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DERMATOLOGIA COSMETOLOGICA

A cura di P. Morganti e L. Muscardin

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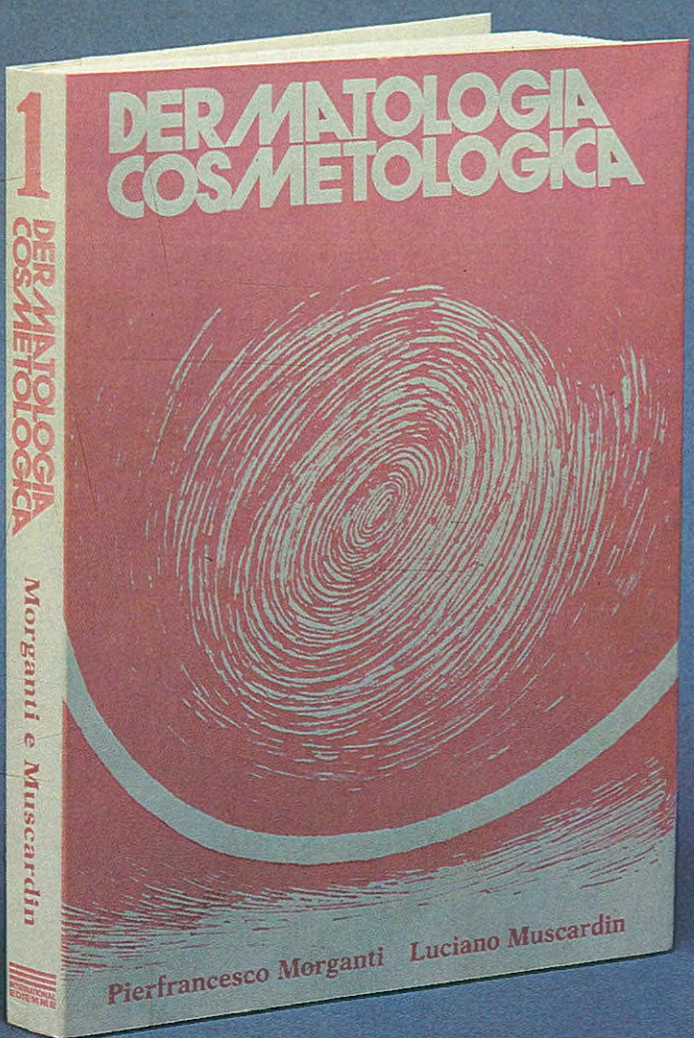

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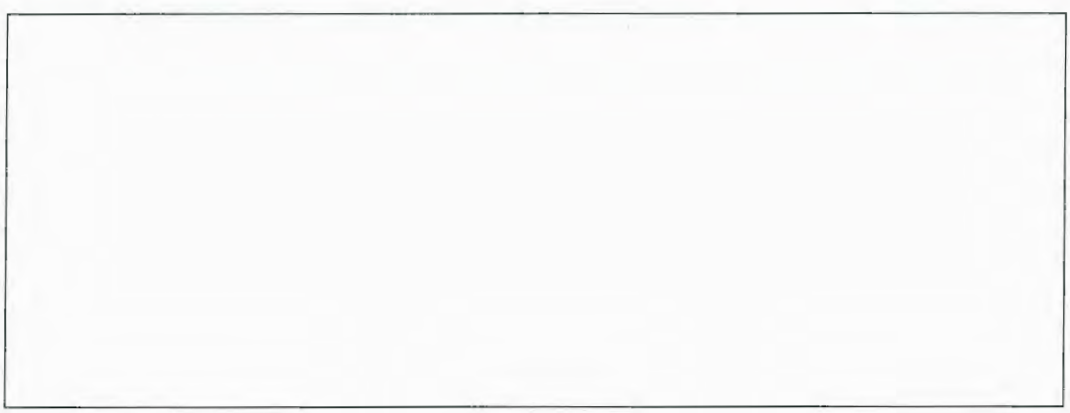

A new look at old skin: A challenge to cosmetology

1st International Meeting on Cosmetic Dermatology, Rome, Italy, March 7-9, 1985

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A CHALLENGE TO COSMETOLOGY**

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Scent glands in mammals: social functions and implications of apocrine gland odours

F. JOHN G. EBLING, D.Sc., Ph. D., University of Sheffield, Sub-Department of Dermatology, Academic Division of Medicine, Royal Hallamshire Hospital, Sheffield, S10 2JF (Great Britain).

Received: May 20, 1987. Presented at 2nd International Meeting on Cosmetic Dermatolgy. Every day Problems in Dermatology: The Cosmetic Connection Rome, Italy May 19-22, 1987.

Key words: Apocrine glands, Human odour, Axillary secretion, Odor voluptatis, Pheromones, Androstenone and Sndrostenol, Odoriferous steroids.

Synopsis

The human apocrine glands and associated structures are scent organs, just as are similar complexes in many other orders of mammals. The units of the axillary organs are precisely equivalent to, for example, those of the oral glands of ground squirrels or the chin glands of rabbits. The rabbit glands are androgen-dependent; clinical and other evidence suggests that this is equally true of the human axillary glands. Amongst other subtsances, the axilla produces 16-androstenes, the same volatile steroids which emanate from boars and induce sexual receptivity in sows. Production of 16-androstenes by the axilla involves the activity of bacteria, particularly of diphtheroids. The recent demonstration that axillary secretions of both men and women can affect the timing of the menstrual cycle clearly establishes the existence of true human pheromones and adds verisimilitude to the numerous entertaining anecdotes on the role of odour in human behaviour and history.

Riassunto

Le ghiandole apocrine umane e le relative strutture sono organi sebacei, proprio come lo sono complessi simili in altri ordini di mammiferi. Le ghiandole ascellari, per esempio, sono esattamente equivalenti a quelle orali degli scoiattoli o a quelle del mento dei conigli. Queste ultime sono androgeno-dipendenti; prove cliniche e non fanno supporre che ciò sia altrettanto vero per le ghiandole ascellari umane. Oltre ad altre sostanze, le ascelle producono 16-androstene, lo stesso steroide volatile secreto dai cinghiali e che produce lo stimolo sessuale nelle scrofe. La produzione ascellare di 16-androstene è legata all'attività di batteri, in particolare di difteroidi. Di recente è stato dimostrato che le secrezioni ascellari di uomini e donne possono influire sulla regolarità del ciclo mestruale e ciò prova inequivocabilmente l'esistenza di autentici feromoni umani e dà verosimiglianza ai numerosi e divertenti aneddoti storici sul ruolo degli odori nel comportamento umano.

Résumé
Les glandes apocrines humaines et ses structures sont del organes olfactifs qui présentent une parfaite ressemblance avec les organes de beaucoup d'ordres de mammifères. Les unités des organes axillaires par exemple, sont identiques à celles des glandes orales d'une espéce d'écureuil (lat. citellus) ou aux glandes du menton des lapins. Parmi d'autres substances, la glande sudoripare axillaire produit des 16-androstènes, c'est-à-dire les mêmes stéroïdes volatils qui sont émis par les sangliers et qui provoquent ainsi la réceptivité sexuelles des laies. La production des 16-androstènes, émis par l'axille, implique aussi une activité de bactéries, en particulier des diphtéroïdes. Récemment on a démontré que les sécretions axillaires masculines et féminines peuvent peser sur la régularité du cycle menstruel; cela prouve l'existence de véritables phéromones humains et rend aussi plus vraisemblable la floraison d'amusants anecdotes à propos du rôle de l'odeur dans le comportement et l'histoire de l'homme.

