PROGRAM

SPECIAL JOINT MEETING

THE SOCIETY FOR INVESTIGATIVE DERMATOLOGY, INC.

AND

EUROPEAN SOCIETY FOR DERMATOLOGICAL RESEARCH

International Congressenrum/RAI
Amsterdam, Netherlands
May 17, 18, & 19, 1972

MORNING SESSION

WEDNESDAY, 9:00 A.M.–12:00 N


1. OPENING REMARKS. OTTO BRAUN-FALCO, M.D., President, European Society for Dermatological Research and ROBERT W. GOLTZ, M.D., president, The Society for Investigative Dermatology, Inc.

2. INHIBITION OF RNA SYNTHESIS AND RNA-DEPENDENT DNA-POLYMERASE BY ETHIDIUMBROMIDE IN MELANOMA. B. R. BALDA, M.D., G. D. BIRKMAYER, PH.D. AND O. BRAUN-FALCO, M.D., Department of Dermatology, University of Munich, 8 Munich, Germany.

Previous studies on the biochemical characteristics of amelanotic hamster melanoma revealed the presence of proflavine-sensitive proteins (Hoppe-Seylers Z. physiol. Chem. 352: 780, 1971). Virus-like particles were detected by electron microscopy in this tumor (Naturwissenschaften 57: 548, 1970). An attempt was made to concentrate these particles by density gradient centrifugation. In vivo labeled RNA of the concentrated fraction was characterized by disc-electrophoresis on polyacrylamide gel. The activity of DNA-polymerase of the fraction was determined simultaneously.

At a density of 1.16 g/cm³ a fraction containing high molecular weight RNA-species (45S-60S), which is characteristic for virus-specific RNA, was isolated. The synthesis of these RNA-species could be inhibited by the phenanthridine dye ethidiumbromide but not by actinomycin D. In the same fraction a RNA-dependent and ethidiumbromide-sensitive DNA-polymerase was demonstrable. The cell-free transmission as well as the results described above indicate viruses as the probable tumorigenic agent which specifically can be inhibited by ethidiumbromide.

3. STUDIES OF ANTI-MELANOMA EFFECT OF POLYINOSINIC-POLYCYTIDYLIC ACID. R. S. BART, M.D., A. W. KOPF, M.D., AND S. LAM, Department of Dermatology, New York University School of Medicine, New York, New York 10016.

Polyinosinic-polycytidylic acid (PIC) inhibits growth of B-16 malignant melanomas (MM) in C-57 black mice. Does PIC augment an anti-MM immune response? Each of 15 mice, 5 weeks old, was implanted with 0.0125 ml amelanotic MM ("sensitizing implant", SI) into the right axilla on day 0; 19 unimplanted mice were controls. On day 7 all mice were transplanted with 0.05 ml amelanotic MM ("definitive transplant", DT) into the left axilla. On day 19 the volumes of each DT tumor was measured. The average volume was less in "sensitized" mice (0.36 vs 1.74 ml), suggesting an immune response. The above experiment was modified to test the effect of PIC on this anti-MM response. Each of 34 mice received SI with melanotic MM on day 0; 15 were injected with 150 μg PIC on days 1–5 (Group I); 19 with saline (Group II). Thirty-nine mice did not receive SI: 20 were injected with PIC on days 1–5 (Group III); 19 with saline (Group IV). On day 7 all mice received DT with melanotic MM. On day 18 the volume of each DT tumor was measured. The average volume for each group was as follows: Group I =
0.71; II = 0.49; III = 0.87; IV = 0.78. In conclusion, prior exposure to MM decreases the growth of subsequent MM implants. PIC does not augment this response.

4. ANTIBODIES TO HUMAN MELANOMA DEMONSTRATED BY A NEW RADIOIMMUNOASSAY. J.-C. Bystryn, M.D., Department of Dermatology, New York University School of Medicine, New York, New York 10016.

