PROGRAM

THE SPRING MEETING

THE SOCIETY FOR INVESTIGATIVE DERMATOLOGY, INC.

Chalfonte-Haddon Hall Hotel
Atlantic City, New Jersey

Friday and Saturday, April 27 and 28 1973

OFFICERS

ROBERT W. GOLTZ, M.D., Minneapolis, Minnesota, President
RICHARD B. STOUGHTON, M.D., La Jolla, California, President-Elect
ALLAN L. LORINCZ, M.D., Chicago, Illinois, Vice-President
JOHN S. STRAUSS, M.D., Boston, Massachusetts, Secretary-Treasurer

WORKSHOPS

(Jointly Sponsored with the National Program for Dermatology)

FRIDAY, APRIL 27, 1973, 7:30 P.M.

Workshop Directors
Acne James E. Fulton, Jr., M.D., Miami, Florida.
Cyclic AMP: Effects on Differentiation and Cell Division
Immunofluorescence

John J. Voorhees, M.D., Ann Arbor, Michigan.
Robert E. Jordon, M.D., Rochester, Minnesota.
W. Mitchell Sams, Jr., M.D., Denver, Colorado.
Irma Gigli, M.D., Boston, Massachusetts.
Kirk D. Wuepper, M.D., Portland, Oregon.
Melanin Pigmentation
Sidney N. Klaus, M.D., New Haven, Connecticut.
Tumor Immunology
Jean-Claude Bystryn, M.D., New York, New York.
William T. Summerlin, M.D., Minneapolis, Minnesota.

MORNING SESSION

SATURDAY, 9:00 A.M.

ROBERT A. BRIGGAMAN, M.D., Chapel Hill, North Carolina, presiding.

1. CATHEPSIN D OF RABBIT SKIN: AN IMMUNOENZYMIC STUDY. G. S. LAZARUS, M.D.* (Introduced by F. PASS, M.D.) Strange-

* by invitation
Cathepsin D (CD), one of the major lysosomal proteinases, has been identified in rabbit skin. Extracts of skin were capable of degrading hemoglobin, at pH 3.2, to peptides that are soluble in trichloracetic acid (TCA). This activity was blocked by the CD inhibitor pepstatin (100 µg/ml) and was eluted from a Sephadex G-100 column at a position identical to that of ultimately pure rabbit liver CD. A line of complete identity between crude skin extracts and ultimately pure CD was found in immunodiffusion studies using sheep anti-rabbit CD (ARCD). The addition of ARCD to skin extracts resulted in the precipitation of 85% of the hemoglobinolytic activity at pH 3.2. Skin extracts which had CD removed by immunoprecipitation lost their ability to degrade both hemoglobin and skin protein to TCA-soluble peptides at acid pH. The removal of CD facilitated the detection of a neutral proteinase which had not been previously recognized in rabbit skin. The location of CD in tissue sections was determined by double layer immunofluorescent microscopy and by autoradiography using H labelled ARCD. By using these sensitive immunoenzymic techniques, CD has been identified in skin and its role in catabolic processes defined.

2. IN VITRO STUDIES OF ALLERGIC CONTACT DERMATITIS TO DNCB. A. E. MILLER, JR., M.D. AND W. R. LEVIS, M.D., Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.

Lymphocyte transformation in vitro is used for the study of dinitrochlorobenzene (DNCB)-induced allergic contact dermatitis in man. Free DNCB, like many contact sensitizers, is highly water insoluble and toxic in leukocyte cultures. DNCB, coupled to peripheral white blood cells, red blood cells, or even some tissue culture cells, forms a complex (DNCB-antigen) that is particulate, storable, has a low degree of toxicity, and induces lymphocyte transformation only in leukocyte cultures from subjects sensitized to DNCB. DNCB-antigen was prepared by incubating cells in dimethyl sulfoxide (DMSO) containing .001-10µg% DNCB for 1 hr at 38°. The resultant DNCB-antigen was washed, lyophilized, and added to peripheral leukocyte cultures (optimal range 10-50 µg/ml). ³HThdR incorporation was measured on the 4th or 5th day of culture (Blood 40: 77, 1972). Sequential studies, utilizing frozen-stored leukocytes, show that specifically reactive lymphocytes as measured by ³HThdR incorporation, are first detected at about 10 days after in vivo application of a primary sensitizing dose of DNCB, reach a peak at about 14-21 days, and remain detectable for at least 2 months following sensitization. Lymphocytes of nonsensitized subjects failed to respond to DNCB-antigen even in cultures containing plasma from sensitized subjects. Sensitive lymphocytes responded regardless of the plasma source. One subject was rechallenged with DNCB in vivo 4 months after sensitization and showed an in vitro boost in peripheral lymphocyte ³HThdR incorporation. Thus, specific lymphocyte transformation to DNCB can be detected in vitro utilizing a system which is useful in the study of cell-mediated immunity in man and may prove applicable to other allergens.

