Abstracts for the IV. International Dermatology Symposium Berlin "Sebaceous Gland, Acne and Related Disorders—Basic and Clinical Research, Clinical Entities and Treatment"
University Medical Center Benjamin Franklin, Berlin, Germany—April 11–13, 1997

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The symposium takes place under the auspices of the president of the Free University of Berlin and the Arbeitsgemeinschaft Dermatologische Forschung (ADF) and is dedicated to Professor John S. Strauss and his work.

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1 THE SEBACEOUS GLAND AND ACNE - 40 YEARS ON W.J. Canfield, Skin Research Centre, Leeds University, UK

There have been several major milestones in our understanding of the pathophysiology of the pilosebaceous unit. The innovative research of Professor John Strain has played an important role in many of these milestones. He and his students have been central to: (1) the hormonal control, in particular androgen control of the sebaceous gland, (2) the importance of comedone formation as an essential feature for acne, (3) the central role of the lymphocyte in acne inflammation, (4) the benefit of long term antibiotics in acne, (5) the dramatic effect of oral isotretinoin in suppressing sebum and in controlling acne.

The purpose of this introductory lecture is to highlight these facts and other aspects of the pathophysiology of the sebaceous gland and thus set the scene for the symposium.

2 CULTURED HUMAN SEBOCYTES: AN IN-VITRO MODEL FOR FUNCTIONAL AND CONTROL STUDIES ON HUMAN SEBACEOUS CELLS.
Department of Dermatology, University Medical Center Benjamin Franklin, The Free University of Berlin, Berlin, Germany

Sebocytes are the active component of the sebaceous gland and sebaceous gland differentiation is species-specific. Therefore, fundamental research on human sebaceous cell function and control studies on human sebocytes in vitro models. In 1989 the human sebocyte culture model has been introduced by our group and was used by us and others in several studies to elucidate the physiology and pathophysiology of the sebaceous gland at the cellular level. Human sebocytes are sensitive to a variety of lipids and lipoproteins, and the response to these may provide insights into the development of sebaceous gland disorders. In this symposium, we will discuss the use of sebocytes in vitro as a model for the study of sebaceous gland function and the potential use of sebocytes in the development of new treatments for sebaceous gland disorders.

3 THE ORGAN MAINTAINED HUMAN SEBACEOUS GLAND. R. Gay, T. Kealey. Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Cambridge, CB2 0QZ, UK.

We have previously reported that human sebaceous glands can be maintained for up to fourteen days in whole organs with full retention of the physiological and structural pattern of new cell formation, but we also reported that the newly formed cells did not differentiate normally, causing a progressive loss of lipogenesis in vitro.

We now show that this abnormal sebocyte differentiation was attributable to the presence of epidermal growth factor (EGF) and phenol red in our maintenance medium. In their absence human sebaceous glands apparently retain in vitro rates of cell division and lipogenesis over seven days' maintenance in addition to a retention of almost perfect in situ morphology. This is reversible on the re-addition of 10ng/ml EGF and 100µM phenol red. The addition of 600µM 17β-estradiol resulted in a significant fall in the rate of lipogenesis over seven days' maintenance, without affecting the rate of cell division. This effect is apparent due to abnormal differentiation of newly formed sebocytes. Neither InM testosterone nor InM dihydrotestosterone (DHT) has any effect on rates of cell division or lipogenesis over seven days. InM 13-cis retinoic acid causes a significant reduction in the rate of lipogenesis over seven days.

These findings, therefore, show that we can apparently model the physiological effects of retinoids, EGF and 13-cis retinoic acid in vitro.


4 MORPHOGENESIS OF SEBACEOUS GLAND IN NEONATAL MOUSE SKIN: EXPRESSION OF NGF, TrkA and TrkC BY DEVELOPING SEBOCYTES. Y.A. Bontcheva, C.M. Peters, Y.A. Bontcheva, S.M. Bontcheva, S.M. Bontcheva, E. Mittler, Department of Dermatology, Virovitica, Humboldt University, Berlin, D-13353, Germany

Expression of neurotrophic factors (nerve growth factor [NGF], brain-derived neurotrophic factor [BDNF], neurotrophin-4 [NT-4], neurotrophin-3 [NT-3]), and their high (tyrosine kinase A, B, and C [TrkA, B, C, respectively] and low (p75) affinity receptor-immunoreactivity (IR) was studied during sebocyte glanogenesis in C57BL/6 back skin of neonatal mice. During stages 6-4 of hair follicle morphogenesis, all keratinocytes in developing hair follicle expressing NGF, TrkA, and p75-IR, while only keratinocytes located in the central part of the follicle showed TrkA-IR. First sebocytes were present in the bulge by their enlarged volume in stage 5, also expressing NGF, TrkA, and p75-IR. Appearance of NT-3-IR keratinocytes in close vicinity to developing sebocytes (outer and inner root sheath of follicle isthmus) was observed in stage 6-7, while in stage 4 TrkA and p75-IR keratinocytes were only in inner root sheath. Sebocytes of fully developed hair follicles in stage 8 of hair follicle morphogenesis also were characterized by the expression of NGF-, TrkA-, and TrkC-IR. No BDNF-, NT-4-, TrkB-, or p75-IR was found in developing sebocytes in any stage of hair follicle morphogenesis, indicating that NGF-receptors are expressed selectively during sebaceous gland morphogenesis. Also, expression of p75-IR nerve fibers were observed at the stage of hair follicle morphogenesis, representing the question whether sebocyte-derived NGF might be involved in development of hair follicle innervation. These results invite one to explore whether NGF plays a role as autocrine factor for sebocyte development, while NT-3, produced by androgenic keratinocytes, may have paracrine functions during sebaceous gland morphogenesis.

5 SEBACEOUS GLAND-BULGE INTERACTIONS IN THE SKIN OF hr/hr HAIRLESS MICE. A.A. Pantaleev*, R. Pinto, T. Rosenbach, *Severstov Inst., 117701, Moscow, Russia and Dept. of Dermatology, Virovitica-Hospital, Humboldt University, D-13353, Berlin,

Since the modulation of sebaceous gland (SG) activity is implicated in the pathogenesis of several skin disorders, the cytokinetics of the SG germinative epithelium, normally the question of stem cells which provide a permanent source of sebocytes, are a fundamental problem in dermatology. Recently the bulge area of the hair follicle has been identified as a site of epithelial stem cells which may be pluripotent and may give rise to sebocytes, as well. To further explore this notion, we have studied normal and dioxin-treated 0.2 µg in acetone solution topically every third day) skin of hairless (hr/hr HRS3) mouse mutants by routine histology. In untreated skin of hairless mice, we found the bulge to become separated from the surrounding follicle epithelium due to the fur mutation, with an almost complete disintegration of the outer root sheath. The bulge remains connected with the SG only, thus providing a unique opportunity to study interactions between these tissue compartments. In untreated hr/hr skin, the distal part of the bulge consists of flattened cells which are in close contact with SG epithelium. Under dioxin treatment, the SG undergoes rapid involution until it completely disappears, while the bulge area retains its integrity, but loses its connection with all epithelial skin structures. After the cessation of dioxin treatment, sebocyte renewal becomes visible in close proximity to bulge cells. These observations support the concept that the bulge structures of both SG and SG are integrated and that, under normal conditions, some bulge-derived cells differentiate into sebocytes. SG renewal after the cessation of dioxin treatment may also arise from the bulge region.

6 SEBACEOUS GLANDS SEEM TO BE INVOLVED IN TGFB-6 REGULATION OF HAIR GROWTH. W. Wellins, D. Lange, K. Rasco, P. Rusch, Department of Dermatology, the Friedrich-Schiller-Universität of Jena, Jena, Germany, Ludwig Institute for Cancer Research, Uppsala, Sweden, and the Department of Dermatology, Alexander-von Humboldt Universität (Clausthal), Berlin, Germany.

TGF-6 is a family of growth factors. We investigated the involvement of different TGF-6 conformers and the TGF-6 receptor during induced hair growth in mice. The model of anagen induction in C57 BL-6 mice has been employed (JD 78 (1995) 122). Skin samples were taken during the hair cycle at different time points. Formalin-fixed sections have been subjected to immunohistochemical staining with antibodies against TGF-6, 2, 3, 5, latent TGF-binding protein (LTBP) and TBR-1 and II.

During anagen and telogen, LTBR and TBR-II were expressed in sebaceous gland epithelium. During catagen, TBR-II could be visualized but LTBR immunoreactivity was lost. TBR-I was expressed in early anagons only. In the hair follicle epithelium, immunostaining was limited to the outer root sheath. TGF-6 was detected in anagen and early catagen, but absent in telogen. TBR-I has been shown to be expressed early during catagen. TGF-6 and TBR-I were expressed by the connective tissue.

These findings for the first time suggest that a bidirectional regulation of hair follicle and sebaceous epithelium is possible. Sebocytes can be considered as a target structure for TGF-6 since they are capable to express both, TBR-I and -II. It is possible to believe that TGF-6 functioning is essential for TGF-6 being involved in anagen/ catagen switch and regulation of sebaceous gland growth.
transit time of intradermal injected 13C labelled acetate up to skin surface.

M.B. Jani, P.G. Agache, Ph. Humbert, Department of Dermatology, University Hospital, 25920 Dijon, France

Introduction: skin transit time between sebum synthesis and excretion (Downing et al., 1972) is in conflict with the 21 days transit time of sebaceous cells (Heywood et al., 1981). To get a new insight into this problem, the concentration of 13C in sebum was followed over 27 days after intradermal injection of 13C labelled acetate in human volunteers.

Materials and methods: 13C labelled acetate was injected intradermally in delimited forehead areas of 5 normo-to-dermatitis men. Every day and every third day, skin surface lipid (SSL) collections were made on 2 injected and 2 un.injected (control) areas to determine and compare 13C concentration, using mass spectrometer. At the same time, SSL levels were measured on 2 nearby (uninjected) areas, by photometric method.

Results and discussion: A peak output of 13C in sebum was found by days 6-8. The 13C excretion course was protracted up to day 27. The amount of 13C was slightly but significantly lower when sebum was collected daily than every three days. These results favour an incorporation of acetate into sebum earlier than the excretion by the sebum acical level and the possibility of the resorption of the accumulated sebum by the skin (Blane et al., 1989).

Intermediary metabolism of the human sebaceous gland.

M.M.T. Downie, T. Kealey, Department of Clinical Biochemistry, University of Cambridge, Addenbrookes' Hospital, Hills Road, Cambridge CB2 0QR, England

We have reported that the isolated human hair follicle is a glutaminolytic, aerobic glycogenic tissue (Williams et al., 1993), so we have now studied the intermediary metabolism of the human sebaceous gland. Glycogen levels were increased during and maintained overnight prior to study (Guy et al., 1996). They were then suspended in bicarbonate buffered saline in the presence of 4mM U-[1-14C]Acetate and 12-hour, [6-14C]Glucose or 4mM U-[2-3H]Acetate or [5-3H]Glucose, or 8mM U-[14C]Glucose. Experiments were performed for 85 patients, 5 glands per subject per time point, at 3 time points of 1, 3 and 6 hours.

Of the total glucose utilized as determined by [5-3H]Glucose hydrolysis, only 6% was oxidized. We confirmed by direct measurement of lactate using a lactate dehydrogenase assay that the remaining 94% was released as lactate. Thus the sebaceous gland engaages in aerobic glycolysis. Moreover, 50% of glucose oxidation could be accounted for by the pentose phosphate pathway.

Interestingly, stimulation of acetate into sebum synthesis was similar to those of glucose oxidation, direct measurement of glutamine in the medium using a combined glutaminase and glutamate dehydrogenase assay showed uptake by the gland was below the limit of detection of the assay. However, it was possible to release of ammonia using a glutamate dehydrogenase assay. In addition, the absence of 2mM glutamine in the medium reduced rates of lipogenesis and cell proliferation by 50%.

In conclusion, the human sebaceous gland is an aerobic glycolytic tissue and although rates of glutamine utilization are small, it does engage in glycolysis.

Sebaceous glands and neoplasms express bilary glycoprotein (BGP), a member of the CEA family.


The carcinoembryonic antigen (CEA) family comprises a group of closely related glycoproteins that have been demonstrated in sweat glands and are involved in sebaceous differentiation. To evaluate the specificity of CEA glycoprotein for sweat gland differentiation their expression was investigated in fetal and adult sebaceous glands or neoplasms with sebaceous differentiation. A polyclonal antibody detecting all members of the CEA family and a panel of monoclonal antibodies highly specific for intestinal CEA, NCA (non-specific crossreacting antigens), and BGP (biliary glycoprotein) were applied for light and electronmicroscopic immunostainings. All the used antibodies showed reactivity with normal and neoplastic sweat glands. Sebaceous glands were exclusively labelled with the antibodies recognizing BGP. The consistent staining of the sebaceous glands was confined to differentiating sebocytes sparing the germinative basal cells. As ultrastructural level reactivity for BGP was demonstrable along small membranes bound vesicles. During fetal development of the sebaceous glands BGP was not found until maturation. A total of 60 cases of sebaceous neoplasms including sebaceous hyperplasia or adenoma, sebaceoma and sebaceous carcinoma revealed staining for BGP. In conclusion, normal and neoplastic sebaceous glands could be demonstrated to express BGP, a glycoprotein of the CEA gene family that can be assured to play a role in sebaceous secretion.

Lipogenesis of the human sebaceous gland.

M.M.T. Downie, T. Kealey, Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QR, England

We have determined the lipid composition of isolated male and female human sebaceous glands maintained overnight (n=5 subjects, 25 glands per subject). Significant sex differences in sebaceous lipids: triacylglycerol was 25% more of male glandular lipids, but 32% in the female (p<0.05), while cholesterol was 11% in the male, and 8% in the female (p<0.005). To account for these differences we have studied the rates and patterns of lipogenesis from different substrates.

Glycogen depleted incubations were loaded with 8mM U-[1-14C]Acetate, 4mM U-[14C]Glucose and 8mM U-[14C]Glyceraldehyde. No sex differences in rates and patterns of lipogenesis were found, so we conclude that the in situ differences might be attributable to exogenous triglyceride or cholesterol uptake in vivo.

There were, however, differences in the lipid patterns with different substrates. The squamous/triacylglycerol ratio was 4.60% higher in male and 1.4 for female. Such substrate-specific differences in ratio could be attributed to competition for NADPH, but the addition of 1mM glycerol to 8mM U-[12]C]Acetate reduced the ratio to 1.7, indicating that some of the difference could be attributed to glycolytic phosphate deficiency. The remaining differences between the three fuels would, however, confirm that glucose can generate NADPH through the pentose phosphate pathway, and that glutamine might through the malic enzyme. This is strengthened by our observations that when non-glycogen depleted glands are studied, the 8mM U-[1-14C]Acetate squamous/triacylglycerol ratio was only 1.6, suggesting that endogenous glycogen breakdown could supply both NADPH and glycerol phosphate.

In conclusion, the pattern of lipogenesis in the sebaceous gland from different fuels appears to be determined by both NADPH and glycerol phosphate availability.

Dioxin-induced chloracne-like lesions in the skin of hairless mice: are sebaceous glands really the primary targets?


Chloracne (CA) is the most common effect of dioxin (Diox) toxicity, thought to be based on squamous metaplasia of sebaceous glands (SG). In order to obtain further insight into mechanisms of D-induced CA we have studied the sequence of histopathological events and the expression of murine keratin 17 (MK17) - a marker of specific keratinocyte populations of the hair follicle, but not of SG by means of in situ hybridization in the skin of intact and D-treated hairless hr/hr R5/5 mice (0.2 μg in acetone, topically every third day for 25 days), which respond to D with the formation of keratinized cysts resembling human CA. D-treatment evoked a rapid involution of SG and adjacent uriculi (epidermis-associated comedones) without any signs of squamous metaplasia or keratinization of sebocytes. In contrast, the dermal cyst underwent epidermal hyperkeratinization, forming CA-like structures. MK17 expression patterns confirmed that dermal cysts originate from the proximal, cycling portion of the follicle outer root sheath (ORS), but not from the SG. Therefore, D-induced chloracne more likely reflects an acceleration of the keratinocyte differentiation program in the proximal ORS, which corresponds to the dermal cyst in hairless mouse skin, than a primary pathogenic event in the SG. Neither the SG nor the distal part of the hair follicle epithelium, from which uriculi arise, appear to be involved. The D-induced SG involution may be caused by an inhibition of sebocyte precursor cell proliferation.
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ISOTRETINOIN TREATMENT OF HUMAN SEBOCYTES IN VITRO RESULTS IN LOW ISO-
RETINOIN, BUT CONSIDERABLY ELEVATED TRETINOIN INTRACELLULAR LEVELS AND ITS EFFECT IS NOT AFFECTED BY THE PRESENCE OF RETINOL.


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Isotretinoin is the most effective drug in reducing sebaceous gland size and suppressing seborrhea, but its use may also lead to severe side effects. Previous work of our group has confirmed the individual inhibitory effects of isotretinoin on proliferation and lipid synthesis of human sebocytes in vitro (J Invest Dermatol 96:792, 1991). Despite its biological activity isotretinoin binds neither CRABP II nor nuclear retinoid receptors. The present study was performed to further elucidate the unique inhibitory properties of isotretinoin on sebocyte activity. Human sebocytes were cultured in DMEM/Ham’s F 12 [1] supplemented with 10% FCS, 10 ng/ml EGF, 6.7 ng/ml KGF and treated with 10⁻⁴ M isotretinoin, 10⁻⁵ M retinoic acid and their concentrations. Isotretinoin significantly inhibited sebocyte proliferation at 10⁻⁴ M with a 50% inhibitory concentration (IC₅₀) of 7.40⁻⁶ M. The IC₅₀ of isotretinoin was detectable at 10⁻⁴ M. In contrast, retinoic acid was only active at 10⁻⁵ M. The isotretinoin/retinoid combinations exhibited antiproliferative effects comparable to isotretinoin. The amount of triglycerides under isotretinoin treatment determined by HPLC was 250% of the triglycerides under isotretinoin treatment. HPLC analysis revealed that after isotretinoin treatment, the intracellular in vitro level was relatively constant (±60 nM) from 3 to 24 h, while the retinoid concentration rose (±500 nM) compared to the endogenous levels. After retinoid treatment, retinoid concentration increased to a constant level (±100 nM) and retinoid palmitate levels more than doubled. After combination, the levels of the retinoids were similar to those after isotretinoin treatment, while retinoid and retinol palmitate were higher than after retinoid exposure alone. In conclusion, the inhibitory effect of isotretinoin on sebocyte activity is not affected by the presence of retinol and could result from low persistent retinoid levels or from the considerably elevated tretinoin concentrations in the sebocytes.

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EFFECT OF 3 STEROISOMERS OF RETINOIC ACID ON THE SEBUM EXCRETION RATE IN SUBJECTS TREATED ORALLY

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Oral 13-cis-retinoic acid (13-cis-RA) has a dramatic sebopressive activity. Two stereoisomers, 9-cis-retinoic acid (9-cis-RA) and all trans-retinoic acid (tRA) have the same activity as 13-cis-RA in preclinical models. The objective of our studies was to evaluate the effect of these isomers of RA on the sebum excretion rate.

One double blind study consisted in comparing 9-cis-RA with 13-cis-RA. 26 male healthy volunteers with a SERR above 1.0 g/cm³/min were treated with either 20mg 13-cis-RA or 9-cis-RA for 4 weeks. SERR was screened before treatment, at week 2 and 4. In a second open study 12 volunteers were treated with 20 mg tRA and SERR was evaluated before treatment and at 2 and 4 weeks of treatment.

In the 9-cis-RA group a median decrease of 37% at week 4 was obtained and 91% in the 13-cis-RA group at week 4. The 12 subjects treated with tRA showed a decrease of 5%.

Of the three isomers tested only 13-cis-RA shows activity on sebopression. 9-cis-RA was very sebopressive (91%) compared to 13-cis-RA and all trans RA showed no activity at all. 13-cis-RA does not bind to nuclear receptors. Therefore, either sebopression is not nuclear receptor mediated or 13-cis-RA is transcribed into a metabolite with receptor-binding properties at the level of the sebaceous gland due to appropriate distribution.

