PROGRAM

THE THIRTY-SECOND ANNUAL MEETING

THE SOCIETY FOR INVESTIGATIVE DERMATOLOGY, INC.

Sheraton-Plaza Hotel
Boston, Massachusetts

Friday, Saturday and Sunday June 18, 19, and 20, 1971

OFFICERS

RICHARD K. WINKELMANN, M.D., Rochester, Minnesota, President
GEORGE W. HAMBRICK, JR., M.D., Baltimore, Maryland, Vice-President
JOHN S. STRAUSS, M.D., Boston, Massachusetts, Secretary-Treasurer

MORNING SESSION

FRIDAY, JUNE 18, 1971

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

TOPIC: MELANOGENESIS, GEORGE F. ODLAND, M.D., Seattle, Washington, moderator.
THOMAS B. FITZPATRICK, M.D., PH.D. Boston, Massachusetts, Development of the melanosome.
MORRIS FORSTER, PH.D. Ann Arbor, Michigan, Genetic aspects of melanogenesis.
ALVIN S. ZELICKSON, M.D. Minneapolis, Minnesota, Clinical anomalies in pigmentation.

8:30 A.M. Business and Executive Session: RICHARD K. WINKELMANN, M.D. Rochester, Minnesota, presiding.

10:00 A.M. SCIENTIFIC SESSION: GEORGE W. HAMBRICK, JR., M.D. Baltimore, Maryland, presiding.


2. EFFECTS OF UV RADIATION ON MICROCIRCULATION IN THE HAMSTER CHEEK POUCH. J. P. BLOCKSON*, P. D. FORBES*, AND F. URBACH, The Skin and Cancer Hospital, Temple University Health Science Center, Philadelphia, Pennsylvania 19140.

Short wave nonionizing radiations, as well as ionizing radiations, cause significant vascular changes, manifested in part by erythema. Several questions have been raised about

* By invitation.
the quantity of mid-range ultraviolet radiation (UVB) that reaches the vessels of the skin, and the extent to which direct irradiation could account for observed erythema. In order to evaluate effects on directly irradiated vessels, we have studied hamster cheek pouches after they were exposed to Westinghouse FS407T12 “sun” lamps. Transmission of the UVB through the membrane was found to be approximately 40%. Hamsters received intravenous infusions of colloidal carbon and Evans Blue dye immediately prior to observation. Microcinematography was used to record changes which included uniform and saccular vasodilation, venous sodium transport is similar in controls. 3. saccular vasodilation, venous saccular vasodilation, venous tortuosity, establishment of preferred channels of flow, reversal of flow, vascular stasis, petechiae formation, increased permeability and endothelial edema, staining of injured vessels with colloidal carbon, and Evans Blue extravasation from severely congested, heavily carbon-stained capillary nets. These reactions appear to be dose-dependent and they are strongly influenced by photosensitizers. The hamster cheek pouch offers the possibility of following in vivo the sequence of damage and repair in irradiated microcirculatory elements.

3. COLLAGENOLYTIC ACTIVITY OF HUMAN SERUM. K. H. NELDNER, M.D., AND C. SOLOMONS, Ph.D.*, University of Colorado Medical Center, Denver, Colorado 80220.

Fresh human serum was investigated for possible collagenolytic activity against tail tendon collagen extracted from young, rapidly growing rats. Four different parameters of investigation yielded confirmatory evidence that serum contains an enzyme with lytic effects on collagen. 1. 14C glycine labeled collagen was separated into 1.0M NaCl soluble and insoluble fractions. Solubilization of radioactivity into the supernate following incubation of the insoluble fraction and serum at 37°C for 6 hours was on the average 2.75 times greater in the serum-collagen mixture than in collagen controls without serum. 2. The reaction products of similarly incubated unlabeled soluble collagen-serum mixtures were analyzed by Sephadex G-200 gel filtration. Collagen breakdown particles in the size range of 100,000-200,000 M.W. were detected in the elution patterns of the reaction mixture but not in the controls. 3. Similar results were observed when 14C glycine labeled soluble collagen was used and the Sephadex G-200 separation products analyzed by scintillation counter rather than spectrophotometry. 4. Electron photomicrographs of the collagen substrate before and after reaction with serum confirm the above described changes and the conclusions that serum contains a collagenolytic factor, the specific nature of which was not determined. The possible clinical significance of this finding will be discussed. The present findings are not believed to be contradictory to the reports of previous authors indicating that human serum inhibits collagenase from some human sources.

4. SEA NETTLE TOXIN. IMMUNOLOGIC PROTECTION FROM CUTANEOUS STINGS AND OTHER BIOLOGICAL EFFECTS WITH STUDIES ON ITS EFFECT ON PERCUTANEOUS SODIUM TRANSPORT, J. W. BURNETT, M.D., W. M. GOULD, M.D., AND R. GOLDNER, M.D., Division of Dermatology, University of Maryland School of Medicine, Baltimore, Maryland 21201.

This study was initiated to define the immunologic and pathogenic properties of sea nettle stings. Chrysaora venom was antigenic to rabbits with complement fixing antibodies and protective factors produced against the lethal, hemolytic and demoneerotic factors. Complement fixing antibodies were found in guinea pigs stung by nettle meuduse. The cutaneous eruption which appears after contact with nettle tentacles was blocked by active immunization but not by local injection of hyperimmune sera. Transport of sodium across frog skin (measured by short circuit currents) was increased by toxin applied to either epidermis or dermis. This increase was blocked by hyperimmune serum but not ouabain. The toxin had no effect on guinea pig kidney ATPase. The toxin's effect on percutaneous sodium transport is similar to vasopressins' and its action may be mediated by cyclic AMP. These studies suggest that the toxin may affect cyclic AMP and that immunologic prophylaxis against the sting may be feasible.
5. LABILITY OF DESMOSOMES IN CELL CULTURE. S. N. KLAUS, M.D., S. H. BRANSON*, AND G. E. MOELLMANN, PH.D.*, Section of Dermatology, Yale University School of Medicine, New Haven, Connecticut 06510.

Recent reports on the movements of epidermal cells in vivo have indicated that desmosomes are readily broken and reformed. In this study lability of these intercellular contacts was examined directly using cell culture techniques.

Trypsin dispersed, dermis free preparations of guinea pig epidermis were grown in Cruikshank chambers. Movements of cells and their membranes were recorded by time-lapse cinemierography. Areas of intercellular contact were examined by electron microscopy.