3. LYMPHOCYTE CYTOTOXICITY—A SYSTEM FOR IN VITRO STUDY OF CONTACT DERMATITIS. S. J. STEGMAN, M.D., K. FUKUYAMA, M.D. AND W. L. EPSTEIN, M.D., Department of Dermatology, University of California, San Francisco, California 94122.

Lymphocyte cytotoxicity has been adapted to study contact dermatitis using in vitro grown epidermal cells conjugated with hapten as targets. Guinea pigs were sensitized to paraphenyamine diamine (PDA). Epidermal cells obtained from ear skin before and after sensitization were grown in vitro, and monolayers were treated with .66 µgm/ml PDA and washed. Splenic lymphoid effector cells were then layered over the epidermal target cells and observed for cytotoxicity. In 15 of 17 trials greater than 80% of epidermal target cells were specifically killed on the 4th day. Control unconjugated epidermal target cells were not killed and lymphoid effector cells from control animals did not kill. PHA stimulated lymphocytes nonspecifically killed all epidermal target cells on day 2. The reactions were not affected by the use of 1) Epidermal target cells taken before or after sensitization 2) autologous or homologous epidermal cells, or 3) complement.
These findings indicate that contact dermatitis can be studied by the in vitro cytotoxic reaction. Use of living epidermal cells for the conjugation of haptens as well as for epidermal target cells more closely approximates in vivo conditions than other in vitro techniques. This method provides a model to further examine the mechanisms involved in contact dermatitis and may be applied for diagnostic clinical use.

4. CONTACT UNRESPONSIVENESS IN CARCINOGEN INFUSED GUINEA PIGS. J. R. POMERANZ, M.D. AND J. F. CARNEY, M.D., Division of Dermatology, Cleveland Metropolitan General Hospital, Cleveland, Ohio 44109.

Young guinea pigs infused with 7,12-dimethylbenz(a)anthracene (DMBA), a carcinogen and contact sensitizer, develop a variety of malignant neoplasms. The following studies were performed to determine if this procedure also induced unresponsiveness to contact sensitization with DMBA. Groups of 3-400 gm guinea pigs were infused with 1 ml of glycofurol-dimethylsulf­oxide 1:1 containing 5 or 30 mg of DMBA; or with 1 or 4 ml of Upjohn fat emulsion containing 5 or 20 mg DMBA respectively. Controls received the solvent alone. Contact testing with 1, 0.5, and 0.1% DMBA two weeks later revealed an occasional animal with a 1 + reaction to 1% DMBA, but most had trace or negative responses. Seven days later the animals were immunized by foot pad injections of 300 µgms of DMBA in complete adjuvant. Tests for contact and PPD reactivity were performed two weeks afterwards. Fifty to 66% of those infused with 5 mg DMBA in either solvent had negative or trace contact reactions to 1% DMBA. Larger doses induced unresponsive­ness in 80 to 100% of the recipients. In contrast, all but a few of the control animals had 2+ or better contact reactions to 1% DMBA. All groups responded positively to PPD.

These studies demonstrate that infusion of DMBA inhibits the capacity to develop contact reactivity to the carcinogen, comparable to the tolerant state obtained with simple chemicals. Studies to evaluate the relationship between contact unresponsiveness and induction of malignant tumors with DMBA are in progress.

5. REFRACTORINESS OF MINIATURE MELANOCYTES TO UL­TRAVIOLET LIGHT. W. M. REAMS, JR., PH.D. AND V. H. HOWARD, Jr., A. B.*, Department of Dermatology, Medical College of Virginia, Richmond, Virginia 23298 and Department of Biology, University of Richmond, Virginia 23173.