17

A NOVEL ENZYMIC ASSAY FOR THE QUANTIFICATION OF SKIN SURFACE LIPIDS

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The applicability of a novel enzymatic assay for quantifying skin surface lipids was investigated experimentally. The standard curves for the assays of glycerol esters, free fatty acids, and cholesterol and its esters were linear over a wide range of lipid concentrations, which ensures the accuracy of measurements. There were significant positive correlations between the quantities of glycerol esters, free fatty acids, and cholesterol and its esters sampled from the skin surface of women when measured by the enzymatic assay compared with the gas chromatographic method. The enzymatic assay was applied to studies of the relationships between age, sebaceous gland activity, and skin surface lipids in women. The quantities of glycerol esters and free fatty acids reached peaks in females in their twenties and increased. Increased quantities of glycerol esters, free fatty acids, and cholesterol and its esters were observed in women with acne compared with women without acne. Among the women with acne, those in the premenstrual phase of the menstrual cycle showed increased levels of glycerol esters, free fatty acids, and cholesterol and its esters compared with those in the menstrual phase. The results suggest that the enzymatic method is a satisfactory new technique for the quantification of skin surface lipids.

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DOES SEBUM MAKE DROPLETS BEFORE SPREADING ON THE SKIN SURFACE?

A calculation of its spreading coefficient from sebum/skin interfacial free energy

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A way to predict the spreading of a liquid on a solid surface is to calculate its spreading coefficient, S. This coefficient is defined as the difference between the energy of adhesion solid/liquid, W_a, and the energy of cohesion of the liquid W_c. When S > 0, the liquid spreads on the surface, while S < 0, the liquid forms a drop with a finite contact angle on the solid surface.

The aim of this work was to calculate S for the sebum on the skin surface, in order to check the use of the assumption of the sebaceous excretion droplets on the skin surface. The S for sebum on skin has been calculated from the determination of the surface tension components thanks to a forced drop on the skin surface and the measurement of the contact angle after degreasing. Surface components of the skin and surface tension components of the sebum were calculated from contact angle measurements and the interfacial acid-base theory.

The S is always positive for each sebum and whatever the skin types, which is in agreement with a degreasing of the skin surface. Only on the forearm, after degreasing, S is negative. These results show that native sebum does spread on the forehead surface. The classical assumption of sebum making droplets at the follicular orifices, before spreading, is not supported.
21 SEBACEOUS LIPIDS ANALYSED BY TWO DIFFERENT EXTRACTION METHODS
S. Motta, G. Sala, M. MONTI
Istituto di Scienze Dermatologiche, IRCCS, Ospedale Maggiore, Milano, Italy
Previous published works showed the lipid compositions produced by cultured sebocytes (1). However, the same analysis is difficult to perform because of the heterogeneity of sebaceous gland cells and the contamination of ductal cells. In this experiment we analysed lipids of human sebaceous glands isolated from abdominal skin. Dermospanolysis was obtained by incubation in a solution of 10 mM sodium edetate in phosphate-buffered saline, calcium and magnesium free. Intact sebaceous glands were isolated from epidural and subcutaneous microsceros. Sebaceous glands underwent overnight extraction with Bligh-Dyer solvent: the supernatant was processed for lipid extraction. Lipids obtained by the two different extractions were separated by thin layer chromatography and quantified by computerized scanning densitometry. The overnight extraction showed a mainly sebaceous lipid pattern: wax esters and squalene account for 30% of total lipids; the pattern of lipids extracted after sonication was characterized instead by the presence of ceramides and free fatty acids accounting for 50% of total lipids. This study demonstrates that in vivo pure sebaceous lipids, without keratinocyte lipids, cannot be obtained even from isolated sebaceous glands. In fact, cultured skin surface lipids, a mixture of sebaceous and keratinocyte lipids is obtained.


23 A NOVEL METHOD FOR SEPARATION OF NEUTRAL LIPIDS AND PHOSPHOLIPIDS FROM CULTURED CELLS AND THEIR SUPERANTIGENS
K. M. FELDO, G. CHAILLEY, D. OSLO, G. C. ZOUBOULIS, Department of Dermatology, University Medical Center Benjamin Franklin, The Free University of Berlin, Berlin, Germany
We developed a reliable method for isolation of lipids from cultured cells in vitro and their supernatants and for the separation into neutral lipids, fatty acids and phospholipids. To extract and purify the lipids we used a two phase separation method in which the cells were harvested in distilled water, then trypsinized and chloroform was added. In contrast to methanol which is classically used, tetrahydrofuran is solely contained in the chloroform phase and allows a higher recovery of polar lipids. We showed this method to be quantitatively reliable in separating lipids from non lipids. Lipids from the media were extracted by adding 10% methanol and applying the solution to reverse phase columns. For separation of the lipid mixtures into individual classes we used a modification of the method according to Kalmaz et al. (1) (J Lipid Res 26:153, 1985) utilizing anion-exchange bonded phase columns. The neutral lipids and fatty acids were eluted as described, the phospholipids by using additionally ammoniumacetate to ensure complete recovery. After having separated the total lipid mixture into its subgroups, these were separated and visualized using high performance thin layer chromatography (HPTLC). We show that using the reverse phase columns we have an extraction of about 20% for neutral lipids and slightly higher for phospholipids. The loss of lipids is probably due to some formation of micelles at a concentration of 10% methanol in water preventing them from binding to the column completely. By using anion-exchange columns we have complete recovery of the separated lipid subgroups. Interestingly medium containing FCS contains some lipids when separated by the method described, in particular bands comigrating with the fatty acid, cholesterol, cholesterol ester and triglyceride standards. One unknown lipid fraction is migrating below the sphingomyelin standard. This has to be taken into consideration when quantifying cellular lipids. In summary a reliable method has been developed to detect and quantify the content of neutral lipids, fatty acids and phospholipids in cultured cells in vitro. Using this method the lipid patterns of human sebocytes and keratinocytes will be presented.
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BIRT-HOGG-DUBÉ SYNDROME AND HORSTEINSKINNECKENBERG SYNDROME ARE THE SAME
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Of the sporadic autosomal dominant inherited syndrome of Birt, Hogg, and Dubé (BHD) is characterized histologically by the multiple occurrence of adenexal hamartomas with sebaceous differentiation, namely fibrofolliculomas and trichodiscomas. Colonic polyps are occasional additional findings in this syndrome. Clinically, the BHD is not discernible from the autosomal dominant inherited Horsteinskin-neckenberg syndrome (HKS). Histologically, however, the skin lesions in the HKS are described as perifollicular fibromas. In the present study we reported on a father and his daughter, in which we for the present diagnosed a Birt-Hogg-Dubé syndrome (BHD).

Material and methods: A greater number of the popular lesions both of father and daughter were examined in H-E-stained sections, taken vertically as well as horizontally to the epidermis.

Results: In sections taken vertically to the epidermis the typical histology of BHDs with fibrofolliculomas and trichodiscomas appeared. However, sections taken horizontally to the epidermis produced transverse cut hair follicle infundibula with only discrete epithelial nodules in a highly fibrocytically, concentrically arranged stroma. These findings were exactly the same to those that Horstein and Knickenberg and also following investigators claimed to be perifollicular fibromas.

Conclusion: We concluded that the histological differences between the skin lesions in the HKS and the BHDs are artificial, caused by different sectioning techniques, and therefore HKS and BHDs are the same. Such diagnostic errors might be expected from the often exceptionally complex architecture of the adnexal tumors of the skin, which cannot be described satisfactorily in two dimensions.

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HUGE SEBACEOUS CARCINOMA OF CENTRAL AREA: A RARE PRESENTATION
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A rare case of sebaceous gland carcinoma is reported occurring in the genital area of a 72-year-old woman. The patient felt a growing nodule on her labia majora for one year but did not seek medical consultation until it had ulcerated and spread over genital, pubic, and inguinal areas. Skin biopsy confirmed the diagnosis of sebaceous gland carcinoma. Radiological and sonographical examinations of her gastrointestinal tract failed of reveal any metastatic lesion.

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The enzymes may play a role in the hyperkeratinization of acne follicles. The goal of our study is to test the hypothesis that infrafundibular keratocyes of acne subjects produce more androgens than those from normal subjects. Thus, the specific activities of 5a-R and 17b-HSD, which are responsible for the production of testosterone (T) and dihydrotestosterone (DHT), were determined in follicular and epidermal keratinocytes.

Skin biopsies were obtained from the foreheads of 32 men and women both wit and without acne. Keratocyes were cultured from epidermis and infranfundibular segments of pilosebaceous follicles. Steroid metabolism studies with radioiodated T and androstenedione were performed in keratocyes homogenates to determine the activity of 5a-R and the oxidative and reductive activities of 17b-HSD. Prior to the biopsies, acne lesion counts were performed and casual sebum output was measured using Sebustage R. Serum levels of T, androstenedione, DHAS, DHEAS and 3α-androstanediol were determined using radioimmunoassay. In all samples tested, activities of 5a-R and 17b-HSD were higher in keratocyes from the infranfundibulum compared to those from the epidermis. Mean activities of 5a-R were higher in infranfundibular keratocyes from acne females (1.5 ± 0.3 pmol/min/mg protein, n=5) vs. acne males (2.3 ± 0.3 pmol/min/mg protein, n=6) vs. nonacne females (2.1 ± 0.3 pmol/min/mg protein, n=10) and acne males (2.3 ± 0.3 pmol/min/mg protein, n=6) vs. nonacne males (1.47 ± 0.3 pmol/min/mg protein, n=10).

A similar relationship was noted for both the oxidative and reductive activities of 17b-HSD with higher activities noted in subjects with acne compared to those without acne. Data on sebum androgen levels, sebum excretion rate and correlations with 5a-R and 17b-HSD activity in follicular and epidermal keratinocytes will be presented.

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EXCRETION OF SERUM IS CHANNELED BY A KERATINOSIL ENVELOPE FROM SEBACEOUS DUCT ORIGIN: THE SEBOEMLIMAL SHEATH.
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The excretion of products of the sebaceous gland occurs in an organized, virtual and acellular tubular conduit formed by a casting of highly lipidized keratin around sebum that flows around stationary commensals. The sebum is not expelled in a "noopposite" manner but as a "sapsage", perfectly contained and isolated from the suprafundibulum, ready to ke out the cutaneous horn.

The casting of keratin that contains the sebum, i.e., the sebomical sheath, is normal, universal, subtle, hyperreplicated, and mobile. It is produced by the rectified, perpendicularly oriented sebaceous duct ("infrafundibulum") in the active gland in concurrent telogens vellus follicle) or by the stunted, obliquely oriented sebaceous duct of glands in terminal or vellus follicles in agan.

The distortion and disorientation of the sebomical sheath is the basis for the formation of a comedo. The comedo is not the perpendicularly sheil product of local hyperkeratinosis of the infranfundibulum, as if the later were follicular rather than sebaceous. I propose that abnormal sebomical sheath, from sebaceous duct origin, slides above some optional reversible suprafundibular hyperkeratinosis (which is probably banal) and does not induce acne) and either looses orientation to the pore and worms itself into a close comedo or delaminates and collapses into a less turgid sheath that stagnates degenerates into an open comedo. Even so, this in itself is not causative of acne without the piercing effect of the seboli, a sebulm concretion described elsewhere.
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AN IN VITRO MODEL FOR THE INFUNDIBULAR CHANGES IN ACNE
Parish, W.E., Barnett, S.E., Kacel, T.
The initial change in acne results from sebum and possibly micro-organisms inducing an irritant or toxic effect on the sebaceous gland infundibular duct epithelium. The living skin equivalent (LSE) culture closely resembles in vivo epidermis. We show that infundibular keratinocytes differentiate in skin culture to form an epidermis identical with that derived from epidermal keratinocytes. The LSE model therefore enables examination of the effects of sebum on differentiated epidermis without involvement of hair follicles or leukocytes.

Addition of artificial sebum containing cholesterol, triglycerides, oleic acid and squalene in 3 days induced hyperkeratosis, increase in keratohyalin granules and granulosum cells, linear morphology of the keratinocytes and oil droplet inclusions. These changes are reported to occur in the early stages of a comedone. They are also similar to changes occurring after mild irritation. Addition of free fatty acids (FFA) in the same concentration assayed in a bulk human sebum sample (18%) induced severe toxic change. Addition of a preparation of human sebum containing FFA induced similar toxic change. It is concluded that accumulation of sebum may initiate the acne lesion, which is promoted by FFA formed by bacterial metabolism.

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TRICHOBLASTOMA IN NEVUS SEBACEOUS - A FREQUENT MIMICRY OF BASAL CELL CARCINOMA?
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While nevus sebaceous (ns), a congenital hamartoma, only forms rudimentary sebaceous glands in childhood, the latter start to proliferate with the onset of adolescence. Eventually, in adults a variety of adnexal skin tumors such as basal cell carcinoma (bcc), sebaceous adenomas, sebaceous hyperplasia, sebaceous cysts, and tricho-blastoma (tb) may develop, among which tb is generally believed to be the most frequent type and tb, in contrast, has previously been reported only occasionally. However, since in ns we never observed aggressive variants of bcc's frequently featuring those types located on the scalp and because some of our tb's were previously found to be bcc's, we reassessed our diagnosis of ns-associated tumors. For this purpose, we retrospectively analyzed all biopsies referred from 1992 - 96, which were formerly diagnosed as ns. Among a total of 22 specimens we recognized 5 tumors, two of which exhibited the characteristic features of sebaceous adenomas or sebaceous hyperplasia. While no tb could be found, we surprisingly diagnosed tb's in the remaining three cases. In conclusion, our results strongly suggest that tb could be a thus far unappreciated tumor developing in ns due to its close similarities resembling characteristic hallmarks of bcc's both clinically and histologically, which easily could have led to false diagnosis in the past. Should this view be confirmed in the future, we will certainly also have to reconsider the widely accepted concept of removing ns already in childhood towards a more restrictive indication for prophylactic excisions.

34 WITHDRAWN

35 WITHDRAWN

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EFFECT OF MINOCYLINES ON PROLIFERATION AND DIFFERENTIATION OF CULTURED NORMAL HUMAN KERATINOCYTES.
G. Meneguzzi (Nizza), O. Partouche, JP Ortonne (INSERM U385, UFR de Médecine, Avenue de Valombrose, Nizza)
The abnormal keratinization of the infra-infundibular zone of the follicular epithelium seems to play an important role in the pathogenesis of acne vulgaris. Clinical studies have shown that the treatment with minocycline correlates with a decrease in the number of the retentional lesions. This observation cannot be explained by the antibacterial and/or anti-inflammatory effect of the treatment. We have therefore evaluated the influence of minocycline on proliferation, keratinization and terminal differentiation of human normal keratinocytes (HNK) in vitro. Secondary cultures of HNK, grown on mouse 3T3 feeder and in high calcium medium, were exposed to increasing concentrations (5, 6, 15, 30, 50 ng/ml) of minocycline. The growth rate of the treated cell cultures, determined after trypsination and enumeration of the living keratinocytes, revealed that at low concentrations (6 ng/ml), the antibiotic exerts a negative effect on cell proliferation. Western analysis of total cellular proteins obtained by lysis of 50% confluent and confluent cell layers (at days 5 and 13 of the treatment, respectively), was then performed using antibodies raised against cytokeratin K14, K10, filaggrin and involucrin. The abnormal expression pattern of these proteins in the presence of minocycline suggested an inhibition of cell differentiation notably at the terminal stages of the process. Concordant with the results of previous immunohistochemical analysis performed on skin biopsies of patients presenting with acne, our results indicate that besides the bactericidal effect, minocycline might exert an inhibitory action on terminal differentiation of the keratinocytes of the infundibular epithelium. Studies on the possible role of this antibiotic on the regulation of transcription of the genes involved in the terminal differentiation of the keratinocyte are in progress.

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MECHANISMS OF ANDROGEN INDUCTION OF SEBOCYTE DIFFERENTIATION.
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Androgens are prerequisites for full sebaceous gland development in vivo and incitants of acne vulgaris. As with other sebaceous glands, the rat prepubertal glands' growth and development at puberty are androgen dependent. We employ prepubertal cells to study the role of androgen in sebocyte development. We use a primary monolayer epithelial cell culture system containing a 3T3-L1 cell feeder layer with 10^{-6} M insulin and cortisol and 10^{-10} M cholescterol in 10% fetal calf serum (FCS). Terminally differentiating (sebum forming) colonies are defined as those containing over 5 cells positive for cytoplasmic fused lipid droplets by Oil Red O staining.

Dihydrotestosterone (DHT) 10^{-6} M has no effect on sebocyte terminal differentiation in this system, in as in other in vitro models. Only 5 ± 2% (SEM) of prepubertal sebocyte colonies cultured with or without DHT underwent terminal differentiation. We have tested the possibility that androgen receptor (AR) expression is attenuated in this system. However, immunochemistry and RNase protection assay studies suggest that the early differentiated sebocytes which predominate in culture express AR.

We hypothesized that androgen induction of differentiation is inhibited by this culture system and that serum is responsible. We substituted serum-free medium for FCS: 11 ± 2% of control and 28 ± 13% of DHT-treated colonies differentiated terminally (p<0.01).

We postulated that synthesis of lipogenesis is missing from this system since only a minority of colonies differentiate. Peroxisome proliferator activated receptors (PPARs) regulate multiple lipid metabolic pathways and are present in sebaceous glands. We found PPAR activators to induce terminal differentiation in up to 79 ± 1% of colonies. These results suggest that sebum formation terminal differentiation is mediated by PPARs.

We have used flak organs of G.S. hamsters as an androgen regulation model system for human sebaceous glands and hair follicles regulated by androgens. To elucidate the molecular action of androgens, we have isolated two androgen-dependent genes, PAR-17a and PAR-17c, from the DNA library of flank organs of male G.S. hamsters by differential hybridization method. PAR-17a was reported previously. Androgens regulated the expression in the flank organs, ear lobe and skin. PAR-17a protein was located in sebaceous gland. PAR-17c has similar expression pattern with that of PAR-17a, but its response to androgen is quicker than PAR-17a. It is expressed strongly in the liver, sebaceous glands and brain. The homology search and mRNA expression pattern suggested that it might encode a stearyl CoA desaturase of G.S. hamsters. The stearyl CoA desaturase is a key enzyme in fatty acids biosynthesis and its expression is controlled by temperature, nutritional conditions and hormones. This is the first report that the mRNA expression of stearyl CoA is regulated by androgens.

39 5α-REDUCTASE TYPE I INHIBITOR MCK386 EXHIBITS A MARKEDLY STRONGER ACTIVITY ON TESTOSTERONE METABOLISM THAN TYPE II INHIBITOR DIGUANYLATE MONOSTEROL IN HUMAN SEBOCYTES AND KERATINOCYTES IN VITRO. M. Fritsch, H. Sellmann, C.F. Orfanes, Ch. Zhou, Zouboulis. Department of Dermatology, University Medical Center, Freiburg, Germany. M. Fritsch, H. Sellmann, C.F. Orfanes, Ch. Zhou, Zouboulis. Department of Dermatology, University Medical Center, Freiburg, Germany.

5α-reductase, a protein which exists in two isoforms type I and type II, is the enzyme converting testosterone (T) to its active metabolite 5α-dihydrotestosterone (5α-DHT) and androstenedione (A) to 5α-androstenediol (5α-A-Diol). 5α-DHT excess is supposed to be among the most important factors in pathogenesis of acne. 5α-reductase has been shown to be abundant in human sebocytes in vivo and by our group in vitro. We present the conversion of 5α- androstenedione to 5α-DHT, 5α-A-Diol, 5α-DHT and 5α-reductase activity in human sebocytes and keratinocytes in vitro. In the first series of experiments, the metabolic products of the supernatant of cell cultures incubated with 20 nM 5α-DHT testosterone were separated by HPLC. The conversion rate was determined using a radioactivity scanner. After 24 hr incubation the amount of 5α-DHT, 5α-A-Diol and 5α-DHT reached a constant level in keratinocytes, about 10%, 4% and 5% of the total androgens respectively. On the other hand, the amount of A and 5α-A-Diol steadily increased. A similar pattern was seen in sebocytes, however, 5α-DHT did not reach a plateau value after 24 hr. These data give rise to the assumption, that in keratinocytes but not in sebocytes, the levels of 5α-reductase activity products is feed-back regulated. In the second series of experiments the metabolism of 5α-DHT in cultured human sebocytes and keratinocytes was assessed in the presence of the specific type I and type II inhibitors MCK386 and diguanylate monosterol, respectively. In both cell types MCK386 led to a nearly complete inhibition of the enzyme activity at a concentration of 2×10⁻⁶ M after 24 hr. Diguanylate monosterol, being ineffective at 2×10⁻⁶ M, exhibited a complete inhibitory effect at 2×10⁻⁵ M. Our results confirm the expression of type I 5α-reductase in cultured human sebocytes and keratinocytes and furthermore prove evidence of type I 5α-reductase being the predominant isoform in both cell types. The type I 5α-reductase inhibitor MCK386 blocked the enzyme activity at low concentrations, while much higher levels of the type II inhibitor were required to block enzyme activity.

