PROGRAM

THE THIRTY-THIRD ANNUAL MEETING

THE SOCIETY FOR INVESTIGATIVE DERMATOLOGY, INC.

Chalfonte-Haddon Hall Hotel
Atlantic City, New Jersey

Friday and Saturday, April 28 and 29, 1972

OFFICERS

GEORGE W. HAMBRICK, JR., M.D., Baltimore, Maryland, President
ROBERT W. GOLTZ, M.D., Minneapolis, Minnesota, President-Elect
NAOMI M. KANOF, M.D., Washington, District of Columbia, Vice-President
JOHN S. STRAUSS, M.D., Boston, Massachusetts, Secretary-Treasurer

WORKSHOPS

(JOINTLY SPONSORED WITH THE NATIONAL PROGRAM FOR DERMATOLOGY)

THURSDAY APRIL 27, 1972, 7:30 P.M.

Workshop
Acne and Endocrinology
Connective Tissue Biology
Immunofluorescence
Photobiology

Directors
Peter E. Pochi, M.D., Boston, Massachusetts
Raul Fleischmajer, M.D., Philadelphia, Pennsylvania
Ernst H. Beutner, Ph.D., Buffalo, New York
Denny L. Tuffanelli, M.D., San Francisco, California
Madhukar A. Pathak, Ph.D., Boston, Massachusetts

MORNING SESSION

FRIDAY, APRIL 28, 1972

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


DAVID AMINOFF, PH.D., Ann Arbor, Michigan, Fundamentals of immunochemistry.

IRMA GIGLI, M.D., Boston, Massachusetts, Fundamentals of immediate hypersensitivity.

MICHAEL J. FELLNER, M.D., New York, New York, Immediate drug hypersensitivity.

WILLIAM L. EPSTEIN, M.D., San Francisco, California, Fundamentals of delayed hypersensitivity as applied clinically.

8:30 A.M. BUSINESS AND EXECUTIVE SESSION: GEORGE W. HAMBRICK, JR., M.D., Baltimore, Maryland, presiding.

1. PRESIDENTIAL ADDRESS: CHALLENGES—THEN AND NOW. G. W. HAMBRICK, JR., M.D., Johns Hopkins University School of Medicine, Baltimore, Maryland.

2. TYROSINASE: THE TYPING OF ISOZYMES BY ISOELECTRIC FOCUSING. J. B. BURNETT, PH.D., AND M. M. LANE-BROWN, M.B., B.S., Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114.

Tyrosinase is present in more than one molecular form in extracts of human and murine malignant melanoma. Two soluble and a macromolecular forms of the human and murine enzyme have been shown to exhibit unique electrophoretic mobilities in standard disc polyacrylamide-gel electrophoretic systems (Burnett and Seiler, J. Invest. Derm., 52: 199, 1969).

Isoelectric focusing on polyacrylamide gels permits far greater resolution of active forms of tyrosinase than is possible with standard disc polyacrylamide gel techniques. At least six distinct bands of active enzyme can be demonstrated in the supernatant from lightly homogenized and centrifuged human or murine malignant melanoma by isoelectric focusing. Only two of these bands of active enzyme (T₁ and T₀) can be demonstrated after further fractionation of the supernatant with ammonium sulfate and acetone. Using a more sensitive pH gradient, however, T₁ is shown to exhibit two separable forms.

Extracts of normal skin, giant hairy nevi, compound nevi, and mature intradermal nevi, as well as primary and metastatic malignant melanoma, have been examined by isoelectric focusing on polyacrylamide gels. The findings suggest that tyrosinase in extracts of melanocytic tissue expresses activity in many previously unrecognized, separable forms.


These investigations in CF, mice were undertaken: 1) to study effects of hydroxyurea (HU) on matrix cell kinetics and 2) to assess synergistic HU-radiation cell damage by measurement of hair loss. HU 100 mgm/kg was administered intraperitoneally and serial biopsies were taken up to 48 hours later. Each animal received ³HdR (0.5 µCi/g) ½ hour before biopsy. Anagen follicles squashes and autoradiographs were prepared. Mitotic indices dropped 30 minutes after drug administration (2.3% in controls vs 1.5% in HU animals). Mitotic indices were 0.6–0.7% at 1–4 hours, returned to normal (2.5%) at 6 hours, and showed striking overshoot (3.2–4.0%) 8–10 hours after HU. Labeling indices were nil one hour after HU, returned progressively to normal (25%) at 4 hours, and at 5–6 hours showed an overshoot (34–36%) preceding the mitotic overshoot. Nuclear necrosis, cell debris, and pyknosis were most marked at 5–7 hours; recovery was almost complete 10 hours after the drug. Proliferative cell compartments showed up to 50% reductions in cell populations at times of maximum cell injury.