Present assays of antibody to melanoma are semi-quantitative or influenced by the class or functional behavior of antibody. A new assay is reported that measures quantitatively the primary interaction between antibody and cell-surface antigens on human melanoma cells.

Cell-surface material on homologous human melanoma cells was iodinated with lactoperoxidase, solubilized in detergent (NP-40) and dialyzed. The lysate was incubated with 20 lambda of serum from patients with or without melanoma. Complexes of tumor antigen-antibody were co-precipitated with goat anti-human Ig. Radioactivity in the precipitate was shown to be directly proportional to quantity of anti-melanoma antibody. An index of antibody activity was calculated from counts per minute bound by test serum vs. counts per minute bound by a standard reference serum. Anti-melanoma antibody activity, indicated by an index greater than 2, was demonstrated in 55% (12 of 22) patients with melanoma and 8% (2 of 25) control patients.

Potential advantages of this assay include ease of quantitation, measurement of individual class of tumor-specific antibody (by using mono-specific anti-human Ig), and measurement of circulating tumor antigen (by inhibition of the assay). In addition, the specific precipitate contains labelled tumor antigen which facilitates its isolation and biochemical characterization.

5. TRANSPLANTATION IMMUNOLOGY OF ADULT SKIN. T. Summerlin, M.D., Department of Dermatology, University of Minnesota Hospitals, Minneapolis, Minnesota 55455.

This study was undertaken to investigate the mechanisms underlying cell-mediated immunity, using a model allowing exploration of cutaneous immunogenicity. With a standard tissue culture technique, both human and mouse whole skin have been maintained viable in vitro for extended periods of time; such skin, maintained in culture for 4–6 weeks, appears to grow without rejection when transplanted homologously to appropriately prepared full-thickness wound sites. These results in eight patients, and in mice, across major histocompatibility barriers, have been confirmed by in-vitro viability and histologic surveys plus continued pigmented and karyotypic marker documentation. In addition, the in-vitro study of the interaction of sensitized lymphoid effector cells and radioactive chromium labelled target cells has been utilized to critically examine the above observational data.

These experimental procedures have permitted both qualitative and quantitative documentation of the events surrounding both normal homologous skin graft rejection and the author's observed lengthy (several months to years) retention of tissue cultured skin by homologous hosts. This paper thus describes definitive work which confirms immunologic change resulting from the culturing process of whole skin.

6. INHIBITION OF IgE-MEDIATED CUTANEOUS ALLERGY BY CYCLIC AMP. M. W. Greaves, M.D., Ph.D., and S. Yamamoto, M.D., University Department of Dermatology, Newcastle upon Tyne, England.

The effect of the adenyl cyclase-cyclic AMP system on IgE-mediated cutaneous allergy has been studied using a new in-vitro method. Skin from rats, actively sensitized from the production of IgE, is divided into 500 μ-thick slices. Triplicate samples are then incubated with antigen and histamine release measured by bioassay. That the observed histamine release is due to sensitization by IgE is indicated by reverse anaphylaxis experiments in which anti-rat IgE serum releases histamine from skin of sensitized, but not normal, rats. The effects of theophylline, and the beta adrenergic amines adrenaline and isoprenaline on antigen-induced histamine releases were then studied. In 10 experiments theophylline 10⁻⁴ M produced a mean inhibition of 18.6% ± 5.1 S.E.M. Although adrenaline failed to inhibit by itself, marked synergism was demonstrated by adding 10⁻⁴ M adrenaline to 10⁻⁴ M theophylline since the mean inhibition in 6 experiments by this mixture was 34.2% ± 5.7 S.E.M. Isoprenaline 10⁻⁴ M produced a mean inhibition of 23.7% ± 4.9 S.E.M. in 9 experiments. Theophylline and beta adrenergic amines increase intracellular cyclic AMP through independent mechanisms. The results therefore support the view that the adenyl cyclase-cyclic AMP system regulates IgE-mediated cutaneous allergy.
7. COLLAGENOLYTIC ACTIVITIES OF BASEL CELL EPITHELIOMA. K. HASHIMOTO, M.D., Y. YAMANISHI, M.D., Ph.D. AND M. DABBOUS, Ph.D., Memphis Veterans Administration Hospital, and Division of Dermatology and Biochemistry, Memphis, Tennessee 38104.