Very small, weakly DOPA-reactive melanocytes have been described at the border of a freckle covering a scar (Breathnach, 1958) and small precursor melanocytes have been described in the bulbs of resting hairs (Silver et al., 1969). Small, weakly DOPA-reactive melanocytes with nuclei less than one quarter the size of normal have been noted in the epidermis of PET/Wmr mice. Their population remains relatively constant against the radically changing population of regular-sized melanocytes during the first postnatal week.

In an attempt to determine the nature of these miniature melanocytes, mice were irradiated daily from birth to ten days with ultraviolet light (UVL) of 2537Å, 3000Å or 3660Å. Epidermal samples were taken from several mice each day of irradiation and for each of the wave lengths. These samples were treated with DOPA and examined. The epidermis showed a normal tanning response to the UVL with an increase in the number of regular-sized melanocytes and in melanogenesis. However, the condition of the miniature melanocytes remained essentially stable. It would appear that these miniature melanocytes of murine epidermis are type specific and are inherently different in response to UVL stimulation which generally provokes hyperpigmentation.

6. CHARACTERIZATION OF MELANOSOMAL PROTEINS. K. JIM­BOW, M.D., H. SUGANO, PH.D.*, J. B. BURNETT, PH.D. AND T. B. FITZPATRICK, M.D., Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114.

Melanosomes, the secretory products of melanocytes, appear to be composed primarily of three distinct chemical components: 1) the biochrome, melanin, 2) an enzyme, tyrosinase, which catalyzes the oxidation of tyrosine and DOPA to melanin, and 3) other proteins which comprise the matrix of the melanosome. This study is directed toward the elucidation of the chemical nature and the mechanism of synthesis of normal and pathological melanosomes. Melanosomes from retinal pigment epithelium of chickens and B-16 and Harding-Passey mouse melannomas
were isolated and purified by sucrose density gradient techniques. These melanosomes were subjected to digestion with trypsin (trypsin: melanosomal proteins = 1:50; 0.1 M phosphate buffer, pH 7.4, 33° C, 30 min) followed by treatment with urea (8 M urea in 0.05 M Tris-HCl buffer, pH 9.0, 33° C, 20 hrs) in order to obtain melanosomal proteins. Using column chromatography, electrophoresis and gel filtration, these melanosomal proteins can be separated into two principal classes: 1) melanoprotein (about 5 species of MW ~ 100,000) associated with a melamin moiety, and 2) nonmelanoprotein (about 15 species of MW ~ 6,000–70,000) which can be further separated into two distinct fractions. Within the extracts of melanosomal proteins, tyrosinase can be discerned in at least two active forms, one which appears to be free from melanin and the other which appears to still have a melanin moiety attached. The “melanin-free” form of tyrosinase has a higher specific activity than the “melanin-attached” form.

7. DIHYDROTESTOSTERONE FORMATION IN SKIN FROM DIFFERENT HAIR-BEARING SITES. L. I. ROSE, M.D.*, R. H. UNDERWOOD, PH.D.*, G. H. WILLIAMS, M.D.*, M. T. W. DUNNING, B. A.*, S. L. PEIETON, M.D. AND P. E. POCHI, M.D. Endocrine-Metabolic Unit, Peter Bent Brigham Hospital, and Department of Dermatology, Boston University Medical Center, Boston, Massachusetts.

The metabolism of testosterone (T) to 5-alpha dihydrotestosterone (DHT) via Δ^5-3-ketosteroid 5-alpha-oxidoreductase (5-reductase) has been demonstrated in the hair follicle. If the formation of DHT is essential for hair growth, then the concentration of 5-reductase might be expected to be greater in skin areas of terminal hair growth. The purpose of this study was to determine whether there are many differences in the conversion of ^14C-T to ^14C-DHT in skin biopsies from an area of terminal hair growth (extensor surface of forearm) as compared to those with vellus hair growth (chin and flexor surface of forearm). Three millimeter punch biopsies of the skin were taken from the three areas of five normal, non-hirsute female volunteers. The biopsy specimens were incubated for 1 hr with ^14C-T and the per cent conversion of ^14C-DHT measured. Purity of the ^14C-DHT was shown by demonstrating constant ^3H/^14C ratios following 3 chromatography steps and the preparation of the acetate derivative. The following results were obtained:

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean % conversion to DHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chin</td>
<td>0.41 (range 0.20–0.94)</td>
</tr>
<tr>
<td>Extensor Forearm</td>
<td>0.09 (range 0.03–0.18)</td>
</tr>
<tr>
<td>Flexor Forearm</td>
<td>0.09 (range 0.05–0.14)</td>
</tr>
</tbody>
</table>

It is concluded that the skin from a terminal hair-bearing area does not show greater 5-reductase activity than that of vellus hair regions and that the greater activity seen in the chin as compared to the forearm sites probably reflects greater density and size of sebaceous glands.

8. STUDY ON IN VITRO LIPOGENESIS OF CUTANEOUS XANTHOMA TISSUE. C. HU, M.D.; R. D. ELLEFSON, PH.D.*; AND R. K. WINKELMANN, M.D., Department of Dermatology, Mayo Clinic, Rochester, Minnesota 55901.

To evaluate whether different mechanisms are responsible for the formation of normolipemic and hyperlipemic cutaneous xanthomas, studies on in vitro lipogenesis of xanthoma tissue were performed. Five patients with various types of rare normolipemic xanthomas (xanthoma disseminatum, diffuse plane xanthoma, xanthelasma), and two cases of type II hyperlipoproteinemia with xanthoma tuberosum and xanthelasma were included in the study. Biopsy specimens of cutaneous xanthoma tissue were incubated at 37° C for 6 hours in 2 ml of Krebs-Ringer phosphate buffer pH 7.4 containing sodium (2-^13C) acetate (1.6 μc/ml, 34 mc/mnmole). The tissue lipids were extracted and then separated by silica gel thin-layer chromatography. The radioactivity was determined by scintillation spectrometry. Control studies were performed on specimens of normal appearing skin on each patient. The incorporation of acetate into all major lipid groups was greater in xanthoma tissue than in normal skin. Among the normolipemic xanthomas, xanthoma disseminatum appeared to exhibit the greatest relative increase in all lipid groups when compared to normal skin. The greatest incorporation occurred in the phospholipid fraction. This finding indicated that local metabolism of lipid contributes to the formation of normolipemic xanthomas. Such distinct data have
not been reported in the literature. The significant amount of acetate incorporation into hyperlipemic xanthomas may be reflective of a role of lipid synthesis \textit{in situ} in the formation of hyperlipemic xanthomas. This finding is in disagreement with work done by others.

9. PERMEABILITY OF HUMAN EPIDERMIS TO HEXACHLOROPHENE. P. H. DUGARD, PH.D. AND R. J. SCHEUPLEIN, PH.D., Department of Dermatology, Harvard Medical School at Massachusetts General Hospital, Boston, Massachusetts 02114.

The reevaluation of the benefit-risk ratio for hexachlorophene (HCP) as a topical agent prompted investigation of its percutaneous absorption. Post mortem samples of isolated human abdominal epidermis were employed in permeability and sorption experiments with \textsuperscript{14}C-HCP to establish permeability constants (k\textsubscript{p}), diffusion constants (D\textsubscript{m}) and membrane-vehicle partition coefficients (K\textsubscript{m}). \[ k\textsubscript{p} \propto K\textsubscript{m} \times D\textsubscript{m}, \] and steady state absorption rate \(~ th) with a high K\textsubscript{m} of 200. Buffer at pH 8.6 with 40\% ethanol content gave a k\textsubscript{p} for HCP of 0.25 \times 10^{-3} cm/hr with a K\textsubscript{m} of 10. In the 40\% ethanol-buffer combination, reducing the pH, and thus lessening ionization of HCP, the k\textsubscript{p} increased progressively; at pH 3.5, k\textsubscript{p} was 4.5 \times 10^{-3} cm/hr with a K\textsubscript{m} over 200 and slightly increased D\textsubscript{m}. From 0.1\% soap solution the k\textsubscript{p} of HCP was 1.6 \times 10^{-3} cm/hr, but the greater damage by 1\% soap gave a k\textsubscript{p} rising to 8.0 \times 10^{-3} cm/hr. The k\textsubscript{p} of HCP from pHisoderm-water (1:1) was <0.1 \times 10^{-4} cm/hr. HCP has a D\textsubscript{m} of 2-8 \times 10^{-11} cm²/sec in stratum corneum and thus takes about 10-25 hours of continuing contact to achieve a maximal rate of penetration. The high affinity that stratum corneum has for HCP in certain vehicles does not immobilize HCP in that tissue, and penetration is enhanced by the high K\textsubscript{m}. Wide variations in k\textsubscript{p} and K\textsubscript{m} show the importance of vehicle composition in optimizing safety and efficacy.