After injection of HU 1200 mgm/kg, the effects of varying the time interval between drug and radiation (650 rads) from 1–12 hours were studied. Hair loss (per sq. mm) was measured 4 days later by photomicroscopy. Maximum alopecia occurred at 4 and 8 hour intervals, with marked “protection” found at 6 hours. Implications of these findings for treatment of benign and malignant hyperproliferative disorders will be discussed.


Surgically obtained human leg skin and hairless mouse skin were used to measure penetration of cold and tritium labeled methotrexate (MTX). Cold MTX was assayed by inhibition of dihydrofolate reductase and tritium labeled MTX was detected by scintillation counting. The

* By invitation
average percentage of MTX penetrating the skin was comparable with both assays and ranged from 0-24%.

The use of two methods to measure MTX provides information not only about its presence after penetration but also about its biological activity.

5. EFFECTS OF METHOTREXATE AND ETHANOL ON RAT LIVER MORPHOLOGY. G. CHOTINER, M.D., P. D. WEBSTER, M.D.*, AND J. G. SMITH, JR., M.D., Medical College of Georgia, Augusta, Georgia 30902.

Fatty infiltration, fibrosis and cirrhosis have been noted in liver biopsies of patients with psoriasis following treatment with methotrexate. It has not been clear whether these liver changes are related to the disease itself, to the drug methotrexate or to other intrinsic or extrinsic factors such as diabetes or ethanol ingestion.

The present study attempted to ascertain the effects of methotrexate and ethanol (alone and in combination) on rat liver morphology. Female Sprague-Dawley rats (Charles River) weighing 150-200 gm were divided into four groups: "A" Control, "B" 10% ethanol in drinking water, "C" 0.1 mg methotrexate intraperitoneally daily and 10% sucrose in drinking water and "D" 0.1 mg methotrexate intraperitoneally daily and 10% ethanol in drinking water. Necropsy obtained at the time of sacrifice after 1-11 days of methotrexate revealed moderate to severe fatty infiltration present in Groups B and D but no significant liver changes in Groups C or A. Mean survival time for animals receiving daily methotrexate was 9 days.

Methotrexate given in a dosage of 0.3 mg intraperitoneally weekly or biweekly to rats maintained for periods up to 12 months in groups similar to the above experiment failed to produce any liver morphological changes in any of the groups except for one instance of mild fatty infiltration in an animal receiving methotrexate alone.

Attempts to reproduce the liver changes attributed to the use of methotrexate in psoriasis were therefore unsuccessful using the rat as a laboratory model.

6. PARAFFIN EMBEDDED BOVINE AND HUMAN EPIDERMIS FOR ANTINUCLEAR ANTIBODIES. S. B. GUSS, M.D., AND A. R. UGELF, M.D., Department of Dermatology, National Institutes of Health, Bethesda, Maryland 20014.

A modification of the standard immunofluorescent technique for antinuclear antibodies (ANA) is described which employs methanol fixed-paraffin embedded tissue instead of cryostat cut sections, which are time consuming to prepare and can not be stored for extended periods.

Bovine hoof and post mortem human heel epidermis were fixed overnight in 80% methanol and paraffin embedded under vacuum by an automatic tissue processor. Prior to ANA testing, sections were quickly cleared of paraffin and rehydrated.
Paraffin sections were easy to interpret due to preservation of tissue architecture, and sections could be stored indefinitely. Of 33 systemic lupus patients' sera tested, 25 (75%) showed equal, 5 (15%) higher, and 3 (9%) lower fluorescent ANA titers on paraffin hoof compared to cryostat mouse liver, the substrate used in many institutions. Paraffin heel epidermal sections showed equal titers in 23 sera (69%) and lower in (30%). Normal controls were negative by all three substrates. All staining patterns on cryostat tissue showed positive ANA on paraffin sections, but only nucleolar staining could be differentiated on paraffin tissue.

The data indicate that paraffin embedded bovine hoof is as sensitive as cryostat mouse liver in detecting ANA and paraffin embedded human heel epidermis less so. The use of paraffin sections makes the ANA test easier and will allow the performance of the ANA test by hospital labs that formerly lacked equipment or manpower to cut cryostat sections.

7. GOLD THIOMALATE THERAPY IN PEMPHIGUS. N. S. PENNEYS, M.D. S. INDDIN, M.D.*, P. FROST, M.D., AND S. KATZ, M.D. Department of Dermatology, University of Miami School of Medicine, Miami, Florida, 33152, The Veterans Administration Hospital and Mount Sinai Hospital, Miami, Florida and Walter Reed General Hospital, Washington, District of Columbia.