41 INCIDENCE OF INCREASED ANDROGEN LEVELS IN PATIENTS SUFFERING FROM ACNE.

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Cells of hair follicles and sebaceous glands are androgen-receptive. Hairloss, seborrhoea and acne are therefore likely to occur in the presence of high circulating androgen levels. The principle androgens in the blood of women are testosterone (T) which is of ovarián and DHEA-sulfate (DHEAS) which is of adrenal origin. Chronically elevated serum androgen levels are known to stimulate the magnitude and frequency of pulsatile pituitary LH release; therefore blood LH levels are often elevated in hyperandrogenemic patients. The frequency of occurrence of elevated T, DHEAS and LH levels in the blood of women suffering from acne is unknown. We analysed therefore retrospectively our hormone data stemming from such patients. Of 223 acne patients (75%) had elevated T, 98 (44%) elevated DHEAS and 78 (35%) increased LH levels. In a population of women not suffering from skin androgenization symptoms elevated levels of these hormones are seen in less than 5%. Of the 223 acne patients 163 were again seen following treatment with an oral contraceptive (containing in most cases 35 μg ethinyloestradiol and 2 mg cyproterone acetate = 0.5 mg levonorgestrel) serum T and LH levels had normalized. Since chronic hyperandrogenism is a common cause for infertility we conclude that androgenization grounds of the skin may be an early indication of future fertility problems. To avoid these problems, treatment of acne patients with an oral contraceptive is indicated.

38 Heterogeneity and Quantitative Differences of the Type 1 5α-Reductase Expression in Cultured Human Skin Cells.

W. Chen, Ch. C. Zouboulis, M. Fritsch, V. Kodolka, C. E. Orfanos. Department of Dermatology, University Medical Center Benjamin Franklin, The Free University of Berlin, Berlin, Germany.

Hormones of steroid 5α-reductase play crucial roles in androgen physiology by catalyzing the conversion of testosterone into the more potent 5α-dihydrotestosterone in androgen-target tissues, like prostate and skin. Type 1 5α-reductase has recently been demonstrated to be mainly distributed at sebaceous glands and epidermis in human skin by using purified polyclonal antibodies that specifically recognize this enzyme on formalin-fixed skin specimens. In this study we used skin cell cultures to better define the intracellular localization and the levels of enzyme protein and mRNA expression. In immunocychemistry using a polyclonal antibody (gift of Dr. Lau-The Van, Quebec, Canada), type 1 5α-reductase was detected in the cytoplasm of human sebocytes, keratinocytes, and fibroblasts from nonsurgical area. In western blot studies, we have shown the possible existence of heterogeneity of the type 1 5α-reductase in all cell types tested, whereas human newborn foreskin keratinocytes and facial sebocytes exhibited the highest rates of expression. In northern blot studies, the amount of mRNA was most abundant in foreskin keratinocytes and facial sebocytes. The mRNA expression was revealed to be 2-10 more abundant in foreskin keratinocytes as compared to that in facial sebocytes by utilizing quantitative PCR coupled with HPLC method. Taken together, it seems very likely that in cultured human skin cells there exist (a) heterogeneity of type 1 5α-reductase protein and (b) quantitative differences in its transcriptional expression.

40 HYPERSENSITIVITY OF SEBACEOUS GLANDS TO ANDROGENS.


There is little direct evidence to support the concept that the seborrhoea of acne is mediated by an end organ hyper response to androgens. To test this hypothesis 10 pre-puberal children (aged 7 to 9 and whose parents had had acne) applied 2% monooxygenase and sebum excretion rate was measured using a direct gravimetric technique. The results demonstrated three groups. Three youngsters showed no change whatsoever in their sebum excretion whereas in 3 others there was a dramatic increase in sebum excretion by a factor of 15 fold. Two subjects had results intermediate between the non-responders and responders. This data provides evidence for the first time that there is likely to be an end organ hyper-response of the sebaceous glands to androgens. It would be of interest to know whether those subjects who showed a response to topical testosterone are those who subsequently develop seborrhoea and acne compared to those who showed no response whatever.

42 BODY WEIGHT AND ACNE EXPRESSION IN MEN.

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While in women androgenic touch is accepted in young men with acne vulgaris it was proved by Krause 1977 and Zimmer 1988. Zimmer found in 54 patients testosterone of 700 +/- 197 ng/dl (mean, sd). Comparing with 32 persons of the same age without acne he found lower serum testosterone of 530 +/- 135 ng/dl. The difference was significant (Student-T-test p < 0.00019).

The mean body weight of control group was 3 kg over the acne group. But only the small group of patients with strong acne vulgaris differ statistically by control group (p < 0.008, Test by Bonferroni-Holm).

In 1989 we checked a group of 101 young men with acne vulgaris only by body weight and length as well as of their expression of acne, and divided in 3 groups: lower grade, medium and strong.

Patients with lower-grade acne (n=19) had a weight of 73,4 +/- 7,05 kg, patients with strong acne 71,12 +/- 8,21 kg. Wilcoxon test ranking shows no significance, but a trend (2 α = 0,1). So we calculated Broca-Index and found significant difference between the two groups (α = 0,02). The peripheral testosterone concentration depends on the weight of the men. Those with higher weight show lower testosterone concentrations in the venous blood. While gonadal testosterone concentration may be an individual marker, body weight is able to vary expression of acne in adolescence. Psycic types may have mild acne, while leptosomous type tends to stronger acne, but is not possible to grade young men by Kretschmer types.
43 A STUDY OF THE HORMONAL ALTERATIONS IN PATIENTS WITH ACNE
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Acne is one of the most common dermatosis to be found. Most people have at some time of their lives suffered acne. It has long been believed that acne starts with adolescence and disappears with time. A multifactorial etiology has been acknowledged in which the hormonal factor is an important element in its genesis. Anderson (1977) thought that its formation could be related to the biosynthesis of androgens in charge of the properties of the sebaceous glands.

We carried out a retrospective study of 300 patients diagnosed with acne. Age, sex and type of acne were taken into consideration. A mini survey was prepared where patients were asked for clinical information of interest, and the relationship between acne and their states of mood, as well as any possible relationship with the ovariatic cycle.

A hormonal study in blood and urine tests was undertaken. The 14-19 age group was the one which presented greater alterations, and these alterations were found in both male and female patients. These results seem to indicate that the hormonal cycling is not such an important element in the formation of acne and that rather than being an alteration of the androgens or the estrogens, it's an imbalance of both.

45 BACTERIAL RESISTANCE IN ACNE
E. A. Eady, The Skin Research Centre, Department of Microbiology, University of Leeds, Leeds, U.K.

Antibiotics play a major role in acne therapy. Persistence of acne from adolescence into adulthood frequently necessitates several prolonged courses of treatment in individual patients. Physicians base treatment choices on personal perceptions of efficacy or cost effectiveness rather than on microbiological grounds. The selection of a predominantly resistant staphylococcal skin flora during antibiotic treatment for acne has been extensively documented. Acne patients represent a considerable community reservoir of resistant strains of these important nosocomial pathogens which can be transferred to untreated close contacts. Resistance in cutaneous propionibacteria has received scant attention in view of the central role of P. acnes in inflammatory acne. Isolates resistant to one or more anti-acne antibiotics (most commonly erythromycin) have been reported in several countries including the USA, UK, France, Germany and Japan. Carriage of resistant strains can result in therapeutic failure. Combined resistance to erythromycin and clindamycin is associated with an A→G transition mutation at base 2058 [E.chei coli numbering] within the peptidyl transferase centre of 23S RNA. This prevents ribosomal binding of both drugs. In our region, skin carriage of resistant propionibacteria by a majority of antibiotic treated acne patients and by 50% of their close contacts suggests that resistant strains are being widely disseminated.

The continued efficacy of antibiotics in the treatment of acne cannot be guaranteed against this background. Further studies are urgently needed to estimate the extent of the global problem associated with carriage of antibiotic resistant propionibacteria by acne patients.

47 ONE IN TWO CLOSE CONTACTS OF ANTIBIOTIC-TREATED ACNE PATIENTS CARRY RESISTANT PROPIONIBACTERIA ON THEIR SKIN SURFACE. Y.W. Miller, E.A. Eady, S. Nyalkrum, C.E. Jones, J.H. Covy and W.J. Caniff, The Skin Research Centre, Department of Microbiology, University of Leeds and Department of Dermatology, Leeds General Infirmary, Leeds, UK.

Antibiotic resistant propionibacteria are now present on the skin surface of a majority of antibiotic treated acne patients seen at our hospital. Carriage of resistant strains is associated with therapeutic failure on some but not all antibiotic regimens. The aim of this study was to determine whether close contacts of acne patients and members of the general public also carry antibiotic resistant cutaneous propionibacteria. Using resident colony counts, samples of propionibacteria were obtained from the faces of 72 antibiotic treated acne patients, 93 of their close contacts (patients, partners, sibs, etc) and from 180 non-acne individuals (age range 13-84 years). Resistant strains were detected by direct plating onto media containing selective antibiotics (erythromycin, clindamycin, tetracycline and trimethoprim). Forty-seven contacts (50.5%) carried resistant strains compared with 52 (72.5%) of patients. In isolates from contacts and patients, combined resistance to erythromycin and clindamycin was the most common phenotype (26.7% of contacts and 50% of patients). Twenty-five (13.9%) non-acne members of the general public carried resistant strains. This study has revealed that antibiotic resistant propionibacteria, especially erythromycin resistant strains, are already widely disseminated and are carried on the skin of individuals in the absence of selective pressure. Contact carriers may act as a source of re-infection of acne patients in whom resistant strains have been eradicated.

44 PROPIONIBACTERIUM ACNES COLONIZATION IN ACNE AND NON-ACNE
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Propionibacterium acnes colonization of skin is seen in some neonates but in general is not found in infants and young children. With onset of adrenal maturation and then gonadal development, acne vulgaris production occurs leading to sebaceous gland enlargement. P. acnes colonization correlates with sebum secretion and appears to require more than 50 mg/day for true colonization. Reduction of P. acnes during isotretinoin therapy follows reduction of sebum, and re-colonization post isotretinoin also follows return of sebum production. Acne patients show earlier onset of sebum production, earlier colonization of skin with P. acnes, and significantly higher levels of P. acnes.

The results of these studies demonstrate the interaction between sebum and P. acnes and suggest that sebaceous lipids are an essential ecological factor in P. acnes colonization of human skin.

46 PROPIONIBACTERIUM ACNES AND ACNE.

Acne is a multifactorial disease restricted to man and usually presents during and immediately after puberty. Inflammatory acne has not been assigned an infectious agent, as Koch's Postulates are not satisfied, but Propionibacterium acnes would appear to play an important role. This bacterium is a resident of normal skin and its involvement with acne is supported by an accumulation of circumstantial evidence, including primarily failure of antibiotic therapy in patients with antibiotic resistant P. acnes. If it is accepted that P. acnes is a key component of the cause of inflammatory acne, then what mechanisms are involved? We will review a variety of hypotheses to account for inflammation. Each hypothesis is based on the premise that chemical signals, directly or indirectly, reach the dermal tissue surrounding follicles from the follicular lumen. These signals invoke the normal host response mechanisms to produce a localised inflammatory response followed by healing. The hypotheses will attempt to explain the increase in P. acnes load on the skin during puberty, and are based on oxygen stress, quorum sensing responses leading to keratinocyte damage, as well as modulation of the host response via cell wall components and heat shock proteins affecting cytokine production. It is not claimed that these hypotheses will stand the rigours of experimental scrutiny but they should provoke a serious discussion on P. acnes and acne.

48 PREVALENCE OF ERYTHROMYCIN RESISTANT PROPIONIBACTERIA AND STAPHYLOCOCCUS EPIDERMIDIS IN ACNE PATIENTS IN FRANCE.
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Topical erythromycin is often used in France for inflammatory lesions of acne vulgaris. This treatment raises the question of emergence of erythromycin (ERY) resistance. The purpose of this study was to determine the prevalence of Ery-resistant (Ery-R) strains of propionibacteria (P. acnes) and staphylococcus epidermidis (S-Epis) in acne patients in France. 40 patients were included. Acne severity were between 0.5 and 3 (Burke and Caniff) and mean duration of acne was 4.36 years. 19 patients had never received Ery and 21 (64%) were previously treated with local ERY. A method of washing (Williams and Kilgman) modified and standardized by Fleurette was used for sampling micro-organisms. 38 patients (95%) carried Ery-R strains of S-Epis (MIC > 512 µg/ml) and 21 patients (52.5%) ERY R strains of P. acnes (MIC > 512 µg/ml). Eighty patients carried both Ery-R strains of S-Epis and P. acnes. Strains of Ery-R staphylococcus epidermidis (100%) and Ery-R P. acnes (42%) were present even in patients without previous treatment with ERY. However, these strains remained without in vitro resistance to zinc salt at 512 µg/ml which is zinc concentration in the skin after 2 months of 30mg glutation zinc therapy (Labcaul) in acne patients. This fact argues for the interest of a combined therapy with zinc and ERY.
49 RESISTANCE TO ERHYSYMOCCUS CLOIDAMYCIN AND CLOIDAMYCIN IN CUTFACE PROPRIONIBACTERIA IS ASSOCIATED WITH MUTATIONS IN 23S RNA.


50 THE PHYSIOLOGY OF CUTANEOUS MICROORGANISMS IN A LIPID- RICH IN VITRO MODEL OF THE PILOSEBACEOUS FOULICLE.


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Pilosebaceous follicles are generally colonized by microorganisms and the environment of individual follicles will determine the expression of inflammatory and comedogenic determinants by these colonizing micro-organisms. However, the interactions between microorganisms and the follicular environment remains unclear as even in the most severe cases of acne only 1% of follicles develop into lesions. An in vitro model system of the lipid-rich follicular environment has been developed using continuous culture technology to identify the physiological conditions which promote colonisation of sebaceous follicles by microorganisms. A stable emulsion, comparable with natural sebum, has been devised containing 80% (w/w) lipid in an aqueous defined medium. The reaction vessel is fed from the bottom by a continuous flow of lipid-rich substrate and an interchangeable solid matrix is provided onto whick the microorganisms are able to adhere. This allows interactions between the microorganisms and a variety of solid substrates to be investigated. The effects of changes in environmental factors such as pH, nutrients and gas mixtures can be evaluated. Biological activity within the reactor is assessed by the degradation of the lipid substrate using TLC. The system will help to identify the physiological conditions which promote microbial colonisation of the sebaceous follicle and the effects of keratinocyte integrity on inflammation in acne.

51 THE ADHESION OF CUTANEOUS MICRO-ORGANISMS TO HUMAN SKIN LIPIDS.


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The sebaceous-rich areas of human skin are colonized by a range of both anaerobic micro-organisms, such as the Propionibacteria, and aerobic flora which includes a range of Staphylococcal species and the diverse group of organisms referred to as the aerobic coryneforms. Certain aerobic coryneforms isolated from the sebum rich axillae have been associated with the production of components of Under Arm Odour (UAD) which include the odoriferous 16 androstene steroids and products of fatty acid degradation. A total of 49 axillary aerobic coryneforms were isolated from 14 healthy adult volunteers. Of this group the growth of 34 isolates (75.6%) was enhanced by the presence of Tween 80 in the culture medium and so were designated as lipophilic. In 28.9% of cases the isolates were shown to bioform transmembrane lipid in vitro. This has previously been shown to indicate the ability of an isolate to produce UAD in vivo.

Four lipophilic isolates, of which 2 were isolated as contaminants in propionibacteria and two non-lipophilic isolates, one of which was active against testosterone, were tested for their ability to adhere to extracted axillary skin lipids. Skin lipids were extracted from the axillae of adult volunteers using the method of Schüchter et al (1995) and the total lipid extract was separated using thin layer chromatography. These plates were treated to block non-specific binding using poly(ethylene)glycol and bovine serum albumin and overlaid with 125I-labelled cells (1 CPM / 50-100 cells). After 2hrs incubation the plates were extensively washed and the degree of adherence to the various lipid fractions assessed using liquid scintillation analysis.

52 MOLECULAR GENETIC ANALYSIS OF THE LIPASES OF STAPHYLOCOCCUS EPIDERMIDIS.


Skin Research Centre, Department of Microbiology, University of Leeds, Leeds, U.K.

Staphylococcus epidermidis is the most prevalent Staphylococcus species on human skin and is found in both normal and inflamed follicles. The lipases produced by Staphylococcus epidermidis have been implicated in colonisation and as a virulence factor. Construction of isogenic mutants will allow their roles to be studied.

Using an allele replacement technique, the lipase gene, gplC of S. epidermidis 9, a volar forearm isolate was inactivated. Studies of this mutant showed it to produce 50% of the extracellular lipase activity of the wild type, indicating the presence of a second lipase. The genome library, from which gplC originating, was rescreened and a second lipase gplD isolated. The nucleotide sequence of gplD was determined and shown to be similar to other staphylococcal lipases. Though gplC and gplD are not identical their active sites are conserved.

Subsequently other isogenic mutants, deficient in gplD and in both gplC and gplD were constructed. In this technique the single mutants were engineered using an erythromycin resistance marker to disrupt the gene in question. In the double mutant a kanamycin resistance marker was used to disrupt gplD and erythromycin to disrupt gplD. Construction of these mutants will allow biochemical analysis of the lipases and their roles in colonisation and as virulence factors to be studied in vitro and in vivo.

53 CONSTRUCTION OF A LUX REPORTER SYSTEM FOR THE ANALYSIS OF THE REGULATION OF STAPHYLOCOCCUS EPIDERMIDIS LIPASES.

C. M. Longshaw and K. T. Holland.

Skin Research Centre, Department of Microbiology, University of Leeds, Leeds, U.K.

Staphylococcus epidermidis is a prominent commensal of the human skin and is prevalent in regions of the skin containing high numbers of sebaceous follicles. The lipase activity of S. epidermidis has been implicated as a possible virulence factor and it has been demonstrated that S. epidermidis encodes and produces two lipase lipases, GplC and GplD, both of which show some homology with lipases from other staphylococcal species. It is not known however, whether there is any difference in the pattern of expression of these two genes, or the mechanism of their regulation.

In order to study the initial expression of the two lipase genes gplC and gplD in greater detail, a LUX reporter system was constructed. Southern blotting and analysis of existing nucleotide sequence data showed that the two lipase genes were not linked as a single regulatory operon. In order to isolate the individual promoters from each gene, approximately 500bp upstream of each gene was sequenced. These regions, containing the promoter elements, were then isolated and amplified by PCR and cloned into separate reporter plasmids, upstream of a luxAB reporter gene. After transformation of wild-type S. epidermidis strains with these two lipase gene reporter systems could be used to monitor the initial expression of gplC and gplD individually and consequently determine when and under what conditions each gene is expressed.

54 THE MICROBIOLOGY OF LATE-ONSET AND PERSISTENT ACNE.


Skin Research Centre, Dept. of Microbiology, University of Leeds, Leeds, UK.

Acne, most evident during the adolescent years and naturally regresses in the majority of patients in the late-teens or early twenties. There is however a small proportion of the population whose acne continues in to adult life. Previous acne studies have concentrated on adolescent acne. In this study we have examined the cutaneous microflora of persistent and late-onset acne patients and compared it with adolescents and age- and sex-matched controls.

Surface and follicular samples were obtained from the face and back using the detergent scrub wash and cyanoacrylate techniques. Individual follicles were isolated from acne patients using punch biopsies and antibody levels to Propionibacteria were measured in plasma. Further analysis of individual strains of P. acnes isolated from patients was carried out using pyrolysis mass spectrometry and a molecular biological approach.

