PROGRAM

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Thirtieth Anniversary 1937–1967

Borton Hall—Dennis Hotel 28th Annual Meeting Atlantic City, N.J.

SUNDAY, JUNE 18, 1967

Morning Session—8:30 A.M. Business and Executive Session

Scientific Session: Dr. Richard B. Stoughton, Cleveland, Ohio presiding:

Presidential Address: THE BIOLOGY OF PSORIASIS.
Eugene M. Farber, M.D., Department of Dermatology, Stanford University School of Medicine, Palo Alto, California.

Behavior of Adult Human Skin in Organ Culture. E. P. Reaven, Ph.D., and A. J. Cox, M.D., Department of Dermatology, Stanford University School of Medicine, Palo Alto, California.

This study has been concerned with defining the behavior and capabilities of human adult skin in organ culture. Two hundred specimens of skin were grown under a variety of conditions and assessed on the basis of their morphologic appearance, mitotic activity (arrested by Vinblastine) and incorporation of thymidine-3H. The formation of keratin granules occurred only between pH 7.4 and 7.5. Mitotic patterns were influenced by the amount of stratum corneum removed, by the oxygen concentration within the incubator, and by temperature. Specifically, specimens completely stripped of stratum corneum showed a burst of mitotic activity 2–3 days after planting at 37° C and 6–8 days at 31° C. Specimens with intact stratum corneum showed no mitotic peak at either temperature at normal oxygen levels, but a striking increase in mitosis following planting in 40% oxygen at both temperatures. Despite a wide variety of conditions, however, adult skin in culture was not modified by the addition of substances such as testosterone, vitamin A, chick embryo extract and ectodermal growth factor.

The Effect of Hydrocortisone on Skin in Organ Culture.
G. A. Caron, M.A., M.B., MRCP, Department of Dermatology, University of Oregon Medical School, Portland, Oregon 97201.

Growth controlling substances have been studied in organ cultures of 150 explants of skin from 16 normal individuals. The explants, 0.4 mm. thickness, 3 mm² obtained with a Castroviejo keratotome in vivo or from fresh surgical specimens were floated on liquid culture media for up to 15 days. Tritiated thymidine (2mc per ml) was added for the final 3 hours of culture. Slides for routine histology, restricted histochemistry and autoradiography were made after Helly's fixation. Criteria of growth include epidermal migration, epidermal cell mitosis and nuclear autoradiographic labelling. Growth occurred in 65 of 92 control explants, in 1 of 3 patterns. (a) Epidermal migration round the cut surface of the dermis, (epiboly 67%), (b) attenuation of the epidermis (25%) and (c) irregular epidermal downgrowth (8%). Failure to grow was partly accountable by lack of dermis in the explant or lack of serum in the culture medium. Explants cultured with hydrocortisone 1 mg per ml for 2, 5 and 7 days differed from controls in having better epidermal cell preservation, smaller epidermal cells, much diminished epidermal cell migration, diminished rate of epidermal mitosis and absence of tritiated thymidine labelling. It is concluded that epidermal cell DNA synthesis in vitro is inhibited by this concentration of hydrocortisone.

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ELECTRON MICROSCOPY OF STRIPPED HUMAN EPIDERMIS. Y. MISHIMA, M.D., PH.D., AND H. PINKUS, M.D., Departments of Dermatology, Wayne State University School of Medicine, Detroit, Michigan 48207 and Veterans Administration Hospital, Dearborn, Michigan.

Stripped human epidermis responds with a blast of mitotic activity. During this period epidermal cell turnover is accelerated. Changes of intercellular relations are to be expected. The ultrastructural details of the desmosome-tonofilament complex give the impression of great stability in normal epidermis. Electron microscopy of epidermis obtained 4 hours after stripping shows that (1) the tonofilaments are separated from the desmosomes, (2) cell contact by the desmosome-tonofilament complex is largely lost and the intercellular space widened, (3) the desmosomes preserve their configuration and do not split at the intercellular contact later, but move as a whole to one side of the intercellular space, (4) the direction of the tonofilaments is changed so that they are arranged around the nucleus in the cytoplasm. Later, evidences of new formation of attachment plates were observed. Simultaneously, the presence of melanosomes within vacuoles resembling lysosomes were observed in Langerhans cells. Results suggest that desmosomes can be dissolved within a few hours and subsequently reformed during rapid cell turnover and that the turnover of symbiotic non-keratinocyte is also affected after stripping. Ultrastructural changes seen after stripping seem to indicate active adaptation for cellular proliferation.

ULTRASTRUCTURAL AUTORADIOGRAPHIC STUDIES OF KERATOHYALIN GRANULE FORMATION. K. KUKUYAMA, M.D., AND W. L. Epstein, M.D., Division of Dermatology, University of California, School of Medicine, San Francisco, California 94122.

"Histidine-rich protein" in granular cells demonstrated by light microscopic autoradiography (Fukuyama et al) was investigated by electron microscopy and autoradiography to further elucidate sites of histidine-H incorporation and the specific relationship between this protein and formation of keratohyalin granules (K-granules). 20 μC of histidine-H was injected intradermally in the dorsal side of newborn rats and biopsies secured at various intervals. Tissues were double fixed in glutaraldehyde and osmium tetroxide and embedded in an Araldite and Epon mixture. Autoradiographs were made with Ilford L-4 employing Caro's method and 5 weeks exposure time. Silver grains were observed over the nucleus, K-granules and other cytoplasmic organelles. At 3 and 6 hours after injection 66% and 75% of the total grains respectively appeared over K-granules which coincides with the observation of Cox and Reaven. At 1 hour after injection, however, only 37% of grains appeared over K-granules; they tended to locate at the edge of K-granules, whereas at the later time intervals many grains were situated in the central portion of K-granules. Grains located outside of K-granules often were seen associated with tonofibrils near cell membranes or K-granules. Precise location of the initial incorporation of histidine-H is being studied at 5, 10, and 30 min. after injection. The available results suggest that injected histidine-H incorporates into a protein within granular cells. The site of protein synthesis seems intimately associated with tonofibrils and other substances, such as lipid.


New histochemical methods have been developed and applied to a study of the chemical composition of components of the transitional zone in guinea pig epidermis. Acidophilic materials can be demonstrated in the keratohyalin granules and lower stratum corneum cells with acid dyes under controlled pH conditions. Specific staining of these structures is accomplished in formalin-fixed sections with the acid dye, Amidoschwarz 10B, at pH 8.0 and
in unfixed sections or those prepared in formalin-free fixatives with Amidoschwarz 10B or Biebrich Scarlet at pH 9.5 (Spicer and Lillie). The latter dye exhibited the unique property of forming a highly birefringent dye-protein complex.

This acidophilic material can be extracted from tissue sections with 0.1 M hydrochloric or acetic acid or 1 M solutions of guanidine hydrochloride, calcium chloride, or sodium chloride. Disc electrophoresis of an acid extract of homogenized guinea pig epidermis reveals a complex mixture of cationic proteins which will stain with Biebrich Scarlet at pH 9.5. The proteinaceous character of the extract was confirmed by quantitative amino acid analyses. Birefringent dye-protein complexes are formed with the extracted epidermal proteins but not with other basic proteins, e.g., calf thymus histones.

A similar but not identical histochemical response is elicited in the keratohyalin granules and stratum corneum of human epidermis.

It is concluded that basic proteins are constituents of the keratohyalin granules and of their initial transformation products in the keratinized stratum corneum cells.

STUDIES ON THE LOCALIZATION OF THE "HISTIDINE-RICH" PePTIDE MATERIAL PRESENT IN THE EPIDERMIS OF THE NEW-BORN RAT. J. Gumucio, M.D., C. Feldkamp, M.S., and I. A. Bernstein, Ph.D., Departments of Dermatology, Industrial Health and Biological Chemistry and the Institute of Industrial Health, The University of Michigan, Ann Arbor, Michigan, 48104.

Radioautography has shown that histidine-3H is initially incorporated in the upper cells of the epidermis in the newborn rat (Fukuyama et al). Unique peptide material, rich in histidine-3H and soluble in 8M urea and 0.1 N HClO4 at room temperature, has been isolated from the entire epidermis of the newborn rat (Hoober and Bernstein). To determine whether this peptide material is localized in the upper layers of the epidermis in the newborn rat, an attempt was made to obtain the peptide from isolated granular cells. Histidine-3H was injected intraperitoneally and 1 hr later the skin was removed, treated with 0.1% trypsin at 0°C for 60 min and soybean trypsin inhibitor added. The epidermis was then separated from the dermis and shaken in 10 M EDTA, pH 7.4 at 37°C. After 15 min, the epidermis was placed in fresh EDTA solution. Histological sections made at this time indicated that the remaining epidermis contained mainly granular cells attached to the stratum corneum. These were removed by shaking in the EDTA solution for 5 more min. The resultant cell suspension was centrifuged and the pellet submitted to the published procedure for isolation of the "histidine-rich" peptide fraction. A radioactive fraction was obtained by solubilization first in 8M urea and then in the 0.1 N HClO4 at 24°C, which behaved on columns of Sephadex G-50 and G-100 similarly to the material previously obtained from the whole epidermis. These data suggest that the "histidine-rich" peptide fraction is present in the upper cells of the epidermis supporting the hypothesis that the synthesis of this peptide material accounts for the initial localization of histidine-3H in the upper epidermal layers.

MECHANISM OF PERCUTANEOUS ABSORPTION. III. THE EFFECT OF TEMPERATURE ON THE TRANSPORT OF NON-ELECTROLYTES ACROSS THE SKIN. I. H. Blank, Ph.D., and R. J. Scheuplein, Ph.D., Department of Dermatology, Harvard Medical School. Address: Massachusetts General Hospital, Boston, Massachusetts 02114.

