melanosomes within keratinocytes. The purpose of this study is to investigate the photobiologic effects of monochromatic and polychromatic light on the pigmented system of cultured cells under different experimental conditions. Brown guinea pig ear epidermis cells were grown in monolayer cell culture systems and in organ culture systems. The cultures were treated with varying concentrations of psoralens and varying doses of ultraviolet irradiation. The cells were observed with light microscopy, microdensitometry and electron microscopy. In the monolayer cell culture system, an increase in size of the melanosomes could not be detected in either the melanocytes or the keratinocytes but an increase in the number of melanosomes could be observed in melanocytes after the treatment with psoralens and longwave ultraviolet irradiation. In the organ culture of the epidermis, the color of the cultured tissue darkened. Electron microscopy, disclosed the presence of large melanosome complexes containing numerous melanosomes and singly dispersed large fully-melanized melanosomes in keratinocytes and large fully-melanized melanosomes in melanocytes. The organized cell structure may have an effect on the size of the melanosome. These findings suggest that pigmentation can occur in the epidermis without an erythema reaction.

6. IMMUNOLOGIC SIGNIFICANCE OF SMALL (1–10 MM) SKIN TEST REACTIONS. H. E. JONES, M.D., J. H. REINHARDT* AND J. GREENBERG, M.D.* Department of Dermatology, University of Michigan, Ann Arbor, Michigan 48104, and Dermatology Division, Letterman Army Institute of Research, Presidio of San Francisco, California 94129.

Recently tuberculin reactions not large enough to be interpreted as positive by established epidemiologic criteria were correlated with in vivo minimal specific lymphocyte responses (Amer Rev Respir Dis 107:350–538). The immunobiologic significance of small (<10 mm) and large (>10 mm) Type IV reactions vs. nonreactivity to trichophytn were investigated by monitoring the course of experimental dermatophyte infections in such subjects. Volunteers exhibiting 72 hour reactions of 1–4 mm (4 men), 5–9 mm (5 men) and greater than 10 mm (10 men) to a purified trichophytn were infected using quantified inoculi of Trichophyton mentagrophytes and a hydration-occlusion technique. The infections were inflammatory in all 19 subjects soon after inoculation. Utilizing an identical technique, 10 trichophytn-nonreactive volunteers were inoculated, however these subjects did not develop an inflammatory infection until 10 to 30 days later (sensitization period). Thus, experimental infections, possibly the most rigorous of immune bioassays, indicate that trichophytn reactivity, even if minimal, reflects prior immunologic experience with related infectious agents. Type IV reactions to purified trichophytn are a continuum from large intense reactions down to small reactions ordinarily considered negative. Such small reactions to trichophytn, and likely other irritant-free test materials, cannot be labeled nonreactive, for it is now apparent that even minimal reactions reflect immunologic sensitivity.

7. LYMPHOCYTE-TUMOR CELL INTERACTIONS WHICH REGULATE CLINICAL APPEARANCE OF A TRANSPLANTABLE MURINE MELANOMA. J. J. NORDLUND, M.D. AND R. K. GERSHON, M.D., Departments of Dermatology and Pathology, Yale University, New Haven, Connecticut 06510.
The concept of immune surveillance implies a system to destroy a small, incipient malignancy. If surveillance is unsuccessful, a tumor grows to a large size and becomes clinically apparent. Studies on the immune response (ImR) indicate that it retards growth of large tumors. It is possible that the type of ImR which can destroy a small tumor differs from that which slows the growth of a clinically apparent tumor. We have studied the kinetics and characteristics of the ImR during the early growth phase of the S-91 melanoma in syngeneic DBA/2J mice. Tumor cells which have been grown in tissue culture produce tumors when injected into mice. There is a dose dependent lag phase during which no tumor is detectable. This period is followed by an explosive growth of the tumor and death of the animal. Animals made immunodeficient by surgical thymectomy and injections of ALS have shortened lag phase, indicating ImR is in part responsible for the duration of this period. This distinct and reproducible lag phase makes the model ideal for studying early ImR to a neoplasm. Preliminary studies indicate the spleen functions to retard or enhance rate of tumor growth depending on the size of the tumor inoculum. Small doses of tumor cells, less than $1 \times 10^6$, grow more rapidly in splenectomized animals than normal controls. Large doses of tumor cells, over $1 \times 10^6$, grow more slowly, in splenectomized animals than in normals. This phenomenon can be explained by enhancing antibody formation or stimulation of suppressor T-cells in the spleen.