The microbiology and antibody level data indicates that there are no significant differences between the four groups, i.e. persistent, late-onset and adolescent acne, and controls. Differentiation of P. acnes isolates showed that there are differences between and within individuals from each group. The results of this study indicate that persistent or late-onset cannot be explained by a microbiological anomaly. Although differences in P. acnes isolates do exist they can offer an explanation for the persistence of acne, since differences are found throughout the groups.
55 AN ULTRASTRUCTURAL STUDY OF ACNE, WITH SPECIAL REFERENCE TO PROPOIONIBACTERIUM ACNES.
M. Morokushi, M. Toyoda, S. Higaki, Department of Dermatology, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Ultrastructural studies were utilized to reveal the morphological characteristics of acne lesions such as microcomedones, closed comedones, open comedones, and inflammatory papules and pustules. Fine structures of Propionibacterium acnes isolated from acne lesions were also examined.
The fine structural characteristics of comedones were cohesion and impaction of keratinized cells with persistence of desmosomes and desmosomal discs and large number of intracytoplasmic lipid inclusions of various sizes. In the infundibulum of pilosebaceous unit, multiple Propionibacterium acnes were observed. They were characterized by the thick cell wall composed predominantly of peptidoglycan, ribosomes and mesosomes in the cytoplasm and a medium without nuclear membrane.

In the inflammatory lesions, extensive infiltrates of macrophages and neutrophils were observed in and around the pilosebaceous unit which revealed widening of interfollicular spaces, disappearance of basal lamina, and degeneration and necrosis of follicular epithelial cells. Macrophages and neutrophils showed large numbers of lipid inclusion bodies. A high density of extracellular fat droplets and neutral fats were observed in the comedones, some of which revealed loss of cytoplasm electron density and degeneration of cell walls.

56 ANTI-BIOTIC RESISTANT PROPOIONIBACTERIA AND ACNE: CRISIS OR CONJUDOM?
C. E. Jones, S. Vykynama, E. A. Eady, J. H. Cove, W. J. Cuffiffe, The Skin Research Centre, Department of Microbiology, University of Leeds and *Department of Dermatology, Leeds General Infirmary, Leeds, UK.

Carriage of resistant propionibacteria is one cause of poor therapeutic outcome in some antibiotic treated acne patients. For five years, we have monitored the carriage of resistant strains by directly inoculating samples obtained with moistened swabs from facial skin onto media containing selective antibiotics. To date, 2,467 patients have been screened. In 1992, 38.1% carried resistant strains. Erythromycin resistance (28.8% of patients) was the most common. The overall prevalence of resistant strains did not change significantly between 1992 and 1994. However, in 1995, 48.5% of patients carried resistant strains and by November 1996 the number had further increased to 61.8% (P<0.001, x²).

The prevalence of tetracycline resistant strains has risen steadily from 17.8% in 1992 to 26.0% in 1996. In contrast, the prevalence of erythromycin resistant strains increased by 8.8% between 1992 and 1995 but by 15.8% between 1995 and 1996. Most of these isolates are cross resistant to clindamycin (88% resistant). MIC determinations on 100 resistant and 25 fully sensitive strains collected during 1996 show changes compared with strains collected up to 1991. Paradoxically, the minimal MIC for strains resistant to clindamycin and erythromycin dropped fourfold whereas the minimal clindamycin MIC has doubled. A significant number of isolates with intermediate MICs have emerged. These changes may reflect the greater use of minocycline compared to other tetracyclines in the last decade.

57 ANTI-LIPASE ACTIVITY OF KAMPO FORMULATIONS (JAPANESE HERBAL MEDICINES), COPTIDIS RHIZOMA AND ITS ALKALOIDS AGAINST PROPOIONIBACTERIUM ACNES.
M. Toyoda, S. Higaki, M. Morokushi. Department of Dermatology, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Anti-lipase activity of Kampo formulations, Coptidis Rhizoma (CR), and its alkaloids against Propionibacterium acnes were examined in vitro. The growth and lipase activity of P. acnes were examined with the amount of propionic and butyric acids in the medium as parameters respectively by using gas chromatography. In tributyryl-phospholipase-Yeast extract Glucose medium with each concentration of Kampo formulation, CR, or the alkaloids added, the production of propionic acid was suppressed remarkably more than that of butyric acid. The suppression of production of these acids by CR was higher than that of the alkaloids. Furthermore, no lipase-negative colonies were found on the medium to which each concentration of Kampo formulations were added.

From these observations, we concluded that not only Kampo formulations and CR, but also their alkaloids, showed suppression of growth of P. acnes, which reduced anti-lipase activity. It was suggested that Kampo formulations and Kampo crude drugs with anti-lipase activity to P. acnes should be synergistic when their ingredients are combined.

58 THE SEBACEOUS GLAND AS AN IMMUNOCOMPETENT ORGAN.
Thomas Jüger and Markus Bohm, Dept. of Dermatology and Ludwig Boltzmann Institute for Cell Biology and Immunobiology of the Skin, University of Münster, Germany.

It is well established that the functional state of sebaceous glands in vivo is a tightly regulated process orchestrated through the interaction of male sex hormones and functional androgen receptors expressed on sebocytes. In the last years, however, it has become increasingly apparent that sebocytes like epidemal keratinocytes express a variety of cytokines which are implicated in inflammatory and immune responses. For example, sebocytes in vivo have been found to highly express alpha-tumor necrosis factor (α-TNF) as determined by immunohistochemical and in situ hybridization methods. Moreover, certain neuropeptides including vasodilating intestinal peptide (VIP) and propiomelanocortin (POMC)-peptides as well as their receptors have been localized within the pilosebaceous unit of murine and human skin. Since POMC-peptides such as α-melanocyte stimulating hormone (α-MSH) have recently been shown to exert important immunoregulatory effects by antagonizing the function of proinflammatory cytokines (e.g. interleukin-1, interleukin-10), modulation of inflammatory molecule expression (e.g. B7-2) or suppression of macrophage-derived nitric oxide, complex interactions between these mediators and their target cells within the pilosebaceous gland seem to exist. The successful cultivation of sebocytes will provide a model which will allow the effect of the mediators to be studied in detail in order to shed light into the various pathophysiological conditions of sebaceous glands.

59 IS ACNE A HYPERSENSITIVITY DISEASE? Gay F. Webster, Department of Dermatology Jefferson Medical College, Philadelphia, Pa, USA.

The great variation in severity of inflammatory disease among acne patients is one of the central unsolved issues in the understanding of acne. Although all post-pubertal individuals have significant levels of Propionibacterium acnes, substantial sebum secretion, and detectable follicular plugging, only a minority have severe inflammatory acne. Hyperandrogenism has been advocated as an explanation and is clearly involved in some patients; yet among virilized women only some have significant acne, although androgen levels are elevated in all, perhaps suggesting that hyperandrogenism serves to mask the basic defect in suitably predisposed individuals. A potential explanation for the variation of acne severity that is consistent with these observations is a differential sensitivity to P. acnes. The evidence for an immune etiology of of inflammatory acne will be presented.

60 THE POSSIBLE ROLE OF REACTIVE OXYGEN SPECIES GENERATED BY NEUTROPHILS IN MEDIATING ACNE INFLAMMATION.
H. Akamatsu, T. Tada, Department of Dermatology, Kansai Medical University, Moriguchi, Japan.

In the pathogenesis of acne inflammation, Propionibacterium acnes (P. acnes) seems to play an important initial role by producing low-molecular-weight chemotactic factors which attract a variety of cytokines which are implicated in inflammatory and immune responses. For example, sebocytes in vivo have been found to highly express alpha-tumor necrosis factor (α-TNF) as determined by immunohistochemical and in situ hybridization methods. Moreover, certain neuropeptides including vasodilating intestinal peptide (VIP) and propiomelanocortin (POMC)-peptides as well as their receptors have been localized within the pilosebaceous unit of murine and human skin. Since POMC-peptides such as α-melanocyte stimulating hormone (α-MSH) have recently been shown to exert important immunoregulatory effects by antagonizing the function of proinflammatory cytokines (e.g. interleukin-1, interleukin-10), modulation of inflammatory molecule expression (e.g. B7-2) or suppression of macrophage-derived nitric oxide, complex interactions between these mediators and their target cells within the pilosebaceous gland seem to exist. The successful cultivation of sebocytes will provide a model which will allow the effect of the mediators to be studied in detail in order to shed light into the various pathophysiological conditions of sebaceous glands.

Antibiotics such as Tetracyclines and Macrolides are widely accepted as an effective drugs in the treatment of acne. The efficacy of these drugs in acne has been found to be not only to the reduction in number of P. acnes, but also to the inhibitory effects on the production of P. acnes-associated inflammatory mediators. Tetracyclines and Macrolides effectively reduced ROS generation by neutrophils, whereas another antibiotics such as Penicillins did not affect the ROS produced by neutrophils. In addition, neutrophils from patients with acne inflammation produced a mLuminogenic greater amount of ROS than those from patients with acne comedones. Amount of ROS from patients with acne inflammation was significantly decreased after the treatment with oral administration of ward doses of minocycline. These findings suggest that ROS generated by neutrophils contribute to the damage of follicular epithium, leading to the excretion of follicular contents into the dermis, and subsequently resulting in a variety of inflammatory processes.

Furthermore, the proportion of lipoic acid, a free fatty acid, has been shown to be markedly decreased in acne skin and comparable with normal hair follicle. Lipoic acid suppressed ROS generated by neutrophils, being like to indicate that a decreased proportion of lipoic acid in acne comedones contribute, in part, to the worsening of acne inflammation.
INFLAMMATION IN ACNE VULGARIS: FAILURE OF SKIN MICRO ORGANISMS TO INDUCE KERATINIZATION IN II-1 PRODUCTION IN IVITRO. E.Ingham, C.E.Walters, E.A.Eady, J.H.Cove, J.N.Kearney and W.J.Cantill". The Skin Research Centre, Deps of Microbiology and Dermatology, U. of Leeds, Leeds, UK. Immunohistological studies of evolving inflammation in acne vulgaris have shown that Cl. acnes lipases for the inflammatory response. We have hypothesised that comedonal II-1 α might initiate the accumulation of T-cells since acne comedones contain sufficient I-1 α to initiate non-specific inflammation if released into the dermis. Micro-organisms are strongly implicated in the pathogenesis of acne vulgaris. Therefore, we have studied the capacity of the cutaneous probiotics to modulate II-1 α production by keratinocytes in vitro. Normal human keratinocytes (NHK, 2 strains) and SVK 14 cells were cultured for up to 72 h with microbial preparations (whole cells: supernatant fluid, cell fractions) from P. acnes, P. girmmogromas, Staphylococcus epidermidis, S.epidermidis, Staphyloccocus and Malassezia globosa. The production of II-1 α was determined by ELISA and bioassay. There were no significant differences in II-1 α levels of the co-cultures compared to controls (culture medium and keratinocytes alone) at 24 and 48 h. At 72 h, S. epidermidis and S.epidermidis cells stimulated significantly more II-1 α (p 0.01) by NHK strain 1 and SVK 14 cells respectively. M.globosa fractionate increased II-1 α production (p 0.05) in co-cultures with NBK strain II. The failure to consistently demonstrate modulation of II-1 α production by keratinocytes in the presence of skin microorganisms suggests that they play no role in the generation of comedonal II-1 α in vitro.

PROINFLAMMATORY INTERLEUKIN-1 INDUCES VEGF/VPF EXPRESSION IN CELLS OF THE HUMAN PILOSEBACEOUS UNIT. U. Kondrolyakova, U. Blume-Petray, V. Kedela, C. Sommer, H. Gerl, S. Jahnkowsky, C. Nozohara, German J. of Dermatology, University Medical Center Benjamin Franklin, The Free University of Berlin, Berlin, Germany. Vascular endothelial growth factor/Vascular permeability factor (VEGF/VPF) influences local inflammatory processes due to its diverse actions on endothelial cells, resulting in vascular hyperpermeability with plasma protein leakage and modulation of endothelial proangiogenic activities as well as due to chemotaxis of monocytes and neutrophils and proliferation of the activated microphages. In regard to its possible role in skin disorders with pronounced inflammation such as acne, we investigated, if strong proinflammatory stimulation influences VEGF expression in various epithelial and mesenchymal cells of the human pilosebaceous unit. The effect of II-1 α and II-1β on VEGF mRNA and protein expression in cultured human sebaceous glands, follicles, papilla cells and follicular keratinocytes was investigated by RT-PCR and radiimmunoassay. II-1α and II-1β strongly induced an immediate increase of VEGF mRNA in dermal papilla cells, followed by rapid synthesis of 46 K VEGF protein; high VEGF RNA levels were still present after 24 h. Follicular keratinocytes also responded to II-1 stimulation with significant VEGF mRNA up-regulation. Based on the observed stimulation of VEGF expression by proinflammatory cytokines in mesenchymal and epithelial cells of pilosebaceous unit we suggest, that modulation of VEGF expression can contribute to the development of the inflammatory cascade having an important role in the pathogenesis of acne.

INVESTIGATIONS INTO THE MODES OF ACTION OF DAPSONE IN INFLAMMATORY ACNE. C.G. Schmid, H. Plechmacher, G. Wozel Klinik und Poliklinik für Hautkinderhautklinikum Carl Gustav Carus, T Dresden, Germany. Recently, interest in dapsone (diaminodiphenylsulfone, DDS) has been renewed owing to its therapeutic value in various opportunistic infections in AIDS patients. It is also being successfully used in cases of severe inflammatory acne (IA) when oral retinoids cannot be administered or fail. Considering its weak antibacterial activity against Propionibacterium acnes (PA), anti-inflammatory effects of DDS seem to be of crucial importance in IA, in our attempts to clarify the anti-inflammatory action of DDS, elasmobase assays failed to detect a suppression of leucotiene B4- induced accumulation of polymorphonuclear leucocytes (PMN) in skin biopsies of healthy volunteers taking 100 mg of DDS daily. In vivo studies using a human whole blood model demonstrated a significant, dose dependent suppression of LPS (1 μg/ml)-induced interleukin 8 (IL-8) production by DDS (1 to 20 μg/ml) up to 50% of baseline levels. IL-1α, a powerful chemoattractant for PMN, has recently been shown to be induced in large amounts by DDS in vitro. Thus, suppression of chemotactic factors might contribute to the therapeutic efficacy of DDS in IA in analogy to the experimental findings of Webster, Akamatsu and others on tetracyclines and erythromycin.

L-ASORBIC ACID INHIBITS UVA-INDUCED LIPID PEROXIDATION AND IL-1α mRNA EXPRESSION IN CULTIVATED HUMAN KERATINOCYTES. INCORPORATION OF THIS ANTIOXIDANT IN SUNSCREENS IS USEFUL. S. Hazenberg, M. Kreidel, C. C. Ofner, S. Kedela, C. E. Orbanz, Dep. of Dermatology and Venerology and of Immunology, Otto-von-Guericke-University, Magdeburg, Germany. An effective sunscreen protection is necessary against skin cancer and in many skin diseases such as photo-sensitizing dermatoses, however, also in acne patients when treated with photosensitizing drugs (e.g. tetracycline, retinoids). The main effect of UVA light on human cells is the induction of free radicals. We tested the antioxidant capacity of L-asorbic acid on lipid peroxidation and II-1α, II-6 mRNA expression in UVA-irradiated human keratinocytes. UVA-induced lipid peroxidation was inhibited by L-asorbic acid in a concentration dependent manner: malonaldehyde (MDA) protein equivalent was reduced by ~7% (0-6M), and the thiobarbituric-acid-reactive substances (TBARS) showed a maximum concentration-dependent decrease of ~9% (0-6 M) in L-asorbic acid-supplemented culture compared to controls (p 0.05). LDL release was decreased by ~45% (0-6 M) in L-asorbic acid-supplemented keratinocyte cultures, indicating protection against cell death (p 0.05). L-asorbic acid was able to downregulate II-1α mRNA expression in both UVA irradiated and non-irradiated cells, however, II-6 mRNA expression remained un influenced. These findings indicate that L-asorbic acid has a cell protective effect on UVA-induced lipid peroxidation and antiinflammatory properties in keratinocytes. Therefore, the application of L-asorbic acid in sunscreens can be recommended.
67 IN VITRO EFFECT OF PROPIONIBACTERIUM ON MACROPHAGE ACTIVATION. W.I. Lee and A.R. Shaltis. Department of Dermatology, SUNY Health Science Center at Brooklyn, Brooklyn, N.Y. U.S.A.

Macrophages exert inflammatory, hormonal, antibacterial and antifungal activity upon activation by cytokines or inflammatory mediators such as IL, TNF-α, LPS. Most of these activities are initiated by nitric oxide (NO) production. In this study, we have examined and compared the role of the cell wall suspension of Propionibacterium granulosum (CWPG) and Carbonylcytotoxic (CMG) with lipopolysaccharide (LPS), a known macrophage activating factor on NO production in the murine macrophage cell lines (RAW 264.7 and J774.GR). Macrophage activation assessed by NO Analyzer (Sievers), was induced with various concentrations of LPS (1-100 ng/ml) with or without addition of CWPG and CMG. Similar to LPS, CWPG and CMG induced macrophage activation in a dose-dependent manner in RAW 264.7 cells, but not in J774.GR. This increase in NO production reached in plates at 2% and 10 ng/ml for CWPG and LPS, respectively. A concentration of 2% CWPG resulted in a stimulating effect as high as 50% (ex. LPS only -21.3 μM; LPS + CWPG -35.7 μM). During the aging process, macrophage and/or skin dendritic cells lose their responsiveness and this phenomenon is amplified by exposure to UV radiation. Our data seem to indicate that bacterial products and polysaccharides may contribute to the preservation of the skin's immune defense system, and to the maintenance of the macrophage "alertness", possibly slowing one of the major aging processes.

69 IMMUNE STATE OF PATIENTS WITH ACNE VULGARIS Jakubčová, B., Bajtálková, J., Bajtálková, K., Dusbábiková, M.

Clinical of Dermatology, University P.J. Safárik, Košice, Slovak Republic

Several factors participate in the pathogenesis of acne vulgaris: increased secretion of sebum and lipo-retinoid, genetic factors, bacterial flora, hormonal and immune factors. The authors decided therefore to examine the actual state of immunity in patients with acne vulgaris and they revealed reduced levels of active E rosettes and elevated values of circulating immuno complexes, as compared with healthy subjects. Elevation of values of immuno globulin G, the S-complement component, complement enzymes alpha-1 antitrypsin and alpha-2 macroglobulin in patients with acne vulgaris is a result of the presence of a non-specific inflammation in repeated relapses of the disease.

The authors recognized examination of the actual immunity state of patients with acne vulgaris focused on the cellular immunity component. Its deficit is indication of immunodeficiency treatment, in particular when there general treatment is ineffective or contraindicated.

71 ANTI-INFLAMMATORY EFFECTS OF ERYTHROMYCIN ON MONOCYEOE CELL ADHESION MOLECULE EXPRESSION IN VITRO C.C. Walters, S. Richards, E.A. Eady, J. H. Cove, W. J. Curtiflle and E. Ingham. The Skin Research Centre, Departments of Microbiology and Immunology, The University of Melbourne, HAMDS, Institute of Pathology, Leeds General Infirmary, Leeds, UK.

Erythromycin (EM) is used in acne therapy and may exert its therapeutic effect, in part, by anti-inflammatory activity. The aim of this study was to investigate the effects of EM on mononuclear cell adhesion molecule expression since extravasation and migration of mononuclear cells is a primary event in acne inflammation.

Freshly isolated mononuclear cells (MNC) from healthy adult donors were cultured with and without antigen or mitogen, in the presence and absence of EM. FAC's analysis of viable cells (propidium iodide negative), was used to determine the expression of ICAM-1 and LFA-1. ICAM-1 expression by unstimulated monocytes after 24 h was constantly and consistently high in controls, but was reduced in the presence of 60 μM EM (p<0.01). ICAM-1 expression on unstimulated lymphocytes after 24 h was not significantly reduced by EM. However, LFA-1 expression on lymphocytes cultured for 8 h was reduced compared with controls, by 80 μM EM and above (p<0.01), in the presence and absence of antigen (tetanus toxoid). This study demonstrates that EM can modulate ICAM-1 and LFA-1 expression by MNC in vitro, which may partially explain why topical EM has a marked anti-inflammatory effect in vivo.