Previous studies (Blank, Scheuplein) of the penetration of water and saturated alcohols showed that lipid-soluble molecules (>C₈) penetrate more rapidly than water-soluble molecules (<C₈) in accord with the corresponding membrane-solvent distribution coefficients. A further study of a wider spectrum of molecules including fatty acids, diols and glycol ethers has led to a more complete understanding of the different molecular mechanisms which are involved in the penetration of polar and non-polar molecules. Techniques for measuring the flux of these substances through excised human epidermis have been
previously described (Scheuplein). Gas chromatography and polarography have been used for the quantitative determination of the compounds. Higher activation energies $16 \pm 2$ KCal mole$^{-1}$ were observed for the polar alcohols as compared to $10 \pm 2.0$ KCal mole$^{-1}$ for the non-polar alcohols. Diols and glycol ethers, strongly polar compounds, have activation energies in excess of $18$ KCal mole$^{-1}$. Lipid extraction with chloroform-methanol destroys the dense structure of the membrane to such an extent that the activation energy for the diffusion of tritiated water is reduced from approximately $16$ to $6$ KCal mole$^{-1}$, i.e., to the free liquid water diffusion value. These studies lead to the conclusion that during steady state diffusion through the stratum corneum polar and non-polar compounds follow different molecular pathways; strong chemical interaction occurs between penetrant and tissue.

SUNDAY, JUNE 18, 1967

Afternoon Session—1:30 P.M.

SCIENTIFIC SESSION: DR. R. K. WINKELMANN, ROCHESTER, MINNESOTA presiding

CUTANEOUS NERVES: DEMONSTRATION BY IMMUNOFLUORESCENCE. H. UEDA, M.D., AND R. D. WILKINSON, M.D., Division of Dermatology, Department of Medicine, Royal Victoria Hospital, Montreal 2, Que.

Indirect fluorescence microscopy has been applied for the first time to the study of human cutaneous nerves. Rabbits were given immunizing injections of bovine sciatic nerve homogenate in incomplete Freund's adjuvant. The antiserum was collected, and the $\gamma$ gamma globulin was purified by Sephadex G-200 and DEAE cellulose chromatography. Cryostat-cut sections of fresh frozen human and monkey skin from the thigh and penis as well as sciatic nerve were exposed to the antibody and reacted with the fluorescent label, with appropriate controls for the non-specific reaction. In nerve tissue, both myelin sheaths and axons were seen. In the skin, both undifferentiated nerve fibres and end-organs fluoresced. The application of this technique should enable more precise determination of innervation of hyperplastic cutaneous processes, such as psoriasis.

EFFECTS OF NERVE-GROWTH PROMOTING PROTEIN ON THE PIGMENTATION OF MURINE INTEGUMENT. W. M. REAMS, JR., B.S., PH.D., Department of Dermatology, Medical College of Virginia, Richmond, Virginia, 23219, and the University of Richmond, 23173.

A protein isolated from mouse sarcoma, snake venom, and mouse salivary gland (Cohen, and Levi-Montalcini) has been shown to have growth promoting effects on sympathetic and spinal ganglia of the chick and mouse. Since sympathetic and spinal ganglia, and pigment cells all have a common ancestry in the neural crest, a investigation is underway to ascertain the effects of the nerve growth factor (NGF) on the pigment cells of mouse skin and its derivatives. NGF was prepared from the submaxillary glands of adult male mice by the CM- and DEAE-cellulose methods of Cohen. Young, postnatal black mice were injected subcutaneously with 0.1 ml of the preparation for 10 consecutive days. Controls were injected with just the syringe needle, physiological saline, or the carrier without NGF. Examination of the integument over the site of NGF injection showed a reduction in the melanocyte population in the dermis and the disappearance of pigmentation in the hair. Integument samples from other areas of the NGF animals, as well as from the controls, appeared normal. Apparently the NGF is specific for promoting the growth of neural crest derived neurons, but has an inhibitory effect on integumentary melanocytes. This melanocyte inhibition may imply the function of NGF as a differentiation factor (Weston).

MELANIN TRANSFER: A PHAGOCYTIC PROCESS. J. H. MOTTAZ, M.S., AND A. S. ZELICKSON, M.D., Division of Dermatology, University of Minnesota Medical School, Minneapolis, Minnesota 55455.

Human hair bulbs were studied in an effort to determine the manner in which melanosomes are transferred from melanocytes to the adjacent cortical cells. Hair was plucked
from the head, immersed in osmium tetroxide, embedded in Epon 812, and sections were examined with an electron microscope.

Many pseudopod-like projections, similar to those seen on phagocytes, extended from the cortical cells. These were found to wrap around dendritic processes, then to pinch off a portion of the dendrite and to include it in the cortical cell cytoplasm, and finally to dissolve the dendritic membrane thus allowing the melanosomes to be dispersed throughout the cortical cell. The stages of this process will be shown in detail thus definitely establishing at least one of the methods whereby melanin passes from the melanocyte to adjacent cells.

**LANGERHANS CELLS IN VITILIGO: A QUANTITATIVE STUDY.** J. BROWN, M.D., R. K. WINKELMANN, M.D., PH.D., AND KLAUS WOLFF, M.D., Department of Dermatology, Mayo Clinic, Rochester, Minnesota.

Langerhans cells, demonstrated histochemically by the method for adenosine triphosphatase, were studied in skin specimens from involved and corresponding uninvolved areas in 10 patients with vitiligo. Cell counts of isolated epidermal sheets with a reticle and calibrated microscopic field revealed an average of 731 Langerhans cells per square mm for involved areas and an average of 697 Langerhans cells per square mm in uninvolved areas. Dopa positive melanocytes were absent or reduced in all involved areas. Studies of vertical sections and epidermal sheets did not reveal any differences in the intraepidermal distribution or morphology of Langerhans cell in vitiliginous and normal appearing areas.

The quantitative and morphologic similarities of Langerhans cells in uninvolved and involved skin from patients with vitiligo as well as the lack of any numerical correlation between the Langerhans cell and melanocyte population indicates that the Langerhan cell does not play an etiologic role in this disease, and that these two cell lines are distinct and perhaps unrelated.

**MECHANISM OF DEPIGMENTATION OF SKIN WITH 4-ISOPROPYLCATECHOL AND MERCAPTOAMINES.** S. S. BLEEHEN, M.B., MRCP, M. A. PATHAK, PH.D., Y. HORI, M.D., AND T. B. FITZPATRICK, M.D., PH.D., Department of Dermatology, Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts 02114.

Depigmentation of skin by exogenous agents should involve selective action on melanocytes and melanogenesis without the induction of an inflammatory response in the epidermis. Selective destruction of melanocytes in black guinea pigs has occurred, only in treated areas, after topical application of 4-isopropylcatechol (4IPC), N-(2-mercaptoethyl)-dimethylamine HCl (MEDA) and \( \beta \)-mercaptoethylamine HCl (MEA). Ten compounds, hitherto unrecognized as depigmenting agents, were evaluated on the epilated skin of the back and the unepilated skin of the ear of pigmented guinea pigs. These compounds were applied daily in concentrations of 1, 2 and 5 Gm% in a vanishing cream. 4IPC and MEDA were potent depigmenting agents; 4IPC was more potent than any other compound yet tested. In areas treated with 5% 4IPC and MEDA, depigmentation occurred in 1—2 weeks; with 1 and 2% preparations in 3—4 weeks. Pigmentation of the hair was not affected. In depigmented areas, almost no melanin organelles were found in the epidermis and the melanocyte count was significantly reduced (from 700—800 to 50—80 melanocytes/mm²). The perikaryon and the dendrites of the melanocytes were markedly altered; their tyrosinase activity and reactivity to dopa were also affected. Acanthosis due to epilation was present in all areas. Five percent 4IPC induced a mild inflammatory reaction; 5% MEDA a more marked dermatitis. Electron microscopy revealed only rare melanocytes in which there were few or no melanized melanosomes, many vacuoles and peculiar dense round bodies with double membranes. The Langerhans cells appeared normal. The depigmentation of skin by 4IPC and MEDA appears to result from the selective destruction and disappearance of melanocytes rather than from the inhibition of melanin biosynthesis.
CHEDIAK-HIGASHI SYNDROME: A GENETIC DISEASE OF MEMBRANES. A. S. ZELICKSON, M.D., D. B. WINDHORST, M.D., J. G. WHITE, M.D., AND R. A. GOOD, M.D., PH.D., Division of Dermatology and Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota 55455.

In the Chediak-Higashi syndrome, the inheritance pattern is that of an autosomal recessive trait. In this disease, hypopigmentation is associated with large lysosomal granules in the blood leukocytes and a common mechanism for these two primary features of the disease is postulated. Electron microscopy of melanocytes revealed that the pigmentation anomaly is also based on the presence of giant melanosomes. Since both types of granules, leukocytic and melanosomal, are characterized by limiting membranes, the Chediak-Higashi syndrome may be a genetic disease of membranes.

Light and electron microscopic study of epidermis, hair, and nevi from a 10-year-old girl with a well-documented history of Chediak-Higashi disease, made it clear that the large melanosomes present in this syndrome are due to both fusion of premelanosomes and also to the formation of giant primary melanosomes. The finding of significant numbers of abnormal melanosomes which were digested and destroyed by the melanocytes which produced them strongly suggests a membrane defect. The melanosomes which reached the neighboring epidermal cells were also engulfed and digested. Presumably the melanosomal membranes are defective thus allowing enzymes to leak to the surrounding cytoplasm, creating an irritative phenomenon with the epidermal cell reacting to wall-off the abnormal granule.

THE LYSOSOME IN CONTACT DERMATITIS. J. H. HALL, M.D., J. G. SMITH, JR., AND S. BURNETT, Division of Dermatology, Department of Medicine, Duke University Medical Center, Durham, North Carolina 27706.