Resumen
Las glándulas apócrinas humanas y las relativas estructuras son órganos sebáceos, precisamente como complejos semejantes en otros muchos ordenes de mamíferos. Las glándulas de los sobacos, por ejemplo, son exactamente equivalentes a las orales de las ardillas de tierra o a las de la barbilla de los conejos. Las glándulas de los conejos son andrógeno-dependientes; hay pruebas clinicas y de otro tipo de que esto es verdadero también para las glándulas de los sobacos. Entre otras sustancias, los sobacos producen 16-androsten, el mismo esteroide volátil que producen los jabalís e induce la receptividad sexual en las cerdas. La producíon de 16-androsten por los sobacos comporta la actividad de bacterias, en particular de difteroides. La reciente demostración que la secreción en los sobecos de hombres y mujeres puede afectar la regularidad del ciclo menstrual establece de manera clara la existencia de verdaderos feromones humanos y añade verosimilitud a las numerosas anécdotas cerca el papel del olor en el comportamiento humano y en la historia.

Synopse
Die menschliche Apokrindrüsen und verbundene Strukturen sind Riechorgane, wie ähnliche Systeme, die in vielen anderen Säugetierarten anwesend sind. Die Bestandteile der Achselhöhlenorgane sind denen der Speicheldrüsen bei eichhörnche ähnlich, der der Kinndrüsen bei Kaninchen. Ergebnisse beweisen, dass Kaninchen und auch Menschendrüsen androgenabhängig sind. Unter anderem produziert die Achselhöhlendrüse 16-Androstenes, d.h. die gleiche volatile Steroidsstoffe die bei Ebern anwesend ist, und die die Geschlechtsempfänglichkeit der Säue fördert. Die Mitwirkung von Bakterien (ins Besondere Diphtheroid) ist dazu notwending, um die Produktion des 16-Androstenes in Achselhölendrüsen zu ermöglichen. Jüngst wurde bewiesen, dass die Absonderungen bei Männern und Frauen einen grossen Einfluss auf die Häufigkeit des Menstruationsflüsses ausüben, demzufolge wurde die Existenz von rein menschlichen Pheronomen bestätigt, und es wurde auch noch einmal bekräftigt, dass der Geruch eine wichtige Rolle spielt was menschliches Verhalten und Leben anbelangt.

Introduction

Dermatologists and cosmetologists often regard the human skin glands as an embarrassment rather than a physiological advantage. Sebaceous glands have no obvious single function except the provision of a site for acne, and though sweat is a proper accompaniment to violent exercise, it must subsequently be removed or deodorized without delay, as must all other potentially odoriferous material. Especially unpopular, at least in theory, are the glandular areas of the axillary and genital regions, which have been and still are subjected to a barrage of cosmetic assaults. Other mammals are less self-conscious; a wide variety of them have scent glands which are overtly utilized in a range of behavioural interactions such as, for example, the establishment of individual identity, social domiance or territory, and sexual attraction. This paper will review some of this phylogenetic background and the evidence that human apocrine glands are, as in other mammals, under hormonal control and have social or sexual functions.

Skin glands

There are two main kinds of glandular unit in the skin of mammals: sebaceous, which secrete lipid, and tubular, with an aqueous product. The first type form their secretion by complete disintegration of the cells in which it is made and are known as *holocrine*. In the second type, known as *merocrine*, the cells are not destroyed, but extrude their product into a lumen. Schiefferdecker (1917); who invented the terminology, further divided merocrine glands into *eccrine*, in which the cells remain completely intact, and *apocrine*, in which he believed secretion involved decapitation of the terminal projections of some, at least, of the cells. Eccrine glands are not associated with hair follicles, and they are found in hairy skin only in some primates and in man, where they function in sweating. In many other mammals, for example in rats and mice, similar glands are found in the footpads and help the animal to grip the substrate. So-called «apocrine glands», whether or not their mode of secretion is truly apocrine, are connected to the ducts of hair follicles and occur in almost all mammalian orders, excepting only whales, sea cows and scaly anteaters. In some large mammals, notably in horses, oxen and camels, apocrine glands are present throughout the hairy regions and function as sweat glands to control body temperature. In a wider range of species, however, apocrine units are aggregated to form scent glands, sometimes, though not always, in association with sebaceous units. For example, the chin and anal glands of the rabbit contain only tubular units, whereas the anal glands in group squirrels contain both apocrine and sebaceous units, and the well-known flank organs of the golden hamster are purely holocrine. Both types of scent gland occur in primates; for example lemurs have «antebrachial» glands, composed of tubular units, on the inner forearms, and paired «brachial» glands, composed of sebaceous units, on the anterior chest. A comprehensive account of scent glands and their function has been compiled by Brown and Macdonald (1985).

The glands of ground squirrels and of rabbits may be considered as model structures. The oral glands of ground squirrels (Kivett, 1978) have several lobes, each composed of branched tubules which coalesce to open into the canal of a hair follicle (Fig. 1). Small sebaceous glands are also present. The anal glands consist of three masses with both apocrine and large sebaceous units, each ope-

ning by a channel to the anal canal. When the animal is alarmed, the channels can be everted to form papillae. The secretions are used to mark territory or in social rituals.

Rabbits have three pairs of apocrine glands: anal, inguinal and submandibular. Mykytowicz (1965, 1966a, 1966b) has described their structure in detail and shown that their development is inhibited by castration in early life. There is clear experimental evidence (Ebling, 1977; Strauss and Ebling, 1970; Wales and Ebling, 1971) that the growth of the glands is stimulated by testosterone and inhibited by oestradiol (Fig. 2).

Chin glands are used for marking vegetation or the ground (Mykytowycz, 1968), and for nose to chin postures in social encounters. Anal glands scent the faeces which are also used for territorial purposes. The inguinal apocrine glands differ from the chin and anal glands, in that they are associated with a pair of discrete glands composed of holocrine units. Their secretion appears to be chemically different, and is probably concerned with sexual encounters.

Human apocrine glands

In man, rudiments of tubular glands are formed in most embryonic hair follicles.

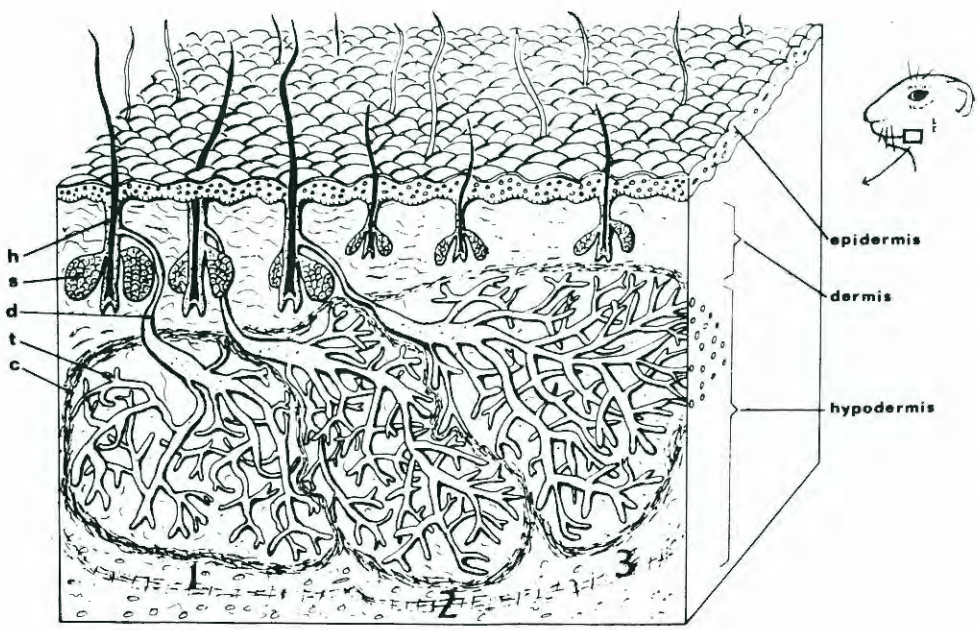


Figure 1

They become canalized and functional in any numbers, however, in only a few areas, including notably the axilla, the perineum, and the areola (Montagna and Parakkal, 1974). The fact that apocrine glands function as sweat glands in some other large mammals, and the evidence from comparative anatomy that eccrine glands appear to have gradually replaced apocrine glands in the hairy skin of nonhuman primates, has engendered a view that apocrine glands were once sweat glands but are now vestigial. It is more appropriate, however, to stress that human apocrine glands are survivors of an archaic system of scent organs which occurs in nearly all mammals and of which the oral glands of ground squirrels and the chin glands of rabbits are appropriate anatomical and physiological models. Each unit of the human axillary organ (Fig. 3) consists of a large coiled tubular gland opening into a hair follicle. The secretory segment is large and compact, with adjacent loops often fusing together and joined by shunts, and extending deep into the subcutaneous fat. There are modest sebaceous glands. The complex is remarkably similar to that of the oral gland of the ground squirrel; the axillary organ differs only in that eccrine sweat glands are also present and contribute substantially to the output.