72 THE SEBACEOUS GLAND AS A TARGET FOR ALLERGIC DRUG REACTIONS U. Jappe, A. Reinhold, H. Gönül, H. Gönül, H. Gönül. (1) Department of Dermatology and Venereology, (2) Department for Experimental Medicine, Otto-von-Guericke University of Magdeburg, Germany.

The report on two cases with a seborrhoeic drug reaction, one is a seventy-year-old male patient undergoing multiple drug exposure including kava-kava extract, the second is a fifty-two-year-old female with kava-kava extract monotherapy. The prominent skin lesions in both cases were confluent or disseminated erythematous patches and papules with a distribution in the sebaceous gland rich regions of the face, the upper chest and back. Dermatopathology of both cases revealed a strongly aggressive CD4+ and CD8-positive lymphocytic infiltrate affecting sebaceous gland lobules but not the follicular infundibulum. Some of the glands or single lobules were disrupted and had become necrotic. Prominent cellular infiltrates were surrounding the adnexal vessels. In contrast to the strong sebocytic attack the epidermis and eccrine sweat glands were not affected. Standardized diagnostic skin tests, allergy testing revealed a specific lymphocyte response in vitro only with kava-kava extract in the first case and a positive reaction for kava-kava extract in the second case as well as in patch-test in our second case. Kava-kava extract is isolated from the roots of the plant Piper methysticum and consists of several kavapyrones with different pharmacological effects. The substances are lipophilic. Nevertheless, the data of kavapyrone pharmacology are still incomplete. Since the lesions were observed in skin with the highest density of sebaceous glands we conclude that lipophilic kavapyrones had enriched in the sebaceous lobes provoking a lymphocytic attack and resulting clinically in an acute drug eruption. The seborrhoeic drug eruption probably is a new entity strongly associated to systemic kava-kava therapy.
ABSTRACTS

73 THE SEBACEOUS GLAND: THE PRIME TARGET IN EOSINOPHILIC PUSTULAR FOLLICULITIS?
U. Blume-Peytavi, W. Chen, N. Djendji, C.C. Zouboulis, S. Goroh, Department of Dermatology, University Medical Center Benjamin Franklin, The Free University of Berlin, Germany

We describe a 29-year-old Caucasian man with eosinophilic pustular folliculitis (Ojului's disease), primarily targeted towards the sebaceous gland, who was successfully treated with isotretinoin. For 7 months the patient had shown recurrent eruptions of sterile papules, papules and plaques on the face and at the neck, erythematous, slightly scaly patches on the upper trunk, accompanied by considerable blood eosinophilia. Histologic examination showed prominent dermal infiltrates with predominant localization around the sebaceous glands and seboglandular ducts presenting severe spongiosis leading to dissolution of the epithelia and infiltration by eosinophils and mononuclear cells. Immunohistochmistry revealed downregulation of differentiation antigen of sebaceous glands and seboglandular ducts presenting severe spongiosis leading to dissolution of the epithelia and infiltration by eosinophils and mononuclear cells. Immunohistochmistry revealed downregulation of differentiation antigen of sebaceous glands and seboglandular ducts presenting severe spongiosis leading to dissolution of the epithelia and infiltration by eosinophils and mononuclear cells.

75 LOCALISATION OF KERATIN 17 mRNA IN ACNE
D.B. Holland, S.G. Roberts and W.J. Culurci, Dept. of Dermatology, Leeds General Infirmary, Great George St., Leeds LS1 3EX, UK.

Keratin (K) 17 is not found in normal epithelium but is aberrantly expressed, together with K6 and K16, in such conditions as pustular and wound healing. Although expressed together, the regulation of the transcription of the K17 gene is specifically induced by IFNγ while transcription of the K6 and K16 genes is induced by EGF or TGFα. Previously we have shown the presence of K6 mRNA in follicular keratinocytes of acne lesions so it was of interest to examine, by in situ hybridisation (ISH), whether K17 mRNA was also present. ISH was carried out using a digoxigenin labelled riboprobe to K17, on 4μm paraformaldehyde fixed paraffin embedded sections of normal follicles and acne lesions. In normal follicles from acne and non-acne skin K17 mRNA was only found in the sebaceous glands. In microcomedones and comedones there was increased expression, with K17 mRNA also being found in the basal and lower follicle, in close proximity to areas of cellular infiltrates around the follicle wall. In clinically inflamed lesions K17 mRNA was strongly expressed along the length of the follicle wall and frequently in the perifollicular epidermis. Three patterns of mRNA expression for K17 paralleled those for K6, although K17 was not located in cells adjacent to the basal layer, as was K6.

This study showed that the presence of K17 mRNA in follicular keratinocytes coincided with the nearby presence of a cellular infiltrate. K17 is specifically induced by IFNγ, a product of Th-1 lymphocytes, suggesting that in acne there is a delayed type hypersensitivity.

77 THE THEORY OF COMEDONE CYCLING

The dynamics involved in the formation of non-inflamed acne lesions remain unexplained. We have proposed a precomedonal cycle theory for the formation of microcomedones from normal follicles, in which we assign clinically normal follicles from acne patients different stages according to their type of K16 and Ki-67 labelling (Aldana et al., 1996. J.ID. 104: 488). We have now investigated proliferation in comedones using the same antibodies, Ki-67 is a marker for abnormal differentiation and Ki-67 which labels cycling epidermal cells. K6 labelling if present was located suprafollicularly in the comedonal wall and occasionally extended into the interfollicular epidermis. Ki-67 positive cells were located in the basal layer of the epidermis and duct. Positive nuclei were counted in the follicle wall, interfollicular and subperifollicular epidermis and then expressed as a percentage of the total number of nuclei in each area. Results fell into 4 groups:

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Thus we conclude that comedones also form part of a cycle which is similar to the precomedonal cycle except that overall Ki-67 percentages are significantly higher in comedones. It is proposed that the precomedonal cycle and the comedonal cycle may be linked. This cycling could explain stages of comedonal development and natural resolution of comedones.

74 CLINICAL ASPECTS OF ACNE
A.R. Shalita, Department of Dermatology, SUNY Health Science Center, Brooklyn, NY, USA.

It is a common myth, shared by the general public and many physicians, that acne is a simple disease which usually disappears with maturity, in the third decade of life. Dermatologists, however, are well aware of the many different manifestations of acne, in addition to the more traditional forms of mild, moderate and severe teen-age acne, there is an increased frequency of visits to dermatologists by adults with acne, particularly women. Furthermore, a variety of different forms of acne have been described as the result of specific factors such as detergents, mechanical agents, cosmetics such as hair powders and drugs such as steroids. Acne can leave both physical and psychological scarring which can have devastating effects on the affected individual. Modern therapeutic intervention can do much to prevent or limit these sequelae.

76 EPITHELIAL DIFFERENTIATION OF DRAINING SINUS IN ACNE INVERSA
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The draining sinus is a late complication of several forms of severe acne, leading to various periodically inflamed lesions, that are undermined by a system of fistulas, supposed to be of follicular origin. We investigated the expression of various cytokinase (CK) and desmosomal proteins of draining sinus in acne inversa using monoclonal antibodies (Mabs) in immunohistochemistry on paraffin embedded sections. CK 5/14 and CK 1/10 Mab showed essentially the same staining pattern as in the epidermis and the upper ploseobaceous duct. CK 6, 17 and 16 were found to be expressed strongly in all cell layers of the sinus. Surprisingly, we also found antibodies against CK 19 positive in some parts of the sinus, whereas a CK 20 antibody remained negative. Mabs against the desmosomal plaque proteins desmoplakin I + II, plakoglobin and plakophilin I stained all suprabasal layers, whereas Mabs against the isoforms of the desmosomal transmembrane protein desmoscin and desmogleins showed differentiation specific staining patterns.

The draining sinus of acne inversa is covered by a stratified epithelium, that is corresponding as indicated by the expression of CK 1/10 and desmoscin I. 1 is proliferative as shown by the strong expression of CK6/16/17 and 17 shows in some parts even simple epithelial type CKs. The sinus epithelium is clearly distinct from the lower outer root sheath of the hair follicle, but shows strong similarities to the upper ploseobaceous duct from which the inflammatory process seems to emerge.

78 RUPTURE OF THE FOLLICLE IN ACNE VULGARIS FOLLOWS THE EROSION AND/OR PIERCING OF THE FOLLICULAR WALL BY SEBUM CALCULUS: THE SEBOITHI.
Aldo Gonzalez, MD Pathology Services, Inc., Cambridge, MA, USA

The popular concept today is that the comedo is the link between a follicle and acne. My different proposal is that in the comedo, or often without one, a pathogenetic concretion of sebaceous and the sebolith. It is this calculus that perforates the follicle and induces the inflammatory stage of acne. The comedo would, in this light, become a minor player in the pathogenesis of acne, just a concomitant (at times) to a harder and more effectively perforating structure. The comedo might just indicate a general state of stagnation and poor drainage of the follicle that also induces the crystallization of sebum into a rocky sebolith.

The sebolith is not calcified but is hard enough to break the containing follicle, potentially at levels that are less epidermal and therefore weaker to the decubital pressure that erodes or pierces the wall. The sebolith is very frequently polarizable and may reach near 1 mm. It has been erroneously called a "cavern", as it appears optically, yet falsely empty in plain preparations not subjected to polarization. In ruptured and inflamed follicles is common to see seboliths thinning or rupturing their walls or amid dermal abscesses, but inflammation may be minimal or nil, almost certainly subclinical, near successfully perforating seboliths. Their ubiquitous but incidental presence in specimens from the face or back of adults suggests that many inflammatory foci are needed to bring into personal or clinical attention the disorder as clinically recognized acne.
THE ROLE OF CYTOCHROME P-450 IN ACNE.

A. Tanaka, N. Kudo, T. Sone, S. Sawada, Y. Hasegawa, K. Kasama, and H. Takahashi. Department of Dermatology, Osaka University Graduate School of Medicine, Japan.

Cytochrome P-450 is a family of enzymes that metabolize drugs and other chemicals in the body. In acne, P-450 enzymes play a role in the metabolism of androgens, which can lead to the formation of comedones. This study investigated the role of P-450 enzymes in the development of acne.

ECLA GRADING SCALE: A NEW SEMI-QUANTITATIVE METHOD FOR ASSESSING THE SEVERITY AND THE EXTENSION OF ACNE LESIONS.

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The ECLA grading scale is a semi-quantitative method used to assess the severity and extension of acne lesions. This study evaluates the reliability and validity of the ECLA grading scale for assessing acne.

ACNE - BASICS, PATHOGENESIS, DIFFERENTIAL DIAGNOSIS, CLINIC AND THERAPY: DEVELOPMENT OF AN INTERACTIVE HYPERMEDIA APPLICATION ON CD-ROM FOR COMPUTER-BASED LEARNING AND INSTRUCTION.

M. Schramm, H. P. M. Gollnick, Department of Dermatology, Otto-von-Guericke-University Magdeburg, Germany.

This study describes the development of an interactive hypermedia application on CD-ROM for teaching acne. The application is designed to be used in a computer-based learning and instruction setting.
ABSTRACTS

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SEBUTAPE INVESTIGATION OF ACNE LESION APPEARANCE.

Individual sebaceous glands secrete sebum at differing rates. High secretors or 'gushers' can be distinguished from low secretors using sebutepe. This study was designed to determine the natural history of acne lesions, testing the hypothesis that 'gushing' sebaceous glands will ultimately develop into acne lesions. It is important to clarify this if individual sebaceous glands are to be investigated in vivo.

Five individuals with facial acne (mean age 18.8 yrs) and five without acne (mean age 28.8 yrs) were followed twice a week for four weeks during which all topical treatments were stopped, but oral medications continued.

On each occasion after degreasing with an alcoholic swab, sebutepe was applied to a flat, spot free area of each subject's temple, forehead and cheek for an hour. An acetate template was made of each area, marked with specific facial characteristics to ensure the same area was investigated each time. Sebutepe was stored on cards at 4°C and permanent photocopied records were made within 24 hours.

Results confirmed those of Pierard (Pierard, G.E., Dermatologia 173: 61-65 1986) of differences in individual gland sebum output and individual gland variability in sebum production over time. They also showed that around an inflamed acne spot there was a clear area on the sebutepe indicating functional failure of sebum output. Preliminary sebutepe observations did not indicate that 'gushing' sebaceous glands always develop before acne lesions occur.

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ACNE AND ATOPIC DERMATITIS.
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That there might be an inverse correlation between Acne vulgaris and atopic dermatitis is an old dermatological opinion, but concerning this, very few data exist in the literature. We asked 241 patients with atopic dermatitis by aid of a questionnaire, if there were an Acne in the past. 19% of the patients had Acne in the history or actually. To compare this figure with the incidence of Acne in a general population, it is realized that former studies are very different. By assuming, that the incidence of Acne vulgaris in a normal population is between 25 and 50%, our findings of 19% in patients with Neurodermatitis is slightly lower, but higher than expected. This result is also interesting for a proper external therapy, because atopic patients don't tolerate skin creams as good as normal acne patients.

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DISORDERS OF SEBACEOUS GLANDS IN THE POPULATION OF TARTU AND TARTU COUNTY.
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Department of Dermatology and Venerology, Tartu University, Tartu, Estonia

In the years 1994 by June 1996 640 patients with acneform eruption were treated at the Out-Patient Department of the Tartu Clinic of Dermatology and Venerology. Their diseases were diagnosed as acne papulo-pustulosa in 412 persons, acne conglobata - 21, acne excorie - 4, rosacea and demodicidosis in 202 persons, acne infantum one person. 270 females and 143 males with the diagnosis acne papulo-pustulosa consulted in a dermatologist. 36.1% of the persons had also trunci acne. Among them were pupils 45% and students 15.3%. Treatment with oral antibiotics was prescribed to 25.6% of the patients. 20.5% of the patients had got treatment in the course of 2 years. The disease of 154 females and 48 males was diagnosed as rosacea and demodicidosis. Microscopic analyses was carried out in 186 persons: 126 patients had Demodex breve, 27 had Demodex longus and 33 had both variants. According to social status there were pupils and students 18,8%, employees 43,6% and workers 14,5%. To 105 patients was prescribed metronidazole, 2 patients 1000 mg, 24,7 of patients needed up to 3 years treatment and 8,4% got in-patient treatment. Patients were assessed with MMPI test. Results of MMPI test aid better understand the psychologival condition of patients and it is very important in the management of acne and related disorders.

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ACNE NEONATORUM: DIAGNOSIS AND TREATMENT.
T Jansen, M Michelsen, G Pleisig. Department of Dermatology, Ludwig-Maximilians-University of Munich, Germany.

Acne is generally considered to be a disease of adolescence, often extending into adulthood. Acne in newborns (acne neonatorum) tends to be overlooked because it is usually minor and transient. A male newborn is reported who developed numerous open and closed comedones intermingled with some papulopustules on the chin and both cheeks until the fourth month of life. The skin was oily, indicating androgen-stimulated sebaceous gland activity. A diagnosis of acne neonatorum was made. Topical treatment including benzoyl peroxide (2.5 %) and tretinoin (0.025 %) led to complete resolution of all skin lesions within six weeks. The exact pathogenetic mechanisms of acne neonatorum remain unclear, but it is probably related to endocrine changes during intra-uterine life. Differential diagnosis includes neonatal sebaceous gland hyperplasia, acne infantum (which starts after three months of life), acne venenata infantum, acneiform reactions following the administration of drugs such as lithium and phenytoin during pregnancy (fetal hyaladystrophy), adenal cortical hyperplasia (21-hydroxylase deficiency). If necessary, topical therapy with retinoid or mild tretinoin preparations may be useful. If inflammatory lesions are predominant, topical erythromycin or benzoyl peroxide may be tried.

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THE SAPHO SYNDROME: DOES IT EXIST?
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We report on five patients presenting to our departments in Magdeburg and Berlin with a pustulosis palmoplantaris, bone and joint pain predominantly of the sternocostoclavicular joints and acniform lesions of the face. In 1987 the term SAPHO was coined by Kahn and Chemtob, designating a group of frequently combined manifestations as Synergistic Acne, Pustulosis, Hyperostosis and Osteitis. They carried out a multicenter study with 85 patients with either musculoskeletal problems combined with palmoplantar pustulosis and severe acne or osteoarticular syndromes mainly affecting the chest wall and found out that the bone involvement in all patients was identical according to the radiologic and pathologic features. The term SAPHO syndrome was proposed to summarize a number of different terms for similar conditions like arthritides pustulosa or Tietze syndrome.

We compared the findings in our patients with the conditions that were found in patients with the called SAPHO syndrome and discussed whether these conditions can be justified by this term, which possible pathogenetic factors may play a role and the therapeutic possibilities.
ACNE CONGLOMATA IN A PATIENT WITH KLINEFELTER’S SYNDROME
A. Wollenberg, T. Jansen, H. Wolff, M. H. Schmid, G. Plevig, Department of Dermatology, Ludwig-Maximilians-University of Munich, Germany.

Acne conglobata has been reported to be more common and more severe in XY chromosomal aberration than in normal subjects, whereas only exceptional cases have been observed in the more common XX Y Klinefelter karyotype. We had the opportunity to study a 17-year-old patient with the combination of acne conglobata and Klinefelter’s syndrome. Serum testosterone level was within normal range, while levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were elevated. Treatment with androgens, as given for growth retardation in excessively tall boys or for substitution in Klinefelter’s syndrome, was carefully ruled out. Skin lesions responded well to a combined therapeutic regimen including oral isotretinoin and prednisolone. A review of the literature substantiates the clinical impression that this is a distinctive combination of two diseases.

ARENEFORM DERMATOSIS
G. Plevig, Department of Dermatology, Ludwig-Maximilians University, Munich, Germany.

Areneform dermatoses are follicular eruptions. The initial lesion is inflammatory, usually a papule or pustule. comedones are later secondary lesions, a sequel to encapsulation and healing of the primary abscess. The earliest histological event is spongiosis, followed by a break in the follicular epithelium. The spilled follicular contents provokes a nonspecific neutrophilic infiltration. Areneform eruptions are almost always drug-induced. Important clues are: sudden onset within days; widespread involvement; unusual locations (forearm, buttocks); occurrence beyond acne age; monomorphic lesions; sometimes signs of systemic drug toxicity with fever and malaise; clearing of inflammatory lesions after the drug is stopped, sometimes leaving secondary comedones. Clinical and sequential histological events will be demonstrated.

MORE DENSELY EXPRESSION OF VIP RECEPTOR PROTEIN IN DERMAL VESSELS MAY CONTRIBUTE TO SEBACEOUS GLAND HYPERTROPHY
U. Wolinska, Department of Dermatology, the Friedrich-Schiller-University of Jena, Jena, Germany.

In local vascular, regulation two systems exist, the endothelial and the perivascular. Rosacea is a cutaneous disease with a disturbed local blood flow regulation leading to persistent erythema, but later on also to sebaceous gland hyperplasia and inflammation. In the present study, the endothelial expression of VIP receptor protein has been investigated and correlated to other morphological features of the disease. Rosacea skin has been obtained during shaving of rhiomata and frozen immediately in liquid nitrogen. Unfixed frozen sections have been subjected to immunoperoxidase staining with monoclonal antibodies against VIP receptor protein, vimentin, glandular-type keratin, neurotensin and angiotensin and S100A. Normal skin was used as control.

In all samples a remarkable amount of vascular and perivascular cells, stained cytoplasmic for the VIP receptor could be identified in rosacea, but not in normal skin. The staining was strongest in the dermal vessels of rosacea. Immature sebocytes reacted positively for S100A and glandular-type keratin. The dermal tissue disclosed a strong immunoreactivity for neurotensin and angiotensin antigens.

The findings suggest an overexpression of VIP receptor protein not only in endothelial but perivascular cells in hypertrophic rosacea which may contribute to the disturbed local blood flow regulation in skin. The type of disturbance seems to be an endothelial one.

SOLID PERSISTENT FACIAL EDEMA IN A PATIENT WITH ACNE VULGARIS
C. Biskar, M. Wolter, R. Kauffmann, University Hospital Frankfurt/M, Department of Dermatology, Frankfurt/M, Germany.