It has been speculated that lysosomal disruption may be involved in the pathogenesis of bullous dermatoses. To test this hypothesis in contact dermatitis, five adult albino guinea pigs, three of which were pretreated with squalene, were topically sensitized to 1-chloro-2,4-dinitrobenzene (DNCB) and subsequently challenged with topical application of 0.2 ml of a 0.1% solution of DNCB in acetone. Biopsies were taken at 0, 2, 4, 8, 12 and 24 hours after challenging. Four more guinea pigs were treated with topical DNCB in acetone in concentrations of 10%, 1%, 0.1%, and 0.01% and biopsied at 0, 1, 2, 4, 6 and 12 hours. Sections were stained for lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), acid phosphatase (AP) and hematoxylin and eosin and examined with the light microscope. The primary irritant and allergic contact reactions were studied in 238 histologic sections. Diminution in stainable AP (a lysosomal enzyme) was observed up to 12 hours after application with concomitant retention of staining for the non-lysosomal enzymes, LDH and SDH. These observations lend credence to the hypothesis that these skin reactions may involve a disruption of lysosomes.

SUCTION BLISTER DEVICE FOR SEPARATION OF VIABLE EPIDERMIS FROM DERMIS. U. KIISTALA, M.D., Department of Dermatology, University Central Hospital, Helsinki, Snellmaninkatu 14, Finland.

Subepidermal blisters are regularly formed when suction of 150 mmHg is applied onto healthy skin. Capillary resistance meters, preliminarily used for blistering, have certain drawbacks. These were avoided by construction of a special device designated for atraumatic production of blisters of predetermined number and size. Broad, grooved suction-cup margins and a concave adapter plate inside the cup ensure air-tightness and avoid deep impression of the margins and excessive shearing and sliding-in of the surrounding skin. Round holes in the adapter plate determine the number and size of the blisters. With this device blistering occurs without discomfort within three hours along the dermo-epidermal junction. The blister roof consists of viable full-thickness epidermis; the clear fluid is a non-inflammatory
transudate, and there are neither petechiae nor scarring damage in the dermal base. Blisters can be produced also on hairy skin of certain laboratory animals and with prolonged suction even on human corpse skin.

This standardized suction blister method provides a versatile tool for investigative purposes.

THE ENTRY OF GLUCOSE INTO THE HUMAN EPIDERMIS. K. M. HALPRIN, M.D., AND A. OHKAWARA, M.D., Division of Dermatology, University of Oregon Medical School, Portland, Oregon.

The question of whether glucose as such enters the epidermal cell has not been answered by determination of human epidermal glucose concentration in vivo. The values obtained (20-75% of the blood glucose) are too low for free entry into the entire cell and too high to be explained by glucose existing only between the epidermal cells.

By floating epidermal sheets on tissue culture media containing high concentrations of substances (glucose-6-phosphate and nicotine adenine dinucleotide) which do not enter the cells, the intercellular volume of the normal human epidermis has been determined to be approximately 15% of the total epidermal volume.

Similar experiments utilizing the simultaneous measurement of the intercellular space (with glucose-6-phosphate) and of the glucose space (with high concentrations of glucose) in the same epidermal slice have revealed that the glucose space represents some 40% of the total epidermal volume. Glucose must therefore enter some intracellular compartment as well as the intercellular space. In addition the kinetics of glucose penetration indicate that glucose enters the epidermis by passive diffusion rather than active transport.

COLLOID MILIUM: A HISTOCHEMICAL STUDY. J. H. GRAHAM, M.D., AND A. S. MARQUES, M.D., Department of Dermatology, Temple University School of Medicine, 3322 North Broad Street, Philadelphia, Pennsylvania 19140.

There is no uniform agreement as to the exact nature of colloid. Some observers favor colloid as a form of collagen degeneration, others conclude that it is derived from abnormal elastic tissue, and a third group considers the hyalin material represents substance derived from serum proteins. In this report, we shall present histochemical observations of biopsies from 3 adult men with typical lesions of colloid milium. Tissue sections were prepared by the following methods: periodic acid-Schiff (PAS), with and without diastase digestion; colloidal iron reaction, with and without bovine testicular hyaluronidase digestion; Snook's reticulum stain; Movat's pentachrome I stain; Gomori's aldehyde-fuchsin, with and without elastase digestion; congo red; crystal violet; and thioflavine-T. Colloid is PAS positive and diastase resistant; has affinity for congo red; stains metachromatically with crystal violet; is not demonstrated with stains for collagen; shows a variable amount of hyaluronic acid separating and surrounding the hyalin deposits; and thioflavine-T stained sections exhibit yellow fluorescence under ultraviolet light. Elastic tissue stains demonstrate abnormal elastic fibers in areas of solar degeneration, but fail to color the colloid. Congo red stained sections examined under polarized light exhibit areas of birefringence and some dichroism. Colloid probably represents an abnormal scleroprotein different from collagen and elastin, and the fibrillar component is embedded in a matrix of solidified serum proteins and mucopolysaccharides. The histochemical properties of colloid show a striking similarity to the hyalin material demonstrated in amyloidosis cutis.

THE RELATIONSHIPS BETWEEN MILIARIA AND ANHIDROSIS IN MAN. T. B. GRIFFIN, CAPT., MC, H. S. WILEY, LT. COL., MC; H. MAIBACH, M.D., AND M. B. SULZBERGER, M.D., U.S. Army Medical Research Unit, Presidio, San Francisco, Calif. 94129 and the Department of Dermatology, Uni-
Although it is known that anhidrosis can follow miliaria, there are no systematic studies of the relationship of anhidrosis to the severity of miliaria nor of the duration of degree of anhidrosis which can be produced over relatively large skin areas. Clinical and histologically confirmed miliaria has been produced in over 50 human male volunteers by the application of a double thickness occlusive wrap to one half of the torso for 48 hours followed by whole-body exposure to 120° F temperature and 40% relative humidity. The nonoccluded side served as a control. The distribution and degree of anhidrosis was evaluated by clinical observation, by a dry heat and bromphenol blue indicator powder technique, and by the use of a modified Bullard sudorometer. Dye and sudorometer testing were done before wrapping; and 15 minutes, 7, 14, 21, 28, 35, and 42 days after removal of wrapping. Relative anhidrosis was observed in all volunteers and persisted for an average of 21 days, the test site returning to normal by an average of 28 days. A mean of 57.5% inhibition of sweating was observed by the sudorometer technique. Complete anhidrosis was not detected in any volunteer by this technique. There was a definite correlation of the severity and extent of miliaria and the degree and extent of the resultant anhidrosis. It is concluded that the duration of clinical anhidrosis following one attack of miliaria is between 21 and 28 days. This raises the question of a possible relationship to the epidermal turnover time. Since long-lasting anhidrosis can follow one bout of miliaria, this potential cause of heat intolerance, previously unrecognized, may be of general and military significance.

MONDAY, JUNE 19, 1967

Afternoon session—1:30 P.M. Business and Executive Session

SCIENTIFIC SESSION: DR. A. B. LERNER, NEW HAVEN, CONN. presiding

LEVELS OF EXONUCLEASE ACTIVITY IN NORMAL AND PARAKERATOTIC SKIN. M. KARASEK, PH.D., F. ZARUBA, M.D., AND E. FARBER, M.D., Department of Dermatology, Stanford University School of Medicine, Palo Alto, California.

Although the hydrolysis of nucleic acids is necessary for normal keratinization to take place, the metabolic fate of nucleic acids in the skin epithelial cell is poorly understood. To test the possibility that parakeratosis is a result of changes in the catabolic nucleases in the epithelial cell, and to study the metabolism of nucleic acids in the normal epithelial cell, we have developed a sensitive assay procedure for the measurement of two exonucleases (enzymes that cleave terminal nucleotides) from microsamples of skin tissue, and have isolated and characterized two exonucleases from skin.

Exonucleases are present in both dermis and epidermis. Skin levels exceed other tissues with high metabolic activity (liver, kidney). The enzymes are found in the microsomal fraction of skin, and have been purified 280 fold by ammonium sulfate fractionation and DEAE chromatography. The enzymes have no distinguishing physical or chemical characteristics.

Parakeratotic skin (psoriasis) shows markedly increased levels of activity when compared with uninvolved skin areas; blood levels parallel skin levels and are correlated with degree of involvement. No example of an inverse relationship between parakeratosis and exonuclease activity was evident.

SURFACE LIPIDS FROM UNINVOLVED SKIN IN PSORIASIS. D. I. WILKINSON, PH.D., AND E. M. FARBER, M.D., Department of Dermatology, Stanford University School of Medicine, Palo Alto, California 94304.

"Non-casual" surface lipids were collected from uninvolved forearm skin areas in 18 psoriatic and 16 control subjects by solvent irrigation 3 hours after defatting of the skin.
surfaces with acetone. A portion of each lipid sample was resolved into free fatty acids (FFA), triglycerides, and remaining neutral lipids using 0.06 N KOH and thin-layer chromatography. FFA were assayed via their copper soaps (Duncombe) and both triglycerides and neutral lipids estimated by a charring method (Marsh and Weinstein). The rest of each lipid sample was subjected to methanolysis and methyl esters analyzed by gas-liquid chromatography. In each case, the amount of FFA was expressed as relative to triglycerides, and to total neutral lipids. There were no statistically significant differences between the mean values for the psoriatic and control subjects. No differences in fatty acid compositions were detected. These results are at variance with published studies which used "casual" lipids, and reported reduced amounts of FFA, as well as a higher incidence of long-chain acids in lipids from lesion-free sites. It is apparent that the use of "casual" lipids may lead to erroneous conclusions in comparative studies.