**AFTERNOON SESSION**

**SATURDAY, JUNE 22, 1974**

2:00 P.M. SCIENTIFIC SESSION: RICHARD B. STOUTHMAN, M.D., presiding.

1. **FOURTEENTH ANNUAL HERMAN BEERMAN LECTURE. MEDIATING AGENTS IN THE INFLAMMATORY PROCESS.** CHARLES G. COCHRANE, M.D., Scripps Clinic and Research Foundation, La Jolla, California.

2. **THE REDUCED LEVELS OF PROSTAGLANDINS AND THE EFFECT OF PROSTAGLANDIN STIMULATION ON cAMP ACCUMULATION IN PSORIATIC EPIDERMIS.** K. ASO, M.D.,* E. K. ORENBERG, PH.D.,* I. N. RABINOWITZ, PH.D.,* AND E. M. FARBET, M.D., Departments of Dermatology and Psychiatry, Stanford University School of Medicine, Stanford, California 94305.

   Decreased levels of cAMP have been reported in psoriatic epidermis. Since prostaglandins are known to be one of the modulators of the cAMP system, we initiated a study of the role of prostaglandins and their relation to the regulation of cAMP in psoriasis. cAMP was quantitated by the protein binding assay procedure of Gilman and prostaglandins by radioimmunoassay. The levels of E type prostaglandins were significantly lower in involved epidermis (129 ± 39.4 ng/Gm) as compared to uninvolved epidermis (303 ± 67.4 ng/Gm wet weight). Similar lowered levels of PGF$_\text{2\alpha}$ were found in involved epidermis. PGE, significantly increased cAMP in psoriatic epidermis in vitro. However, PGE, stimulation of cAMP accumulation was significantly reduced in involved epidermis (2.1 ± 0.1 pmoles/mg wet weight) as compared to uninvolved epidermis (4.2 ± 0.1 pmoles/mg wet weight). The specificity of this stimulation, its occurrence at physiological levels, the lowered prostaglandin content and the decreased responsiveness of the cAMP system in psoriatic epidermis to PGE, stimulation suggest that abnormal prostaglandin metabolism may be one of the factors in the pathophysiology of psoriasis.

3. **RECURRENT STAPHYLOCOCCAL INFECTIONS AND DEFICIENT CELL-MEDIATED IMMUNITY.** L. H. REID, M.D. AND W. L. WESTON, M.D., Division of Dermatology, University of Colorado School of Medicine, Denver, Colorado 80220.

   Four patients presenting with recurrent staphylococcal furuncles were found to have depressed cell-mediated immunity (CMI). CMI was evaluated by intradermal skin tests to streptokinase-streptodornase, monilia, PPD, histoplasmin, trichophytin and mumps antigens, by DNBCB sensitization, by determination of percentage of thymus derived (T) lymphocytes as measured by spontaneous rosette formation with sheep RBC's, by production of macrophage aggregation factor (MAF), and by lymphocyte transformation with phytohemagglutinin (PHA). Two patients had a history of staphylococcal sepsis and another a history of staphylococcal osteomyelitis. A fifth
patient with recurrent staphylococcal abscesses of the lungs and liver, but without skin lesions, was also studied. All five of these patients demonstrated a deficiency of CMI whereas five patients with cystic acne or hidradenitis suppurativa had normal CMI. Neutrophil function studies were performed in two of these patients and were normal.