Time-lapse films showed that after epidermal melanin units had reformed in vitro and contact inhibition of movement had occurred membranes of adjacent keratinocytes continued to ruffle slowly. The membranes overlapped slightly. Electron micrographs showed desmosomes connecting adjacent epidermal cells. No hemidesmosomes were identified. Analysis of time-lapse films indicated that intermediate junctions formed between adjacent keratinocytes within twenty minutes after the cells had made contact. Melanocytes showed no contact inhibition of movement and migrated slowly over the keratinocytes. Specialized junctions did not form between melanocytes and keratinocytes.

This study confirms the lability of intercellular attachments.

6. INDUCTION OF ECTODERM DIFFERENTIATION BY DEAD DERMIS. W. M. REAMS, JR., PH.D. AND P. P. GRECO, M.D., Division of Dermatology, Medical College of Virginia, Richmond, Virginia 23219, and Biology Department, University of Richmond, Virginia 23173.

Previous studies have shown that either living or dead dermis will maintain adult human epidermis or 12-day chick embryo epidermis which had already undergone morphogenesis. Also, it has been shown that ectoderm from early embryos differentiates into a typical epidermis when combined with mesenchyme. The intent of the present study was to determine whether dead dermis could induce the differentiation of embryo ectoderm.

Dermis was prepared by freezing whole skin from 4-week-old mice and then slicing-off the epidermis and hypodermis with a keratome. Other dermis was prepared by trypsin separation. The dermis was frozen-thawed three times and then cut into small pads. Ectoderm was removed from the limb bud area of 10-day mouse embryos by cold trypsin separation. The ectoderm had a single germinative layer plus a simple periderm. The ectoderm sheets were associated with the dermal pads in normal orientation and then transferred to the chorioallantoic membrane of host chick embryos. By transfer, the explants were cultivated up to 18 days before recovery.

Histological examination revealed that the ectoderm had differentiated and produced stratum corneum. Ectoderm grafted to the chorioallantoic membrane without the dermis could not be found at time of recovery of the explants.

Dead dermis not only is able to maintain an intact epidermis, but also can induce embryonic ectoderm to differentiate into epidermis.

7. ULTRASTRUCTURAL LOCALIZATION OF AUTOANTIBODIES IN PEMPHIGUS, PEMPHIGOID AND LUPUS ERYTHEMATOSUS. N. S. WANGER, M.D., AND K. HASHIMOTO, M.D., Division of Dermatology and Rheumatology, University of Tennessee College of Medicine and Memphis Veterans Administration Hospital, Memphis, Tennessee 38104.

The precise ultrastructural localization of autoantibodies in pemphigus vulgaris, pemphigoid and lupus erythematosus (SLE) was determined, using ferritin-conjugated anti-human IgG as a marker. Thin sections of skin from normal individuals were incubated with pemphigus or pemphigoid sera, or serum from SLE patients with antinuclear factor (ANF). The sections were rinsed and incubated with ferritin conjugate. Tissue sections from both uninvolved and involved skin of systemic and discoid lupus erythematosus patients were incubated directly with the ferritin conjugate. In the specimens incubated with pemphigus vulgaris serum, ferritin particles were seen in the intercellular spaces in the epidermis.
There was some concentration around desmosomes and on the cell membranes. Tissue sections incubated with pemphigoid serum showed a heavy ferritin labeling of the basal lamina at the dermo-epidermal junction. There was homogeneous ferritin labeling of both epidermal and dermal nuclei in the sections of normal skin incubated with ANF serum from SLE patients. Sections from involved and uninvolved skin of SLE patients showed distinct ferritin labeling of the basal lamina at the dermo-epidermal junction and basal laminae of dermal blood vessels. Only involved skin of DLE patients showed basal lamina labeling. The basal lamina labeling in pemphigoid and SLE was specific and few other structures in the vicinity were labeled. Basal lamina labeling was minimal in controls.

**AFTERNOON SESSION**

**FRIDAY, JUNE 18, 1971**

2:00 P.M. SCIENTIFIC SESSION: ALFRED W. KOEFF, M.D. NEW YORK, NEW YORK. presiding.

1. HAMSTER MELANOMA CELLS ASSOCIATED WITH VIRUS-LIKE PARTICLES IN CULTURE. T. TANIGAKI, M.D.*, K. FUKUTAMA, M.D., AND W. L. EPSTEIN, M.D., Department of Dermatology, University of California San Francisco, San Francisco, California 94122.

Hamster melanoma cells (HMC) associated with virus-like particles (VLP) were grown in tissue culture for 15+ passages, to further characterize their oncogenic factor(s). In each passage VLP in cells increased in the first 3 to 5 days after subculture, when cells grew rapidly, and tended to disappear when the monolayer was complete. Pigment accumulated late in each passage. HMC from all passages maintained oncogenicity when they were injected into hamsters after 10 freeze/thaw cycles or 6 seconds of sonification. Differential centrifugation of tissue-cultured HMC homogenate at 700, 11,500, 36,250 and 130,000 g did not result in clear separation of oncogenic factor: VLP were seen in all fractions. To develop an in vitro assay for the oncogenic factor, skin or lung of normal newborn hamster was cultured by the plasma clot technic. The resulting cellular outgrowth was treated 2 hours with 700 or 36,250 g supernatant fraction of freeze/thawed cultured HMC. The treated cells, washed and maintained in culture 30 days, transformed into small cells exhibiting loss of contact inhibition by light microscopy and VLP by electron microscopy. These studies show HMC can be grown in tissue culture and continue to produce VLP, and their subcellular fraction(s) cause malignant transformation in vivo and in vitro.

2. PARTIAL ISOLATION OF PAPILLOMA-ASSOCIATED TISSUE ANTIGENS. F. PASS, M.D. AND D. M. MARCUS, M.D.*, Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461.

We have previously reported the properties of a rabbit antiserum that reacted with nuclear and cell surface antigens of human warts, but not with adjacent normal skin. This communication describes the partial purification of these antigens. An aqueous extract of wart tissue was passed through an immunoadsorbent column of Sepharose 4B coupled to the IgG fraction of the rabbit antiserum. After the material not retained by the column (fraction I) was washed through with a neutral buffer, the material bound to the column (fraction II) was eluted with glycine buffer pH 3.0. Agar gel diffusion studies demonstrated two antigens in fraction II, and fraction I contained one of the same antigens that was not completely retained by the column. Rabbit antisera prepared to fraction II had the same specificity as the absorbed antiserum to the whole tissue homogenate, as demonstrated by gel diffusion and immunofluorescence. Absorption of the antiserum with fraction I abolished the nuclear fluorescence but did not affect fluorescent staining of the plasma membrane of wart cells. Additional purification was obtained by gel filtration of fraction II on a column of Sephadex G-100. The two antigens were eluted in the void volume and contaminating material or lower molecular weight was subsequently eluted. The fraction containing the antigens possessed three proteins, as demonstrated by electrophoresis in acrylamide gels.
Additional studies are in progress to complete the purification and chemical characterization of these antigens.