Does the study of percutaneous absorption through the use of in vitro techniques adequately describe, both qualitatively and quantitatively, the in vivo state?

Human abdominal skin obtained at autopsy was mounted as a barrier between two chambers with the inside bathed by buffered, isotonic saline at 37° C., and the outside exposed to ambient laboratory conditions. Thus, the temperature and water concentration gradients that exist \textit{in vivo} were approximated. Ten compounds, whose percutaneous absorption has been studied in living man (Feldman and Maibach), were investigated. The overall correlation was good considering the many problems inherent in this type of work. With compounds whose rate of penetration was high, excellent agreement was found (within a factor of two). Compounds whose rate of penetration was low, consistently gave higher rates \textit{in vitro} than \textit{in vivo} (by an order of magnitude). There are many possible explanations which could explain the discrepancy. Probably most important is the fact that the in vitro studies utilized abdominal skin, whereas the in vivo studies used forearm skin. Electron microscopy has revealed a difference in the thickness of the stratum corneum in the two areas.

One other interesting finding emerging from the study which further corroborates the validity of in vitro work, is the striking similarity of the urine excretion rate/time seen \textit{in vivo} and the flux rate/time seen \textit{in vitro}.

\textbf{AFTERNOON SESSION}

\textbf{SATURDAY, 1:30 P.M.}

\textbf{WILLIAM T. SUMMERLIN, M.D.}, Minneapolis, Minnesota, presiding.

1. THREE GENETIC FORMS OF XERODERMA PIGMENTOSUM. K. H. KRAEMER, M.D.*, J. H. ROBBINS, M.D.* AND H. G. COON, PH.D.* (Introduced by M. A. LUTZNER, M.D.), Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.

Xeroderma pigmentosum (XP) is an autosomal recessive disease in which sun-exposed skin develops actinic damage, pigmentary changes and numerous malignancies. Some patients also
have neurological deficits. Cells from most XP patients are deficient in repairing DNA damaged by ultraviolet (UV) light as shown by a low rate of tritiated thymidine (\( ^3 \)HTdR) incorporation during their DNA repair synthesis. De Weerd-Kastelein, Keijzer and Bootsma have reported the existence of two complementation groups of XP patients (Nature, New Biol. 238: 80, 1972). We now report finding three complementation groups in a series of seven patients. Heterokaryons were made by fusing fibroblasts from different pairs of XP patients using killed Sendai virus. The cells were then irradiated with 150 ergs/mm\(^2\) from a germicidal lamp, incubated at 38\(^\circ\)C with \(^3\)HTdR for 3 hr, washed, and processed for autoradiography. Analysis of the number of grains over the nuclei in binucleate cells revealed that each XP cell line could be assigned to one of three complementation groups. Fusion of cells from any two groups gave 70-100% of the UV-induced \(^3\)HTdR incorporation of normal donors' unfused fibroblasts, whereas fused cells within the same group gave no more incorporation per nucleus than was found in the same cells unfused. The three complementation groups were found to represent three classes of repair rates: unfused cells of group A (2 patients) had < 2% of normal incorporation; group B (1 patient), 3-7%; and group C (4 patients), 15-25%. Additional patients are being studied to determine the relationships, if any, between the complementation groups and both the clinical forms and repair rates of xeroderma pigmentosum.

2. STUDIES ON THE PATHOGENESIS OF RECESSIVE EPIDERMOLYSIS BULLOS A DYSTROPHICA. R. A. BRIGGAMAN, M.D. AND C. E. WHEELER, JR., M.D., Department of Dermatology, University of North Carolina, Chapel Hill, North Carolina 27514.