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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>anergic</td>
<td>+</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>abnormal</td>
<td>0</td>
<td>53</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>normal</td>
<td>+</td>
<td>57.75 ± 1.6</td>
<td>+</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

These studies suggest that CMI is an important host defense against staphylococcal infections.

4. SUBCELLULAR AND MOLECULAR SITES OF STAPHYLOCOCCAL EXFOLIATIVE TOXIN ACTIVITY. P. ELIAS, M.D., P. FRITSCH*, M.D. AND K. WOLFF, M.D. Division of Experimental Dermatology, 1 Hautklinik, University of Vienna, Austria.

Staphylococcal toxin epidermal necrosis (TEN) is now firmly linked to a single exfoliative toxin (ET), elicited by certain phage Group II staphylococci. Prior ultrastructural studies in the newborn mouse and in man indicated that ET produces intercellular separation in the lower granular layer, yet acantholytic cells are not prominent histologically. To ascertain the pathogenesis of staphylococcal TEN on a subcellular level, the following studies were performed: To differentiate between cytology and acantholysis, we injected thorium dioxide (Thorotrast®) or horseradish peroxidase (50 mg/ml normal saline) intracutaneously into newborn mice with early TEN induced by previous inoculation of Group II staphylococci. Cell separation occurred without preceding transmembrane leakage of tracer, although in more advanced lesions, some acan-tholytic cells showed evidence of leakage. A cell-free solution containing ET, prepared as previously described (Brit. J. Dermatol. 85:145, 1971), did not remove ruthenium red-stainable surface coats from keratinocytes of newborn mice undergoing TEN in vivo, nor from mouse or guinea pig keratinocytes exposed to ET for 2 hr. in monolayer cultures. Supernatant ET-containing media retained a constant TEN-producing capacity when tested in newborn mice throughout these experiments. These studies show that the pathogenesis of staphylococcal TEN involves primary cell separation without preceding cytology. While the molecular target on the cell surface remains unknown, cleavage cannot be attributed to removal of stainable surface acid mucosaccharides.

5. SPECIAL LECTURE. POTENTIAL FOR SCANNING ELECTRON MICROSCOPY IN BIOLOGY, ALBERT CREWE, Ph.D., Dean of Physical Science, Enrico Fermi Institute, University of Chicago, Chicago, Illinois 60637.

MORNING SESSION

SUNDAY, JUNE 23, 1974

9:00 A.M. BUSINESS AND EXECUTIVE SESSION: RICHARD B. STOUGHTON, M.D., presiding.

SCIENTIFIC SESSION: CLAYTON E. WHEELER, J.R., M.D., Chapel Hill, North Carolina, presiding.

1. THE EFFECT OF ANTHRALKIN AND ITS DERIVATIVES ON EPIDERMAL CELL KINETICS. L. B. FISCHER, Ph.D. AND H. I. MAIBACH, M.D., Johnson & Johnson Research, New Brunswick, New Jersey, 08903, and University of California, San Francisco, California, 94122.
Anthrakinol, an effective anti-psoriatic agent, also irritates and stains the skin. Anthralin "dimer" and dihydroxanthroquinone, are in the commercial preparation. These and chromato-
graphically pure anthralin were studied to determine which was active in modifying epidermal cell kinetics. The 3 components of commercial anthralin were separated by thin layer chromatography with ethylic acetate (EtAc), 3 groups of 27 male hairless mice were injected with 30 micromoles of tritiated thymidine (H<sup>3</sup>-Tdr) (27.0 mCi/mM). Each group immediately afterwards was treated topically with one compound as a single application of 24 μg in 0.1 ml EtAc. A further group of 18 mice were injected with H<sup>3</sup>-Tdr and treated with 0.1 ml EtAc alone. Animals were killed at intervals, autoradiographs prepared, and the mitotic index and the percent labeled mitoses were determined.