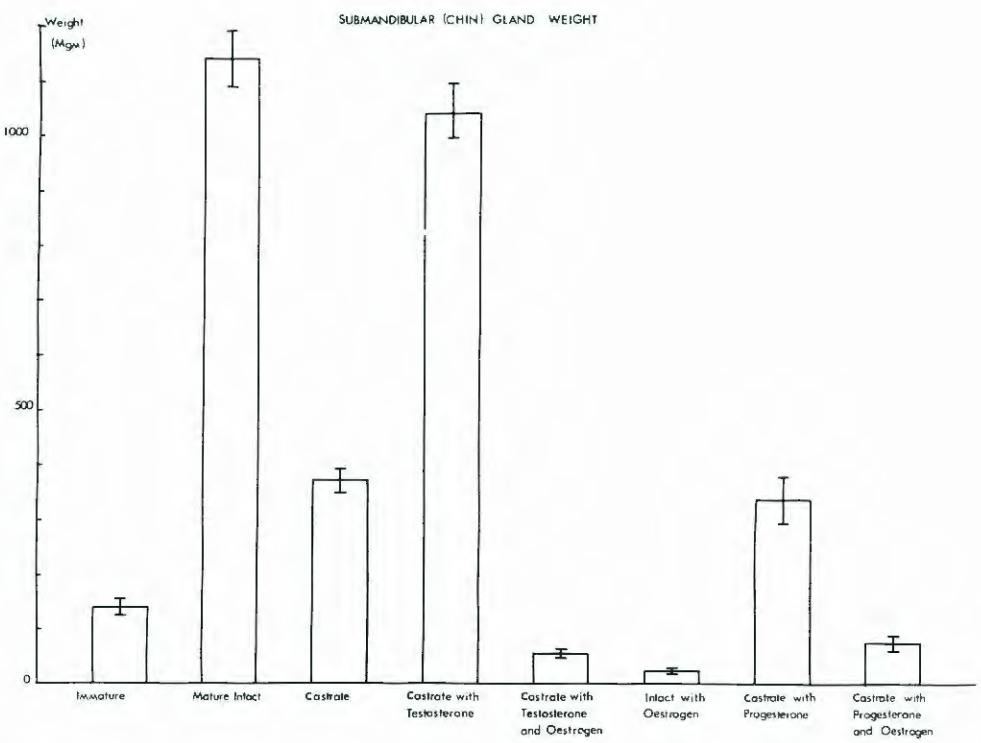


Figure 2

Endocrine control of human apocrine glands

The hormonal dependence of the axillary organs is indicated by the fact that they do not become fully developed and functional until puberty (Hamilton, 1958). Since the pubic hair develops around the same time, it seems virtually certain that apocrine glands in this region also are hormone-dependent as, indeed, are sebaceous glands throughout the body. The role of androgen appears to be firmly established by the demonstration that *hidradenitis suppurativa*, an inflammatory condition of the apocrine glands and associated structures in the axillary and perineal regions, is ameliorated by treatment with the antiandrogen cyproterone acetate (Ebling, 1986; Ebling et al., 1980; Mortimer et al., 1986a; Sawers et al., 1986). The overall pattern of plasma androgen levels suggests that this disease has an androgenic basis (Mortimer et al., 1986b). These conclusions are not contradicted by a reported failure to show any effect of implantation of testosterone into the axilla (Shelley and Hurley, 1957), since like the sebaceous glands (Strauss et al., 1962) the apocrine glands of adult males may already be maximally stimulated by endogenous androgens. It is also worthy of note that the maximal rate of eccrine sweating is greater in men than in women and that this difference starts at puberty (Rees and Shuster, 1980). Mo-

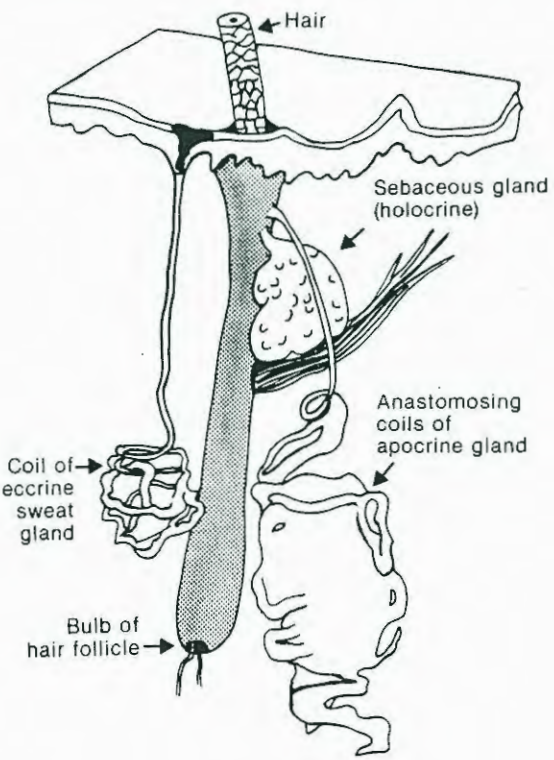


Figure 3

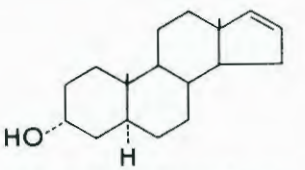
reover, the sweat rate in eunuchs and in females can be increased to the normal male range by treatment with androgen (Walton et al., 1983).

Composition of axillary secretion

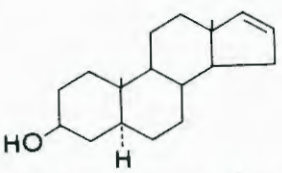
Human odour is usually ascribed to caprylic and similar volatile free fatty acids, which can be obtained from the genital and axillary and some other areas. Such secretions collected from the skin surface may, however, contain material from sebaceous and eccrine as well as from apocrine glands. Pure human apocrine secretion, obtained by cannulating tubules of the axillary glands has been described as a milky fluid containing proteins, reducing sugars and ammonia (Robertshaw, 1983).

Of particular significance is the demonstration that both 5α-androst-16-en-3-ol (Brooksbank et al., 1974) and 5α-androst-16-en-3-one (Bird and Gower, 1981) occur in human axillary secretion. These are the steroids (Fig. 4) which are produced by the boar and induce the sow to adopt a rigid stance, the so-called «standing reaction», in response to his attentions (Booth, 1980; Sink, 1967).