The presence of collagenolytic enzyme was supported in basal cell epitheliomas because the collagenous stroma surrounding the tumor is often digested and forms a clear space. The results of the following experiments suggested that such an enzyme is indeed present in basal cell epitheliomas: (1) Electron microscopy showed thin fragments of collagen in the clear space; (2) tumor homogenate (crude enzyme) released radioactivity from reconstituted acid-soluble collagen prepared from the skin of rat which was injected with C14-proline intraperitoneally. The crude enzyme activity was inhibited by EDTA and normal human serum, but not by trypsin inhibitor (soy bean); (3) disc electrophoresis of tumor homogenate-collagen substrate mixture showed degradation products of collagen such as $\alpha^1$, $\beta^1$ and other bands; (4) the same mixture showed viscosity loss of 30-60% and drop of denaturation temperature midpoint about 5°C below that of native collagen at pH 4; and (5) electron microscopy of segment long-spacing collagen crystalized from the same reaction mixture revealed fragments of tropocollagen molecules severed at $\delta^1$, $\delta^2$ and $\beta_2$ regions. Kinetic studies demonstrated a linear correlation between collagenolytic activity and crude enzyme concentration or the length of incubation time. The optimal pH was 7.85. Collagenase thus seems to contribute to the pathogenesis and local spread of basal cell epitheliomas.

8. THE MECHANISMS OF RELEASE OF COLLAGENASE FROM INFLAMMATORY SITES. T. NIGRA, M.D., M. FRIEDLAND, M.D., R. SNYDERMAN, M.D. AND G. MARTIN, Ph.D., National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014.

The mechanisms of collagen degradation are extremely important to a number of pathologic inflammatory conditions such as destruction of basement membrane in renal glomerulitis, joint degeneration in rheumatoid arthritis and gingival destruction in periodontal disease. To understand the nature of collagen degeneration we have looked at experimental models of inflammation; in the Arthus reaction and the delayed hypersensitivity reaction we have observed an increased rate of collagen degeneration as measured by increased levels of collagenase. With these models we have tried to elucidate which cell types might be responsible for collagenase elevations and how its release is effected.

The peak of collagenase activity in the Arthus reaction coincides with the maximal infiltration of the lesions with polymorphonuclear cells (PMN). We have confirmed that rabbit PMN contain collagenase stored in their cytoplasmic granules. The Arthus reaction begins by formation of immune complexes. Immune complexes are the essence of the Arthus reaction; they activate complement and initiate severe tissue destruction. PMN phagocytose immune complexes and subsequently release their granules containing a number of degradative enzymes. We have shown that PMN in culture will release collagenase upon complement dependent phagocytosis of immune complexes. We propose the following mechanism: Immune Complexes $\rightarrow$ Complement activation $\rightarrow$ Phagocytosis by PMN $\rightarrow$ Collagenase release $\rightarrow$ Collagen degradation.

9. GLYCOSAMINOGLYCAN FORMATION AROUND THE LINEAR WOUND. R. CARLSEN, M.D., AND P. HELINE, M.D., Division of Dermatology, University of Washington School of Medicine, Seattle, Washington 98195, and Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.