We report about a 28-year-old female with a rare case of solid persistent facial edema as a complication of acne. The patient had been treated both topically and systemically for acne papulopustulosa. After a 13-year history of disease she developed a persistent facial edema. While its character was conspicuous on both cheeks and the nose, the edema was nonsclerometric, non-tender, nonproptotic at the forehead and eyelids. Furthermore, atrophic scars and papulocystic lesions were located on the cheeks. Skin biopsy was refusal by the patient but the characteristic clinical appearance was consistent with a diagnosis of a solid persistent facial edema as a very rare complication of acne. The exact pathomechanism has yet to be elucidated, but it appears to be extremely difficult. However, in our case minocycline 100 mg/day, Icthyol-B-Nairum and lymph massage led to significant improvement after five weeks.

ROSACEA: CURRENT CONCEPTS OF PATHOGENESIS
F. C. Powell, Regional Centre of Dermatology, Mater Misericordiae Hospital, Dublin, Ireland.

Rosacea is a common dermatosis about which little concrete information is known. A genetic predisposition is indicated by its frequency in certain populations, fair skin and red cells being particularly susceptible. A role for ultra violet light would seem apparent from its distribution on convex facial skin and the bald scalp of male alseptic patients, but recent studies of ultraviolet sensitivity have failed to show specific abnormalities. Desodex folliculorum has been implicated and undoubtedly they thrive on the rosacea skin. However, successful treatment of rosacea with systemic antibiotics is not paralleled by a decline in the Desodex population. The implication of Helicobacter Pylori in gastric pathology has raised new interest in the gastrointestinal tract in rosacea. Although initial studies appeared to show a higher frequency H.P. in rosacea patients, more recent investigation has failed to support this. Even the pathogenesis of the histopathologic changes found in rosacea are not agreed. Some authors feel that the basic abnormality lies within the cutaneous microvasculature, while others point to the follicular orientation of inflammation as the primary abnormality. These concepts will be reviewed together with a clinical classification of the disease which indicate future directions of research in this cutaneous disorder.

HELICOBACTER PYLORI INFECTION IN ROSACEA
A. Murphy, F. Powell, Regional Centre of Dermatology, Mater Misericordiae Hospital, Dublin, Ireland.

We investigated the incidence of Helicobacter Pylori (HP) infection in rosacea patients and compared this with the incidence in age, sex and socio-economic group matched controls. The 11C urea breath test (Europro Scientific) was used to detect gastric urease activity by HP, which identifies active infection (specificity=93%, sensitivity=96%). HP IgG antibodies were assayed by enzyme-linked immunosorbent assay (ELISA) on blood samples from all patients included in the study. Immature sebocytes reacted positively for S100A and glandular-type keratin. The dermal tissue disclosed a strong immunoreactivity for neurotensin and angiotensin antigens and time.

The findings suggest an overexpression of VIP receptor protein not only in endothelial but perivascular cells in hypertrophic rosacea which may contribute to the disturbed local blood flow regulation in skin. The type of disturbance seems to be an endothelial one.
97 SYMPTOMATOLOGY AND THERAPY OF HIDRADENITIS SUPPURATIVA
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A retrospective review was made of 28 patients (17 male, 11 female) suffering from hidradenitis suppurativa. We studied sex and age at the beginning of the disease, lesions clinical appearance and localization, therapeutic drug effects and laboratory findings.

Women age at the start of the disease was 23 years, men were on average 28 years old. At the beginning of the disease the youngest patient was 14, the oldest patient was 53 years old. Nodules and suppuration were the most frequent findings, followed by cicatricial changes and fistulae. Axillary, inguinal and gluteal localization were nearly equally frequently affected and often different intertriginous areas were involved. Therapeutic attempts with orally given antibiotics gave only poor results and cannot be regarded as successful in hidradenitis suppurativa. We suggest a surgical therapy depending on the clinical findings which leads to a final healing by minimal operative intervention.

99 HIDRADENITIS SUPPURATIVA IN TWO BROTHERS.
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Hidradenitis Suppurativa is a chronic, suppurative, cicatricial and mostly disabling disease that manifests clinically inflammatory lesions and nodules, abscesses and serious tracts on the acral-apocrine-bearing skin. Current data support that follicular hyperkeratosis in different steps in the pathogenesis of the disease. Our patients, 44 and 48-year-old brothers presented with a 25-year history of Hidradenitis Suppurativa involving the axillae, groins, scrotum, buttocks and thighs. HLA findings were A1,A26,B18,Cw6,DR2,DR3,DQ6,DR7,DR8. The degree of severity differed between the two patients but their response to therapy was almost identical. Antibiotics suppressed temporarily the disease. Isotretinoin 1 mg/kg/day provided some relief but therapy was abandoned in 1 month until due to the side effects of chilitis and epistaxis. Oral Cyclosporine A at a dose of 5 mg/kg/day relieved the patients from malodorous abscesses. An effort of drug reduction is now being undertaken. Our presentation may highlight the following: Noninflammatory nodules or comedones were present as precursor or simultaneous lesions to the disease. The presence of the disease in our two brothers speaks in favor of the opinion held that this is a single gene disorder consistent with autosomal dominant inheritance. Although only surgical and CO2 therapy may provide long lasting relief, this require skilled nursing care; instead; Isotretinoin and Cyclosporin A need no special care but give equivocal results. Our findings may help in this direction.

101 ASSOCIATION OF A GENETIC POLYMORPHISM WITHIN THE VITAMIN D RECEPTOR GENE WITH THE FULLMANN COURSE OF ROSACEA CONGLOMERA (ROSACEA FULMINANS).

Rosacea fulminans (RF), which has been previously called pyoderma faciale, is a rare devastating disease characterized by sudden onset of inflammatory nodules and confluent draining sinuses on the face affecting almost exclusively young women past adolescence. Treatment consists of systemic isotretinoin combined with systemic and topical corticosteroids. It is obvious that hormonal regulation and factors leading to inflammatory cascades may be implicated in the development of different stages of rosacea. Recently, a striking finding was the genetic association of a polymorphism in the 1,25-dihydroxyvitamin D receptor (VDR) gene with the heritable component of bone density. Besides the metabolic effects, the calcium-regulatory activity of VDR is involved in proinflammatory and inflammatory responses. One pathway might be the crosstalk with the retinoic acid receptor RXR by formation of VDR/RXR heterodimers which can bind to functional promoter elements. In order to investigate the involvement of the known VDR polymorphism, we isolated genomic DNA of 26 patients with RF, 70 with rosacea III-III and 61 healthy random individuals. The BsmI RFLP of intron 7 was typed using PCR amplification and agarose gel electrophoresis thereafter. VDR alleles were determined according to the absence (1/1) or presence (2/2) of the Band site. No difference in the allele frequencies was detected between healthy controls (genotype 1 = 42 %, allele 1: 0.54) and the patients with rosacea III-III (allele 1: 0.46). An increase of this allele was found in the carrier rate (42 %) and allele frequency (allele 1: 0.58) in patients with RF. Thus far, the functional pathway of the VDR/retinoic acid receptor pathway in a predisposition to this inflammatory disease at the level of hormonal control.

102 BACTERIOLOGIC FINDINGS IN 3 DEPTHS OF HIDRADENITIS SUPPURATIVA DURING CO2 LASER VAPORIZATION AND THERMISC STERILIZATION.

Hidradenitis suppurativa is often a long-standing disease, affecting the areas of axilla, breast, periumbilical, groin and perineum. The first inflammatory and infectious part of the disease is followed by an chronic inflammatory stage fluctuating with more purulent abscesses. We used a method for elimination of diseased with a CO2 laser, eliminating the tissue layer by layer from the surface and downward allowing sterile bacteriological cultures from each layer without any rest products though the tissue and bacteria from the above layer. In this study, 23 women and 3 men were operated by this method and studied, using aerobic and anaerobic cultures, with a sterile technique with swabs and biopsy in superficial and deep levels. Totally 18 different bacterial species were found. Staphylococcus aureus (SA) could be detected in 14 patients and in deep layers too in 6 cases. SA was the only bacteria in 2 deep cultures. Coagulase negative staphylococcus (CNS) was found in 17 superficial and 16 deep samples. In nine of the deep samples cultured CNS was the only detectable bacteria. In eleven superficial cultures could CNS but not SA be found simultaneously. In six superficial cultures SA was isolated but not CNS simultaneously. Only 2 of the 10 cases of CNS that was isolated were found simultaneously with SA. Bacteriologic findings in the deepest layer that can simulate the situation of foreign bodies, indicate that the esclusion with subsequent sequelae of the chronic inflammation is a major component of the HS pathogenesis.

100 AN UNUSUAL CASE OF ACNE INVERSA.
D. Vahradnikova, J. Horvay. Dermatological Department, Charles University, Institute of Endocrinology, Prague, Czech Republic.

Patient 35 years old, treated for extreme obesity since infancy, was affected from the age of 13 by mild inguinal hidradenitis, from 35 an extensive and dense inflammation of the thighs, buttocks, groins and abdomen appeared on her breasts. The usual acne predilection, including perineal area, was spared. Premenstrual aggravation was considerable. Nonphysiological endocrinological findings were: hyperandrogenism with an increase of seato- tropin in hypergynaecic phase of the oral glucose tolerance test and also increased levels of cortisol and dehydroepiandrosterone in urine and serum. Testosterone and SHBG were normal. Cytohistological examination: numerous oestrogenic anovulatory cycles. The influence of adrenal and ovarian androgens to the sebaceous glands, is inferred. Follicular cast formation in follicles and in sebaceous glands can help to explain this unusual acne eruption. The best resolution was reached using higher doses of cyproterone acetate.

102 ASSESSMENT OF METABOLIC HOMEOSTASIS IN STOMACH ULCER PATIENTS WITH ULCERATIVE COLOITIS.

Of 347 stomach ulcer patients under investigation 84 (23.1%) were identified having rosacea. The following parameters were checked — plasma electrolytes, POL, AOS, hormones (growth hormone, cortisol, 3-iodine thyroxine, gastrin, parathormone, testosterone, progesterone, glucocorticoid), cyclic nucleotides. The results obtained were compared to the results obtained from the 23 patients who had rosacea but did not have stomach ulcer. Higher level of parathormone, testosterone, cortison, lactate and lower level of cyclic adenosinmonophosphate was observed in stomach ulcer patients with rosacea. Testosterone level was increases in both groups (12.4+3.5 ng/ml, 11.4+2.05 ng/ml comparing to 3.4+0.59 ng/ml in the control). Increasing of testosterone level in rosacea patients leads to effective lipid absorption and is a compensatory reaction.
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ASSESSMENT OF ULTRAVIOLET THRESHOLDS IN ROSACEA.
A. Murphy, FC Powell, GM Murphy.
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We assessed 40 patients to determine whether the minimal erythema dose (MED) of monochromatic ultraviolet (UV) irradiation in rosacea was different to that in a group of controls. The threshold sensitivity UVB was also determined on facial skin.

Twenty-one rosacea patients (11 females, 10 males) aged range 21-64 years with telangiectasia (n=7) or papulopustular rosacea (n=14) were studied. Phototesting was performed using and Applied Photophysics irradiation monochromator.

Patients were irradiated on the normal skin of the back at 300nm ± 5nm, at 120 ± 10nm and 370 ± 25nm. The MEDs were assessed visually 24 hours later. The 300nm ± 5nm MED (determined on the back) plus one increment above and below was repeated on facial skin of the rosacea patients and readings taken 24 hours later. Fifty-six nonphotosensitive subjects irradiated at the same wavelengths on the back, served as a control group. The mean MEds for all wavelengths tested were comparable to the controls. The MED at 300nm on the face in rosacea patients was greater than that on the back in 17/21 patients tested. Of the remaining 4 patients the 300nm MED on the back was the same as that on the face (80μJ/cm²) in 3, and in one case the MED on the face was lower than that on the back but still remaining within the normal range.

Some clinical features are suggestive of a role for sunlight in rosacea, however UV threshold responses are normal.

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PERSISTENT ERYTHEMA AND EDEMA OF THE FACE ASSOCIATED WITH ROSACEA AND LYMPH VESSEL DYSPLASIA

A 24-year-old woman is reported with congenital lymph vessel dysplasia, developing a continuously growing edema of her legs since her second year of life and a growing edema of her face since she was 23 years old. She also had telangiectasias and purpurae on the background of a diffuse erythema as well as marked seborrhoea on her face. Histopathological examination of a representative facial lesion revealed a granulomatous dermatitis with perivascular distribution mainly consisting of lymphocytes and histiocytes. In addition, there was a moderate fibrosis of the corium with numerous mast cells. By duplex ultrasound, a diagnosis of a slow lymphatic vessel of the legs without evidence for chronic venous insufficiency was made. The clinical and histopathological findings are consistent with solid persistent erythema and edema of the face associated with rosacea and lymph vessel dysplasia. The chronic course, absence of serological abnormalities and nonspecific histopathological features as well as resistance to therapy are the most important diagnostic criteria of this disease also known as Mobach's disease.

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OPHTHALMIC ROSACEA: PATHOGENESIS AND TREATMENT
T. Janzen, G. Plewig. Department of Dermatology, Ludwig-Maximilians-University of Munich, Germany.

Ocular involvement is a common complication of rosacea. The exact prevalence is unknown, but it has been reported to be as low as 3% and high as 58%. In patients who have both skin and ocular manifestations, 20% develop their ocular manifestations first, 53% develop their skin lesions first, and 27% develop both manifestations simultaneously. The course of ocular rosacea is usually chronic and often progressive. Pain and photophobia may be present. The ophthalmitis signs are variable including Mephititis, conjunctivitis, iritis, iridocyclitis, hypopyoniritis, and even keratitis. The ophthalmic complications are independent of the severity of the facial skin involvement. Therefore, all patients with rosacea should be seen by an ophthalmologist to look for other subclinical complications. The topography of rosacea exactly corresponds to the cutaneous area of drainage by the tributary veins of each angularis suggesting that the disease may result from a disturbed microcirculation of the facial angular vessels involved in the brain-cooling vascular mechanism. Ophthalmic rosacea generally responds well to oral tetracyclines. Unfortunately, systemic treatment with tetracyclines may aggravate the ocular complications in some patients and therefore does not seem to be helpful in ophthalmic rosacea.

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LITHIUM GLUCONATE IN SEBORRHEIC DERMATITIS.
Dreiser4, Bayron5, Beyol,3, Bonnefieldl, Ciferei,3, Dercurea,2, Gillmore,2, Humber8, Lamnro10, Lanse11, Leve12, Lotite13, Plasmin14, Sasso15,15

1 Nantes, 2Rothchild, 3Pessac, 4Liogmes, 5Cie, 6Manns, 7Montpelier, 8Nimes, 9Besancon, 9Dijon, 10Besancon, 11Roon, 12Canc, 13Tours, 14Quimper, 15Brest.

Lithium salts is known both to act on emotional factors and to inhibit growth of some fungi. In other rodent, pyrithione and stress are two factors implicated in seborrhoeic dermatitis. Therefore, we performed a double blind controlled study to determine the efficiency of topical Lithium gluconate (Li) Labateal on seborrhoeic dermatitis. 129 patients were included and the evaluation performed on 127 patients. They were either allocated to active (Lithium twice daily) or placebo twice daily for 2 months. A separate assessment was made of redness, scale and overall impression of the efficiency by the patient. A significant improvement was obtained in Lithium arm 2 months later (p=0.001), compared with placebo 2 months later (p=0.019). This difference is highly significant in plates (p=0.001) and placebo (p=0.002). Moreover pruritus is (p=0.019), itching (p=0.013) and burning (p=0.001) were significantly decreased in Lithium arm. No side effects were noted. A significant increase (p=0.001)

Lithium plasma was noted after 2 months of treatment in Lithium arm compared to placebo. However, this value (mean±SD) was largely lower than toxic level. Finally, this study demonstrates that a topic Lithium is efficient in the treatment of seborrhoeic dermatitis.

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The human skin commensal, Malassezia, has been implicated in the pathogenesis of psoriasis verrucosus, seborrhoeic dermatitis, folliculitis and atopic dermatitis. We have demonstrated that Malassezia yeast cells significantly inhibit the production of pro-inflammatory cytokines IL-1, IL-6 and TNFα by human peripheral blood mononuclear cells (PBMC) in vitro (Kesavan et al, 1996). We recently demonstrated that the Malassezia yeast cell wall has an unusually high lipid content (approximately 15-20%), the aim of this study was to investigate the effect of lipid removal from Malassezia cell walls on pro-inflammatory cytokine production by human PBMC in vitro. Lipid extraction on three of the seven species, Malassezia sympodialis, M. globosa, and M. restricta, was carried out by chloroform: methanol (2:1) solvent extraction. Co-incubation (PBMC together with Malassezia species, at a yeast-to-PBMC ratio of 20:1) supernatants (up to 24h), together with LPS (positive) and culture medium (negative) control were assayed by ELISA for IL-1β and IL-6. Levels of IL-6 and IL-1β derived from 3 healthy PBMC donors, were invariably and significantly greater (p<0.05) than constitutive negative control values, the complete reverse of observations prior to lipid removal. These findings suggest that Malassezia cell wall lipid may play a role in the immunomodulatory release from human mononuclear phagocytes, a possible novel mechanism of immunomodulation by Malassezia species.
109
DETERMINATION OF SERUM ZINC LEVEL IN PATIENTS WITH SEBORRHOEIC DERMATITIS AND CONTROL GROUP IN KERMAN S. Saryazdi, M.R. Meshkati, Department of Dermatology, Kerman Medical University, Kerman, Iran.

Seborrhoeic dermatitis is a chronic dermatitis manifests as a erythematous lesion with greasy scale and it seems that many factor has effect in the initiation and progress of the disease one of the cause of the disease is zinc deficiency. In this study 40 persons suffering from seborrhoeic dermatitis at one year in Kerman hospital were selected and then history taking being sure that none of them had not already taken zinc orally, 5 cubic centimeter of their blood catched and the serum of the samples were separated. 30 member were the control group in this study. Following the determination of serum zinc level it was observed that none of the individuals suffered from zinc deficiency. In patients average serum zinc level amount 103.68±g/dl and 11.9±g/dl in control group. It is recommended that in the case of seborrhoeic dermatitis therapeutic effort with zinc put into action after being sure of serum zinc level.

111
Psychological Impact of Acne
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There is no characteristic personality profile for the acne patient, so it will depend largely on the prevailing psychological subtype. Stress however is the major trigger of exacerbations; and particularly those exacerbations characterised by an inflammatory component and acte have a profound psychological effect on the sufferer. In case in which early childhood experience has failed to establish a solid body-image and a positive self-esteem, acne during the psychologically vulnerable period of adolescence can be an especially severe blow. Studies have shown that children who are physically impaired are most vulnerable to adverse psychological effects if these imperfections are sustained during adolescence. The consequences are diminished self-esteem and in severe cases also a distortion of the body image. They include a depressive affect, social withdrawal, difficulties in peer relationships, and poor school performance. In more severely affected young people, depressive symptoms may lead to disciplinary problems, social phobia and severe family conflict: substance abuse may become a problem. Not infrequently treatment compliance becomes a weapon in bitter struggles between adolescent and parent, even social is not excluded.

In studies it could be shown that during time of enhanced psychosocial strain subjectively assumed by the patients, the lesions increased and the patients were dinated in social interaction and communication. Surprisingly, there are no correlations between the clinical status and significant psychiatric findings. Our results show that in acne vulgaris, the individual experience of wanting physical attractiveness, associated with a predominantly neurotic depressive personal structure, may play a central part in a disturbed process of interaction with the environment and suggest the influence of psychic factors in the pathogenesis of acne vulgaris.

113
A NEW CONCEPT OF DRUG DELIVERY FOR ACNE THERAPY

Bhram Shroot, CIRD Galderma, Sophia-Antipolis, France

The use of retinoids in acne therapy has been extensive over the past 30 years. The pathology of the disease is well described even if the cause remains obscure. The selection of new substances which could be new therapies for this disease is based on a paradigm of controlling cellular proliferation and differentiation in the pilosebaceous unit. Data will be presented, which identified adipocyte, a naphthoquinone derivative, which had a marked effect on keratinocyte differentiation both in vitro and in vivo. Careful selection of animals gave rise to a formulation which resulted in rapid delivery of the drug to the pило-sebaceous duct. Taken together this research resulted in a therapy for acne.