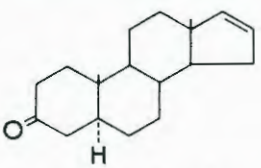
5α-Androstenone levels are higher in men than in women (Bird and Gower, 1981). In tests with alcoholic extracts of axillary secretion, that of males was considered to have a «strong» or «musky» odour,



5α-Androst-16-en-3α-ol



5α-Androst-16-en-3β-ol



5α-Androst-16-en-3-one

Figure 4

whereas that of women was more generally rated as «sweet» (Gower et al., 1985). The smell of pure 5α-androstenone was described as «strong», «musky» or «urinous» by men and «repellant» or «unpleasant» by 90% of women. The evidence thus suggests that the musky or strong smells of male axillary extracts, as compared with the sweeter smell of those from women, are related to production of 5α-androstenone.

Fresh axillary secretion is generally considered to have little odour. The pungence appears to develop as a result of the action of anaerobic diphtheroid bacteria on the native secretion. Leyden et al. (1981), while unable to detect differences in the skin surface lipids between smelly and non-smelly axillae, found clear bacteriological differences. Odour was associated with lipophilic diphtheroids. In an experiment, they first sterilized volar forearms of human subjects with compresses of 70% ethanol for two minutes. After evaporation, 3 μl of sterile apocrine sweat, collected after local injection of adrenalin, was spread over a small area. This was then inoculated with 0.1 ml of a suspension of one or other strains of bacteria isolated from human axillae. Odour was produced only by diphtheroids, not by micrococci (Fig. 5). Subsequently, Bird and Gower (1982) have produced evidence that 5α-androst-16-en-3-one is, indeed, a product of the bacterial action.

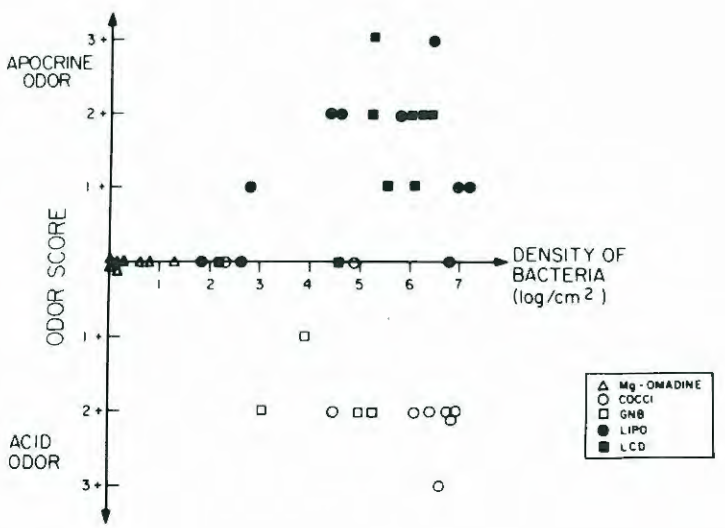


Figure 5

Biological and social functions of human odour

If the role of odour in human behaviour is sparsely documented in science, it is richly illuminated by anecdote. Female odour has, indeed, affected the course of history. King Henry III, while at the betrothal feast of the King of Navarre and Margaret of Valois, is said to have dried his face with a garment which was wet with the perspiration of Maria of Cleves, for whom he developed an irresistable passion, notwithstanding that she was the bride of the Prince of Condé (Doty, 1976).

Havelock Ellis (1905), in his book on the psychology of sex, laid stress on the sexual aspects of odour. He points out that the characteristic body odours develop at puberty and may become exaggerated in sexual and other emotional states. Men frequently, if not normally, emit an odour during sexual excitement which may last for several hours. So do women. According to the ancient medical writers, this phenomenon was especially marked in harlots and in the newly married. A case has been recorded of a woman who emitted a rose odour for two days after coitus, and it is said that there was a monk in Prague in the seventeenth century who could recognize which women were chaste by their smell. A somewhat more practical use of female odour was that of Asiatic princes who sometimes «caused a number of ladies to race in the seraglio garden until they were heated; their garments were then brought to the prince, who selected one of them solely by the odour». Ellis quotes other authors as attributing the source of the female sex attractant to the glands at the vulvar orifice.

Iwan Bloch (1934) has surveyed the field, with many historical references, in his book «Odoratus Sexualis». Bloch was particularly intrigued by the belief that the natural odour of the body becomes more intense immediately before, during, and after sexual intercourse, and he attributed this mainly to secretion from the axillae. According to Bloch, the philosopher Democritus was well acquainted with this *odor voluptatis*. «When Hippocrates visited him once, the physician was much surprised that the sage greeted a female who was accompanying the father of Greek medicine, one day as a virgin, and the next as a woman. La Motte le Vayer regards it as highly probable that in these judgments Democritus was following not his eye, as Diogenes Laertius supposes, but his nose. Ever so often we hear the story of the blind man who, upon his return home, recognized that in his absence his daughter had suffered herself to be seduced».

If Bloch paid most of his attention to female odours, Ellis was well aware of the possible effects of male odour on the female. He refers to an Austrian peasant who found that he was aided in seducing young women by dancing with them and then wiping their faces with a handkerchief he had kept in his armpit, and he mentions also the case of a young lady, not usually sensitive to body odours, who on one occasion «when alrealdy in a state of sexual erethism, was highly excited on perceiving the odour of her lover's axilla».

The historical evidence thus suggests that oodur, especially that from the axillary and genital regions, plays a part in social and sexual communication in man, just as it does in other animals. It is important, however, to recognize that the associations of odour may be cultural rather than biological or, at least, that any biological mechanisms may be heavily overlaid by cultural tradition. Ellis (1905) clearly recognized this. He pointed out that axillary odours are anathema to the

Japanese and that at one time an odorous axilla constituted a disqualification for military service. He illustrates the ambivalence of reactions to axillary odour by relating a story about a man who on a hot day entered a steamboat with a woman to whom he was attached and seated himself between her and a male stranger. He soon became conscious of an axillary odour which he concluded to come from the man and which he feltwas disagreable; but a little later, when he realized it proceeded from his own companion, the odour at once lost its disagreable character.

How far are these beliefs of history justified by experimental evidence? It seems clear that individual axillary odours can be recognized by their owners, and that discrimination can be made between male and female odours. Russel (1976), for example, had 13 women and 16 men wear T-shirts for 25 hours without bathing or using deodorants. The subjects were then asked to sniff the armpit regions of his or her own shirt, a strange male's shirt and a strange female's shirt. They were each asked to identify their own shirt and to report which of the other two shirts came from a male. Nine out of the 13 females and 13 of the 16 males correctly made both judgments.

Doty (1985) accepted that individual identification could be made on the basis of olfactory cues. (The conclusion, incidentally, adds verisimilitude to Ellis's report that Chinese can correctly sort items of laundry by their smell). However, Doty and his associates showed that there was a perfect correlation between perceived maleness and odour intensity. Thus discrimination may be based solely on intensity, not on any qualitative difference between males and females. Doty further found that, in American subjects at least, there were no differences between males and females in their ratings of the intensity and pleasantness of different odours, which would seem to vitiate against sex-specific roles in odour communication. However, regardless of their actual origin, odours which were believed to come from sexual partners were rated more pleasant than those believed to come from strangers. This finding clearly adds weight to the conclusion Ellis (1905) drew from the ambiguous reactions of a man to the odours of his girl friend on the steamboat trip.

Evidence about the effects of vaginal odour on behaviour is difficult to assess. Doty et al. (1975) took samples of vaginal secretions from women at different stages of the menstrual cycle and invited both male and female judges to rate the odours. Secretions from the preovulatory and ovulatory phases of the cycle were generally rated as weaker and less unpleasant in odour than those from the menstrual and luteal phases, but there was great variation.

One difficulty is that of identifying the nature and source of the important compounds, since a number have been isolated. For example, the view of several authours (for example, Curtis et al., 1971) that certain aliphatic acids are «pheromones» which elicit male attention is not, according to Doty (1985), satisfactorily established.

Another major question in all human studies is whether attitudes or responses to odour result from an underlying physiological mechanism or are culturally conditioned. Recent experimental evidence that human axillary secretions can affect the menstrual cycle appears to establish a clear biological as distinct from a psychological effect.