In 6 patients with pemphigus who required corticosteroid therapy to control their disease, the intramuscular administration of gold thiomalate (GTM) permitted cessation of steroid therapy. Two patients had active skin lesions which resolved during chrysotherapy. The antiepithelial antibody titer decreased in all 6 patients, reaching 0 in 4 patients. All patients have since been on intermittent doses of GTM. One developed gold dermatitis and another mild albuminuria during the course of chrysotherapy. These complications resolved when the drug was stopped and did not reoccur with resumption of GTM therapy. In one patient in whom GTM therapy was stopped, blisters developed 7 weeks later; reinstitution of GTM controlled the eruption. In another, blisters developed when her maintenance doses of GTM were decreased to 25 mg monthly and corticosteroids had to be reinstituted to control her eruption.

AFTERNOON SESSION

FRIDAY, APRIL 28, 1972

2:00 P.M. SCIENTIFIC SESSION: RAYMOND R. SUSKIND, M.D., Cincinnati, Ohio, presiding.

1. DIFFERENTIAL SUPPRESSION OF MACROPHAGES AND LYMPHOCYTES BY CORTISOL IN DELAYED HYPERSENSITIVITY REACTIONS. W. L. WESTON, M.D., AND H. N. CLAMAN, M.D., University of Colorado Medical Center, Denver, Colorado 80220.

Lymphocytolysis had been thought to be the mechanism by which cortisol suppressed delayed hypersensitivity reactions. However, recent evidence reveals that guinea pigs and humans are cortisol resistant and their lymphocytes are not lysed by cortisol. We therefore investigated the cellular effect of cortisol on tuberculin reactions in guinea pigs.

Tuberculin-sensitive guinea pigs were treated with cortisol intraperitoneally daily for four days, and biopsies of these and control animals were fixed for electron microscopy. Differential counts of a biopsy specimen revealed that cortisol treatment resulted in an 81 per cent reduction in the macrophages and a 64 per cent reduction in the small lymphocytes. Other cells were not affected. This disproportionate reduction in macrophages, viewed from the macrophage inhibitory factor (MIF) model of delayed hypersensitivity, shows that either the sensitized lymphocyte is unable to produce and release MIF or the macrophage itself cannot respond to MIF.

2. INHIBITION OF DNA SYNTHESIS IN CHICK EMBRYO AND MOUSE SKIN BY EPIDERMAL EXTRACTS. S. ROYTHBERG, PH.D., AND B. C. ARP, B.S.*, Division of Dermatology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23219.

Although we are aware of the accelerated turnover in the psoriatic epidermis (Rothberg, Crounse, and Lee, J. Invest. Derm. 37: 497, 1961) the mechanism for this stimulation of the proliferative process in psoriasis or for the maintenance of homeostasis in the normal skin is unknown. Previous reports suggest that an aqueous epidermal extract (chalone) is essential for maintaining homeostasis in regard to epidermal mitosis. (Bullough and Laurence, Exp. Cell Res. 33: 176, 1964)
The present report indicates that the epidermal aqueous extract from newborn mouse and cow snout epidermis inhibits thymidine-\(^3H\) incorporation into epidermal DNA. The inhibition is not tissue specific (as in mitosis) for DNA synthesis in both the epidermis and the dermis is inhibited by the extract. In hairless mouse skin, newborn and adult, the inhibition does not require epinephrine, hydrocortisone or both, as does the epidermal mitosis inhibition enzyme. In the 19 day chick embryo, inhibition of DNA synthesis in the epidermis, dermis or whole skin requires epinephrine in addition to the epidermal extract.

It is not known from present studies whether the factor or factors influencing these two essential parts of the cell cycle are similar or different.

3. SPECIAL LECTURE. XERODERMA PIGMENTOSA: DNA REPAIR AND CARCINOGENESIS. JAMES E. CLEAVER, PH.D., Laboratory of Radiobiology, University of California, San Francisco, California, 94122.

4. STRATUM CORNEUM CULTURE TECHNIQUE. ARTHUR KNIGHT, M.B., M.R.C.P.*, (Introduced by R. B. STOUGHTON, M.D.), Division of Dermatology, Scripps Clinic and Research Foundation, La Jolla, California 92037.

A tape culture technique has been devised and tested for investigating dermatophytic growth on stratum corneum (SC) alone.

Method: 3M\(^\text{TM}\) surgical tape, which is translucent and has an inert copolymer adhesive, is used to strip off the superficial SC. This is applied to a slide, SC uppermost. It is placed on a bent glass rod in a covered petri dish and sterilized with ethylene oxide gas. The tape is inoculated with a spore solution, water is run into the dish and incubated at 31° C. T. mentagrophytes spores were used for the present studies.