3. FINE STRUCTURE OF "CLEAR" CELLS IN FROG EPIDERMIS. R. M. Lavker, Ph.D.*, (Introduced by A. G. Matoltsy, M.D.), Department of Dermatology, Boston University School of Medicine, Boston, Massachusetts 02118.

Previous studies on the fine structure of the epidermis of Rana pipiens described the presence of cells which contain numerous filaments and mucous granules. These cells comprise the bulk of the epidermis, and form a keratinized protective layer.

The present study revealed the occurrence of another cell type which constitutes approximately 5% of the frog epidermal cell population. This cell type is easily distinguished from the predominant epidermal cells at both the light and electron microscope level by their "clear" appearance. "Clear" cells contain relatively few filaments when compared with adjacent epidermal cells. Mitochondria, ribosomes, Golgi bodies, endoplasmic reticulum and desmosomes are abundant within the "clear" cells. Numerous small membrane-bound granules are present and contain an amorphous substance. These granules correspond in density to the small mucous granules present in the other epidermal cell type of the frog. Lysosome-like bodies are often seen within the "clear" cells.

"Clear" cells do not appear structurally different when observed at various levels of the epidermis, and apparently do not differentiate in a manner similar to the other epidermal cells. No evidence of "clear" cells has been seen in the stratum corneum, thus, these cells are probably not involved in forming the outer protective layer. However, those "clear" cells adjacent to the completely keratinized epidermal cells have highly convoluted apical membranes suggestive of some type of secretory involvement.

4. HYPOMELANOSIS RESULTING FROM MELANOSOMAL AUTO-PHAGOCYTOSIS AND MELANOCYTE DESTRUCTION. K. Jimbow, M.D.*, K. Toda, M.D., Ph.D.*, AND T. B. Fitzpatrick, M.D., Ph.D., Department of Dermatology, Harvard University and Massachusetts General Hospital, Boston, Massachusetts 02114.

Congenital hypomelanosis results from several mechanisms (absence or destruction of melanocytes; decreased melanization of melanosomes; or failure of transfer of melanosomes to keratinocytes). To elucidate the mechanism of hypomelanosis, the feather germs of developing White Leghorn chick embryos were investigated by electron microscopy and histochemistry. Melanocytes in the early period of embryonic development were found to be actively synthesizing melanosomes, which were abundant in Stages I, II and III. Before these melanosomes developed into Stage IV, they aggregated into autophagic vacuoles, most of which later fused with each other and then, by the middle of the incubation period, were enclosed in one large membrane-limited vacuole. Most of the melanocytes neither retained these aggregates of melanosomes nor survived, and degenerated melanocytes, organelles such as endoplasmic reticulum and aggregates of melanosomes were identified in the keratinocytes. From this time on until hatching, a few melanocytes remained, although they contained only a small number of disorganized melanosomes. These results show that hypomelanosis of the feathers of White Leghorn embryos can be attributed to (1) autophagocytosis of melanosomes in melanocytes, resulting in prevention of the full development of melanosomes and even of melanosomal synthesis, and (2) to destruction of melanocytes. A similar autophagocytosis of melanosomes has been seen by us in an acquired melanoleukoderma in man.

Forty-eight patients with severe intractable psoriasis, warranting methotrexate therapy have been studied by liver biopsy, as well as by clinical evaluation and liver function tests. Sixteen had never had methotrexate (or aminopterin). Only eight of these had completely normal pre-methotrexate liver biopsies. Of the remaining 8, one had cirrhosis, 1 had greatly increased cellular infiltration and 6 had various "borderline" abnormalities.

Of the thirty-two patients who had received previous methotrexate, three had histological cirrhosis. All of these had received a total dose of methotrexate exceeding 1.0 gram. All 3 were alcoholics and 2 of the 3 had abnormal liver function tests before methotrexate was given. Seven patients had hepatic fibrosis in the absence of clinical liver disease. Two of these had abnormal function tests prior to therapy but none was alcoholic. Of the other 22 patients, 4 had increased cellular infiltration and only 11 had completely normal biopsies. Seven patients had at least 2 biopsies at intervals. Progression from increased cellularity to cirrhosis was seen in one alcoholic (included above) but progression from fibrosis to cirrhosis has not been observed.

There is a suggestion (not statistically significant) that risk of fibrosis is related to total dosage and is greater in patients treated with daily oral doses rather than weekly schedules.

In summary, this study has produced no evidence yet that methotrexate causes cirrhosis in non-alcoholic patients whose livers were previously normal. It does suggest a significant risk of hepatic fibrosis from the drug, as was previously found in leukemic children, but this fibrosis has not been observed to progress to cirrhosis and its prognosis is quite uncertain. The study has confirmed the findings of others that minor abnormalities of hepatic structure and function are to be found in untreated psoriasis.

6. PROPERTIES OF AN EPIDERMAL MITOTIC STIMULATING FACTOR. S. W. MELBYE, PH.D.*, AND M. A. KARASEK, PH.D., Department of Dermatology, Stanford University School of Medicine, Stanford, California 94305.

Trypsin-released epidermal cells plated on collagen gels undergo a rapid degeneration and detachment from the gel surface within three days after initial attachment. When plated in the presence of intact skin fibroblasts or the medium from skin fibroblast cultures, a marked stimulation in mitoses and growth is observed. The purpose of the present study was to characterize the molecules produced by the dermal connective tissue cells which induce mitosis and growth of epidermal cells in culture.

Rabbit epidermal cells were released from 0.1 mm keratotome slices of shaved skin by gentle trypsinization and allowed to attach overnight to collagen gels in foam-stoppered scintillation vials. Growth of cells after feeding with regular or fibroblast conditioned media was followed by microscopic observation and "P uptake. Incubations were carried out at 37° in a humidified 5% CO2 atmosphere.

The mitotic stimulating factor is a small dialyzable molecule stable to boiling (5 min) and treatment with RNase or DNase. It is not removed from fibroblast conditioned media by either charcoal or ether extraction.

7. THE MITOTIC CYCLE OF THE MAMMALIAN EPIDERMAL CELL. S. K. OLVEY*, AND C. J. MCDONALD, M.D., Department of Medicine, Roger Williams General Hospital, Brown University, Providence, Rhode Island 02908.