Ovulatory menstrual cycles of normal length (29.5 ± 3 days in North America) are known to be more frequent in women who have weekly coital activity than in those who do not. The act of coitus may

not be essential, provided a male is present and there is genital stimulation; self-masturbation is ineffective. Cutler et al. (1985) collected axillary secretions from males in which large numbers of lipophilic diphtheroids and micrococcaceae were present. Fifteen women aged 19 to 22 and one aged 30, with cycles of less than 26 or more than 32 days were then used as test subjects. Each woman received either an alcoholic extract of male axillary secretion, or pure ethanol, on her upper lip three times a week, and was instructed not to wash the area for at least six hours. The study was double blind in the sense that neither the administrator nor the receiver knew whether extract or placebo was being used. The results (Fig. 6) showed unequivocally that male axillary extracts reduced the incidence of menstrual cycles of aberrant length.

In a somewhat similar experiment (Preti et al., 1986) axillary secretions were collected from female donors and applied to recipients with normal length menstrual cycles. The onset of menstrual bleeding in the recipients moved significantly towards synchrony with that in the donors within two cycles (Fig. 7). Moreover, frozen axillary secretions from a group of women were able to alter the menstrual cycles of another group when thawed one year later.

Whether or not these experiments lead to

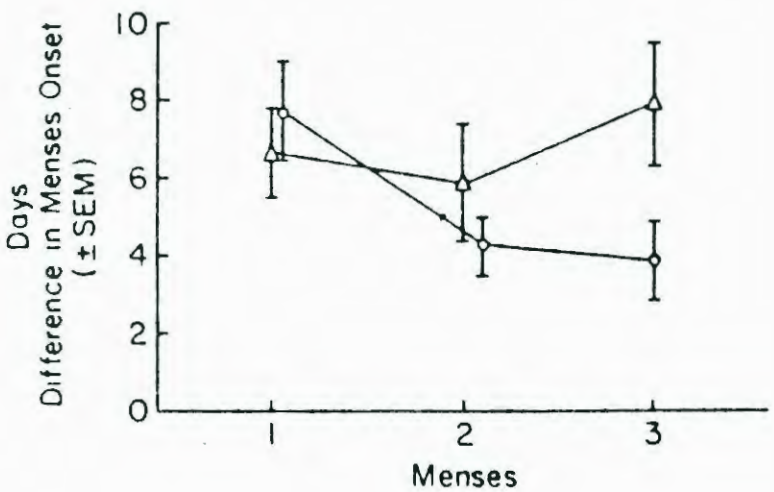


Figure 6

advances in therapy, they appear for the first time to show that human apocrine glands produce a true pheromone, that is to say a substance which produces a biological response in another individual, as distinct from a scent which may have a culturally conditioned, purely psychological effect.

Conclusions

The apocrine glands of man are scent organs, as are those of many other mammals, and the human axillary organ closely resembles structures in, for example, ground squirrels and rabbits. Scent glands are, in general, androgen-dependent and the evidence suggests that the axillary organ is no exception. The glands of the axilla, with the aid of bacteria, particularly diphtheroids, produce androstenone and androstenol, the same odoriferous steroids with which the boar induces sexual receptivity in the sow. The experimental demonstration that the human menstrual cycle can be affected by extracts of male and female axillary secretion, perceived by smell, suggests that a true pheromone is produced and adds weight to anecdotal beliefs that the axillary and other body odours have a functional role in behaviour.

The suppression of copious eccrine sweating with antiperspirants may be rational, but should deodorization be the aim of cosmetology? Suppression of all odour certainly eliminates social risks, but a more subtle approach to the understanding of the ways in which natural odour might be modified rather than eliminated is worth consideration.

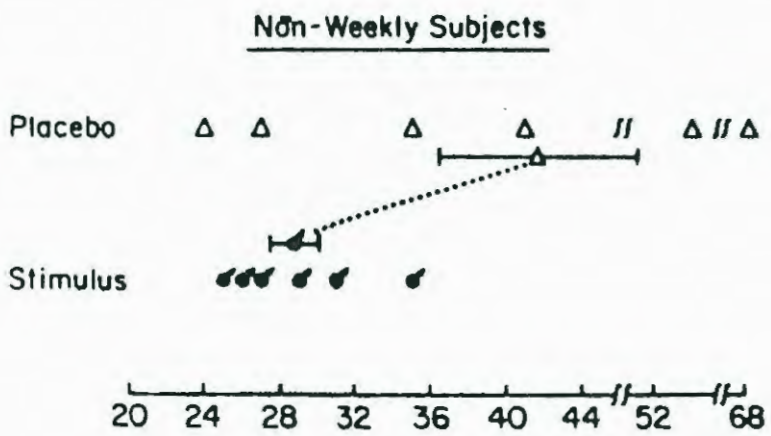
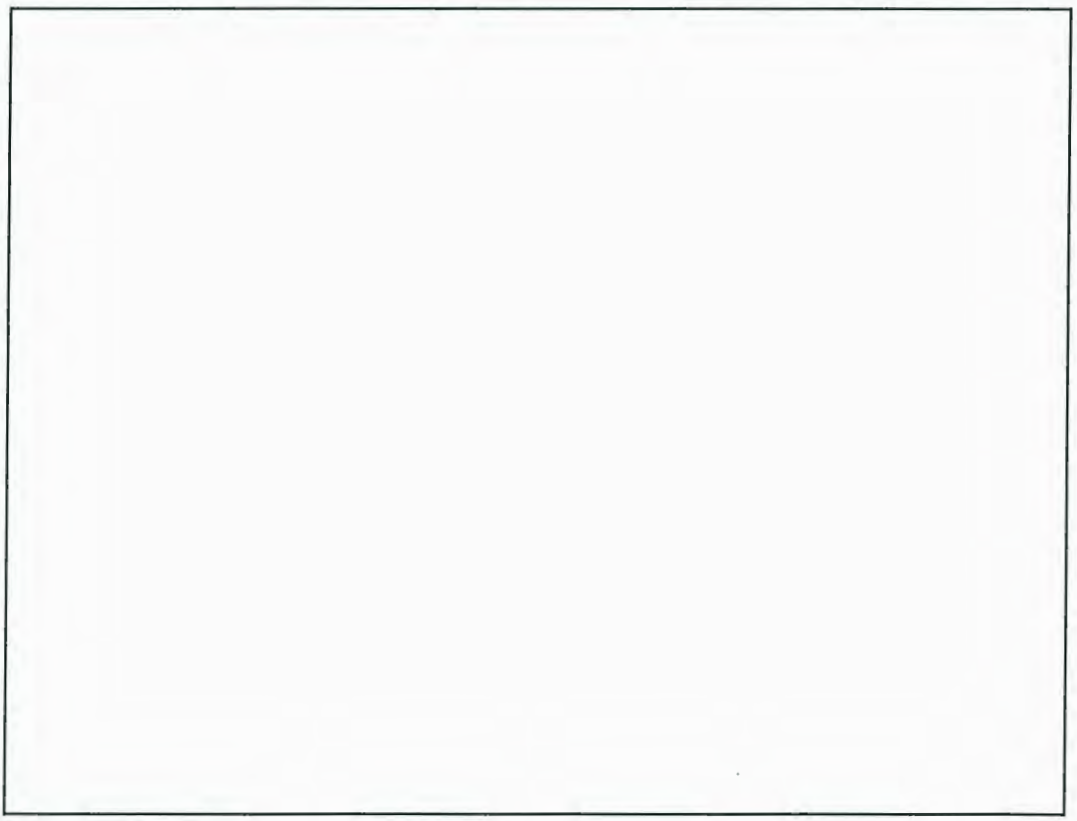