Observations were made on 100 tape cultures at 10 days and a grading system of I–III is used. Duplication of observations showed a 90% reproducibility. 600 spores consistently gave a grade II or III growth on normal SC. Using tape alone (no SC) or no water there was no growth. Pretreatment of the skin was 2% sodium omadine prevented fungal growth on the tape for 24 hours and at 48 hours it had returned to normal. Using patients receiving oral griseofulvin 500 mg daily, it was possible to demonstrate inhibition (not total) within 24 hours of starting which returned to normal within 72 hours after stopping the drug. Differential stripings of the top, middle and lower layers of SC during therapy showed almost complete inhibition at the bottom layer.

5. BIOMECHANICAL PROPERTIES OF DELIPIDIZED STRATUM CORNEUM, M. WOLFRAM*, PH.D., N. WOLEJSZA*, B. S., AND K. LADEN, PH.D., Gillette Research Institute, Rockville, Maryland 20850.

Alterations of the mechno-physical properties of stratum corneum induced by ether extraction were studied. Mechanical work to stretch (Instron extensometer) was measured at various pH's before and after ether extraction. Before extraction, skin exhibited its greatest mechanical stability at neutral pH values. A decided weakening of the skin occurs at both acidic and alkaline pH's. After ether extraction, a pronounced weakening was observed at all of the pH values studied. In addition, ether extraction produces a marked increase in the cross-sectional as well as transverse swelling of skin when it is immersed in water. It is postulated that ether extraction results in the unmasking of hydrophilic sites in stratum corneum. Differences observed in the hysteresis behavior of extracted vs untreated skin support this hypothesis. The enhanced swelling of skin after ether extraction was also observed in vivo using a replica technique and scanning electron microscopy. These results suggest that removal of lipids from the surface of the skin produces a pronounced weakening and swelling when the skin is subsequently immersed in water. This may be reflected in the appearance of cracks, upturned stratum cells and overall conditions associated with dry and chapped skin.

6. TIME COURSE LABELLING OF SKIN POLYUNSATURATED FATTY ACIDS WITH \(^{14}C\)-ACETATE. D. I. WILKINSON, PH.D., Department of Dermatology, Stanford University School of Medicine, Stanford, California 94305.

The variation of labelling with time may provide insight into interconversions of polyunsaturated fatty acids. Newborn mouse skin and human preputial skin were used. Minced skin was incubated (37°) in buffer solutions with 1-\(^{14}C\)-acetate for time intervals up to 9 hours. Lipids were extracted with CHCl\(_3/\text{H}2\text{OH},\) methylated (CH\(_3\)OH/H\(_2\text{SO}_4\)), and fatty acid methyl esters
purified and resolved by silver nitrate thin layer chromatography. Individual polyunsaturated fatty acids were isolated by preparative gas liquid chromatography, and their radioactivity assayed by scintillation counting.

The uptake of acetate depended on the incubation medium used. Distribution and rate of change of radioactivity with time varied widely among the polyunsaturated fatty acids. Synthesis of linoleic (ω6) and linolenic (ω3) classes were observed. Both linoleic and linolenic ("essential") acids were labelled, and their activity increased with time in proportion to other polyunsaturated fatty acids, e.g., the 18:3ω6/18:3ω3 ratio remained constant. This would hardly be expected if 18:3ω3 were labelled by carboxyl exchange (the classical view). The pathway 18:2ω6 → 20:4ω6 via 20:2ω6 is preferred to that via 18:3ω6 although both may be in use. Levels of labelled 20:4ω6 sharply increased after about 6 hours’ incubation. This may be significant for prostaglandin E₂ formation.

Similar results were observed for both types of skin.


Differential thermal analysis and x-ray diffraction were utilized to study the stability of the crystalline lipid structure of the stratum corneum of neonatal rat and of middle age human. The DTA results for both rat and human indicate the presence of two melting endotherms below 100°C—one at approximately 45 and a stronger one at 70°C. Superimposed is a broad endotherm which results from the presence of water. The identification of all of these endotherms was verified by further DTA experiments involving samples preconditioned at specified relative humidities. Further identification of the lipid melting points and degree of recrystallization was established by noting the thermal dependence of x-ray diffraction patterns taken on the corneum. It was verified that lipid recrystallization occurs with or without the presence of water and is essentially complete in neonatal rat relative to its initial structure. The reversibility in human corneum is not as apparent. It was also demonstrated that the lipids (their presence and thermal stability) affect the mechanical behavior of corneum at small deformations. All of these data will be correlated.

Friday, April 28, 1972
35TH ANNIVERSARY BANQUET
7:00 p.m.
(For those members and guests who have made reservations)

MORNING SESSION

SATURDAY, APRIL 29, 1972
9:00 A.M. BUSINESS AND EXECUTIVE SESSIONS: GEORGE W. HAMBRICK, JR., M.D., presiding.

SCIENTIFIC SESSION: ROBERT W. GOLTZ, M.D., Minneapolis, Minnesota, presiding.