That glycosaminoglycan (GAG) and histamine changes occur around wounds is well-established. Previous studies have not determined the exact dimensions of these changes. It is important to know these limits when studying chemical changes around wounds. To delineate these, levels of newly formed GAG were determined sequentially at measured distances laterally from a linear skin wound on the back of rats. Forty-eight hours prior to collection of tissue, the rats were given an intraperitoneal injection of 0.5 $\mu$ curies of S-35. On scheduled days after wounding, consecutive skin strips 2.5 mm wide and 4 cm long were excised parallel to the wound. This tissue was dissolved in papain and GAGs were precipitated with CPC. Liquid scintillation counting was used to determine the radioactive label that had been converted into sulfated GAGs.
The results indicate that sulfated GAGs, newly formed in response to wounding, are limited to the zone within 5 mm from the wound edge. Increases in GAG content begin at day 2 and reach peaks at days 7 and 26 after wounding; by day 50, the values have returned to near control levels. The results are correlated with other parameters of wound healing.

**LUNCHEON WORKSHOPS**

(Jointly sponsored with the National Program for Dermatology)

**WEDNESDAY, 12:15 P.M.—1:45 P.M.**

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**AFTERNOON SESSION**

**WEDNESDAY, 2:00 P.M.—5:00 P.M.**

**GEORGE W. HAMBRICK, JR., Baltimore, Maryland, U.S.A., AND CHARLES LAPIERE, M.D., Liege, Belgium, presiding.**

1. **ROLE OF SWEAT IN ACCUMULATION OF GRISEOFULVIN IN SKIN. V. P. SHAH, PH.D., W. L. EPSTEIN, M.D., AND S. RIEGELMAN, PH.D., School of Pharmacy and Department of Dermatology, University of California, San Francisco, California 94122.**

A gas-liquid chromatography method has been developed for the analysis of griseofulvin in stratum corneum. The drug appears in skin in 4-8 hours after oral administration. Griseofulvin distribution was found to be higher in superficial layers (Level I, 20.8 ± 1.5 ng/mg) than in the inner layers (Level II, 10 ± 1.5; III, 7.5 ± 2.2 ng/mg). These findings suggest that factors other than normal diffusion or cell turnover must effect drug transfer to stratum corneum. In order to study the precise mechanism of griseofulvin transfer to stratum corneum, the role of sweat in accumulation of griseofulvin was considered. Heat induced total body sweating depleted the stratum corneum of griseofulvin by a mean drop of 55% (9 subjects) and 200-300 ng/ml of griseofulvin accumulated in sweat. Prevention of transepidermal water and sweat loss by: 1) topical application of 15% methenamine cream to one palm 2) wearing a rubber glove for 24 hrs. 3) 2 x 2 cm. occluded patch on arm showed a lower griseofulvin concentration when compared to control areas in the same subjects. A total of 40-700 ng/glove and 50-200 ng/patch of griseofulvin was detected.
Griseofulvin concentration (ng/mg) and % drop

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<td>8</td>
<td>Methenamine cream</td>
<td>12.1</td>
<td>6.5</td>
<td>27.3</td>
<td>11.3</td>
<td>55</td>
<td>40</td>
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<td>9</td>
<td>Glove</td>
<td>5.5</td>
<td>3.6</td>
<td>22.8</td>
<td>9.5</td>
<td>76</td>
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<td>6</td>
<td>Occluded Patch</td>
<td>11.2</td>
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These findings suggest that sweat and transepidermal fluid loss play an important role in griseofulvin transfer in stratum corneum.

2. THE MODE OF ACTION OF DITHRANOL AND DITHRANOL LIKE COMPOUNDS ON PSORIASIS. G. SWANBECK, M.D., Department of Dermatology, Karolinska sjukhuset, S-104 01 Stockholm 60, Sweden.

Dithranol forms complexes with DNA and induces respiration deficient (RD) mutants in yeast. These mutations are in the mitochondrial DNA. No chromosomal mutations have been observed after dithranol treatment of different microorganisms. The mitochondrial DNA codes for the protein of the inner membrane of the mitochondria. The respiration deficient mitochondria of the mutant yeast cells have incompletely developed inner membranes as seen by electron microscopy. An electron microscopic study of psoriatic lesions treated for two weeks with a dithranol paste showed similar changes in the epidermal mitochondria. It is therefore postulated that dithranol acts on the psoriatic epidermis by interfering with the mitochondrial DNA making a large portion of the mitochondria respiration deficient.