THE EFFECT OF PREDNISONE ON SEBACEOUS GLAND SECRE-TION. P. E. Pochi, M.D., AND J. S. Strauss, M.D., Department of Dermatology, Boston University Medical Center, Boston, Massachusetts 02118.

Previously it was shown that prednisone fails to counteract estrogen-induced suppression of sebaceous gland secretion and thus lacks an androgenic-like effect. To determine whether corticoids might instead exert a suppressive effect on sebaceous glands, prednisone was given orally to 3 groups of adult subjects: (a) 10-20 mg daily for 10 weeks, to 6 normal males; (b) 20 mg daily for 8 weeks, to 8 castrate males; and (c) 10-20 mg daily for 8-10 weeks, to 12 normal females. Measurements of sebum production were made before and approximately weekly during drug administration. The results were as follows: a) in intact males, no significant change in sebaceous gland secretion was observed; b) in the castrate males, suppression of sebum production occurred in 7 of 8 subjects, ranging from a 20.5% to 64.5% decrease (mean, 44.0%); and c) in the 12 normal females, sebum output was lowered in the majority of cases (18.3% mean decrease, p < .01), with those receiving 20 mg daily showing a greater response than those from 10 mg (23.0% vs 11.7%). These data demonstrate sebaceous gland suppression from prednisone administration and suggest that it results from inhibition of adrenal androgen. In the castrate male, the adrenal cortex is the sole androgen source, and the greatest response to prednisone suppression occurred in this group. In the intact male, no effect is seen due to the overriding stimulatory effect of testicular androgen. Thus, the variable but significant lowering of sebaceous gland secretion by prednisone in females offers evidence that adrenal androgen may be of physiologic importance in addition to gonadal androgen.

THE EFFECT OF EMOTIONAL STRESS ON SEBUM FREE FATTY ACIDS. S. J. Kraus, M.D., Dept. of Dermatology, Western Reserve School of Medicine, Cleveland, Ohio 44106.

Recent studies on the pathophysiology of acne have emphasized the role of sebum free fatty acids (ffa). The most irritative components of sebum are the ffa and the therapeutic effectiveness of tetracycline can be correlated with a fall in sebum ffa. It is a widely held clinical impression that emotional stress exacerbates acne. This study measured the sebum ffa in seven medical students with an acne history—three of whom had active lesions at the start of the study. A 14.5 cm² area of skin was washed three times with reagent grade acetone introduced into glass cylinders held on the forehead. Three hours later the central 62 cm² of the larger area was washed twice with 3 ml. of an acidified heptane-isopropyl alcohol mixture and the ffa content was measured by a non-aqueous titration system utilizing tetrabutylammonium hydroxide as the titrant. Two collections on consecutive days were made three weeks before the exam, two days before the exam, two days before the results of the exam were published, and two weeks after the results were known. A statistically significant increase in sebum ffa occurred (p < 0.05 using the Sign Test) when the mean of the stressful periods (two days before the exam and two days before the results were returned) was compared to the mean of the control periods (three weeks before the exam and two weeks after the results were posted). The rise and fall of sebum ffa was paralleled by exacerbation and clearing of clinical acne lesions.
TETRACYCLINE IN SEBUM. RELATIONSHIPS TO TIME, DOSE AND BLOOD LEVELS. P. L. Rashleigh, M.D., E. Rife, B.S., and R. W. Goltz, M.D., Division of Dermatology, University of Colorado Medical Center, Denver, Colorado 80220.

Because of the beneficial effect of tetracycline on acne, and conflicting reports on its presence in the skin after oral administration, it was thought worthwhile to look for this drug in surface and comedo sebum of persons taking amounts in the therapeutic range. Surface sebum was collected by the cigarette paper method, and comedo sebum by manual expression. The presence of tetracycline was determined by in vitro bacterial inhibition, using B. subtilis and C. acnes, and by fluorimetry, after the method of Hayes and DuBuy. Studies were made on the relationship of the concentration of the drug in the sebum to dose, time of administration and blood levels.

Significant amounts of tetracycline were found in surface sebum, averaging .12—.15 μg per mg after a dose of 500 mg/day, and .06—.08 μg/mg after a daily dose of 250 mg. In comedo sebum the concentration was considerably higher, averaging near 1.0 μg/mg. There appeared to be a significant correlation between dose, blood levels, and concentration of the drug in the sebum. The time relationships are now being studied.

The results of this study indicate that effective amounts of tetracycline are found in the sebum after oral administration of doses commonly employed therapeutically, and that the concentration is considerably higher in comedo sebum than in that collected from the surface.

RED FLUORESCENCE OF COMEDONES; PRODUCTION OF PORPHYRINS BY CORYNEBACTERIUM ACNES. C. E. Cornelius III, M.D., and G. D. Ludwig, M.D., Departments of Dermatology and Medicine, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104.

It has been suggested in the literature that the red fluorescence noted in facial comedones might be a porphyrin produced by C. acnes. An investigation was carried out to support this hypothesis. Examination of smears of fluorescent comedones by a fluorescent microscope showed that the amorphous material of the comedones was the source of the fluorescence. Gram stains of these revealed an abundance of gram positive diphtheroid organisms. Extraction of 50 mg of fluorescent comedones according to the method of Wranne, but with slight modification, for coproporphyrin revealed in these solutions a fluorescent peak at 600 μm on an Amico Bowman spectrophotofluorometer using an excitation wave length of 401 μm. This is characteristic of coproporphyrins and demonstrated the probable presence of coproporphyrin in comedones.

Cultures of C. acnes grown in thioglycolate broth were analyzed in a similar fashion (as noted for comedones) and found to contain both coproporphyrin and lesser amounts of protoporphyrin. Paper chromatography of methyl esters of this material, using known standards, showed these porphyrins were coproporphyrin III and protoporphyrin IX. Recordings of the absorption spectrums of these porphyrins on a Cary spectrophotometer yielded absorption peaks which corresponded well to the peaks given by Falk for coproporphyrin III and protoporphyrin IX. It is concluded that the fluorescence noted in comedones is due to the porphyrin produced by C. acnes. Coproporphyrin III and protoporphyrin IX are the porphyrins produced in vitro by C. acnes.

REDUCTION OF SKIN FREE CHOLESTEROL BY ULTRAVIOLET LIGHT. E. W. Rauschkole, M.D., and John M. Knox, M.D., Dermatology Research Laboratory, Veterans Administration Hospital and Department of Dermatology, Baylor University College of Medicine, Houston, Texas, 77031.

A systematic investigation is being carried out into the biochemical alterations induced in human skin by ultraviolet light. An environmental chamber was designed which permits rigid control of temperature, humidity, and oxygen tension, equipped with a near infrared
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(NIR) optical silica window through which the skin specimen is irradiated. All wavelengths between 2,200 and 27,000 Å are transmitted. Skin is obtained from the lower abdominal wall immediately post mortem, kept chilled in the dark until irradiated. A control specimen obtained from the same source is subjected to the identical incubation procedure but not irradiated. Irradiation is carried out with a broad spectrum Hanovia ultraviolet lamp, exposure time 2 minutes at 40 cm from the skin. After irradiation, skin specimens are frozen, sectioned into 20 mμ slices, suspended in 5 ml distilled water and extracted with 30 ml chloroform. In the present study, attention was focused upon the lipid soluble fraction. In preliminary experiments, the chloroform extract was transferred quantitatively and subjected to chromatography on thin layer silica gel G plates using 5 different single phase solvent systems. Visual inspection of the developed plates clearly suggested a decrease in skin sterols following irradiation. A micromethod for the quantitation of sterols was then devised (modification of the Liebermann-Burchard reaction). Quantitation was at 620 mμ in a spectrophotometer. The method is sensitive to 2 μg; recovery averaged 88.69%. Following exposure to ultraviolet light, it has been possible to confirm that prompt and significant quantitative reduction in free skin cholesterol content occurs. Chloroform soluble cholesterol content in 6 control nonirradiated specimens of caucasian skin was on the average 69.08 ± 13.3 μg/Gm. After 2 minutes exposure to ultraviolet light, the average level declined to 34.66 ± 10.9; a change of —47.5 ± 7.6% (t = 6.25 p < .01). It is evident that highly significant events in sterol metabolism occur with rapidity when ultraviolet light strikes the skin.

TESTING DRUG PHOTOTOXICITY IN HAIRLESS MICE. A. E. Ison, M.D., AND H. Blank, M.D., Department of Dermatology, University of Miami School of Medicine, Miami, Fla. 33136.

Compounds to be tested were suspended or dissolved in a sodium carboxymethylcellulose preparation and injected intraperitoneally into albino hairless mice. All drugs were given at the levels of 660, 66, 33, and 6 mg/kilogram except when the higher doses were toxic, and then intermediate doses were used. The animals were exposed to the light source in individual glass beakers covered with window glass. The radiation source consisted of two General Electric F40BL lamps at a distance of 10 in. and the exposure time was 48 hr.

Irradiated mice injected with the vehicle alone, when compared with nonirradiated animals, show practically no visible reaction. Compounds were evaluated for their minimum phototoxic dose (MPD). Representative minimum phototoxic doses in this system are: demethylchlortetracycline 66 mg/kg; tetracycline 200 mg/kg; chlorpromazine 33 mg/kg; prochlorperazine 66 mg/kg; 8-methoxypsoralen 33 mg/kg; and an antimalarial, 6,8 dichloro-2-phenyl-a-2-piperidyl-4-quinoline methanol (SN 10275) 33 mg/kg. Others will be reported. The results with this technic parallel human experience, are reproducible, and may have predictive value with new drugs.

THE EFFECTS OF GRISEOFULVIN ON PORPHYRIA CUTANEA TARDA. J. M. Spiro, M.D., AND D. J. Demis, Ph.D., M.D., Division of Dermatology, Washington University School of Medicine, St. Louis, Missouri.