The epidermal cells of guinea pigs were studied autoradiographically to determine the lengths of the G1, S, G2, and M periods of the cell cycle. Initial processing was as described by Klaus (J. Invest. Derm. 48:352, 1967). Suspensions of cells in Fischer's medium containing 15% calf serum were placed in specially prepared glass culture chambers and cultured at 37° C; H-thymidine was added to a final concentration of 0.21Mc/ml. Progress in the cell cycle was monitored hourly, from 2-24 hours, by stopping growth in a portion of the chambers and preparing cover glasses with attached monolayers of epidermal cells for autoradiography. Kodak AR-10 Stripping film was used. Exposure time was 7 days. Azure-B dye was used for staining purposes. 1000 cells on each slide were scored to determine the percentage of labeled and unlabeled 1) cells, 2) cells in mitosis, and 3) cells in metaphase. The total length of the cell cycle (T) was found to be 15 hours; G1 was minimally 3 hours.
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(Ys Tl; S lasted 5.4 hours (½ T); the duration of M was 0.4 hour; G, was 6.2 hours in length (nearly ½ T). A marked linear rate of increase in DNA synthetic activity at hours 7 to 8 probably indicates the achievement of partial synchrony. A constant mitotic index was noted in hours 2 to 10; peak mitosis was noted at 12 hours. These data indicate that the in vitro mitotic cycle of the mammalian epidermal cell, as represented by guinea pig epidermis, closely approximates the cell cycle of other mammalian cells that undergo stimulated DNA synthesis in vitro.

8. DEOXYURIDINE KINASE: A DEVELOPMENTALLY REGULATED ENZYME IN SKIN. B. A. HITT, Ph.D.*, J. T. MOORE, Ph.D.*, AND M. A. KARASEK, Ph.D., Department of Dermatology, Stanford University, Stanford, California 94305.

The rapid incorporation of pyrimidine deoxynucleosides into the skin DNA suggests that salvage pathways play a role in the neutralization of deoxynucleosides during epidermal differentiation. The purpose of this investigation was to study the behavior of three key enzymes in the salvage pathway (deoxycytidine kinase, deoxyuridine kinase, and thymidine kinase) in epidermis and dermis at two stages in post-natal development of the skin.

The pathway was followed by the incorporation of the appropriate isotope into epidermal and dermal DNA and by the assay of the enzymes in homogenates of epidermis and dermis. Both assays revealed a unique reaction for deoxyuridine in the epidermis of the newborn mouse and rat. Conversion of deoxyuridine to thymidylate took place only in the dermis. No deoxyuridine was incorporated into the epidermis by the newborn animals nor was deoxyuridine kinase activity present in the epidermis. In contrast, the adult mouse and rat showed similar uptake of all isotopes in both epidermis and dermis.

These findings show that in the newborn epidermis deoxyuridine kinase is separately controlled, that it is different from thymidine kinase, and that it appears as a result of post-natal development.

9. ULTRA STRUCTURE OF MIGRATING EPITHELIAL CELLS FOLLOWING INCISION WOUNDS. I. R. MARTINEZ, JR., M.D., PH.D., AND A. PETERS, PH.D.*, Departments of Dermatology and Anatomy, Boston University Medical Center, Boston, Massachusetts 02118, and The Ochsner Clinic, New Orleans, Louisiana 70121.

The ultrastructure of migrating cells following incision wounds in keratinizing gingival epithelium is presented for the first time. Incision wounds, 0.5 mm deep were made in the incisor gingiva of albino rats. Animals were sacrificed at 3, 6, 9, 12, 24, 48 and 72 hours after wounding, and wounds were prepared for electron microscopy. Migration of cells begins between 3 and 6 hours. Cells migrate as a group, connected by small, infrequently occurring desmosomes. They move along the fibrin strands and through the inflammatory exudate by means of pseudopods and long microvillous projections. Some cells appear to engulf fibrin and cellular debris and contain inclusions considered to be phagolysosomes. Also present within cells are: whorls of filaments, lysosomes, lipid droplets and increased amounts of smooth and rough endoplasmic reticulum and Golgi complexes. At 9 hours, leading cells from opposing sides are touching and desmosomes are formed between them. By 12 hours, migration has ceased and the wound defect is covered by an epithelium 10 to 15 cell layers thick, which rests upon a fibrin base. The epithelium is anchored to the clot by hemidesmosomes which are present opposite patches of basal lamina occurring at irregular intervals.


A new technique of affinity chromatography has been developed, using methotrexate coupled to agarose. Tissue homogenates are passed through columns, and the dihydrofolate reductase is retained completely. Elution occurs with a steep pH gradient alone, above 9.0,
but yields of active enzyme are poor. Yields of up to 56% are obtained using folic acid or leucomorin as counter-substrates.

Enzyme forms from methotrexate-resistant L. casei have been studied, and it has been shown that they may be interconverted by incubation with NADPH and dihydrofolate.

Homogenates of rat skin have been passed through such columns, and dihydrofolate reductase has been purified approximately 3,700-fold in a single step. Properties of the enzyme are reported. Extracts of rat brain, spleen, liver, kidney, and intestine have also been studied, and the enzyme obtained with high purity.

This technique has also shown conclusively, that other proteins with affinity for methotrexate are present in tissues, besides dihydrofolate reductase. These proteins do not have enzymic activity when stained with MTT-tetrazolium on polyacrylamide gels. They may be relevant to the known long persistence of methotrexate in tissues, and also to the toxicity of the drug.

MORNING SESSION

SATURDAY, JUNE 19, 1971

9:00 A.M. Business and Executive Sessions: RICHARD K. WINKELMANN, M.D., presiding.

SCIENTIFIC SESSION: ROBERT W. GOLTZ, M.D., Denver, Colorado, presiding.

1. EFFECT OF VITAMIN A ACID ON MITOTIC INDEX AND THICKNESS OF THE HAIRLESS MOUSE EPIDERMIS. J. Zil.* (Introduced by R. B. STROUGHTON, M.D.), Division of Dermatology, Scripps Clinic and Research Foundation, La Jolla, California 92037.

Vitamin A acid is said to be effective in psoriasis, acne vulgaris, and certain ichthyoses. The reason for the effect of this form of the vit. is unknown. Mitotic index (MI) (no. mitotic figures/10^6 epidermal cells) and epidermal layers were measured in a blind study of serial biopsies after topical application of vitamin A acid in ethanol to adult male hairless mice.

One application of 667 IU of vitamin A acid increased MI at 2 hours.