1. PASSIVE INDUCTION OF PEMPHIGUS-LIKE LESIONS IN MONKEYS WITH SERA OF PEMPHIGUS VULGARIS PATIENTS. M. V. GAUTO, M.A.*, E. H. BEUTNER, Ph.D.*, and T. CHORZELSKI, M.D.*, (Introduced by H. MILGROM, M.D.), State University of New York at Buffalo
School of Medicine, Buffalo, New York 14214, and Warsaw Academy of Medicine, Warsaw, Poland.

Previous studies on the passive transfer of pemphigus sera have yielded some evidence of lesion formation with Brazilian pemphigus foliaceous sera but none to date with pemphigus vulgaris sera or with their IgG fractions.

One or two intramuscular injections of monkey lips with two fold concentrated pemphigus vulgaris sera (titer 2560) yielded some in vivo binding of pemphigus antibodies but little or no intraepithelial changes. However, pemphigus-like lesions developed in each of eight sites in two monkeys which received three intramuscular injections of a given site with the same sera at time 0, 3, 8 hours. Gross lesions appeared before 14 hours following the third injection. These appeared to be indistinguishable from those of pemphigus in both histopathologic and immunofluorescent (IF) studies. Injection of the IgG fraction of a pemphigus serum (titer 1280) also elicited histologically typical lesions. However for some unknown reason these were not typical in immunofluorescence in 22 hours. All control sites which received three injections of comparable material failed to develop either gross or microscopic lesions or in vivo fixation of IgG. In essence these preliminary observations suggest that pemphigus antibody containing sera from pemphigus vulgaris patients are pathogenic and that their IgG fraction also causes typical lesion formation but that the latter may differ in the immunofluorescent reaction from those elicited by whole serum.


When guinea pigs were sensitized to picric acid (PA) by a split adjuvant technique (J. Invest. Derm. 49: 460, 1967), the initial contact reactivity to PA ascends slowly over 3 days. But after 3 successive tests with PA, the reactions are sharply heightened and become maximal by 24 hours. Cross-reactions are noted with pyrrol chloride (PCI), a covalently-linking hapten. As sensitivity to PA increases, reactivity to PCI also ascends, but not as much. In experiments involving transfer with oil-induced peritoneal exudate cells (approximately 1 x 10^9), cells taken from donors after one contact with PA conferred a slowly ascending reactivity to PA over 48-72 hours, and contact sensitivity to PCI also. Following three boosts with PA, the donors, now sensitive to contact with 0.003% PA, yielded cells which at 1 x 10^9 stimulated maximal reactivity to PA and to PCI in 24 hours, proceeding to crusting. Both the highly boosted donors and the recipients of their cells responded to PA contact not only within the site of application but, with a different quality, perifocally. Thus the special features of reactivity of the donor animals are preserved in transfer. This suggests that a qualitative change in the population of antigen-reactive cells has been secured through induction of an anamnestic response in the area of delayed hypersensitivity. The unique histology (polymorphonuclear leukocyte infiltrate, epidermal abscesses) as well as the characteristic evolution (follicular papules, crusts) of the challenge reactions of guinea pigs sensitized to picric acid were seen also in the recipient animals.

3. SPECIAL LECTURE. CLINICAL AND IMMUNOLOGICAL ASPECTS OF PENICILLIN HYPERSENSITIVITY. BERNARD B. LEVINE, M.D., Department of Medicine, New York University School of Medicine, New York, New York 10016.

4. BASEMENT MEMBRANE ANTIBODY IN THE SKIN OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS. M. LANDRY, M.D., AND W. MITCHELL SAMS, JR., M.D., Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901.

Immunofluorescent techniques reveal granular deposits of immunoglobulin along the skin basement membrane zone of patients with systemic lupus erythematosus (SLE). Similar granular deposits are seen in kidney glomeruli, and Koffler et al, using acid elution, demonstrated them to be antinuclear antibodies. Our studies were designed to investigate the nature of the antibodies of the skin.

Skin and kidneys were obtained at autopsy from three patients with SLE. After homogenizing and washing the tissue, bound antibody was eluted with pH 2.2 or 3.2 buffer, concen
trated, reacted with appropriate tissue sections, and stained with fluorescein-tagged antihuman IgG. All three kidney and skin eluates showed an antinuclear antibody; in addition, two skin eluates showed a skin but not a kidney basement membrane antibody:

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This antibody gave a linear rather than a granular pattern and blocked staining by a fluorescein-tagged pemphigoid antibody, indicating a common antigenic site.

These elution studies show two antibodies in the basement membrane zone of SLE skin: one a granular deposit of presumably antigen-antibody complexes derived from serum and the second a linear deposit that appears to be an antibody for the skin basement membrane.