110
Effect of acne on the quality of life
A.Y. Finlay, Dept. Dermatol, Univ. Wales Coll. Med., Cardiff, CF4 4XN, UK

Acne can have major effects on the lives of sufferers: anxiety, depression, embarrassment, shame, problems with relationships and unemployment have all been reported. These effects can be measured using questionnaires that record psychological well-being or that assess health related quality of life (QOL). Disease specific questionnaires such as the Acne Disability Index (ADI), the short Cardiff Acne Disability Index (CADi) and the Assessments of the Psychological and Social Effects of Acne (APSEA) have or are being cross-validated. Other methods used include general health measures such as the UK Sickness Impact Profile and the SF-36, and dermatology specific measures, the Dermatology Life Quality Index (DLQI) and the Children's DLQI (CDLQI). Utility measures can be used to assess patients' views of the 'value' of their acne, and the Quality Adjusted Life Year (QALY) concept has been applied to acne. These methods have been used to assess the impact on QOL of isotretinoin and of oral antibiotics. A recent Oxford study has confirmed the major and persisting improvement seen following isotretinoin. The results of such studies give excellent supporting evidence for resource allocation to acne management.

112
PSYCHOTHERAPEUTIC APPROACH FOR ACNE TREATMENT

E. Pannocchia, Dept. of Dermatology, Univ. of Florence, Florence, Italy

From a psychological medical point of view acne vulgaris can be schematically divided into two clinical pictures.
1. The common adolescent eruption, more mind-influencing and thus psychosomatic.
2. The less frequent acne of adults (young adults for the most part), both as a continuation of adolescent acne, and more rarely, as a never before experienced cutaneous affection and thus psycho-somatic in a strict sense.

We believe that the dermatologist can treat both of these clinical pictures, even from a psychological aspect, from the very first visit with the patient using the first step in psychotherapy: counselling.

The principal points of this approach will be presented, with special attention to the differences to be considered in the two clinical pictures specified well as to the opportuneness and timing of eventual liaison-consultation with psychologists/psychiatrists in realizing other therapeutic strategies.

114
RETIINOID AGONISTS AND INVERSE AGONISTS - A NEW GENERATION

R. A. S. Chandrarsing, S. M. Thacker, E. S. Klein
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Retinoids bind to retinoic acid receptors (RARs) and by altering the equilibrium interaction of RARs with coactivator and corepressor proteins effect the transcriptional activity of a multaitude of target genes. We have designed acetylated retinoids of three distinct classes: RAR agonists, neutral antagonists and inverse agonists. Inverse agonists bind to the RARs and repress their basal transcriptional activity while neutral antagonists do not change basal activity. Neutral antagonists can inhibit the transcriptional activation effects of agonists as well as the transcriptional repression effects of inverse agonists.

We show that both RAR agonists and RAR inverse agonists regulate markers of abnormal differentiation and inflammation in human keratinocytes. An RAR neutral antagonist has no effect by itself but can antagonize the effects of both RAR agonists or RAR inverse agonists. Our data indicate that both RAR agonists and inverse agonists are of potential therapeutic utility in the treatment of skin diseases.
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FOLLICULAR TARGETING IN ACNE THERAPY

Hans Schaefer, L'Oréal Cheley France

The follicle and sebaceous gland are the only affected sites in acne. Since any action of a drug in a non affected area (as acne the interfollicular epidermis and dermis) is a side effect by definition, topical acne therapy should ideally be confined to the follicle. However, although follicular penetration is known since long, its specificity and importance was hitherto underestimated. Thus it is only recently that the basis and rationale for drug targeting to the follicular lesions could be established. The following principles pertain:

- The more lipophilic a molecule, the more it accumulates in the sebaceous gland.
- Such molecules can enter deeply into the lumen as well as into the outer content of the follicle.
- Percutaneous penetration into the follicle is very selective in regard to receptor expression. This selectivity is of pivotal importance for follicular targeting and can be taken advantage of: unexpectedly high amounts of drug can be directed almost exclusively to the follicle, as can be visualized by fluorescence for example.
- Retinoids are expressed in the skin in a site specific manner. By choosing molecules with appropriate receptor specificity, their site of action can be predetermined i.e. targeting can be refined.
- The often neglected retinoid s irritation s has little in common with classical irritants in that its mechanism as well as its time course are distinctly different. Thus modern retinoids can be screened according to their antinflammatory potential.

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TOPICAL DRUG TREATMENT IN ACNE

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The main part of acne treatment is going via the topical route. More than 50 % of acne patients belong to the group presenting with acne comedonicum and papulo-pustulosa. Whenever small nodes or scars are occurring systemic co-involvement is indicated. However, Topical treatment is affecting at least three of the four main pathogenetic factors responsible for the development of acne i.e. hyperkeratosis, microcomedo formation, and inflammation. The agents currently available have only a limited influence at least one of these factors, but often have additional properties. Those with act comedolytic and antiinflammatory are the retinoids tretinoin, isotretinoin, tazarotene and tazarotene and azelaic acid (AZA), as well, some of the retinoids having additional antiinflammatory potency. AZA has strong antiinflammatory potency without inducing bacterial resistance similar to benzylperoxide. Unfortunately, the bacterial resistances are beginning to emerge as a significant problem. P. acnes resistance to the commonly used erythromycin can also be transferred to clindamycin, whereas no resistance has been reported to rifadinocium, so far.

Today, more and more evidence comes up, that topical antiandrogenic agents will soon be available to treat the important factor seborthoea, because patients with marked hyperseborrhoea are frequently relapsing. Finally, liposome encapsulation of agents incl. phospholipids can enhance penetration and efficacy, but particular with regard to retinoids can lead to higher absorption and adverse drug reactions.

118

TOPICAL SEBUM SUPPRESSION

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Sebum released from the sebaceous gland is collected in the infundibular reservoir before reaching the skin surface. As it is a viscous liquid, it might be expected that the follicular reservoir is depleted at a rate proportional to the size of its opening at the skin surface. Topical sebostatic agents may have two main targets which are the sebaceous gland and its infundibulum. Topical antilipidogenerating evidence for sebum suppression are not yet available. Other molecules, such as astrogenin, zinc and ketoconazole decrease skin oiliness without induction of microcomedogenesis. Etilulol which is a dichlorophenyl- imidazolidinol is a relatively new addition to the category of sebum control agents. Long term use of etilulol results in a progressive decrease in skin oiliness on the forehead, as compared to the collateral control site. At the same time, the function of the follicular reservoir appears to be affected without significant increase in the sebaceous gland activity, excluding the possibility of a systemic effect of the ingredient. The effect is not immediate, and requires administration over a number of weeks. Ongoing studies are looking into the mechanism of action of etilulol on the surface of the skin or in the upper part of the follicular reservoir.

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TOPICAL ANTIBIOTICS IN ACNE TREATMENT

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Topical use of antibiotics is a widely accepted effective and safe treatment for acne. Review of articles in the past 30 years revealed that topical formulations such as tetracycline, erythromycin and clindamycin showed clinical effectiveness for mild to moderate inflammatory acne. The mechanisms of action of topical antibiotics are considered to be due to inhibition of microbial flora, alteration of surface lipid composition and suppression of leukocyte chemotaxis. In order to show their effectiveness, the topical antibiotics must penetrate into the sebaceous follicles. Various methods including radioautograph, fluorescent microscopy, or bioassay have been applied for measuring penetration of antibiotics. To enhance antibiotic penetration, vehicles such as DIMSO or follicular irritants have been applied. Use of topical antibiotics avoids the possibility of the adverse effects of systemic therapy. The adverse effects of topical preparations are usually minor and negligible. However, there is a report of an increased systemic absorption of clindamycin up to 4% under topical application. With an increased use of topical antibiotics, we should also consider the risk of development of resistant strains of Propionibacterium acnes, since topical clindamycin therapy occasionally produces erythromycin-resistant Propionibacterium. Newly developed topical agent of synthetic fluoroquinolone derivative, mifloxicin is widely used in Japan. It shows excellent clinical response, good penetration into sebaceous follicles, anti-inflammatory effects as well as anti-microbial effects to Propionibacterium.

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COSMETICS IN ACNE


The empirical state of cosmetics in the past, together with false claims of existing "lines" of "antiseborrhoeic" products and on the other hand the concept of cosmetic acne have created much confusion to both patients and the dermatologists. In modern times the development of a wide range of cosmetics, technically improved, permits, as an adjunctive therapy, a better quality of life of the acne patients by:

- Hydric products selected for their gentle effect on the acneic skin before, during and after the topical or systemic treatment.
- Appropriate vehicles for antiacne topical drugs addressed to oily, dry, sensitive, fragile or hairy skin.
- Non-comedogenic moisturizers exerting a beneficial effect on dry or irritated skin pre-treated by topical or systemic antiacne drugs.
- Oil-free coloured products (make up etc) to cover bleaches.
- Non-greasy photoprotective agents for the acneic or the acne-prone individual.
- Peeling agents for post inflammatory acneic skin etc.

Selection of cosmetic products for acne using the above numerous prepararions, should be based on their non acne promoter ingredients with regard to the acne's grade each time and the condition of the skin.
121 NADIFLOXACIN (OPC-7251) IN ACNE VULGARIS: CLINICAL EVALUATION AND IN VITRO ANTIBACTERIAL ACTIVITY. P. Nenoff, U.-E. Haustein, N. Hilt*. Department of Dermatology, University of Leipzig, Germany. NADIFLOXACIN (OPC-7251) is a new topical fluoroquinolone which inhibits the conf- guration of the supercoiled DNA by the DNA gyrase. Previous studies showed the effi- cacy of nadifloxacin in acne vulgaris and bacterial skin infections. The purpose of this double-blind, controlled, phase II study was to investigate the clinical efficacy and tolerance of nadifloxacin 1% lotion compared with erythromycin 2% by topical applica- tion in 90 patients with acne vulgaris. Efficacy was assessed by counts of open comedo- nes, papules, and pustules. Furthermore, the antibacterial activity of nadifloxacin against isolated bacterial strains was tested in vitro by an agar dilution technique. Both nadiflox- acin and erythromycin treatment led to a statistically significant reduction in the counts of open comedones and pustules. Nadifloxacin was effective against all aerobic and anaerobic isolates. Before treatment, minimum inhibition concentrations (MICs) were in a range of 0.005-1.0 (MIC90 0.1) g/ml for Staphylococcus (S.) aureus (n=9), 0.1-0.39 (MIC90 0.2) g/ml for Propionibacterium (P.) acnes (n=51), and 0.2-0.39 (MIC90 0.39) g/ml for P. granulosum (n=16). All organisms isolated from the lesions before and at the end of the study were highly sensitive and none was resistant to nadifloxacin. On the con- trary, MIC values for erythromycin indicated diminished in vitro susceptibility or even resistance in 47% of CNS, 24% of P. acnes, 50% of P. granulosum, and 22% of S. au- reus strains. For CNS, MIC values were 0.006-2.08 (MIC90 0.16) g/ml and for S. au- reus of 0.1-1.00 (MIC90 0.16) g/ml, and both for P. acnes and P. granulosum each 0.006-1.00 (MIC90 0.39) g/ml.

123 INFLUENCE OF DIFFERENT FORMULATIONS ON THE EFFICACY OF TOPICAL NADIFLOXACIN (OPC-7231) CM Smith, G. Schuster, W. Koning, M. Pack, G. Gelnick Department of Dermatology and Venerology, Department of Microbiology, Otto-von- Guericke University, Magdeburg, Germany. Antibiotics are extensively used to treat acne via the topical or oral route. Antibiotic resistant propionibacteria have therefore been isolated with an increasing frequency leading to a demand for new antibiotics. Nadifloxacin is a new fluoroquinolone derivative with broad spectrum activity which has been assessed with regard to clinical efficacy in previous studies. The aim of this study was to determine whether the efficacy of topical nadifloxacin may be increased by a suitable formulation. Nadifloxacin 1% cream was compared to nadifloxacin 1% lotion in a single blind study and both treatments were given twice a day for a period of 4 weeks. Clinical improvement was assessed after two and four weeks. Quantitative bacterial evaluations were carried out before and after four weeks of treatment. Samples were taken at identical sites using a standardized washing procedure. Propionibacteria were isolated, counted, subtyped and calculated per cm² skin sample. Coagulase negative staphylococci were also isolated, counted and calculated per cm² skin sample. Both treatments reduced the number of inflammatory acne lesions and led to clinical improvement. After 4 weeks both staphylococci and propionibacteria were decreased with both formulations. The lotion produced slightly better results suggesting that a suitable formulation may increase efficiency of topical nadifloxacin.

125 THE COMPARATIVE SAFETY AND EFFICACY OF BENZOYL PEROXIDE 5% / ERYTHROMYCIN 3% GEL AND ERYTHROMYCIN 4% / ZINC 1.2% SOLUTION IN THE TREATMENT OF ACNE VULGARIS A. Chu, Hennemanst Hospital, London, UK; F. J. Huber, Jr, and B. T. Plot, Dermik Laboratories, Collegeville, Pennsylvania, USA. This randomized 10-week study compared the safety and efficacy of benzoyl peroxide 5%/erythromycin 3% gel (benzoyl peroxide 4%/zinc 1.2% solution in 72 acne vulgaris patients. Physician global evaluations were significantly (p<0.05) more improved in the benzoyl peroxide 5%/erythromycin 3% gel treatment group compared to erythromycin 4%/zinc 1.2% solution at the Week 2 and at each subsequent biweekly clinical visit. Inflammatory lesions (papules / pustules) were significantly (p<0.005) more reduced in the benzoyl peroxide 5%/erythromycin 3% gel treatment group than the erythromycin 4%/zinc 1.2% solution at Weeks 2 and 4. Comedones were significantly (p<0.001) more reduced in the benzoyl peroxide 5%/erythromycin 3% gel treatment group than in the erythromycin 4%/zinc 1.2% solution group at Weeks 8 and 10. Patient efficacy evaluations significantly (p<0.001) favored benzoyl peroxide 5%/erythromycin 3% gel to erythromycin 4%/zinc 1.2% solution.

126 MEASUREMENT OF THE INHIBITION OF SKIN LIPASES USING FOURIER-TRANSFORMED INFRA-RED SPECTROSCOPY B. Tarroca, A. M. Cauman, D. Redoudes, Y. Gall. Institut de Recherche Pierre Fabre I.R.P.F., Service de Chimie-Cutanae - BP 74 - 11322 Canalet Tolosan. Using the technique of fourier-transformed infra-red spectroscopy, absorption by carbonyl groups indicates the presence of free fatty acids (1712 cm⁻¹) and triglycerides (1741 cm⁻¹). The ratio of absorption at these two frequencies may be used to quantify the lipolytic activity of certain skin microflora bacteria from sebum samples. Bacterial lipases produce free fatty acids from triglycerides of skin lipid origin. Using the cigarette paper sampling method as applied to forehead sites, the absorption at these frequencies was measured by placing the paper sample directly in the infra-red beam. This simple technique has been validated with the aim of having a method for evaluating the effect of certain ingredients in topical skin formulations on surface bacterial activity. One application of the technique, involved a study demonstrating such an inhibitory activity with retinolaldehyde, the active ingredient contained in one of our dermocosmetique preparations : Yateal. The study comprised six volunteer subjects who applied both the active formula (with retinolaldehyde) and the placebo. An intratrained site was included as a control. The results showed the active formula to have a greater effect than the placebo, thus demonstrating the true inhibitory activity of this retinoid. This simple test is of potential use in establishing the anti-bacterial profile of all topical skin preparations.

127 CLINICAL AND MICROBIOLOGICAL EFFECTS OF TOPICAL NADIFLOXACIN AND ERYTHROMYCIN IN ACNE Y. Greulich, W. C. Rateike, G. Pieck, R. Bojar, R. F. Holland, Dept. Derm. Leeds Gen Inf., Leeds UK & *Dept. Derm., Univ. Munich, Germany. There is an increasing incidence of clinically relevant resistant P. acnes. Thus, there is a need to develop new antimicrobials. Nadifloxacin in vitro is effective against bacteria including P. acnes. We wish to report a double blind study comparing the effect of 1% Nadifloxacin against 2% erythromycin cream in 474 acne patients from 28 Western European centres. Patients were seen at a control visit, and at 2, 6, 10, 12 weeks on therapy. Both treatments significantly reduced the number of inflamed lesions at all time points. The greatest reduction was seen at the 6th week. Thereafter there was a gradual and slower rate of improvement with maximum suppression at 12 weeks (p<0.001). There was no difference between group differences. For non-inflamed lesions the reduction was 17% and 18% at 12 weeks. Patient self assessment at 12 weeks gave a preference for Nadifloxacin (61% vs 53%; p=0.014). Both products were well tolerated. Both therapies equally reduced P. acnes significantly at 12 weeks (p<0.001). At 12 weeks, there was no Nadifloxacin resistance in P. acnes or micrococcus. In the erythromycin group 27.9% of the P. acnes and 97.7% of the micrococci were resistant (p<0.01). Systemic absorption of Nadifloxacin was negligible. Thus, topical nadifloxacin could be an effective therapy in acne.

128 AN INVESTIGATOR-BLIND, RANDOMIZED STUDY COMPARING A 3% ERYTHROMYCIN/5% BENZOYL PEROXIDE COMBINATION IN GEL VERSUS 20% AZELAIC ACID CREAM IN THE TREATMENT OF ACNE VULGARIS F.E. Dunlap, Argus Research, Tucson, Arizona; J.M. Maloney, Cherry Creek Dermatology, Denver, Colorado; S. Levy, Dermik Laboratories, Collegeville, Pennsylvania, USA. One hundred and fifty male and female patients, ages 13 to 30 years, with acne vulgaris (Grades II or III, Pillsbury classification) were enrolled in this trial. Patients were randomized to topical treatment with 3% erythromycin/5% benzoyl peroxide in a gel vehicle or 20% azelaic acid cream applied twice daily for 8 weeks. On each visit (baseline and Weeks 2, 4, and 8), the physician counted the number of comedones and inflammatory lesions (papules/pustules). Global evaluations assessing the overall effectiveness of treatment, compared to baseline, were done by the physician at each follow-up. One hundred forty-four patients were considered evaluable for efficacy (69 erythromycin/benzoyl peroxide, 65 azelaic acid). The results of the study demonstrated significant differences favoring 3% erythromycin/5% benzoyl peroxide over 20% azelaic acid for the following parameters: 1) reduction in inflammatory lesions (papules/pustules) at each follow-up evaluation (Weeks 2, 4 and endpoint); 2) reduction comedones at Weeks 2 and 4; 3) Improvement in overall acne condition at each follow-up evaluation (Weeks 2, 4 and endpoint), as measured by Physician Global Evaluations.
LACK OF AVERSION OF TOPICAL ISOTRETINOIN

Oral retinoids are teratogenic. Concern has been expressed about their systemic absorption when topically applied. We therefore determined the percutaneous absorption of reduced retinoid levels in healthy volunteers during and after the use of isotretinoin gel (0.025% w/w), with a gel base n=40/group). Lesion counts performed at 4.8 and 12 weeks. At most visits the active therapies were significantly superior to placebo. The percentage reduction in total lesions increased with time reaching a maximum of 12 weeks of 29.6% with isotretinoin, 21.5% with isotretinoin, 25.2% with isotretinoin, and placebo was 8.8%. Between group comparisons demonstrated that isotretinoin was significantly more effective than 0.05% isotretinoin alone for inflamed lesions at week 4 and acne grade at weeks 4 and 8. There was also a trend of improved efficacy of isotretinoin over the 2% retinoloychin with mean reduction in total lesions, total non-inflammatory lesions and acne grading being greater for isotretinoin. All products were well tolerated.

Conclusion: combined product did show increased efficacy over the individual components and can be recommended as a suitable alternative topical therapy to either constituent alone.


This study was aimed at evaluating in man the percutaneous absorption of clindamycin from three topically applied gel formulations containing clindamycin phosphate (1% w/w as clindamycin) with tretinoin (0.025% w/w) (Vel Ballard), in a comparison with a clindamycin phosphate lotion (1%; Dalacin T8) and with a clindamycin HCI/tretinoin gel.

Formulations were applied once daily for 5 days on the facial skin of 12 volunteers at a dose of 1 ml per day. On the 5th day of each treatment, the skin of the cows was scored visually, and blood and urine were collected for 12 h following the last topical application for subsequent clindamycin analysis.