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<th>MI after Treatment</th>
<th>Hours after Treatment</th>
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<td>2</td>
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<tr>
<td>Vitamin A acid in Ethanol</td>
<td>2.4</td>
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<tr>
<td>Ethanol Control</td>
<td>1.1</td>
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<tr>
<td>No-Treatment Control</td>
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Excesses over controls were significant at P < .005 for all times through 74 hours. The effect declined after 74 hours, but still persisted at 170 hours. Vitamin A acid thickened the basal and prickle layers by 50 hours (P < .005), and increased the number of cell layers by 84 hours (P < .0125).

Multiple application of 667 IU vitamin A acid at 0, 24, and 48 hours also increased MI, which reached a peak of 5.2 at 26 hours, then plateaued at about 4.8 until the mice died after 122 hours.

The rapidity of response suggests an initial stimulation of a cell population in G2.

2. STRATUM CORNEUM DAMAGE BY IONIC SURFACTANTS. P. H. DUGARD, PH.D.*, R. J. SCHEUPLEIN, PH.D.*, AND I. H. BLANK, PH.D. Department of Dermatology, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts 02114.

Commonly used ionic detergents and soaps may damage the “diffusion barrier” of
human skin. In an investigation of the nature of this type of damage, the rates of increase of in vitro permeability of stratum corneum have been studied.

Since a linear relationship is demonstrable between the electrical conductivity of an isolated epidermal membrane and permeability constants for tritiated water, the low frequency, low voltage AC conductivity of a stratum corneum membrane provides a sensitive and instantaneous reflection of the permeability of the membrane during progressive surfactant damage. N-alkyl surfactants of the form R-COONa, R-OSO$_3$Na and R-NH$_2$HCl were employed in buffered solution to permit assessment of the effects of anionic or cationic nature, carbon chain length, concentration and pH on rates of damage.

Several consecutive members of each homologous series were shown to damage the stratum corneum, in comparison to buffers alone, and to produce more rapid damage with increasing concentration. The often extensive alterations in conductivity and permeability to water were found to be largely irreversible despite prolonged washing with buffer alone. Within appropriate ranges of ionic dissociation, no pH dependent variations were apparent in the rate of damage by a given surfactant.

3. KERATIN AND KERATOHYALIN GRANULES OF MAMMALIAN EPIDERMIS. T. TEZUKA, M.D., Ph.D.*, AND I. FREEDBERG, M.D., Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215.

The synthesis of keratin filaments and keratohyalin granules are central to the function of mammalian epidermis. To relate previous data on the general pathways and controls of epithelial protein synthesis to the specific synthesis of structural components, this study has been aimed at the isolation of keratin filaments and keratohyalin granules from newborn rats.

Tetraphenylboron separated epidermis has been homogenized, extracted with buffered desoxycholate or urea, fractionated by differential centrifugation and purified by chromatography on Sephadex G200 or Sepharose 6B. Two fractions have been obtained, each of which is homogeneous by disc electrophoresis. The first arises from the keratohyalin granules, contains hexose, hexosamine, complex lipids and protein, is amorphous when reconstituted in vitro, has a predominantly random configuration by circular dichroism or x-ray diffraction and elicits antibodies to keratohyalin granules following injection into rabbits. In contrast, the second fraction arises from the filamentous intracellular material, can be reconstituted in vitro to form electron microscopically identifiable filaments, contains $\alpha$ helix by circular dichroism and x-ray diffraction and elicits antibody directed at keratin filaments following injection in a foreign species. Amino acid compositions and molecular weights of the fractions differ.

From these results we have been able to characterize identifiable subfractions of the two major structural components of mammalian epidermis.

4. THE REPEATING SUBUNIT OF EPIDERMAL FIBROUS PROTEIN. D. SKERRow, Ph.D.* (Introduced by A. G. MATOLTSY, M.D.), Astbury Department of Biophysics, Leeds University, England, and Boston University School of Medicine, Boston, Massachusetts 02118.

The fibrous protein extracted from cow's nose epidermis by citrate buffer, pH 2.6, has been studied in order to determine the size of any repeating subunit.

Amino acid analyses, tryptic peptide maps and tryptic + chymotryptic peptide maps were obtained and calculations of subunit size made from the content of the least-occurring amino acids and from the numbers of peptides produced.

The least-occurring amino acids were tryptophan 4.4 res/1000; $\frac{1}{2}$-cystine 8.8 res/1000, histidine 8.8 res/1000 and the lysine and arginine contents were 52.1 and 58.6 res/1000 respectively. Twenty-eight tryptic peptides were detected and forty-four tryptic + chymotryptic peptides, of which one was Ehrlich positive and nine were Pauly positive.

From these data, the presence of a repeating subunit was established which was calculated to contain 227 amino acid residues and to have a molecular weight of 24,300.
5. SKIN PERMEABILITY IN VIVO: RAT, RABBIT, PIG AND MAN. M. J. BARTEK, PH.D.*, J. A. LABUDEDE, PH.D.*, AND H. I. MAIBACH, M.D., Mead Johnson Research Center, Evansville, Indiana, 47721, and University of California School of Medicine, San Francisco, California 94122.

For the interpretation of pharmacology and toxicology studies, there is little data available to compare the permeability of animal and human skin in vivo. The amount of radioactivity excreted in urine for 5 days following application of a labeled compound (¹⁴C or ³⁵S) to the skin was employed as the index for quantifying percutaneous absorption on the back of the rat, rabbit, pig and the forearm of man. The results obtained from the skin penetration studies were corrected for recovery of radioactivity following intravenous administration of the radioactive compound. A special non-occlusive foam pad device was developed to prevent contamination of the animal metabolic cage with chemical from the skin. The results are presented as percent of applied dose excreted in urine in five days. Two to four animals of each species and six men were studies for each chemical. The dose was 4γ/cm² of the following:

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<thead>
<tr>
<th>Penetrant</th>
<th>Rat</th>
<th>Rabbit</th>
<th>Pig</th>
<th>Man</th>
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<tbody>
<tr>
<td>Haloproglin</td>
<td>92.0</td>
<td>113.5</td>
<td>19.7</td>
<td>11.0</td>
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<tr>
<td>N-acetylcysteine</td>
<td>5.0</td>
<td>2.0</td>
<td>0</td>
<td>2.4</td>
</tr>
<tr>
<td>Cortisone</td>
<td>22.0</td>
<td>30.2</td>
<td>4.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Testosterone</td>
<td>54.9</td>
<td>63.8</td>
<td>25.4</td>
<td>13.2</td>
</tr>
<tr>
<td>Caffeine</td>
<td>53.1</td>
<td>75.6</td>
<td>32.3</td>
<td>47.6</td>
</tr>
<tr>
<td>Butter Yellow</td>
<td>48.1</td>
<td>84.6</td>
<td>41.8</td>
<td>21.6</td>
</tr>
</tbody>
</table>

Rat and rabbit skin was several fold more permeable than pig and man for most compounds; N-acetylcysteine was an exception. The pig has the closest permeability characteristic to man with this series; extrapolation to other compounds must be done with caution.