A controlled study was undertaken to delineate the effects of griseofulvin on patients with porphyria cutanea tarda (PCT). Clinical and laboratory evaluations, with particular reference to skin and hepatic status, were made of three hospitalized patients. Griseofulvin (500 mg.) daily was administered for a ten day period and laboratory studies including liver function tests, measurement of porphyrin excretion products, and hematograms were performed at approximately two day intervals. In addition, serum griseofulvin levels were measured spectrophotofluorometrically.

Serum levels of griseofulvin in therapeutic range were found in all patients during the course of this study. However, at no time were exacerbations of the symptoms of PCT noted, and each patient experienced clearing of the vesicles of the hands while taking griseofulvin. Repeated measurements of liver function showed no changes other than those detected prior to griseofulvin administration. No other laboratory abnormalities were noted,
although all these patients demonstrated an abnormal diabetic glucose tolerance test; one patient had an elevated serum iron and a high transferritin saturation; two patients had red-pink fluorescence of liver biopsy tissue, but none had evidence of hepatoma on radioactive iodine liver scans.

It is concluded that griseofulvin does not adversely influence porphyrin metabolism in patients with PCT. In addition, there was no evidence of drug-induced abnormalities in hepatic function. These findings are in sharp contrast to reports of precipitation of PCT by griseofulvin in laboratory animals.

THERMOLUMINESCENT RADIATION DOSIMETRY. I. X-RAY DOSE RECEIVED BY HUMAN OCULAR TISSUES DURING IRRADIATION OF EYELID CANCERS. A. W. KOPF, M.D., E. N. GRISWOOD, A.B., M.A., R. BART, M.D., AND M. PETRATOS, M.D., Departments of Dermatology and Physics, New York University, New York, 10016.

X-rays interact with lithium fluoride (LiF) to excite electrons to higher energy levels where they fall into electron traps. These trapped orbital electrons can be brought back to their ground state by the application of energy in the form of heat. This fall in energy level of the LiF electrons is accompanied by the emission of light which can be measured in photomultiplication and read-out circuitry. The amount of light emitted is directly proportioned to the dose of radiation absorbed by the LiF. In the experiments reported a LiF thermoluminescent dosimetry system was used in order to study the amount of X-ray received by human ocular tissues during irradiation for eyelid cancers. The advantage of this dosimetry system is that the phosphor (LiF) can be incorporated into tiny (1 x 5 mm) Teflon rods which can be strategically placed throughout the orbital tissues. Using a standard lead eyeshield (0.92 mm thickness) and considering the air dose at the eyelid skin surface as 100%, the dose of X-rays received by the palpebral conjunctiva is 75%; by the cornea 1.4%; by the lens 1.2%. It was further shown that the amount of radiation received by these structures could be significantly reduced (20 to 33%) by the use of a newly constructed eyeshield (lead core electroplated with copper and layered with aluminum) designed to cut down back scatter radiation.

PHYSIOLOGIC STUDIES OF THE PERIPHERAL CIRCULATION IN HEREDITARY ANGIONEUROTIC EDEMA. C. HYMAN, PH.D., N. E. LEVAN, M.D., W. H. WONG, M.D., AND H. JANKLOW, M.D., Department of Dermatology, University of Southern California School of Medicine, Los Angeles, California, 90033.

In recent years a deficiency in the inhibitor of C1 esterase and the permeability globulins, kallikrein and PF/dil, has been established in hereditary angioneurotic edema. The pathophysiology of this disorder including the pathway from enzymatic defect to clinical manifestations remains largely uncertain.

We therefore carried out a group of physiologic studies on the cutaneous circulation on a patient with hereditary angioneurotic edema. The blood flow through the skin of the forearm, as measured plethysmographically, proved to be within normal limits. Tissue clearance, an estimate of the exchange function of the cutaneous circulation, was likewise normal.

Capillary filtration coefficient was determined by a new method involving continuous plethysmographic determinations during a prolonged (10 minute) venous occlusion. The method was validated by 12 measurements on normal subjects, which gave a mean value of 0.0039 ± 0.00014 ml/100 ml tissue/min/mm Hg. This agrees with published values. Immediately after an episode of edema a similar measurement on the patient gave a value of 0.0137; one week later, 0.0103; and one month later, 0.0097 ml/100 ml/min/mm Hg. These values differ significantly from normal and suggest a mechanism which could explain the accumulation of tissue fluid (edema).
THE EFFECT ON STEROIDS ON ISOLATED VASCULAR SMOOTH MUSCLE. W. M. SAMS, JR., M.D., AND R. K. WINKELMANN, M.D., Mayo Clinic, Rochester, Minnesota 55901.

Vasoconstriction, as evidenced by blanching, is an established phenomenon of corticosteroids applied to the surface of the skin. Whether this is a direct effect of steroids on the blood vessels in the dermis or is due to the action of a mediator has been largely unknown. The present study was designed to resolve this problem. The vessels used were from the distal portion of the median artery of the rabbit ear. At this level the outer diameter of the vessels is 200—300 microns. All manipulations were carried out under a dissecting microscope. The vessels were dissected free of their surrounding connective tissue and mounted on a fine wire obturator so that they could be cut into a spiral 2—3 mm. long. They were then mounted with 200 mg tension in a physiologic measuring device employing a transducer and a paper strip recorder. The vessel was mounted in a heated bath so that the physiologic salt solution could be changed at will. Any desired concentration of steroid could be added to the bath to determine its effect on the vascular smooth muscle. It was found that the steroids studied caused a relaxation of the vessel contraction induced by epinephrine, histamine or KCl. The following equivalent amounts of steroids were used: Hydrocortisone in a concentration of 0.5 mg/ml was the most potent relaxer followed by methylprednisolone, 0.1 mg/ml, and by dexamethasone, 0.02 mg/ml. Similarly, addition of steroid to the bath prior to addition of epinephrine greatly decreased the response of the latter. The smallest equivalent concentration which caused a detectable effect was 0.05 mg/ml of hydrocortisone. From these studies on isolated skin arteries, it appears that the direct effect of steroids on the vessel is relaxation rather than the constriction seen clinically. It is thus probable that the vasoconstrictive effect is due to mediators released by the steroids.

TUESDAY, JUNE 20, 1967

Afternoon Session—1:30 P.M.

Scientific Session: Dr. Eugene M. Farber, Palo Alto, Calif. presiding

 SEVENTH ANNUAL HERMAN BEERMAN LECTURE: BULLET AND TARGET—AN ANALYSIS OF ANTIGENIC STIMULATIONS.
G. J. V. NOSSAL, M.D., PH.D. Director, The Walter and Elizabeth Hall Institute of Medical Research, Victoria, Australia

STUDY OF AUTOIMMUNE DISEASE IN NZB/NZW HYBRID MICE.
I. M. BRAVERMAN, M.D., Section of Dermatology, Yale University School of Medicine, New Haven, Connecticut 06510.

NZB/NZW (B/W) hybrid mice exhibit a syndrome mimicking lupus erythematosus: positive LE cell phenomena, antinuclear factor, Coombs' positivity and membranous glomerulonephritis. The serologic abnormalities and renal disease are transmitted independently to successive generations. The adult hybrid may have a spontaneous remission with disappearance of LE cell phenomena and antinuclear factor. LE cell phenomena may occur in the absence of demonstrable antinuclear factor. The extensive renal perivascular accumulations of lymphocytes and plasma cells which have been emphasized by other investigators are not related to the pathogenesis of the glomerulonephritis but represent a response to environmental infectious agents. B/W hybrids develop normal delayed hypersensitivity reactions to PPD, egg albumin and bovine serum albumin. Parabiosis of old B/W hybrids to young B/W mice does not accelerate the appearance of renal disease in the young parabionts. It was not possible to produce renal lesions or serologic abnormalities
in Swiss albino mice injected at birth intracerebrally with sterile cell free extracts or homogenates of kidney and lymphoid tissues from an old B/W mouse. Swiss albino mice spontaneously develop severe glomerular lesions identical to those found in B/W hybrids. The less severe glomerular lesions seen in B/W mice can be found in a number of other murine strains. Experiments designed to study the transfer of renal disease from B/W and NZB mice to other strains will have to be carefully controlled.

EXAGGERATED DELAYED-TYPE HYPERSENSITIVITY TO SIMPLE CHEMICAL ALLERGENS IN THE GUINEA PIG. H. C. MAGUIRE, JR., M.D., AND M. W. CHASE, Ph.D., Laboratory of Immunology, The Rockefeller University, New York, N.Y. 10021.

Guinea pigs can be made exquisitely sensitive to peryl chloride or dinitrochlorobenzene (DNCB) by the "Combination Method" (Intern. Arch. Allergy 5, 163, 1954). That technique requires initial sensitization by parenteral emulsion which contains allergen coupled to guinea pig RBC stromata in Freund's complete adjuvant, followed by a series of contact applications of allergen. We now can secure similar exquisite sensitivity by injecting allergens—dinitrochlorobenzene (DNCB), picric acid (PA) or formaldehyde—into sites prepared with intradermal injections of killed mycobacteria suspended in paraffin oil, or vice versa. For example, 0.05 ml paraffin oil containing 2.5 µg killed human tubercle bacilli was injected into each of 5 sites, into which DNCB (2.5 µg/site) or PA (100 µg/site) was injected on the following day. Contact application of sensitizer on days 12, 18 and 25 gradually boosted hypersensitivity to very high levels, as in the Combination Method. This report appears to describe a unique anamnestic response with delayed hypersensitivity. The method can perhaps exaggerate the response to weak sensitizers and be applicable for screening substances of widely varying solubilities.

ON THE TISSUE CELL CULTURE OF THE WART VIRUS. W. N. MACK, Ph.D., Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48823.