In a preliminary clinical trial the antipsoriatic activity of a number of RD-inducing substances has been studied. A good correlation between RD inducing properties and antipsoriatic activity has been found for hydroxylsubstituted aromatic compounds.

3. A RAPID IN VITRO ASSAY OF EPIDERMAL CHALONE. M. G. C. DAHL AND S. SHUSTER, Department of Dermatology, University of Newcastle-upon-Tyne, England NE1 4LP.

A rapid method is required for assay of epidermal chalone since counting of mitoses or radioactively labelled cells is laborious and time-consuming. Mouse ear is incubated at 37° C in glucose saline and incorporation of ³H thymidine (Tdr) into epidermis is measured by liquid scintillation counting as an index of DNA synthesis. ³H-Tdr is incorporated in this system for at least 10 hours though the rate falls gradually. A nitrogen gas phase significantly depressed ³H-Tdr uptake but no significant difference was found between air and oxygen up to 5 hours incubation. Cellophane adhesive tape stripping caused a 70% increase in ³H-Tdr uptake 24 hours later (p < 0.002). Hydroxyurea 4 × 10⁻³ mg/ml reduced ³H-Tdr uptake by 23% and 4 × 10⁻² mg/ml by 84% (p < 0.001). Bovine epidermal chalone reduced ³H-Tdr uptake after 2-3 hours incubation. The percent reduction varied in different groups of animals but appeared dose related in individual groups. A chalone solution containing 4 mg protein/ml produced a 12-40% reduction in ³H-Tdr uptake in different experiments. The effect was abolished by preheating at 100° C for 10 minutes. Pig epidermal extract caused similar depression of ³H-Tdr uptake which was dose related and heat labile. This system provides a rapid assay for the effect of epidermal chalone on DNA synthesis. A single assay takes 3 days and several may be done per week.

4. SPECIFIC EFFECT OF EPIDERMAL EXTRACTS ON DNA SYNTHESIS IN TISSUE CULTURE. C. DELESCULUSE, M. REGNIER AND M. PRUNIERAS, M.D. Rothschild Foundation, 29 rue Manin, Paris 19e, France.

If epidermal extracts (chalones) are tissue specific in vivo, do they exhibit the same specificity in tissue cultures? Coverslip primary cultures of cells dissociated with collagenase have been established from epidermis, lung and spleen of adult Hartley guinea pigs. Mechanically dispersed bone marrow cells have also been cultured as controls for collagenase dispersion. All seed lots of cells were studied by electron microscopy. At day 7, cultures were challenged for 3 hours with 2.5 mg/ml of lyophilized crude pig skin extract (Boldingh and Laurence, 1968). After
a 30 min pulse with \(^{3}H\)-thymidine, cpm were counted in a liquid scintillation analyser. The coverslip cultures were then stained with Giemsa and optical densities (OD) of cell populations (proportional to the number of cells attached to the coverslip, Delescluse and Prunieras, 1971) were estimated by scanning with a Joyce and Loebl chromoscan. Thymidine incorporation was expressed as a cpm/OD ratio. In the four series there was a statistically significant depression of thymidine incorporation in the epidermal cells as opposed to each of the other three cultures of mesenchymal origin. Therefore, it is concluded that DNA synthesis is specifically inhibited by epidermal extracts in epidermal cells in culture.

5. ABNORMALITIES IN ADENYL CYCLASE OF PSORIATIC SKIN.
S. L. Hsia, Ph.D., R. Wright, M.S., S. H. Mandy, M.D. AND K. M. Halprin, M.D., Departments of Dermatology and Biochemistry, University of Miami, Miami, Florida 33136.