Stratum corneum macromolecular components play a major role in determining the structural forces involved in its stability. Analysis of the membrane's temperature dependent properties should aid in elucidating the molecular mechanisms responsible for its biomechanical behavior.

Samples of neonatal rat and human stratum corneum were examined with a commercially available thermomechanical analyzer which affords rapid and reproducible measurement of thermally induced viscoelastic and dimensional changes in both the longitudinal and transverse directions.

Rat corneum exhibited a transverse softening at 45° C accompanied by a 0.5% longitudinal contraction, elongation at 117° C, reversible melting at 155° C, a 404% expansion at 213° C and complete loss of modulus at 285° C. Human corneum behaves similar to the rat with the exception of an increase of 20° C in the 155° C melting, a reduction in the 213° C expansion and a second longitudinal contraction at 171° C.

Rat corneum extracted with organic solvents alter the thermogram indicating thermal transitions which are lipid and protein related. Crosslinking with formaldehyde is revealed by an increase in the transition temperature as well as the magnitude of the mechanical deformation.

Cell envelopes, or modified cell membranes, have been isolated from callus, stratum corneum, and psoriatic scale following proteolytic digestion with Subtilopeptidase A. This enzyme solubilizes intracellular material of these cells, but does not appear to attack the cell envelopes. Final purification of the envelopes was achieved by sucrose density gradient centrifugation.

Equilibrium isodensity centrifugation yielded density values of 1.136, 1.176, and 1.158 gm/ml for envelopes of normal stratum corneum, callus, and psoriatic scale, respectively. By this method envelopes of normal stratum corneum were distinguishable from envelopes of either callus or psoriatic scale.

No marked differences were noted in the relative quantities of protein, lipid, and carbohydrate. Thin layer chromatography of the envelope lipids revealed the presence of sterol, sterol esters, triglycerides, free fatty acids, and a number of unidentified polar lipids. The chromatographic patterns were similar for all three preparations. Little or no phospholipid was detected.

A major difference among the three preparations was in the amino acid composition of the envelope proteins. Envelopes of psoriatic scale contained approximately twice the amount of proline, but only one-half the amount of serine, and one-third the amount of glycine of normal envelopes. All preparations had a relatively high cysteine content compared to intracellular proteins.

8. AUTOIMMUNITY IN VITILIGO. L. J. DORMEIER, M.D., AND W. M. SAMS, Jr., M.D., Department of Dermatology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901.

Patients with vitiligo tend to have increased formation of autoantibody. Evidence for the inclusion of vitiligo as an autoimmune disorder would be provided by the demonstration of antimelanocyte antibodies in such patients. Direct immunofluorescent antibody studies using 4μ frozen sections of skin biopsies from 10 patients, and monospecific fluorescein-conjugated antisera to IgG, IgA, IgM, IgE, and β₁,₂, showed the absence of in vivo-bound γ-globulin and complement. Indirect immunofluorescent antibody studies to detect circulating antimelanocyte antibodies in the sera of 10 patients with vitiligo, using 4μ frozen sections of skin biopsies from the patient's own normally pigmented skin, and from melanomas, as substrates, failed to reveal the presence of circulating antimelanocyte antibodies. Two of the 10 vitiligo patients also had pernicious anemia and circulating gastric parietal cell antibodies in their sera. Low titer (1:25 to 1:250) thyroglobulin antibodies were present in 5 of the 10 patients. Thus, the lack of antimelanocyte antibodies in patients who clearly develop other circulating antibodies fails to support an autoimmune pathogenesis for vitiligo.

9. SURVIVAL OF PATHOGENIC MICROORGANISMS ON HUMAN SKIN. R. ALY, PH.D.*, H. I. MAIBACH, M.D., H. SHINEFIELD, M.D., AND W. STRAUSS, M.D.*, Department of Dermatology, University of California School of Medicine, San Francisco, California 94122.

This study was designed to establish in vivo the presence of antimicrobial substance(s) in human skin. If such agents are present on the skin surface, their removal should allow increased multiplication of applied organisms.

One forearm was washed repeatedly for 5 minutes with lipid solvents; the other served as the control. The organisms applied (1.5 X 10⁶ X 7 X 10⁴ in 0.01 ml Ringer's solution) on the forearms were not allowed to dry and covered immediately with plastic protective caps for 5 hours. The microorganisms were harvested using a modified Patchman method (1954). With Staphylococcus aureus and Streptococcus pyogenes, acetone washed forearms had 2-500 fold (average 1:62) more organisms than the control arms (20 subjects, p = 0.002).
Similar results were noted with ether (average 1:90) (10 subjects, \( p = 0.011 \)). *Candida albicans* increased 2-300 fold (average 1:66) (10 subjects, \( p = 0.0004 \)). This effect did not occur with *Escherichia coli* and *Pseudomonas aeruginosa* (20 subjects, \( p = 0.8 \)). To determine the time for natural replenishment of the antimicrobial substances, bacteria were applied 2, 3 and 5 hours after washing with acetone. Increased numbers were seen at 2 (\( p = 0.07 \)) but not at 5 hours (\( p = 0.43 \)). Regional variations studies showed that the forehead did not inhibit *S. aureus* persistence (as did the forearms) (10 subjects, \( p = 0.05 \)). These in vivo human data suggest the biological relevance of previous *in vitro* demonstration of antimicrobial substances in skin.

10. **METABOLIC AND ULTRASTRUCTURAL STUDIES IN A PATIENT WITH PUSTULAR PSORIASIS (VON ZUMBUSCH)**. I. M. Braverman, M.D., I. Cohen, M.D., and E. O'Keeffe, M.D., Section of Dermatology, Yale University School of Medicine, New Haven, Connecticut 06510.