An attempt was made to establish the wart virus in tissue cell cultures. Cell-free extracts were made from 78 samples of verruca vulgaris tissues and tested on 12 different lines of cell cultures. There was no evidence that infection was established in any of the cell lines. Although an additional cell line (AU) of secondary human skin cells did not react to cell-free inoculum, when fragments of wart tissues were placed in contact with monolayers of these cells, 3 of 23 samples gave some evidence of infection. Infection constituted rounding and aggregation of the cells with release from the tube wall. Infection could be continued only by the passage of infected AU cells, indicating a cell to cell transfer of the virus. Fluorescent antibody and acridine orange studies on infected cells showed intranuclear inclusions 48 hrs. after the cultures were infected. Deoxyribonuclease treatment of the intranuclear inclusions did not remove the particles unless they first were treated with a proteolytic enzyme. After 10 or more passages in tissue culture, electron micrographs of infected cells showed nuclear inclusions of dense staining material with particles which resemble viruses without capsid or coats.

The interpretation of these findings was that a latent infection was established with the wart virus and its host cells. Matured virus, if produced, was in limited amounts. No information was gleaned as to why cell to cell infection is necessary in the passage of the virus.

EXPERIMENTAL INFECTION WITH STREPTOMYCES MADURAE AS A FUNCTION OF COLLAGENASE. G. L. PECK, M.D., AND J. W. RIPPON, Ph.D., Department of Medicine, University of Chicago, 950 East 59th Street, Chicago, Illinois 60637.

In experimental infection with normal collagenase producing Streptomyces madurae, myceloma like grains were produced in mice with subsequent loss of vast areas of skin and
eventual death. In mice injected with a collagenase-deficient mutant of S. madurae, granules were poorly formed and no skin loss or mortality occurred. The experimental procedure was as follows: 12-18 week old Swiss white mice were divided into groups of seven and injected with cortisone 4, 3, 2, 1 and 0 mg/animal and subsequently injected in the right groin with washed S. madurae (Macotela-Ruiz) at 400, 200, 100, 50 and 25 mg of organisms/animal. It was found that 200 mg of organisms were necessary to establish infection in mice without cortisone and that mice pretreated with 1 mg cortisone would become infected with 50 mg of S. madurae. The course of infection was similar in all cases. Large subcutaneous swellings occurred at the site of injection. Histologic examination showed mixed pyogenic and granulomatous reaction and the formation of large 100-500 μ granules. S. madurae was recultured and collagenase was assayed in the tissue. Between day 14 and 18 large areas of dermis separated from the underlying fascia, fell off and the animals subsequently died. A collagenase-deficient mutant of S. madurae, produced by exposure to nitrosoguadinidine, was also injected as above. Few granules were produced, all animals lived, none showed skin loss or collagenase in assay of tissue.

BACTERIAL INVASION OF THE STRATUM CORNEUM IN ERYTHRASMA: ULTRASTRUCTURAL EVIDENCE FOR A KERATOLYTIC ACTION EXERTED BY CORYNEBACTERIUM MINUTISSIMUM. L. F. MONTES, M.D., University of Alabama Medical Center, Department of Dermatology, Birmingham, Alabama, S. H. BLACK, PH.D., AND E. McBRIDE, PH.D., Baylor University College of Medicine, Departments of Dermatology and Microbiology, Baylor University, Houston, Texas.

The purpose of this investigation was to observe at the submicroscopic level the process of bacterial invasion of the stratum corneum in erythrasma. Ten culturally proven untreated patients with fluorescent crural and/or axillary lesions were used. Negative mycological cultures revealed that these subjects had no superimposed fungal infection on the patches of erythrasma. Multiple 1 mm punch biopsy specimens from every patient were fixed using simultaneously a procedure for bacteria (Ryter-Kellenberger) and a method for adequate preservation of the skin (Caulfield). They were embedded in a mixture of Epon and Araldite or in Maraglas. Thin sections were cut perpendicularly to the skin surface and observed in an RCA EMU3F electron microscope using an accelerating voltage of 50 Kv.

Large numbers of C. minutissimum were seen in different locations within the upper third of the stratum corneum: a) on the skin surface; b) in the intercellular spaces; c) within cells; d) penetrating the cells from the intercellular spaces or directly from the skin surface. Whereas most of the free organisms showed a homogeneous fine structure, intracellular bacteria were quite pleomorphic. Dividing organisms were numerous on the surface, but they were also abundant within cells. The stratum corneum was considerably thicker than normally, with the cells disrupted at the sites of bacterial penetration. In the invaded keratinized cells, cytoplasmic areas of decreased electron density, observed regularly around intracellular bacteria and in immediate contact with the bacterial cell wall, showed clearly a dissolution of the normal keratin filaments.

It seems likely that this change is the result of a keratolytic action exerted by C. minutissimum.

CELL-WALL DEFICIENT FORMS OF DERMATOPHYTES PRODUCED IN VITRO. S. A. ROSENTRAL, PH.D., H. L. FINE, M.D., AND R. L. BAER, M.D., Department of Dermatology, N.Y.U. Medical Center, New York, New York 10016.

Cell wall deficient forms of bacteria and saprophytic fungi ("protoplasts," "spheroplasts") have been produced in vitro by a number of investigators using various methods. The study reported here is an application of one of these methods to the production of such forms from dermatophytes. Six species of dermatophytes (T. rubrum, T. mentagrophytes, T. schoenleiniti,
M. canis, M. gypseum and M. audouini) were fragmented and suspended in osmotically stabilized (0.5 M sucrose) buffer containing 10% snail enzyme and incubated at room temperature. After 1 to 24 hours' incubation, "protoplast-like" forms were seen in the specimens from all species tested. These forms were spherical, 3-10 μ in diameter and contained granules. Lowering of osmolarity resulted in a swelling and subsequent disruption of these forms. However there seemed to be a wide variation in susceptibility to osmotic shock, with some of the "protoplasts" retaining their morphology in solutions of reduced osmolarity. To date, attempts to obtain these "protoplasts" free of unaltered filaments have been unsuccessful. It would seem that the protoplasts produced from dermatophytes, although similar in morphology to those of bacteria and saprophytic fungi, differ to some extent in susceptibility to osmotic shock. This may indicate a stronger limiting cell membrane or a lower internal osmotic pressure.

TOPICAL CONTROL OF SKIN BACTERIAL GROWTH WITH HEXACHLOROPHENE IN DIMETHYLACETAMIDE. R. B. STOUGHTON, M.D., AND G. S. STOUGHTON, PH.D., Section of Dermatology, Western Reserve University, Cleveland, Ohio 44106.

A previous publication showed the ability of dimethylacetamide (DMAC) to enhance the deposition of hexachloropheene (Hex) C-14 in human stratum corneum when compared to many standard vehicles. The stratum corneum so treated inhibited bacterial growth when implanted on inoculated culture plates. This study concerns in vivo quantitation of bacteria on the forearms and hands of humans before and after application of 3% Hex in 25% DMAC compared to 3% Hex in a widely used detergent vehicle. When applied to forearms in a paired comparison study, Hex in DMAC was superior in decreasing the number of bacteria. The results were statistically significant. The same agents were compared in hand studies using serial basin washing techniques. 3% Hex in the detergent vehicle applied daily for 7 days did not reduce bacterial counts as much as 3% Hex in 25% DMAC applied only one time. The rate of recovery to normal bacterial counts was slower in the 3% Hex in 25% DMAC group than in the 3% Hex in detergent vehicle. Once applied, 3% Hex in 25% DMAC controls bacterial growth in vivo in spite of vigorous washing and scrubbing of the skin.

Fifteen of the 39 abstracts of manuscripts, submitted but not accepted for the program, follow:

STUDIES OF THE LIPIDS OF DOG SKIN IV. THE IN VIVO INCORPORATION OF BLOOD LIPIDS INTO THE LIPIDS OF ISOLATED PERFUSED DOG SKIN. G. LIPKIN, M.D., V. R. WHEATLEY, PH.D., T. H. Woo, M.D., AND C. MARCH, M.D., Department of Dermatology, New York University Schools of Medicine, New York, N.Y. 10016.

While it is undoubtedly true that dietary, and hence circulating, fats can influence the lipids of the skin, it has proved difficult to obtain unequivocal evidence or quantitative data. The critical step in the chain of evidence to prove that a dietary fat can be utilized in the formation of cutaneous lipids is to demonstrate that such a fat can reach the sites of lipogenesis in the skin. We have used the in vivo dog skin perfusion model to show that typical lipids can be transported from the circulation to these lipogenic sites. Perfusions were performed as follows: palmitic acid-14C (3 perfusions); triolein-14C (6); cholesterol-14C (3); cholesteryl palmitate-14C (2). In each the labelled lipid was added to the blood just prior to perfusion. The perfused skin flaps were separated into subcutaneous adipose tissue, dermis, and epidermis-plus-adnexae, utilizing mechanical separation and treatment with 2 M KCNS. The lipids were isolated from each tissue source, the incorporated radioactivity determined on the total lipid and on lipid fractions isolated by thin-layer chromatography. Significant uptake of all four compounds into the epidermis and adnexae was observed (0.1 to 1.4% of perfused activity). Of the incorporated lipids 50% of the palmitic acid was further metabolized, 10% of the cholesterol esterified, while triolein and cholesterol palmitate showed
littie change in the duration of our experiments. Hence circulating blood lipids are utilized by the skin for the formation of cutaneous lipids to a significant extent and certain of these lipids may reach the skin surface with little or no metabolic change.

CUTANEOUS CARCINOGENESIS IN THE RAT: A HISTOPATHOLOGIC AND HISTOCHEMICAL STUDY. By K. NAKAMURA, M.D., AND W. JOHN-son, M.D., The Skin and Cancer Hospital, Department of Dermatology, Temple University School of Medicine, Philadelphia, Pa. 19140.