5. CYCLIC 3',5'-NUCLEOTIDE PHOSPHODIESTERASE: SUBCELLULAR LOCALIZATION IN RAT SKIN. L. E. KING, JR., M.D., PH.D.* (Introduced by K. HASHIMOTO, M.D.), Memphis Veterans Administration Hospital and Divisions of Dermatology and Anatomy, University of Tennessee, Memphis, Tennessee 38104.

Evidence that 3',5'-adenosine monophosphate (c-AMP) is an important regulator of epidermal proliferation has been suggested (Voorhees et al, Arch. Derm. 104: 353, 1971). The enzyme which hydrolyzes c-AMP is c-AMP phosphodiesterase. The activity of this enzyme thus regulates the magnitude and duration of c-AMP activity in the tissues. A cytochemical method to detect c-AMP phosphodiesterase activity at the ultrastructural level was recently developed (Florendo et al, Science 173: 745, 1971). In the present study this method was applied to the rat's skin to determine if sites of c-AMP phosphodiesterase activity could be detected.

The study showed that 1) the enzyme activity was found in the epidermal keratinocytes (particularly in the basal cells), melanocytes, dermal fibroblasts, mast cells, and blood vessels; 2) precise location of the lead phosphate precipitates, the final reaction product in this method, was on or near the plasma membranes; 3) on the plasma membranes the reaction product was limited to spotted sites and was not in a diffuse pattern; 4) aggregations of lead phosphate were seen in the blood vessel lumens; and 5) control tissues, including those incubated with theophylline, an inhibitor of c-AMP phosphodiesterase, showed no recognizable precipitates noted in areas comparable to the experimental tissues.

6. ISOLATION OF SEA NETTLE NEMATOCYST TOXINS. G. J. CALTON, PH.D.* AND J. W. BURNETT, M.D., Division of Dermatology, University of Maryland School of Medicine, Baltimore, Maryland 21201.

Sea nettle (Chrysaora quinquecirra) nematocysts were isolated from tentacular debris and its possible toxic agents by differential centrifugation. All types of nematocysts had a similar density (1.22-1.24). Higher yields of toxin lethal to mice were obtained when nematocysts were ruptured by pressure (28,000 psi) rather than by sonic treatment. Purification of the toxin thus obtained by repeated gel diffusion resulted in at least 10 fractions lethal to mice which had molecular weights over 100,000 and 1 lethal fraction with lower molecular weight. Only two of these fractions are in the eluate containing the majority of the protein. The fractions which ruptured in vitro rat liver lysosome preparations were not lethal to mice and immediately preceded or coincided with the peak of the protein curve. These toxins did not alter rat liver microsomal glucose-6-phosphatase nor affect the respiratory control index of mitochondria. These studies indicate that sea nettle nematocysts contain many toxins which may have several independent actions.

7. SENILE PURPURA. R. FEINSTEIN, M.D., K. HALPRIN, M.D., N. PENNEYS, M.D., AND J. R. TAYLOR, M.D., Department of Dermatology, University of
Miami School of Medicine, and the Veterans Hospital, Miami, Florida 33152.

We attempted to study senile purpura in elderly male in-patients by using intradermal injections of autologous red blood cells which had been labelled by Chromate-Na 51. Removal of activity was measured by a scintillation counter probe pressed against the skin over the injection site. Data was analyzed and then an IBM 370 computer which we had programmed for linear regression, was used to calculate half life values for various experimental situations.

For all cases we found a biphasic curve with rapid removal of activity in the first 72 hours, and a more gradual loss over the next week. Loss of activity was as rapid from areas that are prone to senile purpura (dorsal arm) as from usually uninvolved locations (ventral arm and abdominal wall). The rate of removal was unaffected by application of Cordran® or Blenderm® Tapes. Ingestion of bromelains, which are proteolytic enzymes recommended for use by patients who have sustained intradermal bleeding from trauma, did not accelerate the rate of removal of activity.

We concluded that removal of activity associated with intradermally injected Chromate-Na 51 tagged red blood cells is as rapid in areas where senile purpura occurs, as in areas not usually involved. Stripping the epidermis, occluding with medicated or non-medicated tapes, and ingesting bromelains, appeared to have no positive effect on the rate of removal. Senile purpura does not appear to be associated with delayed removal of extravascular red blood cells.

**AFTERNOON SESSION**

**Saturday, April 29, 1972**

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

1. **TWELFTH ANNUAL HERMAN BEERMAN LECTURE. IMMUNE COMPLEX DISEASE.** FRANK J. DIXON, M.D., Department of Experimental Pathology and Biomedical Research Departments, Scripps Clinic and Research Foundation, La Jolla, California 92037.