The quinone and dimer both showed a diurnal mitotic rhythm and cell cycle similar to the control. However, the second mitotic peak, seen between 18 and 34 hours, was eliminated by application of either commercial or pure anthralin (P < 0.01). The cell cycle was also altered by these two compounds in a way which indicated an increased duration of the 'S' phase from 6 hours to 12 hours.

These results suggest that pure anthralin and not its derivatives—the dimer and anthroqui-
none—is the active agent in this system.


The photosensitizing dye, acridine orange (AO) and a continuous wave argon laser were used to induce tumor destruction in mouse epithelial tumors.

Phototoxicity was demonstrated using Ehrlich's ascites tumor cells. After incubation in the dark with AO, these cells could be killed by argon laser irradiation. The amount of cell death was dependent both on the concentration of AO and on the intensity and length of the laser exposure. AO fed to C3H mice was shown by fluorescence microscopy to be selectively localized to benzo(a)pyrene induced epithelial tumors (papillomas and carcinomas). Tumor destruction produced by subsequent laser exposure in mice fed 15 to 75 mg AO (total dose) over 3 to 5 days was assessed 48 hours after irradiation and compared to destruction produced by the laser alone. Hour long irradiation at 65 mW/cm<sup>2</sup> and at 165 mW/cm<sup>2</sup> produced partial or complete tumor destruction in AO fed mice. Adjacent normal skin was not damaged (65 mW/cm<sup>2</sup>) or showed mild inflammatory changes (165 mW/cm<sup>2</sup>). The degree of tumor destruction appeared dependent on dye dosage. Hour-long irradiation at 280 mW/cm<sup>2</sup> produced tumor destruction with gross damage to adjacent skin in both control and AO fed animals, presumably reflecting thermal damage.

3. CORRELATION OF HUMAN IN VIVO AND IN VITRO CUTANEOUS ANTIMICROBIAL FACTOR(S). R. Aly,* Ph.D., H. Maibach, M.D., H. Shenefield, M.D. AND A. Mandel, M.D., Department of Dermatology, University of California, San Francisco, California 94143.

This study investigates the presence of antimicrobial substances in human skin. We noted in pilot studies that the ability of skin to destroy pathogenic microorganisms varied from individual to individual. Some subjects readily and consistently destroyed applied Staphylococcus aureus on their skin; others did not.

10<sup>6</sup> colony forming units of S. aureus were applied on the forearm of 50 subjects and covered with a semipureocclusive device for 24 hours; thus subjects allowing persistence or inhibition of S. aureus were delineated. Fifty-four percent allowed persistence and 34% demonstrated inhibition of S. aureus. This relationship was not noted with Streptococcus pyogenes. Subjects demonstrating S. aureus persistence also demonstrated persistence of Candida albicans and vice versa.

Skin lipids from these two populations were extracted with acetone and assayed against S. aureus, S. pyogenes and C. albicans in vitro. Skin lipid extracts (0.1 ml) were added in duplicate tubes containing 0.25 ml of peptone and 0.1 ml of bacterial suspension (10<sup>3</sup> CFU). The percent recovery of S. aureus or C. albicans was higher (83 and 65% respectively) in subjects who demonstrated persistence of microorganisms on their skin than those who did not (40 and 37% respectively, p = 0.05).

Thus, there is a direct correlation between killing of S. aureus and C. albicans in vivo and the presence of an acetone-soluble substance (presumably lipids) which kills the same organisms in vitro. The relationship of this acetone-soluble substance(s) and cutaneous infection will be discussed.
4. SPECIAL LECTURE. HERPESVIRUSES, LATENCY, AND CANCER: A BIOCHEMICAL APPROACH. BERNARD ROIZMAN, Sc.D., Professor, Department of Microbiology and Biophysics, University of Chicago, Chicago, Illinois 60637.