Basal cell carcinomas (BCC) and other skin tumors have been reported to occur in a high percentage of rats treated with anthræmine. This study was designed to evaluate changes occurring prior to and during development of tumors in rats painted with anthræmine, dimethylbenzanthracene (DMBA) and to determine the effect of ultraviolet light (UVL). Groups consisting of 15 female albino rats (Fisher strain 344) were treated as follows: (1) 1% anthræmine; (2) 0.5% DMBA; (3) acetone control; (4) 1% anthræmine & UVL; (5) 0.5% DMBA & UVL; (6) acetone and UVL; (7) UVL; (8) and untreated controls. Biopsies were taken from each group at 2 week intervals. At 44 weeks only 20 tumors had developed in the rats treated with anthræmine and none were BCC. The rats treated with anthræmine and UVL developed a total of 6 tumors including 1 BCC before dying between 25 and 37 weeks. At 28 weeks 93 tumors had developed in the DMBA treated group and 108 in the DMBA and UVL treated group. These tumors included squamous papillomas, squamous carcinomas and fibrosarcomas but no BCC. Significant histochemical changes prior to tumor development included marked proliferation of alkaline phosphatase (AP) positive fibrocytes; accumulation of AP positive material in the superficial corium; an increase in acid mucosaccharide (AMS) in the upper corium; and dilatation, proliferation, and anastomosing of capillaries. Rats treated with UVL showed a marked increase in AMS in the superficial corium but this area did not show the usual staining of senile elastosis. BCC did not occur in this study as frequently as expected from previous reports. Tumors were preceded by vascular changes, connective tissue changes and cellular enzymatic changes. UVL did not cause or significantly increase tumor formation by 44 weeks, except for 2 tumors of the ear, but caused a striking increase in AMS in the superficial corium.

DIFFERENTIATION OF CONNECTIVE TISSUE IN CHICK SKIN. R. FLEISCHMAJER, M.D., AND J. M. BROWN, M.A., Section of Dermatology, Department of Medicine, Hahnemann Medical College and Hospital, Philadelphia, Pa. 19102.

Although considerable data is available on epidermal and feather differentiation, little is known as to the events taking place simultaneously in the dermis. Sequential biopsies were taken from White Leghorn embryos at 24 hours intervals between days 3-20. For comparative purposes, biopsies were also taken from follicular, interfollicular, and scale areas of adult skin. Specimens were fixed in Bouin's and cetylpyridinium chloride and the following stains performed: hematoxylin and eosin, PAS with diastase digestion, Alcian blue pH 0.5 and 2.5, aldehyde fuchsin, Gomori's reticular, trichrome, Verhoeff's, and acid orcein. The basement membrane of the epidermodermal junction is present at the 4th day and contains a mixture of acid mucopolysaccharides (AM) (sulfated and non-sulfated), PAS positive material, and reticular fibers. Between the 12th and 14th day, the AM disappear and the final structure consists of PAS positive, reticular fibers. Up to the 14th day, the dermis consists of a dense net of fibers (silver positive, trichrome negative) embedded in a ground substance rich in cells and sulfated and non-sulfated AM. At the 14th day, the AM disappear abruptly (except in the feather papillae) and this coincides with the fibers losing their affinity for silver stains and reacting positively with the trichrome. This study suggests that (a) AM may participate in the early stages of basement membrane formation and fibrillogenesis and (b) that reticular fibers may represent an early stage during collagen maturation.
MOLLUSCUM CONTAGIOSUM CYTOTOXICITY IN PRIMARY HUMAN AMNION CELLS—AN ELECTRON MICROSCOPIC STUDY.
J. W. BURNETT, M.D., AND J. S. SUTTON, M.D., Division of Dermatology, Dept. of Medicine, University of Maryland School of Medicine and Dept. of Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland.

The cytotoxic effect of suspension molluscum contagiosum lesions upon primary human amnion cell cultures was investigated by electron microscopy. Cultures exposed to low and high titers of molluscum suspensions were studied 1, 24 and 48 hours as well as 21 days after inoculation. No recognizable forms of poxviruses were detected intracellularly at any stage. Cells examined one hour after inoculation showed no abnormality. In markedly altered cells fine structural changes include: massively enlarged nucleoli; dispersed aggregates of chromatin; a reduction or disappearance of tonofilaments, granular endoplasmic reticulum and lysosomal bodies; swollen fragmented mitochondria and multicentric Golgi regions. Desmosomal disruption, widening of intracellular spaces and cell separation occurred. Cytoplasmic membrane systems were severely affected. Varying degrees of cytopathic effects between different cells of the same culture were observed. This study supports the hypothesis that the molluscum cytopathic effect is not dependent upon the presence of infectious intracellular virus.

EFFECT OF PROLONGED ADMINISTRATION OF TESTOSTERONE AND HYDROCORTISONE ON THE SKIN OF THE RAT. H. S. ZACKHEIM, M.D., Department of Dermatology, Stanford University School of Medicine, Palo Alto, California 94304.

The effect of the long term parenteral administration of testosterone and hydrocortisone on the epidermis, sebaceous glands and cutaneous connective tissue of young albino Sprague-Dawley rats was studied. Testosterone was administered to 2—3 month old females as an aqueous suspension, 2.5 mg, i.m., 3×/week; as testosterone cypionate in oil, 1 mg/100 gm body weight, i.m., weekly; and as a 75 mg pellet, implanted subcutaneously every 3 months. Treatment and observations have been recorded up to 20 weeks. All forms of testosterone produced hyperplasia of both the epidermis and the sebaceous glands. However, the effect on the sebaceous glands was more consistent than that on the epidermis. Testosterone also produced an increased number of fibroblasts and other connective tissue cells. The aqueous suspension of testosterone had the greatest stimulatory effect on the skin.

Hydrocortisone as the aqueous suspension has been administered 1 mg, i.m., 3×/week, to 2—3 month old male rats for up to 12 weeks. No observable differences were noted as to the thickness of the epidermis, size of sebaceous glands, or character of the dermal connective tissue, as compared to controls.

ALLERGIC CONTACT DERMATITIS TO HYDROCORTISONE. R. D. WILKINSON, M.D., E. M. MCGARITY, M. D., AND S. SOLOMON, Ph.D., Royal Victoria Hospital, Montreal 2, P.Q.

Allergic contact dermatitis to hydrocortisone cream complicated the treatment of chronic otitis externa in a menopausal housewife aged 50. Eczematous reactions occurred to patch tests and intradermal injections of hydrocortisone acetate and succinate. Hydrocortisone and 13 related steroids with 11 beta hydroxy and/or 21 hydroxy groups also gave eczematosus patch test reactions. 9 alpha fluoro compounds were without effect. 1-2 unsaturated and 6 alpha methyl compounds induced a delayed eczematosus response. Aldosterone, desoxycorticosterone, and cortisone were among the endogenously produced steroids which cross reacted. Indices of adrenal function were measured to detect possible anomalies produced by this sensitivity. Blood and urinary corticoids, urinary 17 ketogenic steroids, estrogens, and aldosterone secretion rates were all normal.

It was concluded that the contact allergy to hydrocortisone did not influence adrenal physiology.
THE IN VITRO TRANSFORMATION OF CORTISONE TO HYDROCORTISONE IN HUMAN SKIN. S. L. HSIA, PH.D., AND YU-LEE HAO, M.S., Departments of Dermatology and Biochemistry. University of Miami, School of Medicine, Miami, Florida.

Previous studies in our laboratory established the transformation of hydrocortisone to cortisone in human skin (Biochem 5: 1469, 1966). The reverse reaction, reduction of cortisone to hydrocortisone has now been demonstrated. Cortisone-4-14C was incubated with small pieces of human skin excised from the abdominal wall at autopsy. Analysis of the recovered radioactive materials by paper chromatography revealed the formation of several metabolites. Addition of pyridine nucleotides, especially NADPH to the incubation medium increased the metabolism several-fold. Dilution of the radioactive metabolites with carrier steroids, preparation of the acetates and crystallization to constant specific activity established that the major metabolite was hydrocortisone and that Reichstein’s Substances U (17α,20β-21-trihydroxy-4-pregnene-3,11-dione) and epi-U (the 20α-isomer) were also formed.

RELATIONSHIP OF GLYCOGEN CONTENT OF ECCRINE GLANDS AND SODIUM STATE IN THE RAT. H. L. WECHSLER, M.D., AND E. R. RISHER, M.D., Department of Dermatology and Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Morphological changes similar to man have been described in the rat’s sweat glands following induced sweating. Since no previous work has dealt with abnormal states in the animal, this study was undertaken to observe the effect of pathophysiological conditions on the sweat glands of the Wistar rat. This included salt loaded and depleted, fluid restricted and loaded, Doca and renal hypertensive, and acute and chronic uremic states. The sweat glands were studied histologically including tinctorial, histochemical and enzymatic techniques; and also ultrastructurally. The most striking finding was diminution in glycogen content of the secretory cells in salt depleted, water loaded, renal hypertensive and chronic uremic animals. The salt loaded group had a higher glycogen content. These findings support the concept that glycogen is related to sodium transport. The fact that the glycogen changes occurred in the secretory cells in the rat as opposed principally to the ductal cells in the human is best explained by the absence of the proximal convoluted duct in the rat.

THE INFLUENCE OF AGE, UVL, AND FREEZING ON AUTOANTIBODY FORMATION IN A/J MICE. D. L. TUFFANELLI, M.D., AND J. H. EPSTEIN, M.D., Division of Dermatology, University of California School of Medicine, San Francisco, California 94122.