Figure 7

REFERENCES

1. **Bird S., Gower D.B. (1981)** «The validation and use of a radioimmunoassay for 5α-androst-16-en-3-one in human axillary collections» *J. steroid Biochem.* **14**, 213-219.
2. **Bird S., Gower D.B. (1982)** «Axillary 5α-androst-16-en-3-one, cholesterol and squalene in men; preliminary evidence for the 5α-androst-16-en-3-one being a product of bacterial action» *J. steroid Biochem.* **17**, 517-522.
3. **Bloch I. (1934)** «Odoratus sexualis» *Panorge Press, New York.*
4. **Booth W.D. (1980)** «Endocrine and exocrine factors in the reproductive behaviour of the pig» In: Olfaction in Mammals, Stoddart D.M. *Symp. Zool. Soc. Lond.*, **45**, 289-311.
5. **Brooksbank B.W.L., Brown R., Gustafsson J.A. (1974)** «The detection of 5α-androst-16-en-3-one in human male axillary sweat» *Experientia*, **30**, 864-865.
6. **Brown R.E., Macdonald D.W. (Eds.) (1985)** «Social odours in mammals» Vols. I and II. *Clarendon Press, Oxford.*
7. **Curtis R.F., Ballantine J.A., Keverne E.B., Bonsall R.W., Michael R.F. (1971)** «Identification of primate sexual pheromones and the properties of synthetic attractants» *Nature, London*, **232**, 396.
8. **Cutler W.B., Preti G., Krieger A., Huggins G.R., Garcia C.R., Lawley H.J. (1986)** «Human axillary secretions influence women's menstrual cycles: the role of donor extract from men» *Hormones and Behav.* **20**, 463-473.
9. **Doty R.L. (Ed.) (1976)** «Mammalian olfaction, reproductive processes and behaviour» *Academic Press, New York.*
10. **Doty R.L. (1985)** «The primates. III. Humans» In: Social Odours in Mammals, Brown R.E. and Macdonald D.W. *Clarendon Press, Oxford*, 804-832 (Vol. II).
11. **Doty R.L., Ford M., Preti G., Huggins G. (1975)** «Human vaginal odors change in pleasantness and intensity during the menstrual cycle» *Science, New York* **190**, 1316-1318.
12. **Ebling F.J. (1977)** «Hormonal control of mammalian skin glands» In: Chemical Signals in Vertebrates, Müller-Schwarze D., Mozell M.M., *Plenum Press, New York*, 17-33.
13. **Ebling F.J.G. (1986)** «Hidradenitis suppurativa: an androgen-dependent disorder» *Br. J. Derm.* **115**, 259-262.
14. **Ebling F.J.G., Cooke I.D., Randall V.A., Sawers R.S., Thomas A.K., Skinner J. (1980)** «The influence of cyproterone acetate on the activity of hair follicles and sebaceous glands in man» In: Androgenization in Women, Hammerstein J., Lachnit-Fixon U., Neumann F., Plewig G. *Excerpta Medica, Amsterdam*, 239-245.
15. **Ellis H. (1905)** «Studies in the psychology of sex. IV. Sexual selection in man» *F.A. Davies, Philadelphia.*
16. **Gower D.B., Bird S., Sharma P., House F.R. (1985)** «Axillary 5α-androst-16-en-3-one in men and women: relationships with olfactory acuity to odours 16-androstenes» *Experientia* **41**, 1134-1136.
17. **Hamilton J.B. (1958)** «Age, sex and genetic factors in the regulation of hair growth in man: a comparison of Caucasian and Japanese populations». In: The Biology of Hair Growth, Montagna W., Ellis R.A., *Academic Press, New York*, 399-433.
18. **Kivet V.K. (1978)** «Integumentary glands of Columbian ground squirrels (*Spermophilus columbianus*): Sciuridae» *Can J. Zool.* **56**, 374-381.
19. **Leyden J.J., McGinley K.J., Hölzle E., Labows J.N., Kligman A.M. (1981)** «The microbiology of the human axilla and its relationship to axillary odour» *J. invest. Derm.* **77**, 413-416.
20. **Montagna W., Parakkal P.F. (1974)** «The structure and function of skin» 3rd edition. *Academic Press, New York.*
21. **Mortimer P.S., Dawber R.P.R., Gales M.A., Moore R.A. (1986a)** «A double-blind cross-over trial of cyproterone acetate in females with hidradenitis suppurativa» *Br. J. Derm.* **115**, 263.
22. **Mortimer P.S., Dawber R.P.R., Gales M.A., Moore R.A. (1986b)** «Mediation of hidradenitis suppurativa by androgens» *Br. Med. J.* **292**, 245.
23. **Mykytowycz R. (1965)** «Further observations on the territorial function and histology of the submandibular cutaneous (chin) glands in the rabbit, *Oryctolagus cuniculus* (L.)». *Animal Behav.* **13**, 400-412.
24. **Mykytowycz R. (1966a)** «Observations on odoriferous and other glands in the Australian wild rabbit, *Oryctolagus cuniculus* (L.), and the hare, *Lepus europaeus* P. I. The anal gland» *CSIRO Wildlife Research* **11**, 11-29.
25. **Mykytowycz R. (1966b)** «Observations on the odoriferous and other glands in the Australian wild rabbit, *Oryctolagus cuniculus* (L.), and the hare, *Lepus europaues* P. II. The inguinal glands». *CSIRO Wildlife Research* **11**, 49-64.
26. **Mykytowycz R. (1968)** «Territorial marking by rabbits» *Scientific American* **218**, 116.
27. **Preti G., Cutler W.B., Garcia C.R., Huggins G.R., Lawley H.J. (1986)** «Human axillary secretions in-

fluence women's menstrual cycles: the role of donor extract of females» *Hormones and Behav.* **20**, 474-482.
28. **Rees J., Shuster S. (1980)** «Maximum rate of cholinergic sweating is greater in men than in women and this difference occurs at puberty» *Clin. Sci.* **60**, 689.
29. **Robertshaw D. (1983)** «Apocrine sweat glands» In: Biochemistry and Physiology of the Skin, Goldsmith L.A. *Oxford University Press, New York*, 642-653.
30. **Russel M.J. (1976)** «Human olfactory communication» *Nature, London* **260**, 520-522.
31. **Sawers R.S., Randall V.A., Ebling F.J.G. (1986)** «Control of hidradenitis suppurativa in women using combined antiandrogen (cyproterone acetate) and oestrogen therapy» *Br. J. Derm.* **115**, 269.
32. **Schiefferdecker P. (1917)** «Die Hautdrüsen des Menschen und der Säugetiere, ihre biologische und rassenanatomische Bedeutung, sowie die Muscularis sexualis» *Biol. Zbl.* **37**, 534-562.
33. **Shelley W.B., Hurley H.J. (1937)** «An experimental study of the effects of subcutaneous implantation of androgens and estrogens on human skin» *J. Invest. Derm.* **28**, 155-158.
34. **Sink J.D. (1967)** «Theoretical aspects of sex odour in swine» *J. Theor. Biol.* **17**, 174-180.
35. **Strauss J.S., Ebling F.J. (1970)** «Control and function of skin glands in mammals» *Mem. Soc. Endocr.* **18**, 341-371.
36. **Strauss J.S., Kligman A.M., Pochi P.E. (1962)** «Effect of androgens and estrogens on human sebaceous glands» *J. Invest. Derm.* **39**, 139-155.
37. **Wales N.A.M., Ebling F.J. (1971)** «The control of apocrine glands of the rabbit by steroid hormones» *J. Endocr.* **51**, 763-771.
38. **Walton S., Cbadwick L., Kendall-Taylor P., Shuster S. (1983)** «The effect of testosterone on maximal sweat rate in eunuchs» *Br. J. Derm.* **108**, 245.



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Ethyl lactate and benzoyl peroxide in acne vulgaris

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Key words: Ethyl lactate, Benzoyl peroxide, Acne vulgaris, Seborrhea.