2. **DEVELOPMENT AND REGRESSION OF ACUTE CONTACT DERMATITIS AT THE CELLULAR LEVEL.** B. C. FRICHTH, III, M.D., J. H. MOTTAZ, B.S.*, AND A. S. ZELICKSON, M.D., Department of Dermatology, University of Minnesota, Minneapolis, Minnesota 55455.

The purpose of this study was to visualize, at the ultrastructural level, the development and resolution of acute contact dermatitis, with and without topical steroid therapy. The skin of symmetrical areas of the mid-back was touched with the broken stem of a poison ivy plant. Serial 2 mm paired punch biopsies were obtained before therapy (16 hours) and at 4 and 10 days. Additional biopsies were obtained from the treated lesion at 3 and 5 days. The tissue was processed for direct and histochemical examination with the electron microscope.

The major findings in the untreated dermatitis were primarily in the epidermis; they included extra- and intracellular edema and invasion of the epidermis by inflammatory cells. With therapy, recovery began in the granular layer, proceeded downward through the prickle cell layer and appeared last in the basal layer. Intracellular edema was seen in keratinocytes, in the form of large para-nuclear vacuoles containing residual bodies, and demonstrating acid phosphatase reaction product, indicating that they were lysosomes. In the steroid-treated specimens, these lysosomes maintained their membrane boundary and progressively disappeared (superiorly to inferiorly) with subsequent return of normal cellular architecture.

An acute contact dermatitis appears to resolve following topical steroid therapy in a superficial to deeper cell-layer sequence.

3. **PHOTOSENSITIZED THYMINE DIMERIZATION IN DNA BY CARBONYL COMPOUNDS.** M. A. PATHAK, PH.D., AND A. KORNHAUSER, PH.D.*, Department of Dermatology, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts 02114.

Recently we reported (Photochem. Photobiol, 15: 177, 1972) that in vivo irradiation of skin (290-320 nm) induces cyclobutyl thymine dimers (TT). It is not known whether in vivo, DNA is the primary chromophore for 290-320 nm absorption or dimerization results by a photosensitization mechanism involving triplet-triplet energy transfer from some other UV-absorbing
molecule. We investigated the possibility of several biologically important carbonyl and non-carbonyl compounds acting as photosensitizers in $\text{T}$ formation. Benzophenone (BP), acetophenone (AcP), 4-methoxy acetophenone (MAcP), ethylacetocacetate (ETA), acetone (Ac), dihydroxyacetone (DHA), Xantheine-9-one (XO), psoralens (Ps), and urocanic acid (UCA) were examined for their ability to induce $\text{T}$. Solutions of the thymine $2\text{-}$IC (2 x $10^{-2}$ M) with and without these potential sensizers ($10^{-4}$-$10^{-2}$ M) were irradiated (300-365 nm; 1.8 x $10^7$ ergs $\cdot$ cm$^{-2}$). Thymine dimers were separated chromatographically and their radioactivity estimated in a liquid scintillation spectrometer. The percentage of $\text{T}$ formed by each of the sensizers was BP = 8; AcP = 10; ETA = 30-40; DHA = 25%; UCA, Ps, and other agents produced negligible $\text{T}$. ETA and DHA, hitherto unknown as photosensitizers, were found to be more effective than Ac or AcP. The results indicate that: 1) epidermal DNA is the primary UV-absorber in $\text{T}$ formation; 2) in vivo, $\text{T}$ formation may also result by a photosensitization mechanism and triplet-triplet energy transfer; and 3) topical preparations containing BP, DHA, or Ac may be potentially harmful to skin.

4. LYMPHOCYTE REACTIVITY IN MYCOsis FUNGOIDES AND SOME MALIGNANT LYMPHOMAS. A. KANAIDE, M.D.*, A. E. POWELL*, V. J. KULAK, M.D., AND B. MICHEL, M.D., Division of Dermatology, and Department of Surgery, Case Western Reserve University, Cleveland, Ohio 44106.

Depressed lymphocytic responses to phytohemagglutinin (PHA) in tumor patients have been reported. We compared responses to PHA with those elicited by concanavalin A (ConA). Lymphocytes were examined from six mycosis fungoides (MF) patients, one with Hodgkin's disease (HD) secondary to MF, one with Sezary's syndrome, two with chronic lymphocytic leukemia (CLL), one with lymphosarcoma and one with HD. Lymphocytes were treated with PHA or ConA in normal or autologous plasma. DNA synthesis was measured at 72 hours by $\text{H}$-thymidine incorporation. The MF patients except those in tumor or HD stage, responded normally. The tumor stage MF gave depressed responses. Both HD patients showed depressed responses to ConA only in autologous plasma, suggesting an inhibitory plasma factor. Sezary and lymphosarcoma patients showed depressed responses especially to ConA in autologous plasma. In CLL, either slight or marked depression occurred, reflecting severity of illness. Thus in MF, depressed responses occurred only in advanced tumor stage or systemic involvement, as with Sezary, CLL and lymphosarcoma patients. ConA proved a sensitive indicator of lymphocyte depression and revealed the existence of a ConA-specific inhibitory plasma factor which would have been missed with PHA alone. PHA specific plasma factors were not evident, but some depressed responses were detected only with PHA. The inhibitory factor is heat stable ($56^\circ$ C) and appears to be active in the mixed lymphocyte reaction.