5. ANALYSIS OF SOLUBLE PROTEINS IN COMEDONES. C. W. LEES, M.D., J. S. STRAUSS, M.D. AND P. E. POCHI, M.D., Department of Dermatology, Boston University Medical Center, Boston, Massachusetts 02118.

Because it is possible that structural proteins, enzymes such as proteases and lipases, and serum proteins may play a role in the pathogenesis of acne, the soluble proteins of comedones from acne subjects have been analyzed and compared to the soluble proteins from non-inflammatory nasal sebaceous follicles. Ten to 50 comedones (approximately 5-25 mg, fresh weight) were homogenized (10 mg/ml) in 0.1% Triton X-100, 5 mM CaCl2 0.05 M TrisHCl, pH 7.9, centrifuged at 37,000 x g to remove bacteria and epithelial cells, and the supernatant concentrated. Samples were subjected to electrophoresis in a discontinuous Tri-glycine system at pH 9.5, stained for protein, and scanned with a recording spectrophotometer. Slices of unfixed, unstained gels were crushed, and assayed for protease and lipase activity. In addition, longitudinally-sliced gels were studied by immunodiffusion.

In 16 samples from 10 acne patients, generally similar protein band patterns were obtained, but with the variable presence of some bands at characteristic Rf values. Protease and lipase activities have been detected in the whole concentrated supernatant prior to electrophoresis, and protease activity has been found to be associated with at least two different electrophoretic bands. Samples of comedones from two patients have been shown to contain alpha-globulins and albumin by immunodiffusion.

The data that will be presented demonstrate that a detailed protein fingerprint can be obtained from the contents of a small number of pilosebaceous units.


A comparative study of immediate tanning (IT) and delayed tanning (DT) stimulated by ultraviolet radiation: UV-B (290-320nm) and UV-A (320-400nm), with and without oral 8-methoxysporalen (8-MOP), was carried out. 101 biopsies from 27 Caucasoid, 6 Mongoloid and 6 Negroid volunteers were obtained from unexposed and UV-exposed skin at 0, 30 minutes, 24 hours, 5 and 10 days after irradiation. Dopap reaction and differential staining with AgNO₃ and routine H&E were used to study the effects of single and multiple exposures on the differentiation of the melanocytes (MC) and keratinocytes (KC). Ultrastructural changes in MC, and the size, degree of melanization, transfer and degradation of melanosomes (MS) were compared. They are caused primarily by UV-A and involve selective changes in filaments (100 A) of MC and in the distribution pattern of MS in MC and KC. Single exposure-stimulated DT after UV-B, UV-A, or UV-A plus 8-MOP irradiation is manifested by a minimal increase in the number of functional MC, (b) a definite increase in the synthesis of MS and their increased transfer from MC to KC. Multiple exposure-stimulated DT by UV-B, UV-A, or by UV-A plus 8-MOP is characterized by at least two-fold increase of MC. The number, degree of melanization and transfer of MS are also increased. Oral 8-MOP appears to enhance not only the rate of synthesis, but the degree of melanization and transfer of MS without causing apparent changes in the size, or in the distribution pattern of MS in the MC and KC.

7. ROLE OF MELANOCYTE-KERATINOCYTE INTERACTION IN PIGMENT TRANSFER. M. SEJJII, M.D., PH.D. K. TODA, M.D., PH.D., K. OKAZAKI, M. UZUKA, AND F. MIRIKAWA, M.D.,* Department of Dermatology, Tohoku University, Sendai, Department of Dermatology, Tokyo Teishin Hospital, Tokyo, and Shisedo Laboratory, Yokohama, Japan.