Synopsis
In a double-blind study involving patients with mild to moderately severe acne vulgaris, a 2.5% Benzoyl Peroxide formulation was compared with a 5% Ethyl Lactate anhydrous gel, with a Benzoyl Peroxide-Ethyl Lactate Gel and with a new vehicle. This new vehicle seems to develop a considerable antiseborrheic activity per se, since it reduces the «casual level» by some 10% ($p<0.05$) after two weeks of therapy, gradually reaching 40% ($p<0.01$) after three months of continuous use.
The 2.5% Benzoyl Peroxide formulation showed to be more effective than the 5% Ethyl Lactate Gel, but less effective than Benzoyl Peroxide-Ethyl Lactate Gel in reducing the numer of papules and pustules.
Both Benzoyl Peroxide and Benzoyl Peroxide-Ethyl Lactate formulations significantly reduced propionibacterium acnes and free fatty acid percentage in surface lipids after 2.5 weeks of topical use.
On the contrary, Ethyl Lactate alone showed only a bacteriostatic activity, whereas, in compounds, it appears to be capable of strenghtening the effect of the sole Benzoyl Peroxide.
Desquamation, erythema and symptoms of burning were observed only with benzoyl Peroxide formulations.
Finally, what is to be noticed is the exceptionally interesting activity of this new anhydrous vehicle consisting of a highly volatile special lipidic mixture. This vehicle results to be able to strongly reduce surface lipidic films and, at the same time, it facilitates the penetration of both Ethyl Lactate and Benzoyl Peroxide through the pilo-sebaceous ducts.

Riassunto
Nel corso di uno studio a doppio cieco su pazienti con acne vulgaris da leggera a moderatamente severa, si è comparata una formulazione al 2,5% di perossido di benzoile con un gel anidro al 5% di lattato di etile, un gel di lattato di etile e perossido di benzoile e un nuovo veicolo.
Tale veicolo sembra sviluppare una considerevole attività antiseborroica, poiché riduce il «livello occasionale lipidico» di circa il 10% ($p<0,05$), arrivando progressivamente al 40% ($p<0,01$) dopo tre mesi di uso continuato.
La formulazione al 2,5% di perossido di benzoile si è dimostrata più efficace del gel di lattato di etile al 5%, ma meno del gel di lattato di etile e perossido di benzoile, nel ridurre il numero di papule e pustule. Sia la formulazione di perossido di benzoile che quella di lattato di etile e perossido di benzoile hanno ridotto significativamente il numero di propionibatteri, nonché la percentuale di acidi grassi liberi nei lipidi di superficie, dopo 2,5 settimane di applicazione locale. Il lattato di etile da solo ha dimostrato di possedere, al contrario, un'attività batteriostatica, mentre sembra essere in grado di rafforzare l'attività del perossido di benzoile. Disquamazioni, eritemi e sintomi di bruciature sono stati notati solo con le formulazioni di perossido di benzoile. In conclusione, ciò che si rivela di eccezionale interesse è l'attività svolta da questo nuovo veicolo anidro, composto da una speciale miscela lipidica altamente volatile, che sembra in grado di ridurre energicamente il mantello lipidico di superficie e, allo stesso tempo, di facilitare la penetrazione sia del lattato di etile che del perossido di benzoile, attraverso i dotti pilo sebacei.

Resmen
En un estudio doble ciego sobre pacientes con acné vulgaris de leve hasta moderadamente severa, se ha comparado una formulación de 2,5% de peróxido de benzoilo con un gel anhidro de 5% de lactato de etilo, con un gel de lactato de etilo y peróxido de benzoílo y con un nuevo venhículo.
Ese nuevo vehículo parece desarrolar por si mismo una considerable actividad antiseborrea, ya que reduce el «nivel casual» de más o menos el 10% ($p<0,05$) despés de dos semanas de tratamiento, llegando gradualmente al 4*% ($p<0,01$) después de tres meses de uso continuo. La formulación de 2,5% de peróxido de benzoílo se ha probado más eficaz que el gel de 5% de lactato de etilo, pero menos eficaz que el gel de lactato de etilo y de peróxido de benzoílo en reducir el número de pápulas y pústulas.
Tanto la formulación de peróxido de benzoílo como la de lactato de etilo y de peróxido de benzoílo reducieron significativamente la acné de propionibacterias y el porcentaje de ácidos grasos libres en los lípidos de superficie después de 2,5 meses de applicación tópica. Al contrario, el lactato de etilo solo ha manifestado una actividad bacteriostática solamente, mientras que, en los compuestos, parece ser capaz de reforzar el efecto del peróxido de benzoílo. Descarnaciones, eritemas y síntomas de quemaduras se han observado sólo con las formulaciones de peróxido de benzoílo. En fin, lo que hay que notar es la actividad sumamente interesante de ese nuevo vehículo anhidro que consiste en una especial mixtura lipóidica altamente volátil. Ese vehículo resulta capaz de reducir furtemente la película lipóidica de superficie y, a la vez, facilita la penetración tanto del lactato de etilo como del peróxido de benzoílo a través de los conductos pilo-sebáceos.

Résumé
Dans une étude double aveugle sur des patients avec l'acnée vulgaris, de sa forme légère jusqu'à une forme moderément sévère, une comparaison a été efectuée entre une formulation à 2,5% de peroxyde de benzoyle et un gel anhydre à 5% de lactate d'éthyle et peroxyde et un noveau vehicule. Le vehicule semble donner lieu à una activité antiseborrhéique remarquable, car il réduit le «niveau casuel» d'environ 10% après trois mois d'emploi continu.
La formulation à 2,5% de peroxyde de benzoyle s'est révélée plus efficace que le gel de lactate d'éthyle et peroxyde de benzoyle, dans la réduction du nombre de papules et pustules.
Aussi bien la formulation de peroxyde de benzoyle pour la formulation de lactate d'éthyle et peroxyde de benzoyle ont réduit d'une façon significative l'acnée causée par les propionbactéries, ainsi que le pourcentage d'acides gras libres dans les lipides de surface après 2,5% semaines l'application locale.
Au contraire, le lactate d'éthyle tout seul n'a démontré q'une activité bactériostatique, tandis que quand il est combiné, il semble pouvoir renforcer l'effect du peroxyde de benzoyle. Les desquamations, les érythèmes et les symptömes de brûcures n'ont été remarqués qu'avec les formulations de peroxyde de benzoyle.
En conclusion, ce qui se révèle très intéressant, c'est l'activité de noveau véhicule anhydre composé par un mélange lipidique spécial très volatil. Le dernier est à même de réduire énergiquement la pellicule lipidique superficielle et, en même temps, de faciliter la pénétration aussi bien du lactate d'éthyle que du peroxyde de benzoyle par les canaux pilo-sébacés.

Synopse
Während einer «doppel-blind» Forschung auf Patienten mit leichter oder gemäßig scwerer Akne Vulgaris, wurde eine 2,5% Dose von Benzoylperoxid mit den folgenden Stoffen miteiandergeprüft: Anydrel mit 5% Ethyllaktat, Ethyllaktat - Benzoylperoxidgel, und einem neuen Mittel. Es scheint als ob dieses Mittel eine betrachtliche antiseborheische Aktivität entwickelte, als eine rund 10% ($p<0,05$) Verminderung des «zufälligen Niveaus» verursachte. Nach 3 monaten forgesetzter Anwendung, erreichte dieses Niveau ein 40% ($p<0,01$) Wert.
Die Wirkung der 2,5% Dose von Benzoylperoxyd war höher als diejenige des 5% Ethyllaktatgels, aber niedriger als die des Ethyllaktat - Benzoylperoxydgels, was die Verminderung der Knötchen - und Pustelanzahl anbelangte.
Nach 2,5 Wochen örtlicher Anwendung, haben Benzoylperoxyd und Benzoylperoxyd - Ethyllaktat zu einer betrchtlichen Verminderung der von Propionbakterien bewirkten Akne gebracht, zusammen mit einer Ermäßigung des Prozentsaztes freier Fettsäure als in oberflächigen Fetten.
Ethylaktat allein hat, dagenen, nur eine bakteriostatische Aktivität bewiesen, aber wenn kombiniert, verstärkt es die Auswirkung des Benzoylperoxyds.
Hauttrötunge, Abschieferungen und Brandsymptomen, wurden nur von der Anwendung von Benzoyl - Peroxydmischungen verursacht.
Zum Schluß, ist die Aktivität dieses neuen Anydrmittels sehr interessant. Die hochvolatile Fettmischung kann die oberflächige Fettschicht verringern, und gleichzeitig die Durchdringung des Ethyllaktats und Benzoylperoxyds durch den Haaartalgkanal begünstingen.