In general, clindamycin plasma levels did not exceed the limit of quantification (5 ng/mL) in all three formulations, and the subject concentrations up to 13 mg/mL were observed following clindamycin HCI/tretinoin gel. Urinary excretion of clindamycin after the clindamycin phosphate/tretinoin gel was comparable to values with the clindamycin phosphate lotion, whereas the clindamycin HCI/tretinoin gel gave significantly higher values. Concerning adverse events, 3/12 subjects reported light to moderate irritation with clindamycin HCI/tretinoin gel, and 1/12 with clindamycin phosphate lotion. No adverse events were reported for clindamycin phosphate/tretinoin gel.

Clindamycin phosphate in the gel formulation resulted in reduced skin irritation and reduced percutaneous uptake of clindamycin, compared to clindamycin HCI. The results did not indicate an enhancing action of tretinoin on percutaneous uptake of clindamycin.

ORAL RETINOIDS IN ACNE TREATMENT
Claire Bently

Acne is a multifactorial disorder where hormonal influences on the pilosebaceous unit play an important role. The role of hypersebaceous tissues, hair follicle damage, and silver bionasal are implicated in the pathogenesis of acne. In women with acne, the androgen receptor expression increases in the sebaceous glands. The androgen receptor is a nuclear receptor that regulates gene expression in response to androgenic stimuli. The androgen receptor expression is upregulated in the sebaceous glands of women with acne compared to women without acne.

Androgens stimulate the development of sebaceous glands in the skin, leading to increased sebum production. Sebum production is controlled by the androgen receptor in the sebaceous gland, and this receptor mediates the effects of androgens on sebaceous gland activity.

Androgens have been associated with increased sebum production in acne-prone skin. The increased sebum production can lead to inflammation and clogged pores, which are characteristic features of acne. Therefore, targeting sebum production and reducing sebum levels is an important treatment strategy for acne.

In conclusion, oral retinoic acid is a promising treatment option for acne, particularly in women with acne. Its efficacy in reducing sebum production and improving skin texture makes it a valuable addition to the armamentarium for treating acne in women.
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CLINICAL EFFICACY OF OTHER ANTIANDROGENS
J.B. Schmitt

The extent of androgenic action at the cellular level depends on 2 major mechanisms. First, the activity of the cytoplasmic enzyme 5α Reductase (5α R) that transforms the inactive androgenic DHT, which binds primarily to the cytoplasmic Androgen Receptor (AR) and second, the concentration of available AR.

Antiandrogens - by classical definition competitors for the binding at the AR - include besides Cyproterone acetate the following substances: Spironolactone, Ketokonazole, Cimetidine and Flutamide. Due to the extent of side effects, Spironolactone which has also 5α R inhibitory effects, and Flutamide, a pure, non steroidal antiandrogen, are preferentially indicated in Histrum. Ketokonazole and Cimetidine exhibit weaker antiandrogenic properties and side effects and therefore may be used in acne.

Furthermore, recent experimental data show AR-binding capacities of isotretinoin and thus raise the discussion upon antiandrogenic properties of the substance (1).

By amplifying the view on substances that are effective against androgenic action at the cellular level, also 5α R inhibitors have to be included in the therapeutic regimens. The group of 4-Androstanols are potent agents, of which Finasteride - a selective 5α R inhibitor - is effective in androgenetic alopecia. Recent investigations show, that of the 2 isomers of 5α R, mainly the 15-α is to be found in sebaceous glands (2). According to this finding, 5α R inhibitors should be specified and for acne substances, that show 15-α R inhibition under experimental conditions, (3) should be investigated clinically.


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COMEDONAL DIFFUSION OF MINOCYCLINE IN ACNE VOLUNTEERS.
O. Chosidow*, F. Poff, E. Naline, C. Advenier, J. Revuz, "Department of Internal Medicine, Groupe Hospitalier Pitié-Salpêtrière, Paris, France.

Efficiency of minocycline has been demonstrated in acne vulgaris. The purpose of the study was to investigate comedonal diffusion of minocycline in acne volunteers, using high-performance liquid chromatography (HPLC). Sufficient and methods: Ten acne volunteers were included in a monocentric open prospective study. All the volunteers received minocycline, 100 mg/day for 30 days, at the same morning time. A blood sample was drawn before treatment (day 0) and then on day 15 and 30 days (day 30) after the onset of comedonal treatment. Comedonal samples were taken at day 0, day 15 and day 30. Minocycline was assayed by HPLC other in blood and comedonal samples. Results: At day 0, the minocycline level was zero. At day 15, the mean blood level was 2.4 ± 0.4 µg/ml. The mean minocycline ratio comedonal levell/blood level was 1.1 ± 0.4. At day 30, the mean blood level was 2.6 ± 0.6 µg/ml. The mean minocycline ratio comedonal level/blood level was 3.0 ± 1.6. The values of comedonal weights and mean minocycline ratio comedonal level/blood level at day 15 and day 30 were not statistically different, respectively.

Conclusion: The comedonal diffusion of minocycline was demonstrated 15 and 30 days after a daily intake of minocycline, 100 mg/day, in a acne volunteers population.

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ZINC THERAPY FOR ACNE: RESULTS OF A COHORT STUDY
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The purpose of this study was to assess the long-term benefit of zinc therapy in acne patients.

Patients and Method: We carried out a retrospective analysis based on the medical file of 60 patients treated with zinc in a French General Hospital (CHU Nantes). Initial dose of treatment was zinc gluconate 200mg/day.

Results: 60% of the acne cases were classified as grade 6 or 7 according Samuelson's photographic method (1). 53% of patients (mean age: 19) had experienced treatment failures before zinc therapy. Therapeutic response after 2 months of treatment was considered as complete for 25% of the patients and partial for 50%, 30% of patients in complete remission were relapsing within 6 months after the end of zinc therapy. For patients with complete response the mean duration of zinc therapy was 4 months. For patients in partial remission after 4 months of treatment a prolonged treatment did not improve the clinical result.

Conclusion: Zinc therapy in acne with an improvement in 90% of patients appears as efficiency as cyclic. Prolonged treatment with zinc (superior 4 month) appears without interest, for obtaining a better clinical result. No severe side effects were noted.


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WHY AND WHEN THE TREATMENT OF ACNE FAILS. WHAT TO DO.
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The vast majority of acne patients show a good response to the proper acne treatment. However a small percentage not exceeding 10-15% do not respond as satisfactorily as expected. For the following reasons:

1) Poor compliance because of improper advising and tasting. The doctor must follow a strategy in the treatment of acne and discuss many important points with the patient.
2) Selection of the inappropriate formulation of the appropriate topical drug.
3) Poor responders, despite proper treatment. The reason for poor response is a) the very high sebum excretion rate which results in the lowering of the concentration of the antibiotics in the hair follicles and b) Gram-negative flora.
4) Patients suffering serious side effects. All the systemic anti-acne medications (Antibiotics - Isotretonin, etc.) can, very rarely, cause serious side effects, necessitating proper manipulations and perhaps complete change of therapy.
5) Patients suffering from cystic acne. Cystic acne needs a very specific management.
6) The medicator's lack of attention to treated acne. Patients suffering some initial forms of acne need a much more aggressive treatment than their acne severity would need.
7) Patients with scars. The treatment of scars is not so successful as the treatment of active acne. Scars can be either hypertrophic (keloid scars) or atrophic and the treatment varies accordingly. Closing, it has to be emphasized that all acne cases can be adequately controlled if the relationship between doctor and patient has been built on trust and confidence.

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ACTUAL TRENDS FOR THE TREATMENT OF NODULO - CYSTIC ACNE
N. Berova, A. Nikolova

Our strategy is based on the combination of effective systemic and local drugs for the most resistant form of acne vulgaris - the nodulo - cystic acne. We treated 30 patients of nodulo - cystic acne with a combination of 1) 13-cis retinoic acid administered orally as Rosaccinat tabl. (Hoffman La Roche) 1 mg/kg/d for one month and then 0.5 mg/kg/d for 6 months to one year, depending on the improvement and the therapeutic effect, 2) Locally applied azeluk acid twice daily as Skinoren (Schering, Berlin), 3) Aftersalves - peeling with glycolic acid each day for 4 months, finished the procedure. Results: Complete remission was observed in 12 patients and improvement in 11, so that the positive effect could be estimated as 77% achieved between the 4th and the 6th month. From the theoretically possible side effects, we did not observe any, neither for the systemic, nor for the local treatment.

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THE EFFECTS OF AN ORAL CONTRACEPTIVE CONTAINING CPA ON ACNE, SEBORRHEA AND HIRSUTISM - RESULTS OF AN OPEN - LABEL MULTICENTER STUDY IN 800 WOMEN.

Background: Cyproterone acetate (CPA) is a potent antiandrogen used in combination with ethinylestradiol, acne, seborrhea and hirsutism.

Objective: The aim of this open-label, multicenter study was to determine the efficacy and tolerance of CPA and ethinylestradiol (EE) in women with various grades of facial acne, seborrhea and hirsutism.

Patients: 890 women (15 to 50 years) with grade I-IV (Peligri & Kligman) facial acne.

Methods: Patients received 6 cycles of 35 µg EE and 2 mg CPA. Changes from baseline counts of comedones, papules, pustules, nodules and cysts were monitored. Reduction in the number of all and individual lesions were classified as: 75-100%, very good; 50-75%, good; 25-50%, moderate; ≤ 25%, absence of therapeutic effect.

Results: A good or very good response (i.e.reduction in counts ≤50%) was seen in 82.6% (95% CI: 80.1-85.5) of patients after 6 cycles of EU/CPA. A significant decrease of acne lesions occurred in all groups throughout the study (p<0.05). A greater than 50% reduction in open and closed comedones, papules, pustules, nodules and cysts was observed in 75.6%, 80.0%, 88.4% and 85.1, respectively. By the end of the study, 64.3% of women displayed a lower grade of acne and only 4.9% experienced exacerbation of the condition. Seborrhea resolved in 70.1% of sufferers and the incidence of hirsutism decreased from 8.7% to 3.6%.

Conclusion: EE/CPA is an effective treatment for SAA-syptoms.
ACNE, THERAPEUTIC EFFECTS OF MITOXANTRONE
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In a period of six years we have examined twelve hundred-twenty-eight patients with Multiple Sclerosis, seven hundred-twenty-eight of which were female. Twenty-eight female patients of our pool have been suffering from a moderate to severe case of progressive Multiple Sclerosis. These 28 patients were found to also be suffering from chronic resistant to treatment acne. In the context of their treatment, for Multiple Sclerosis, the patients received 80-100 mg of mitoxantrone every six months during the period. The clinical evaluation was made using the EDSS scale before and after treatment. In all 28 cases there has been a clinical improvement of the Multiple Sclerosis symptoms using the EDSS parameters. At the same time an improvement has been realised in their acne problem. In 26 of the patients presented an amelioration in their acne condition analogous to that of the Multiple Sclerosis symptoms.

If we would consider the low amount of the doses, further research is warranted to further inquire the possibility of a new alternative treatment.

MONITORING ISOTRETINOIN THERAPY BY MEASURING PLASMA LEVELS: A USEFUL TOOL IN ACNE MANAGEMENT
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Isotretinoin (13-cis-RA) for oral therapy in severe acne congestiosa and acne nodulocystica in ten patients was given a course of treatment. Isotretinoin reduces sebum production, causes involution of sebaceous glands and modulates cell proliferation and differentiation by interacting with nuclear retinoid receptors. It exerts several dose-dependent mucocutaneous and systemic adverse effects, besides its teratogenic potency.

33 patients (29 males, 4 females; age 14-53 years) with severe acne and acne related disorders were studied under long term oral intake of isotretinoin. Therapeutic effects and side effects were evaluated prior therapy and in monthly intervals, and subsequently correlated to the administered oral dose /kg body weight. The corresponding plasma levels of isotretinoin and its metabolite 4-epo-isotretinoin were measured by reversed-phase HPLC. Dose dependent and important interindividual differences of isotretinoin plasma levels were detected: Plasma levels of isotretinoin at a mean dosage of 0.6 mg/kg body weight ranged between 89-231 ng/ml, with 3 to 5 times higher plasma levels for its metabolite 4-epo-isotretinoin. Plasma levels correlated well with the oral administered dose of isotretinoin and the observed mucocutaneous side effects, and oral dosage was adapted accordingly. Our data demonstrate that measuring plasma levels may be a helpful tool to determine the individual therapeutic dose regimen in patients with severe acne in order to minimize undesired side effects.

DIFFUSE SEBACEOUS GLAND HYPERPLASIA: SUCCESSFUL TREATMENT WITH ORAL ISOTRETINOIN
T. Janzen, C. G. Schäfer, A. Limper, P. Kind, G. Plewig, Department of Dermatology, Ludwig Maximilians-University of Munich, Germany
We report the case of a 43 year-old woman with sebaceous gland hyperplasia that occurred in a diffuse pattern of aggregated yellowish or whitish papules involving the entire face, neck and upper chest. Comedones, pustules and inflammatory papules were absent. The eruption was accompanied by marked seborrhoea. The histopathological examination revealed enlargement of the sebaceous acini, in a focally increased number of immature sebocytes and a dilated follicular infundibulum. Oral therapy with isotretinoin, 25 mg (0.5 mg/kg body weight) per day, resulted in remarkable improvement within three months. A dosage of 2.5 mg daily was established as the lowest possible dose necessary to control the sebaceous hyperplasia. Isotretinoin was stopped after six months. No recurrence has been observed for more than eight months after treatment. Diffuse sebaceous gland hyperplasia is a rare variant of seboplaudral proliferative disorders which is different to the well-known circumscribed type.

PRESCRIPTION OF ZINC THERAPY IN ACNE: STUDY OF A SAMPLE OF GENERAL PRACTITIONERS AND DERMATOLOGISTS
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Objective: The specificities of zinc therapy (non-invasivity and no photosensitive effect) are well-known, but the use of such a treatment isn't well defined. The survey conducted by the CRESGE on a sample of French dermatologists and general practitioners (GPR) allows a better understanding of the treatment modalities of acne in an ambulatory setting.
Method: A prospective survey on 1500 dermatologists and 230 GPR was initiated at two different periods (summer and fall 1993) and gathered data about type, former treatments (before zinc therapy) and co-treatments of acne, and also patient’s profile.
Results: 497 zinc prescriptions were analyzed. Mean age of GPs' patients is 19 years old, vs 22 for patients followed by dermatologists. Acne has an inflammatory component in 67% (GPR) vs 87% (dermatologists) of the cases. In 2 cases out of 3, zinc therapy is in a second line treatment, mostly after cyclins (treatment considered as a failure in nearly 50% of the cases). Concerning motives of prescription, the type of acne (inflammatory) and good tolerance to zinc treatment are mentioned by 4 doctors out of 5. Zinc therapy is associated with local antibiotics or antibiotics in 3 cases out of 4.
Conclusion: Zinc therapy is mostly considered as a second line treatment in acne, after failure of antibiotic treatment or as a relay treatment. The choice of zinc therapy is motivated by the type of lesion, the good tolerance and lack of contra-indications of zinc treatment.

ORAL ISOTRETINOIN IN HIV POSITIVE WOMEN WITH ACNE: REPORT OF 3 CASES
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Three HIV positive women (30-45 y) presented with papulo-pustular eruption on the face. One patient had a typical polymorphous acne and two patients presented an acneiform eruption without comedones. Two were severely (respectively 4 and 10 CD4) immunosuppressed and had developed at least one opportunistic disease. Systemic antibiotics showed no efficacy. Treatment with isotretinoin 0.5 mg/kg resolved in rapid response and clearing was obtained within 2 to 7 weeks of treatment with a total dose of respectively 10,83 and 90 mg/kg. No recurrence was noted 6 months after the end of the treatment. Systemic retinoids have been used in different skin diseases associated with HIV infection and were well tolerated. In our experience with 3 HIV immunosuppressed patients systemic isotretinoin seems to be extremely efficient and well tolerated.

DIFFUSE SEBACEOUS GLAND HYPERPLASIA: SUCCESSFUL TREATMENT WITH ORAL ISOTRETINOIN
T. Janzen, C. G. Schäfer, A. Limper, P. Kind, G. Plewig, Department of Dermatology, Ludwig Maximilians-University of Munich, Germany
We report the case of a 43 year-old woman with sebaceous gland hyperplasia that occurred in a diffuse pattern of aggregated yellowish or whitish papules involving the entire face, neck and upper chest. Comedones, pustules and inflammatory papules were absent. The eruption was accompanied by marked seborrhoea. The histopathological examination revealed enlargement of the sebaceous acini, in a focally increased number of immature sebocytes and a dilated follicular infundibulum. Oral therapy with isotretinoin, 25 mg (0.5 mg/kg body weight) per day, resulted in remarkable improvement within three months. A dosage of 2.5 mg daily was established as the lowest possible dose necessary to control the sebaceous hyperplasia. Isotretinoin was stopped after six months. No recurrence has been observed for more than eight months after treatment. Diffuse sebaceous gland hyperplasia is a rare variant of seboplaudral proliferative disorders which is different to the well-known circumscribed type.

EXTERNAL TREATMENT OF ACNE VILIGANS BY NEW SORBENT SILLARD AND LASEROTHERAPY
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We studied the influence of original sorbent Sillard and new technique of laserotherapy in the treatment of patients with acne vulgaris. Two groups of patients were taken for trial research. The first group received laserotherapy and Sillard. The second group received only traditional methods of treatment. Sillard was prescribed as monotherapy 10 days, 5g daily. The traditional treatment included antibiotics, vitamins, sedatives and herbal medicines. Steroids and immunotherapy were not used. Laserotherapy was conducted by gallium arsenide laser in infrared range, with power of 15 mWt, exposure time 15 min, for two weeks. Both groups of patients received applications of penetrating action vitamin including diacetilhyaluron 20%, sulfur 3%, salicilic acid 5%, theezylcin 6%. A significant improvement of clinical course and immunological indexes of blood were noted after three weeks by 40% of patients of the first group. It was 1.8 times higher then in the second group. It may be accounted for, by the fact, that Sillard conjugates effectively xenobiotics and their derivatives in the diisoprase organs. Laserotherapy brings to normal the disorders of system and local immunity, neurovegetal disfunction of skin glands and megacirculatory.
TREATMENT OF ACNE SCARS
S.F. Marin, Dermatological Unit, Calatayud Hospital, Calatayud, Spain.

We carried out a retrospective study between March '94 and March '95 on a group of 24 patients, 19 of them being women with acne scars, mostly located in cheeks. With ages understood between 16 and 31 years.

All of them were treated with sessions of Peelings with Neostrata Glicolic Acid (70% free) every two weeks.

The number of sessions that were necessary to get the desired effects oscillated between 6 and 15 depending on the depth and number of scars as well as the degree of satisfaction of the patients, the majority requiring between 6 and 10 sessions. A marked improvement was noticed by the proper patients between the 2nd and the 5th session.

We can conclude saying that this method is simple, not aggressive, and comfortable for the patients, together with the fact that very positive results are obtained in the treatment of acne scars.

CRYOSURGICAL TREATMENT OF ACNE KEOLOIDS
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The management of acne keloids is difficult. They tend to be refractory and frequently recur. We used cryosurgery for the treatment of 16 patients with 26 acne keloids (median: age 23 years, size 0.23 cm², duration of lesion 60 months). The lesions were mostly localized at the chest (46%) and the back (42%). Cryosurgery was mainly applied with the contact method (85%), single freeze-thaw cycle, whereas nitrous oxide (50%) and liquid nitrogen (50%) were equally used as refrigerants. Excellent and good results with flattening of the lesions to the skin level or slightly persisting hypopigmentation were obtained in 57% of the lesions. Resistance to the treatment occurred in 12% of the lesions. The excellent and good results were particularly observed in cases with short duration (median 59 months); poor results were shown by older keloids (median 94 months). Younger patients respond better (median age with excellent and good results: 23 years in contrast to patients with poor results: 38 years). Resistance was mostly observed in cases of larger keloids (median 1.13 cm²). Method of freezing and refrigerant had no influence on the results. The frequency of sessions was decisive (≤4 sessions: 100% excellent and good results; 1-3 sessions: 50% poor results). Only minor side effects occurred (slight local pain 50%, lesional changes of pigmentation 12%). Neither progression nor recurrences were observed. Cryosurgery was found effective in the treatment of acne keloids, especially, young smaller lesions exhibited satisfactory results. To obtain best results early repeated treatment is required.
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