We have examined a number of enzymes in the psoriatic plaque and found abnormalities in adenyl cyclase. Skin specimens were obtained with a keratome having the depth of the blade set at 0.2 mm, and adenyl cyclase activity was demonstrated by the in vitro incorporation of \(^{3}H\)adenine into cyclic AMP. This activity in skin of control subjects was stimulated by epinephrine (1 ug/ml) and NaF (10 mM). Psoriatic plaques had less activity than normal skin and the enzyme was unresponsive to NaF and less responsive to epinephrine. The uninvolved skin of the patients had normal activity and normal responses to epinephrine and NaF. In further studies, the 650 x g pellets were prepared from skin homogenates and incubated with 2 mM [\(\alpha^{32}P\)]-ATP in the presence of theophylline. The amounts of \(^{32}P\)cyclic AMP formed by preparations from the unaffected skin were 7.5 pmoles of cyclic AMP per min/mg protein, while those from the psoriatic plaque had approximately half the capability. These results indicated defects of adenyl cyclase in psoriatic skin.

6. INTRACELLULAR DESMOSOMES. E. C. Wolff-Schreiner, M.D., AND M. J. Karnovsky, M.B., B.Ch., Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115.

Intracellular organelles with features of desmosomes (ICDs) have recently been observed in some epidermal tumors. We have found that epidermis regenerating at the floor of intact blisters represents a reproducible model to study their origin and formation. Blistering was induced by liquid nitrogen in 3 human volunteers and 6 guinea pigs, and 24 to 144 hour specimens of the regenerating epidermis were studied by conventional electron microscopic and tracer techniques (horseradish peroxidase (HRP), Graham and Karnovsky, 1966). ICDs were observed regularly after 48 to 72 hours in up to 20% of the regenerated keratinocytes of all cell layers at the margin of the blister, individual cells containing up to 100 ICDs in one plane of section. ICDs lacked connections with the cell surface, except very rare ones which were part of infoldings of the cytomembrane. ICDs were present in multinucleated keratinocytes but were absent from the immature cells at the leading edge of the regenerating epidermis. Vesicular and partly collapsed, racket-shaped organelles with typical layers, attachment plaques and inserting tonofilaments suggested stages of formation within the cytoplasm. ICDs were never surrounded by a lysosomal membrane. Injected HRP was identified in some ICDs which provides evidence that their membranes initially were in contact with the extracellular compartment and that they originate from the cell surface.

7. LANGERHANS CELLS IN CULTURE: SURVIVAL AND IDENTIFICATION. P. Fritsch, M.D., AND E. Diem, M.D., Department of Dermatology I., University of Vienna A-1090 Vienna, Austria

Langerhans cells (LC) have not yet been identified in epidermal cell cultures. The purpose of the present study was to elucidate the fate of LC subjected to cell culture techniques and to establish a suitable and simple method for their identification. Shave biopsy specimens from white ears of spotted guinea pigs were trypsinized and processed as follows:
1. Cryostat sections were stained with the nucleosidetriphosphate (NTP) method. NTP-positive LC were found in usual numbers and distribution but, like NTP-negative keratinocytes, were detached from the surrounding cells.
2. Epidermis and dermis of the trypsinized skin were separated and epidermal cell suspensions were dried on coverslips. NTP-positive LC contrasted with the non-reactive keratinocytes.
3. Coverslip cultures were made using standard techniques and subjected to NTP-staining at
daily intervals. Most LC failed to spread but some settled extending short and blunt processes. LC stayed NTP-reactive until final detachment. After 4 days no LC were recognized.

4. These procedures were monitored by electron microscopy. The NTP-technique thus proves suitable for the identification of LC in vitro. LC are brought into primary cultures in large numbers but grow only poorly under routine conditions.

8. COMPARATIVE PENETRATION OF T. MENTAGROPHYTES IN NORMAL AND THE LOW SULFUR-DEFECTIVE CUTICLE HAIR OF TRICHOSCHISIS. A. C. BROWN, M.D., L. HALEY, PH.D., AND R. J. GERDES, PH.D., Division of Dermatology, Emory University School of Medicine, Atlanta, Georgia 30322.