5. QUANTITATION OF LYSOSOMES IN MURINE KERATINIZING EPITHELIA BY ELECTRON MICROSCOPY. G. ROWDEN, PH.D., Medical School, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

The extent of the involvement of lysosomes in keratinization is not clear. Measurements of the absolute numbers of lysosomes in the various epidermal layers may prove valuable in resolving this problem. Measurements were made using a series of grid lines superimposed on sections, that had been incubated to demonstrate a number of acid hydrolases. Counts of the number of lysosomes/cell were converted to lysosomes/stratum, by the application of the previously determined cell volume measurements. Values were obtained for mouse epidermis and oesophageal epithelial samples. The accuracy of the stereological methods was assessed. The results indicate that it is extremely unlikely that the release of hydrolases from pre-existing lysosomes when keratinocytes reach the granular layer, could account for the extremely rapid metamorphic events occurring in the transformation from this layer to the cornified layer.

6. MECHANISM OF DECREASED PIGMENTATION IN TUBEROUS SCLEROSIS, NEVUS DEPIMENTOSUS AND PIEBALDISM. K. JIMBOW, M.D., T. B. FITZPATRICK, M.D. AND G. SZABÓ, PH.D., Department of Dermatology, Harvard Medical School and Electron Microscopy Laboratory, Harvard Dental School, Boston, Massachusetts 02114.

Hypomelanosis of the skin and hair can result from alteration in one or more of the four major processes that occur in normal melanin pigmentation in man. These four processes in-
volve the synthesis, melanization, transfer, and degradation of melanosomes. This study, based on fifteen patients and using electron microscopy and histochemistry, was directed at elucidating the decreased pigmentation occurring in three types of congenital, circumscribed hypomelanosis: tuberous sclerosis, nevus depigmentosus, and piebaldism. In 8 patients with tuberous sclerosis, the hypomelanosis appears to result from a decrease in the synthesis of melanosomes (less than 0.7 x 0.25 μ) in all patients resulted in an aggregation of melanosomes in keratinocytes in the hair and also in the non-follicular keratinocytes of one Negro patient. In 3 patients with nevus depigmentosus, the number of melanocytes is not reduced, and the melanization of melanosomes is unchanged, but the number of melanosomes in keratinocytes is reduced and aggregates of melanosomes are often seen in melanocytes, suggesting a block in the transfer of melanosomes from melanocytes to keratinocytes. In 4 patients with piebaldism, the hypomelanosis results from an absence of functioning melanocytes; in the islands of pigmented skin that characteristically occur in the hypomelanotic areas of piebaldism, a marked changed in the structure of melanosomes was observed. In these pigmented islands, the melanosomes were spherical and granular in lieu of the normal ellipsoidal and filamentous structure.

7. DENDRITIC CELLS IN DEVELOPING MAMMALIAN EPIDERMIS.
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The purpose of this investigation was to study the primary appearance and subsequent maturation of dendritic cells in developing epidermis. Skin biopsies were obtained at 24-hour intervals from the mid-dorsolateral regions of the trunk of C57B1/6 mice ranging in age from embryonic day 8 through postnatal day 4 inclusive. Biopsies were also obtained at postnatal day 18. The skin was fixed in buffered osmium, processed routinely, stained with uranyl acetate and lead citrate and studied with an RCA-3G electron microscope. Fontana-Masson's ammoniacal silver technique with eosin counterstain was used on tissue fixed in Bouin's solution for light microscopic studies of melanocytes.

Epidermal melanocytes, which lacked processes and contained few premelanosomes, were observed first in the 15 day embryo. By day 18, dendritic processes and premelanosomes in various stages of melanization were present. The number of melanocytes appeared to increase progressively from prenatal day 15 to birth (day 21). Numerous melanocytes, which contained many premelanosomes and melanosomes, were present during the first 3 days after birth. On postnatal day 4, fewer of these organelles were present and membrane-limited vacuoles appeared within the melanocyte. Phagocytosed melanocytes, in which organelles and nuclear remnants could be identified, were observed completely within the cytoplasm of basal keratinocytes. On postnatal day 18, no melanocytes were observed but Langerhans cells could be identified for the first time. Indeterminate cells were noted first in the 16 day embryo. Mitotic dendritic cells or cells crossing the basal lamina were not observed.

CLOSING EXECUTIVE SESSION: Installation of New Officers
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