The purpose of this study was to define the ultrastructural defects and to determine any abnormalities in formation of structures at the epidermal-dermal interface in recessive epidermolysis bullosa dystrophica (EBD-R).

Ultrastructural abnormalities in patients with EBD-R were confined to the sub-basal lamina area where anchoring fibrils were very sparse and those rare fibrils which were present were abnormal. Some collagen fibers in the sub-basal area had an abnormal smudged appearance whereas others had typical collagen cross-banding. Mechanically induced epidermal-dermal separation consistently occurred immediately beneath the basal lamina.

In previous studies (J. Cell Biol. 51: 384, 1971), both basal lamina and anchoring fibrils reformed in grafts composed of isolated and recombined epidermis and dermis derived from normal adult skin grown for periods of 9 days on the chick chorioallantoic membrane (CAM). Recombinants of isolated epidermis and dermis from normal subjects and patients with EBD-R were prepared and cultured on the CAM. In recombinants of EBD-R epidermis and EBD-R dermis, basal lamina was present, but normal anchoring fibrils were never seen. In recombinants of normal epidermis and EBD-R dermis, basal lamina reformed normally, but anchoring fibrils were consistently absent. Recombinants composed of EBD-R epidermis and normal dermis showed reformation of both basal lamina and anchoring fibrils. These studies indicate that there is an impairment in the formation of anchoring fibrils in EBD-R and that the defect resides in the dermal component.

3. HAIR DEFECTS IN CARTILAGE-HAIR HYPOPLASIA. L. A. GOLDSMITH, M.D. AND H. P. BADEN, M.D., Department of Dermatology, Harvard Medical School, Boston, Massachusetts 02114.

Cartilage-hair hypoplasia (CHH) is an autosomal recessive disease with metaphyseal dysostosis, dwarfism, fine hair and frequent infections. Physical and chemical studies of hair from three Amish and one non-Amish sibships were undertaken to investigate the molecular defect.

Stress strain curves of CHH hair in water showed a lowered breaking strength and decreased slope of the Hookean region, although no abnormality was observed in non-aqueous solvents. The ultrasonic modulus of elasticity, sulfur content and x-ray diffraction pattern were normal. These data suggest that the major abnormality in CHH hair may be the coupling between filaments and matrix.

Since disulfide bonds couple matrix and filaments, the stability of these bonds in CHH and control hair was compared. CHH hair extracted at pH 11 with 6 M urea and 0.1 M mercaptoethanol yielded less protein, which was relatively deficient in the filamentous components as judged by disc electrophoresis. When dithiothreitol, a more efficient reducing agent, was substituted for mercaptoethanol, there was a much smaller difference between CHH and control hairs.
Minor structural changes near disulfide bonds could be responsible for the altered physicochemical properties which have been observed and the various components of CHH hair are now being studied.

4. PROLINE ANALOGUES INHIBIT THE FORMATION OF HELICAL COLLAGEN. J. Uitto, M.D., Ph.D. AND D. J. Prockop, M.D., Ph.D.*, Department of Biochemistry, Rutgers Medical School, New Brunswick, New Jersey 08903.

Matrix-free cells from embryonic tendon were incubated with \( ^{14} \text{C}-\text{proline} \) or \( ^{14} \text{C}-\text{lysine} \) to study the synthesis and secretion of procollagen. The effects of the following proline analogues were tested: azetidine-2-carboxylic acid, cis-4-hydroxyproline, cis-4-fluoroproline, 3,4-dehydroproline and cis-4-bromoproline. All five proline analogues in concentrations of 10 to 400 \( \mu \text{g} \) per ml decreased the amount of intact procollagen secreted into the medium. The small amount of protein-bound \( ^{14} \text{C} \) still recovered in the medium in the presence of the analogues consisted of small peptides which were degradation products of procollagen. The cells were homogenized directly in acetic acid and treated with 100 \( \mu \text{g} \) per ml of pepsin at 4° for 15 hrs and then at 15° for 6 hrs. Gel filtration of the products on agarose in SDS indicated that in control cells about 60% of the intracellular \( ^{14} \text{C}-\text{collagen} \) was resistant to pepsin and therefore presumably triple-helical. In cells incubated with proline analogues in concentrations which inhibited the secretion of intact procollagen, essentially none of the intracellular \( ^{14} \text{C}-\text{collagen} \) was resistant to pepsin. Previous data demonstrated that the proline analogues are incorporated into collagen and the present results suggest that the presence of the analogue prevents the polypeptides from becoming helical. The proline analogues appear therefore to provide a novel mechanism for preventing the deposition of collagen in tissues and they may prove useful in clinical situations.