Dermatophyte penetration of human hair may be related to a defective cuticle or the hair sulfur content. To verify this hypothesis, blind in vitro hair penetration tests were performed on normal human hair with an intact cuticle and normal sulfur content and compared to the defective human hair of Trichoschisis with absent cuticle and low sulfur content.

In vitro tests for perforation of human hair appears to be a reliable way of differentiating T. mentagrophytes from T. rubrum. Control and defective human hairs were autoclaved in dry petri dishes for 10 minutes. To each plate was added 25 ml of sterile distilled water, 0.15 mm of 10% Yeast Extract and fragments of the fungi. T. mentagrophytes. More than 25 hairs were added to each petri dish. The hairs were examined at 5 to 7 day intervals for in-situ perforation for 30 days.

The normal control hairs were perforated by T. mentagrophytes within 7 days. In situ penetration was not demonstrated in the low sulfur and defective cuticle hairs of Trichoschisis after 30 days. Spores and hyphae were noted surrounding the defective hair. However, penetration did not occur even though the cuticle cell layer was incomplete or absent.

9. STUDIES ON RAT HAIR CULTURE: III. THE EFFECTS OF CHONDROITIN SULFATE. Y. TAKAKI, M.D., G. MORETTI, M.D., A. REBORA, M.D., AND E. RAMPINI, M.D., Dermatologic Clinic, University of Genoa, 16132 Genoa, Italy.

Since the acid mucopolysaccharide (AMPS) content of rat-skin increases in anagen, AMPS may influence the development of hairs in cultures of rat-embryonal skin. Therefore, we added to hair cultures chondroitin-sulfate (CSA) A, B and C in 4 progressively higher concentrations than those previously extracted with Schiller's technique (1961) from the basic medium (cock plasma and minced chicken-embryo extract) (Hardy, 1951). The culture volume, the number of follicles and the volume of the follicles were then evaluated through a combined histologic and histophotometric technique partially based on the measurement of fluorescence produced by growing follicles in serial sections treated with acridine orange. All data were statistically analyzed. The results were: a) CSA-A increases the number of developing follicles and their volume as much as 5 times with the highest concentration. b) CSA-B increases the follicle volume only at the intermediate concentrations. c) CSA-C does not favorably influence the number and volume of follicles.

10. CELL PROLIFERATION AND MIGRATION IN THE ACROSYRINGIUM. E. CHRISTOPHERS, M.D. AND G. BLEWIG, M.D., Department of Dermatology, University of Munich, 8 München, Germany.

The formation of the human intraepidermal eccrine sweat duct coil (acrosyringium) was investigated. Twenty-six specimens from the back and the forehead were obtained 45 minutes after local $^{3}H$-thymidine injections. Autoradiographs prepared from serial sections were analysed by relating labelled cells to epidermal surface length as well as planimetry. Tissue volume and proliferative activity of the adjacent epidermis were compared. The acrosyringial volume was nearly twice as large as the epidermis per unit surface length. However, in the acrosyringium the number of labelled cells per unit surface length was slightly lower compared to the adjacent epidermis of both body areas and even more than 30% lower when calculated per tissue volume. To explain this discrepancy, turnover measurements were carried out after $^{3}H$-TdR-labelling. The results show, that the acrosyringial epithelium is synchronously shed together with the adjacent epidermis and that cells from the subepidermal portion of the duct move upward transepidermally. Coiling therefore results from a surplus of newly produced cells derived from the duct.
MORNING SESSION

THURSDAY, 9:00 A.M.-12:00 N

Otto Braun-Falco, M.D., Munich, West Germany, and Richard B. Stoughton, La Jolla, California, presiding.

1. PEMPHIGOID ANTIBODY: STUDY OF THE SUBTYPES. W. M. Sams, Jr., M.D., and P. H. Schur, M.D., Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901; Robert B. Brigham Hospital, Boston, Massachusetts 02120.