A 56 year old white woman with pustular psoriasis was studied for 3 months in our Clinical Research Center. Acute flares occurred every 3 to 7 days, and mild activity was almost always present during the relative remissions. Hypocalcemia, 8.3–8.9 mg% (normal 9.1–10.6 mg%) and hyposalbuminemia, 2.9–3.6 gm% (normal 3.7–4.8 gm%) were invariably present during the illness. The half time disappearance rates of \( ^{125} \text{I} \)-albumin was determined on 2 occasions. The results were 4.7 and 4.0 days (normal 10–11 days). \( ^{51} \text{Cr} \)-albumin was injected IV to determine if the albumin was being lost into the gastrointestinal tract, but no radioactivity was found in the stool. Urinary protein excretion was \( < 0.1 \) gm/day. It was concluded that the albumin was being lost into the skin or other organs. Electron microscopy of the skin lesions revealed normal appearing neutrophils migrating from the vessels into the dermis through gaps between the endothelial cells. In some sections there were gaps between endothelial cells without visible neutrophil migration. Identical gaps were seen in the dermal vessels of skin which had been involved in earlier flares but now appeared normal. It is postulated that "leaky" vessels are responsible for the rapid disappearance of serum albumin with the resultant hyposalbuminemia and associated hypocalcemia. The cause of the endothelial gaps is undetermined. No evidence for malabsorption was found in this patient.

**AFTERNOON SESSION**

Saturday, June 19, 1971

2:00 P.M. **SCIENTIFIC SESSION**: Richard K. Winkelman, M.D., presiding.


2. **PHYSIOLOGIC AND HISTOLOGIC STUDIES OF CHROMIDROSIS DUE TO OCHRONOSIS**. A. W. Hoke, M.D., H. I. Maibach, M.D. and W. L. Epstein, M.D., United States Public Health Service Hospital and Department of Dermatology, University of California School of Medicine, San Francisco. California 94122.

A patient with ochronosis, a rare disease characterized by precipitation of homogentisic acid polymers in the tissues was studied to determine the source of his chromidrosis. Intradermal injection of 0.1 ml of 1:1000 epinephrine into the axilla produced large droplets of bluish-black apocrine sweat, while intradermal injection of 1:10,000 acetylcholine elicited only clear eccrine sweat.

Punch biopsy of the axilla revealed: 1) pigment outlining apocrine coils and ducts; 2) brownish variably sized granules in apocrine cells and sometimes below them by light
microscopy; and 3) apocrine granules indistinguishable from normal by electron microscopy, but deposits of similar dense staining amorphous material in myoepithelial cells.

The results suggest that intradermal injection of epinephrine into the axilla may prove useful diagnostically and electron microscopy has detected a new site of deposition of the pigmented polymer (Lichenstein and Kaplan, Am. J. Path. 50: 99, 1954).


A current hypothesis of the etiology of acne states that bacterial lipolytic action in the sebaceous follicles releases free fatty acids (FFA) from triglycerides (TG) in sufficient amounts to initiate the sequence of events which leads ultimately to comedones and pustular acne. We have isolated, purified, and partially characterized a lipase produced by C. acnes (the major component of the skin flora) to aid in a test of this hypothesis. The enzyme was purified approximately 200-fold from the culture supernatant of a static culture of C. acnes. The optimal pH for activity was 7.5–8.5. Tributyrin was the best substrate for the enzyme, although considerable activity was displayed against TG of longer chain length fatty acids. The action pattern of the enzyme against TG indicated non-specific cleavage to glycerol and FFA, with little accumulation of diglyceride and monoglyceride intermediates. The enzyme did not catalyze the hydrolysis of wax esters, sterol esters, or lecithin. Agar diffusion precipitation with antibodies to a whole-cell suspension of C. acnes demonstrated that the enzyme preparation was one of the major antigenic determinants.

4. SEBACEOUS GLAND SUPPRESSION BY ETHINYL ESTRADIOL AND DIETHYLSTILBESTROL. P. E. Poehl, M.D., and J. S. Strauss, M.D., Department of Dermatology, Boston University Medical Center, Boston, Massachusetts 02118.

The degree of sebaceous gland inhibition induced in women by various dosages of ethinyl estradiol or diethylstilbestrol was studied. These estrogens were administered orally to 54 women in 72 separate drug trials for 3 weeks of each month for an average period of 16 weeks. The dosages of ethinyl estradiol ranged from .02 mg to .25 mg daily, and for diethylstilbestrol from 0.5 mg to 5.0 mg daily. Sebum production was measured weekly by gravimetric assay of the lipid secreted to the surface of the forehead in a 3-hour period. The results, expressed as the percent decrease of sebum production from the average of values beyond the 9th week of drug administration, were as follows:

<table>
<thead>
<tr>
<th>Ethinyl estradiol</th>
<th>Diethylstilbestrol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose, mg</td>
<td>% Decrease</td>
</tr>
<tr>
<td>.02</td>
<td>8.4</td>
</tr>
<tr>
<td>.06</td>
<td>20.1</td>
</tr>
<tr>
<td>.10</td>
<td>28.9</td>
</tr>
<tr>
<td>.25</td>
<td>33.7</td>
</tr>
<tr>
<td>0.5</td>
<td>12.8</td>
</tr>
<tr>
<td>1.0</td>
<td>18.9</td>
</tr>
<tr>
<td>2.0</td>
<td>25.0</td>
</tr>
<tr>
<td>3.0</td>
<td>30.0</td>
</tr>
<tr>
<td>5.0</td>
<td>35.4</td>
</tr>
</tbody>
</table>

The significant findings were the following: (1) sebaceous gland inhibition was dose-related although only 3–4 fold over a 10-fold dose range; (2) there was no correlation between the pretreatment sebum level and the degree of sebaceous gland inhibition achieved; and (3) the average reduction in sebum secretion for any dose failed to exceed 35%, but there was considerable variation with a given dose. For example, with 3.0 mg of diethylstilbestrol sebum suppression ranged from 10% to 60%.
5. SEBUM EXCRETION: ANALYSIS BY INFRARED SPECTROSCOPY.

J. E. Fulton, Jr., M.D., A. Anderson, B.S.*, and S. Randall*, University of Miami, Miami, Florida 33136, and Westwood Pharmaceuticals, Buffalo, New York.

A recent development in infrared (IR) spectroscopy makes it possible to sample sebum excretion in situ and to overcome some of the sampling and procedural difficulties inherent in the gravimetric assay. The method involves the use of a Wilk’s “Skin Analyser” used in conjunction with a mixed crystal of thallium bromide-iodide (KRS-5). As skin is pressed against this crystal a sample of the surface film is deposited. The forehead lipid film remains on the crystals in droplets corresponding to the follicular orifices. The optical density at 1740cm⁻¹ (carbonyl stretch band of free fatty esters), at 1710cm⁻¹ (carbonyl stretch band of free fatty acids), and at 1460cm⁻¹ (CH bending of hydrocarbons in —CH₃) is monitored.