The factors influencing the modes of pigment transfer between melanocytes and keratinocytes have been studied, using several cell culture systems. Black guinea-pig ear epidermal cells and
new-born C-57 mouse hair follicular cells were grown in a monolayer cell culture system and in a semi-organ culture system respectively. All cultures were treated with varying concentrations of cytochalasin B and dibutyryl cAMP. Cells were observed with time-lapse cinematography, electron microscopy and scanning electron microscopy. In the monolayer cell culture, several large groups of melanosomes were found to be either melanosome complexes or constricted dendrites. These dendrites could not be found after treatment with cytochalasin B. Tips of several dendrites accumulated numerous melanosomes, forming huge balloon-like structures which were then pinched off by the keratinocyte. Such structures have never been identified in the ear of a living animal. Thus, the mechanism of melanosome transfer in vitro may differ from that in vivo. In the hair follicular cell culture, the tips of dendrites which contained several melanosomes were pinched off by cortex cells, Then melanosomes were dispersed into cortex cell cytoplasm singly or in complexes of a few melanosomes. Keratinocytes and melanocytes in the hair follicular cell culture are arranged in an organized fashion, thus melanosome transfer appears to be carried out in vitro similar to that in vivo as a continuous regular minor event.

8. ERYTHROCYTE PORPHYRINS—A RAPID QUANTITATIVE MICROFLUOROMETRIC ASSAY. M. B. POH-FITZPATRICK, M.D.,* S. PIOMELLI, M.D.,* P. YOUNG, B.,* H. HSU, M.S. * AND L. C. HABER, M.D. Department of Dermatology and Department of Pediatrics, New York University School of Medicine, New York, New York 10016, and Department of Dermatology, Columbia University College of Physicians and Surgeons, New York, New York 10032.

The application of a new quantitative assay for protoporphyrin and coproporphyrin in RBC as an aid in the diagnosis of erythropoietic protoporphyria (EPP) is presented. At present, the diagnosis of EPP is primarily based on demonstration of abnormally elevated quantities of protoporphyrin in RBC of affected individuals assayed by the method of Wrann or that of Grinstein which require venipuncture to obtain 5 ml or more of blood and tedious repetitive chemical extractions requiring close to 90 minutes for each determination. The new microtechnic extraction and fluorometric measurement procedure can be performed on 40 lambda or less of blood in a few minutes. Although a wet specimen is preferred, a few drops of blood collected and dried on special filter paper can be submitted for quantitative analysis by this new method.

129 determinations of RBC porphyrins were performed on 15 EPP patients, 8 lead poisoning patients, 2 porphyria cutanea tarda patients and 8 normal controls. Data indicated similar values in comparing a classical method with the new rapid assay. Average recovery using the new method approached 95% with a loss range of only 6–9%. With the new method, quantitation followed the Baer-Lambert law from 30.5 to 2441.0 μg of free erythrocyte porphyrin/100 ml RBC. In no case was a false negative result obtained with either method. Marked advantages of the new microfluorometric assay are speed, simplicity, superior reproducibility and lower cost as compared with standard methods.

9. PROSPECTIVE LIVER STUDY OF 6-AZAURIDINE (AZARIBINE) FOR PSORIASIS. R. A. KEFFER, M.D. AND H. H. ROENICK, JR., M.D., Department of Dermatology, Cleveland Clinic, Cleveland, Ohio 44106.

An open study of 6-Aza uridine has been carried out on 43 psoriatic patients at the Cleveland Clinic for 2 years. Even patients who took the drug for a period of 7 to 16 months had pre-Azuridine and post-Azuridine needle liver biopsies performed. No differences in the pre and post treatment biopsies were seen. Abnormalities in liver function tests did not develop or worsen while on the medication.

Clinical response to the drug was slow, but good to excellent in 70% of the patients.

Side effects included a 1 to 2 gram decrease in hemoglobin, mild nausea and lethargy in most patients. Peptic ulcer reactivation occurred in 2 patients. Two patients had myocardial infarctions and one patient had a superficial phlebitis while on the drug. One patient died of multiple pulmonary emboli 2 months after discontinuing the drug. The relationship of these thromboembolic episodes to the medication and/or psoriasis is discussed.
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