Pemphigoid antibody is an immunoglobulin of the IgG class; it fixes complement in about 75% of patients and is noncomplement fixing in the remainder. This study examines the subtypes of IgG antibody involved in these 2 groups; 10 ml of serum from each of 3 patients from each group was applied to a DEAE-cellulose column, and stepwise elution was done with four phosphate buffers of increasing ionic strength and decreasing pH. Each peak was concentrated by positive pressure dialysis followed by dialysis against phosphate-buffered saline. Immunofluorescence was performed on each of the four peaks using guinea-pig esophagus as the substrate and fluorescein-tagged anti-human IgG or Bu/Bsl as the conjugate. In noncomplement-f辛xing sera there was no basement membrane fluorescence in the first two peaks with an IgG concentration of 10 mg/ml, but the last two peaks contained strong immunofluorescence with IgG concentrations of 0.26 mg/ml. But in complement-f辛xing sera there was basement membrane fluorescence in all peaks, strongest in the first two, and complement-fixing activity only in the first peak. In subtyping, with pure fluorescein-tagged anti-human IgG, IgG, IgG, and IgG, noncomplement-f辛xing serum contained only IgG antibody in the last 2 peaks, whereas complement-f辛xing sera contained IgG in peak 1, IgG, and IgG, in peak 2, IgG, in peak 3, and IgG, in peak 4. Thus there are at least two types of IgG antibody in pemphigoid; the specific subtype involved determines whether complement fixation occurs.

2. HYPERPIGMENTATION, MELANOSOME SIZE, AND DISTRIBUTION PATTERNS OF MELANOSOMES. K. Konrad, M.D., and K. Wolff, M.D., Department of Dermatology I., University of Vienna, 1090 Vienna, Austria.

This study was performed to determine if (1) different states of hyperpigmentation exhibit differences in melanosome distribution, i.e. single melanosomes or melanosome complexes (MC), within keratinocytes; (2) if size influences the distribution of melanosomes; (3) if melanosome distribution correlates with the degree of pigmentation. Various hyperpigmented conditions (melasma, porphyria, lentigo, café au lait spots, Becker's nevus, etc.) in 15 Caucasoids of different ethnic background were investigated by electron microscopy. Electron micrographs of lesional and control skin were evaluated using the following parameters: (a) total numbers of melanosomes, (b) ratio of single melanosomes to MCs, (c) melanosome size. The results reveal that (1) numbers, sizes and distribution patterns of melanosomes vary in the different pigmen-
tary lesions, (2) there is a definite trend for small melanosomes to be complexed and for large melanosomes to occur singly; in melanosome populations with average (transversal) melanosome diameters of 0.25μ, 80% of melanosomes occurred singularly; in melanosome populations with average diameters of 0.1μ, only 5% of the melanosomes were single and 95% of the melanosomes occurred as MCs; in mixed melanosome populations the larger melanosomes were singly dispersed, the smaller melanosomes were complexed; (3) there is no definite relationship between degree of pigmentation and the distribution pattern of melanosomes. Hence, the size of melanosomes is important for the type of melanosome distribution but the distribution pattern alone does not correlate with the degree of hyperpigmentation.

3. INVERSE CHLORPROMAZINE PHOTOSENSITIZATION AND PERSISTENT LIGHT REACTION IN GUINEA-PIG. E. G. Jung, M.D., Department of Dermatology, University of Heidelberg, Germany.

Chlorpromazine (CHP) photosensitization of white guinea pigs (groups of 15 animals) was evoked by the application of 5% CHP in 70% ethanol followed by ultraviolet exposure for 30 minutes (Kromayer lamp, 2 × 10⁶ erg mm⁻²). This procedure was repeated every 2 weeks on an uninvolved skin area. Inverse preparations in which ultraviolet light was given at variable intervals before CHP application were carried out in 3 other groups. Elicitation was done with the
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