The data reveal: (1) One hour rates of sebum excretion are adequate for routine evaluation, although the excretion rates are usually linear for up to three hours. (2) Casual sebum levels are 40-100% higher than three hour levels. (3) Free fatty acids to fatty ester ratio varies from subject to subject but is constant for the same subject from day to day. (4) This ratio differs with anatomical areas (nose > cheek > forehead). (5) Sebum excretion is temperature dependent but has no diurnal variations. (6) No qualitative or quantitative difference between patients with acne vulgaris and controls. This method is faster, less uncomfortable and more qualitative than the gravimetric assay.

6. THE EFFECT OF CASTRATION, HORMONE AND TETRACYCLINE ADMINISTRATION ON DIHYDROTESTOSTERONE PRODUCTION IN THE PREPUTIAL GLANDS OF MICE. G. S. Bazzano*, D. Cummings*, and R. M. Reisner, Department of Medicine, Division of Dermatology, Harbor General Hospital, Torrance, California, and UCLA School of Medicine, Los Angeles, California.

It has been demonstrated that conversion of testosterone (T) to dihydrotestosterone (DHT) is greater in acne-bearing skin when compared to non-acne-bearing skin. The 5α reductase enzyme which converts T→DHT is present in the preputial glands of rodents. In order to determine whether or not this enzymatic conversion is affected by castration, prednisone or tetracycline administration, we have studied conversion rates in the preputial glands of castrated as well as normal adult male mice fed diets containing tetracycline, or prednisone. We have also studied the effects of testosterone and estradiol administration on the conversion of T→DHT in the preputial glands of the animals. The preputial glands were excised and incubated with testosterone 1,2 ³H in Krebs Ringer phosphate buffer. Steroid extracts were analyzed by thin layer and gas liquid chromatography. Our results indicate that normal animals have higher conversion rates of T→DHT per mg. of tissue than castrated animals and that estradiol appears to depress T→DHT conversion in the preputial glands of normal and castrated animals. However no significant depression of T→DHT conversion could be demonstrated in the prednisone or tetracycline treated groups. This may indicate a possible alternate mode of action of estradiol in suppressing sebum production.

7. NETHERTON’S SYNDROME: AN ULTRASTRUCTURAL STUDY. E. G. Thorne, M.D., J. H. Mottaz*, and A. S. Zelickson, M.D., Department of Dermatology, University of Minnesota, Minneapolis, Minnesota 55455.

Netherton’s syndrome is a rare genodermatosis characterized by abnormal keratinization. To examine the morphologic alterations of keratinization at the ultrastructural level biopsies were obtained from normal appearing skin and from areas showing the typical psoriasis-form dermatosis of Netherton’s syndrome. In addition, hairs were plucked from the scalp, axilla and pubic area for scanning electron microscopy. Electron microscopic examination revealed the most striking abnormalities confined to the granular layer—a small (1µ) elec-
tron-opaque granule not associated with tonofilaments and a larger (3μ) granule contiguous with tonofilaments. Both types of granules were surrounded by ribosomes. Tonofilaments found in the keratinocytes of the granular layer were short and were often arranged haphazardly in small bundles. The stratum corneum contained large (10-20μ) bodies filled with a granular electron dense material which seemed to distend the intercellular spaces of the horny layer. Similar material was noted in vacuoles (1-2μ) both in the extracellular space and within keratinocytes of the upper granular layer. Examination of the hairs with the scanning electron microscope showed abnormalities of the cuticle accompanying the bamboo defects of the hair shafts. The major ultrastructural alteration in Netherton’s syndrome appears in the granular layer and stratum corneum suggesting faulty keratohyalin synthesis may be occurring.

8. THE SUBCUTANEOUS TISSUE IN SCLERODERMA. R. FLEISCHMAJER, M.D., V. DAMIANO, Ph.D.*, AND A. NEDWICH, M.D.*, Division of Dermatology, Department of Medicine, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania 19102, and the Franklin Research Laboratories, Philadelphia, Pennsylvania 19103.

Evidence is presented in this investigation that the most striking pathology in scleroderma takes place at the level of the subcutaneous tissue. Four patients with localized scleroderma, 10 with the systemic form and 5 normal controls were studied. Skin biopsies including the subcutaneous tissue were taken from indurated areas and studied by histochemistry (trichrome, aldehyde fuchsin, PAS, Verhoeff’s elastic tissue, Alcian blue and Snook’s reticulum). Seven specimens from systemic and two from localized scleroderma were studied by electron microscopy (EM). The actual dermis showed compact collagen with normal staining properties and the EM revealed normal, mature collagen. The subcutaneous area showed marked replacement of fat by hyalinized connective tissue which consisted of fine collagen fibers, with increased glycosaminoglycans, reticulum fibers and fibroblasts. EM of the subcutaneous tissue revealed a) fine collagen fibers in random fashion, 200-400 Å and some below 100 Å in diameter (normal 700-900 Å), b) a massive increase in ground substance represented by granular or structureless material and c) abnormal fibroblasts. It is suggested that the replacement of the subcutaneous fat by altered connective tissue is responsible for the induration of the skin in scleroderma.

Saturday, June 19, 1971
7:00 p.m. COCKTAILS AND DINNER
ESCAPE TO REALITY
ISAAC ASIMOV, Ph.D.
Department of Biochemistry
Boston University School of Medicine, Boston, Massachusetts
(For those members and guests who have made reservations)

MORNING SESSION
SPECIAL SYMPOSIUM
MEDICAL FRONTIERS—1971

SUNDAY, JUNE 20, 1971
9:00 A.M. RICHARD K. WINKELMANN, M.D., presiding.

THE PUBLIC POLICY DEBATE ON THE FEDERAL SUPPORT OF MEDICAL RESEARCH AND TRAINING. ROBERT H. EBERT, M.D. Dean, Harvard Medical School.
AMYLOID—DISEASE AND FIBRIL. ALAN S. COHEN, M.D. Professor of Medicine, Boston University School of Medicine.

COLLAGEN AND COLLAGENASE. STEPHEN M. KRANE, M.D. Associate Professor of Medicine, Harvard Medical School.

AFTERNOON SESSION

SUNDAY, JUNE 20, 1971

2:00 P.M. GEORGE W. HAMBRICK, JR., M.D., presiding.

PROSPECTS FOR GENETIC INTERVENTION IN MAN. BERNARD D. DAVIS, M.D. Adele Lehman Professor of Bacterial Physiology, Harvard Medical School.

MECHANISM OF RELEASE OF CHEMICAL MEDIATORS OF HYPERSENSITIVITY REACTIONS IN MAN. K. FRANK AUSTEN, M.D. Professor of Medicine, Harvard Medical School.

VIRUSES AND AUTOIMMUNITY. ROBERT S. SCHWARTZ, M.D. Professor of Medicine, Tufts University School of Medicine.

Closing Executive Session: Installation of New Officers.