5. HISTIDINE-RICH PROTEIN AS A MAJOR COMPONENT OF KERATOHYALIN IN THE NEWBORN RAT EPIDERMIS. L. A. Sibrack, M.S.* AND I. A. Bernstein, Ph.D., Department of Environmental and Industrial Health, The University of Michigan, Ann Arbor, Michigan 48104.

Synthesis of histidine-rich protein (HRP) which takes place in the epidermal granular cells of the newborn rat and accounts for much of the total \( ^{3} \text{H}-\text{histidine} \) incorporated in these cells in vivo, represents one molecular event in the process of keratinization. Since \( ^{3} \text{H}-\text{histidine} \) is first seen in the extra-granular cytoplasm and later accumulates in the keratohyalin (KH), it is reasonable to expect that HRP is synthesized outside the granule and then becomes a component of KH. HRP has now been obtained from KH isolated from newborn rat epidermis by the method of Ugel (J. Cell Biol., 49: 405, 1971) and has been compared with HRP from KH-free residual epidermis. Both samples of HRP had similar amino acid compositions and had subunits of the same molecular weight (15,000). Each subunit was homogeneous as determined by chromatography on Sephadex and polyacrylamide disc gel electrophoresis in SDS. Samples of HRP from KH and from the residual tissue were obtained by chromatography on Sepharose 6B with molecular weights of 390,000 and 190,000, respectively. Kinetic data on incorporation of \( ^{3} \text{H}-\text{histidine} \) into the two forms of HRP in vivo suggest that HRP in the extra-granular cytoplasm is converted to KH-HRP by aggregation from a particle with 13 subunits to one with 26 subunits. Identification of HRP, a special gene product, as a major component (50–60%) of KH provides a handle for studying control mechanisms in keratinization.

6. PROSTAGLANDINS AND SUNBURN. D. Sekura Snyder, Ph.D.* AND W. H. Eaglstein, M.D., Department of Dermatology, University of Miami, Miami, Florida 33152.

Prostaglandins (PG) have been implicated as mediators of ultraviolet (UV) erythema. In humans oral aspirin will delay the onset and decrease the intensity of UV-induced redness. This study was undertaken to determine the effect of some anti-PG agents on UV erythema.

A Westinghouse FS 20 sunlamp was used to irradiate the volar forearms of volunteers and the backs of depilated albino guinea pigs. Test agents were injected intradermally at various times before or after irradiation. The test agent was judged effective if it caused greater blanching than did saline.

In both humans and guinea pigs, indomethacin and aspirin (inhibitors of PG synthesis) can blanch UV erythema if injected before or after redness appears. Indomethacin exhibits a dose
response and can blanch redness if injected up to five hours after erythema has developed. Since PG are rapidly metabolized by the skin, this suggests that PG may be continuously produced for some time after UV irradiation. A PG antagonist, 7 oxa 13-prostynoic acid, was tested in guinea pigs and was effective. Triamcinolone acetonide was effective if injected before or after redness appeared but only in human skin. The ability of anti-PG agents to decrease and delay UV induced redness lends further support to a probable role for PG in sunburn.

7. THE SYNTHESIS OF PROSTAGLANDINS \( E_2 \) AND \( F_{2\alpha} \) IN PSORIATIC SKIN. K. Aso, M.D.*, N. Sakamoto, Ph.D.*, E. Farber, M.D., D. Deneau, M.D., L. Krulig, M.D. AND D. Wilkinson, Ph. D., Department of Dermatology, Stanford University, Stanford, California 94305.