Clinical observations suggest that environmental factors influence antinucleoprotein antibody formation in susceptible individuals. In the present study we examine the effect of age, UVL, and freezing trauma on a number of autoimmune parameters in the A/J mouse. 75 inbred female A/J mice were divided into 3 equal groups. The posterior half of the backs of the mice in Group I received 20.12 × 10^6 ergs/cm2 of mid-UVL energy from a hot quartz contact lamp three times a week for 12 months; Group II received similar exposures through windowglass; and the mice in Group III were treated with tri-weekly 3 second applications of liquid nitrogen on the posterior half of their backs. Venous blood collected every 3 weeks from each mouse up to the age of 15 months was examined for ANA (indirect immunofluorescent technique, 1,129 determinations), and L.E. factor (858 determinations).

UVL and freezing trauma had no influence on autoantibody formation; however, age appeared to be an important factor. At 2 months, 7% had a positive ANA and none had L.E. factor. By 6 months 24% had positive ANA tests and one animal had L.E. factor. At 9 months, 58% showed positive ANA tests and 3 had L.E. factor. At 12 months 69% had positive ANA tests and 14% had L.E. factor; by 15 months positive ANA tests were present in 93% and L.E. factor was demonstrated in 20% of the animals. Histologic fluorescent antibody studies done on tissues from 20 mice revealed chronic glomerulonephritis and
deposits of mouse gamma globulin in the glomerular basement membranes. Germinal cells in the spleen also fluoresced. No significant fluorescence was noted in the lungs, heart, liver, muscle, and skin of these mice.


Differentiation of acid mucopolysaccharides (AMP) in normal and pathologic human skin including senile elastosis, myxoma, urticaria pigmentosa, fibromyxoma, alopecia mucinosa and myxedema have been studied. The principle of controlling the binding of cationic dyes to specific anionic AMP by varying ionic concentrations and pH, as developed by Scott (Scott, J. E. and Dorling, J., Histoehimie, 5: 221, 1965) for Alcian Blue (AB) was applied by us to the metachromatic dye Toluidine Blue (TB). The TB-AMP complex is much more readily distinguished from the free dye, and the resolution of mast cell granules is far superior to that obtained with Scott's technique. Hyaluronic acid (HA), chondroitin sulfate (CS), heparin (Hep) and keratosulfate (KS) were studied. Microsections were stained for 4 hours in 0.01% TB in MgCl2 solutions ranging from 0.0, 0.1, 0.2—1.5 M in 0.05 M acetate buffer, pH 5.8 and pH 2.5. The TB-AMP complexes were stabilized with potassium ferricyanide and ammonium molybdate. Normal and elastotic skin showed a high concentration of AMP in the sub-epidermal dermis which was identified as CS; in senile elastosis there was an increase of HA and mast cells. In myxoma only HA seemed to be present. The presence of Hep in the granules of mast cells in urticaria pigmentosa was confirmed. KS was not in evidence between the connective tissue fibers of normal or pathologic skin. The total AMP were chemically isolated from some of these tissues by enzymic proteolysis, fractionated chromatographically and quantitated via uronic acid, hexosamine and sugar analyses. The chemical analyses correlated with the histochemical observations.

HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF THE LASER REACTION IN PORT WINE ANGIOMAS. H. SOLOMON, M.D., B. HENDERSON, M.D., AND L. GOLDMAN, M.D., Department of Dermatology, College of Medicine and Department of Pediatric Surgery Children's Hospital Medical Center, University of Cincinnati.

To explain the differences of the laser reaction in port wine lesions, the whitening or lack of whitening effect was correlated with the color qualities and biopsy of the port wine, the various parameters of the lasers used, and the histological and histochemical studies of pre- and post-treatment biopsies. Histochemical studies included, PAS, alkaline phosphatase, elastase and collagenase. These showed non-uniform significant connective tissue changes with secondary occlusion of blood vessels as well as direct coagulation necrosis of vessels through color absorption of the transmitted and reflected laser beam by the red cells. These changes were distinct from those induced by the controls of electro-surgery, tattooing, and grenz radiation. These studies demonstrated why the darker colored lesions with more superficial vascular net-works showed greater response to the laser therapy. These studies also showed that the good cosmetic results in the localized area was due to the relative resistance of the skin appendages to the laser beam.

The selective action of pulsed laser radiation at 6943 Å and 10600 Å on pigmented structures in the skin of (black) C-57 mice was compared with the effects of other physical agents on pigment formation. Laser irradiation during the active phase of hair growth (7-9th day) when melanin production is most marked in the hair bulb, resulted in interruption of melanogenesis for one hair generation. There were no permanent histological changes and hair growth, per se, was not affected. This temporary depigmentation differed from pigment loss produced by continuous laser radiation sources (N_{2}CO_{2} gas laser at 10600 Å) and other types of physical trauma (heat, diffuse light, ionizing radiation) which are permanent and associated with destruction of the melanocytes. The effect of X-radiation differs further from laser irradiation in that maximum depigmentation occurs during the resting phase. Laser irradiation carried out on each day of the hair cycle indicated progressive increase in energy threshold levels for induction of depigmentation, as the resting phase was approached. Conversely, injury to the underlying tissues by pulsed laser radiation was most severe during the resting phase and became less marked as the amount of melanin increased with the activity of this cycle. The protective action of melanin was demonstrated against radiation at higher intensities than previously available. Temporary depigmentation for the duration of the hair cycle suggests the loss of melanogenetic components which are reconstituted only at the beginning of a new hair cycle. The inhibition of melanogenesis without inhibition of cell division indicates other than thermal effects of laser radiation.

STUDIES ON LOCAL CHEMOTHERAPY IN LEUKOPLAKIA. By E. KLEIN, M.D., H. MILGROM, M.D., P. CALAMEL, M.D., J. E. BERGER, M.D., AND P CHAROENVEJ, M.D., Department of Dermatology, Roswell Park Memorial Institute, Buffalo, New York 14203.

The local administration of several chemotherapeutic agents resulted in disappearance of lesions lasting for observation periods of up to 5 years in premalignant keratoses, superficial basal cell carcinoma, and squamous cell carcinoma in situ with widespread multifocal cutaneous involvement. Subsequently two of the agents, 5-Fluorouracil (5-FU) and 2,3,5-Tri-ethylene imino benzoquinone (TEIB), were investigated in 12 patients with oral leukoplakia. After topical application the sites were covered with occlusive dressings. In the case of 5-FU, the initially studied drug, regression or ablation of lesions on the lips, gingivae, and hard palate lasted for observation periods of up to 6 months. The manner of use of TEIB was different, in that the agent was applied to the skin until cutaneous hypersensitivity was induced before application to the mucosal lesions. Regression and in some cases disappearance of treated areas of leukoplakia became apparent within 2-5 days. Systemic toxicity was not produced by local application of these agents. Adverse effects were limited to superficial mucosal erosion in 5 patients, which became insignificant after 48 hours. In the remainder of the group local adverse effects did not occur. Further study of local chemotherapy in leukoplakia to determine long range effects and mechanisms of action appear warranted.

LOCAL AND SYSTEMIC CHEMOTHERAPY OF KAPOSI'S HEMORRHAGIC SARCOMA. By E. KLEIN, M.D., H. MILGROM, M.D., E. EZDINLI, M.D., P. CALAMEL, M.D., J. E. BERGER, M.D., AND P. CHAROENVEJ, M.D., Departments of Dermatology and Medicine, Roswell Park Memorial Institute, Buffalo, N. Y. 14203.

Vinblastin (Vb), Vincristin (Vc), Mithromycin, and Mitomycin C were administered intralesionally in patients with advanced Kaposi's hemorrhagic sarcoma resulting in rapid regression or resolution of tumors in 4 patients. Dimethyl urethemide (AB 132) and epsilon-amino capric acid induced partial regressions. AB 132 increased the sensitivity of the tumor to X-radiation, but induced severe cutaneous hypersensitivity precluding further administration. A number of other chemotherapeutic agents did not show effects. Intravenous administration of Vb (0.15 mg per kgm) to 3 patients at intervals of 10 to 14 days resulted in marked improvement with epithelial repair of extensive neoplastic ulcerations. Side effects
consisted of neutropenia lasting from 3 to 5 days, and increase in uric acid levels. Concurrent infections (with or without local or systemic antibiotics) may prove to be limiting contraindications. Uric acid levels promptly returned to normal on administration of allopurinal. In two of three patients, who were treated with systemic Vb, simultaneous local administration of Vc to indolent ulcers appeared to increase the rate of (local) tumor regression and to accelerate re-epithelialization. It appears that local and systemic chemotherapy are methods of value in the management of Kaposi's sarcoma.

EXPERIENCES WITH HYPNOTHERAPY IN TRICHOTILLOMANIA AND MULTIPLE WARTS. J. H. BECKLEY, M.D., AND R. A. BERGER, M.D., Orentreich Medical Group, 909 Fifth Avenue, New York, N.Y. 10021.

Earlier work has established the usefulness of hypnosis in the treatment of a number of disorders of unquestioned psychosomatic origin. The present study contrasts results obtained in the treatment of four patients with such a disorder, namely trichotillomania, with results obtained in four patients with a viral disease, multiple warts, in which good results with hypnosis have also been claimed in the past. The eight patients in the study were all children, and received roughly the same number of hypnotic sessions. Differences in autosuggestive technique in the two diseases evolved from considerations of the essential differences in the nature of the conditions. An unsuccessful attempt was made in all of the patients with warts to treat certain lesions and spare others. The results suggest that hypnototherapy is a useful modality for the treatment of purely psychocutaneous disorders, but of doubtful value in warts.

ANNOUNCEMENT

Skin Research Club

The Skin Research Club will meet on Saturday, April 29, 1967, at 8:30 A.M. in the Mandarin Room, Haddon Hall, Atlantic City, New Jersey. There will be 31 papers presented.