Prostaglandins have been implicated in epidermal growth control. The excessive epidermal proliferation found in psoriasis appears to indicate a disturbance in this control mechanism. This study was undertaken to estimate and compare the biosynthesis of prostaglandins in the involved and uninvolved psoriatic epidermis. Strips of psoriatic plaque epidermis 0.4 mm thick were obtained with the Castroviejo keratome. Similar specimens 0.2 mm thick were removed from uninvolved skin. The depth into the epidermis of all the specimens was evaluated histologically. The specimens were homogenized in ice cold modified Bucher medium, centrifuged at 900 g and the supernatant was incubated with 1 \( \mu \)Ci of \( C^14 \)-arachidonic acid (Sp Act 55.5 mCi/mM), 0.65 mM glutathione and 0.55 mM hydroquinone. Radioactive prostaglandins were extracted with dichloromethane and subjected to silicic acid chromatography. \( \text{PGE}_{2} \) and \( \text{PGF}_{2\alpha} \) were separated on thin layer chromatography and expressed as picomole/mg protein. Average levels of synthesis of \( \text{PGE}_{2} \) and \( \text{PGF}_{2\alpha} \) in uninvolved epidermis were 5.02 \( \pm \) 0.79 picomole/mg protein and 1.24 \( \pm \) 0.45/mg picomole/mg protein, respectively. In involved epidermis these values were 0.89 \( \pm \) 0.41 picomole/mg protein and 0.35 \( \pm \) 0.35 picomole/mg protein, respectively. The lowered synthesis of prostaglandins in the psoriatic plaque may indicate that these substances play a significant role in the control of epidermal growth.

8. IDENTIFICATION OF HUMAN EPIDERMAL CHALONE. D. P. Chopra, Ph.D. AND B. A. Flaxman, M.D., Department of Dermatology, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140.

Crude human skin extract containing mitotic inhibitory activity (chalone) was treated by ethanol fractionation in order to purify and identify the epidermal chalone. The greatest mitotic inhibitory activity was concentrated in the 81% precipitate, the active principle being purified about 30 times. Disc gel electrophoretic analysis of the 81% precipitate revealed only 2 bands, one of which was albumin; the second band is believed to represent the epidermal chalone. This finding was confirmed by studies in which various groups of proteins from the crude extract were removed from electrophoretic gels and tested for mitotic inhibitory activity. The group of proteins which contained the chalone activity corresponded to the protein bands of the 81% ethanol precipitate.

9. DERANGED EPITHELIAL HOMEOSTASIS IN PSORIASIS ASSOCIATED WITH IMBALANCED CYCLIC AMP AND CYCLIC GMP LEVELS. J. J. Voorhees, M.D., E. Duell, Ph.D., M. Stawiski, M.D.*, M. Haddox* AND N. Goldberg, Ph.D.*, Department of Dermatology, University of Michigan, Ann Arbor, Michigan 48104, and Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota 55414.

Since epinephrine raises epidermal cyclic AMP (cAMP) and can slow cell division, we suggested that rapidly dividing psoriatic epithelium might be associated with decreased cAMP and subsequently showed a significant decreased cAMP in 25 patients. This decrease may be analogous to rapidly dividing cAMP-deficient "malignant" transformed cultured cells. In this current study, to confirm our previous report that cAMP is significantly decreased, we used Gilman's assay in an additional 25 patients to show that cAMP is 36% decreased (p = .001) based on DNA in involved epithelium (IE) vs uninvolved epithelium (UE). Synthesis of cAMP in IE may be normal since the basal rate and stimulation by isoproterenol of adenylate cyclase activity in epithelial slices are similar in IE and UE of 23 patients. Hydrolysis of cAMP in IE may also be normal since the maximum catalytic capacity of soluble cAMP-phosphodiesterase, measured
with saturating cAMP concentrations, were no different in IE and UE from 18 patients. Since cGMP can have the potential to decrease cAMP and because mitogen stimulated proliferation of lymphocytes is associated with strikingly increased cGMP, we measured and found a 94% increase (p = .001) in cGMP based on DNA of 12 pts.

In conclusion: 1) the data definitely do not exclude but militate against a defect in cAMP synthesis or hydrolysis to explain the decreased cAMP in IE. 2) Rather, increased cGMP may decrease cAMP in IE. 3) Normal keratinization may require cAMP and cGMP levels to be within some “normal”, perhaps very narrow, homeostatic range.