Introduction

Benzoyl Peroxide and Ethyl Lactate are both used as active principles for the treatment of acne vulgaris (1-4).
Ethyl Lactate is used, without any problems, at concentrations of 10%, whereas Benzoyl Peroxide is generally used at concentrations of 5, 10 and 20%. The higher concentrations, however, are frequently associated with irritative and desquamative side effects (6-10). Furthermore, the activity of both Ethyl Lactate and Benzoyl Peroxide always directly depends upon the type of vehicle used.
Recent studies have demonstrated that when the concentration of Benzoyl Peroxide is reduced to 25%, similar therapeutic results can be obtained in terms of reduction of inflammatory lesions of acne, while markedly decreasing the irritative effect (11).
The present study is aimed at controlling the activity of 5% Ethyl Lactate, of 2.5% Benzoyl Peroxide and of a combination of both these components when incorporated into a special anhydrous vehicle. In order to define the activity of these formulations, sixty patients suffering from acne vulgaris were examined with regard to the total number of inflammatory lesions, and the total quantity of sebum and fatty acids in the horny layer.

Materials and methods

Materials:
Formula 1: Special anhydrous vehicle.
Formula 2: Vehicle+5% Ethyl Lactate.
Formula 3: Vehicle+2.5% Benzoyl Peroxide.
Formula 4: Vehicle+5% Ethyl Lactate+2.5% Benzoyl Peroxide

Equipment:
Sebumeter SM 410.

Clinical Study

Sixty voluntary subjects (30 men and 30 women) aged from 14 to 25 years, who had all been suffering from polymorphous acne vulgaris for a period ranging from 8 months to 6 years, were subjected to experimentation. All the subjects, divided into three groups of twenty persons each, were given two small jars of cream. The creams given to the groups were labeled as follows: formula 1 and formula 2, were given to the first group; formula 1 and formula 3, were given to the second group; formula 1 and formula 4, were given to the third group. The contents of the jars was known neither by the voluntary patients, nor by the researchers.
All the subjects were given detailed instructions — twice daily each washed their faces with a special non — medicated glycerine soap, rinsed with normal water and dried with a cotton towel. They then applied the two creams under examination, the first formula being applied on the right cheek and the second formula on the left cheek. This was continued, morning and evening, for twelve weeks. Four weeks before starting the experiment, the subjects suspended all pharmaceutical or cosmetic treatments, both topically and systemically. All the subjects were checked before starting the experiment and at 2, 4, 6, 8, 10 and 12 weeks. The acne lesions were checked in a specified area of 9 cm^2, both on the right and on the left cheek. Comedones, microcysts, pustules and nodules were quantified by separately calculating them by means of a transparent millimeter grid of cellophane. The resulting data are shown in Tables I-III and Figures 1-3. At each evaluation the presence of erythema at various intensities was also observed, with or without evident desquamation, and each observation was given a score as per the following scheme:

Table I
Comparison of effect of 5% ethyl lactate and vehicle

	5% Ethyl lactate							Special anhydrous vehicle					
Weeks	Number of subjects	Comedones and Microcysts (Mean N.)	Mean % Reduction	Pustules and Nodules (Mean N.)	Mean % Reduction	Sebumeter	Mean % Reduction	Comedones and Microcysts (Mean N.)	Mean % Reduction	Pustules and Nodules (Mean N.)	Mean % Reduction	Sebumeter	Mean % Reduction
0	20	19.1	—	12.3	—	189	—	18.8	—	11.7	—	185	—
2	20	18.6	3.0	11.2	8.5	173	8.0	17.9	4.5	11.5	1.5	167	10.1
4	20	16.7	12.8	10.5	14.6	160	15.1	17.6	6.2	11.6	0.8	151	18.0
6	20	15.5	19.1	9.6	22.0	145	22.9	17.1	9.0	11.3	3.3	137	26.3
8	20	13.1	32.0	8.4	31.4	126	33.0	17.2	8.5	11.5	1.4	122	34.1
10	20	9.3	51.2	8.5	30.9	113	40.3	16.4	12.6	11.4	2.5	108	42.3
12	20	8.1	58.0	8.1	34.0	101	45.7	16.0	14.9	11.0	5.9	99.7	46.3

Table II
Comparison of effect of 2.5% benzoyl peroxide and vehicle

	2.5% Benzoyl peroxide							Special anhydrous vehicle					
Weeks	Number of subjects	Comedones and Microcysts (Mean N.)	Mean % Reduction	Pustules and Nodules (Mean N.)	Mean % Reduction	Sebumeter Lectures	Mean % Reduction	Comedones and Microcysts (Mean N.)	Mean % Reduction	Pustules and Nodules (Mean N.)	Mean % Reduction	Sebumeter Lectures	Mean % Reduction
0	20	22.3	—	11.5	—	177	—	21.4	—	10.3	—	169	—
2	20	15.7	22.1	10.0	13.2	151	14.1	20.1	5.9	10.4	- 2.5	152	10.0
4	20	12.5	44.0	8.3	27.6	134	24.4	19.7	7.8	10.1	1.8	140	16.8
6	20	10.6	51.9	6.1	47.0	110	38.0	19.5	8.9	10.5	- 3.6	126	25.1
8	20	9.1	59.2	4.5	60.3	97.8	44.3	19.8	7.5	10.0	2.8	116	31.3
10	20	7.3	66.9	3.6	68.7	84.3	51.7	18.2	14.1	10.2	0.8	98.1	42.0
12	20	6.9	69.0	2.8	75.1	76.7	56.3	17.4	18.5	9.8	4.5	92.4	45.7

Table III
Comparison of effect of 5% ethyl lactate + 2.5% benzoyl peroxide and vehicle

	Benzoyl peroxide + Ethyl lactate							Special anhydrous vehicle					
Weeks	Number of subjects	Comedones and Microcysts (Mean N.)	Mean % Reduction	Pustules and Nodules (Mean N.)	Mean % Reduction	Sebumeter	Mean % Reduction	Comedones and Microcysts (Mean N.)	Mean % Reduction	Pustules and Nodules (Mean N.)	Mean % Reduction	Sebumeter	Mean % Reduction
0	20	20.8	—	11.9	—	201	—	19.7	—	10.8	—	194	—
2	20	13.5	35.1	10.3	13.5	169	16.2	19.0	3.3	11.0	- 1.0	171	11.3
4	20	10.7	48.0	7.5	33.4	112	44.1	18.6	5.6	10.7	0.9	149	22.7
6	20	8.2	59.1	6.1	48.5	93.1	54.3	17.4	11.3	10.5	2.5	123	35.9
8	20	6.4	68.9	4.7	60.6	77.5	60.7	16.9	14.1	10.6	1.8	111	42.5
10	20	5.8	72.1	3.3	72.1	68.1	65.9	16.0	18.5	10.4	3.5	96.4	50.1
12	20	5.0	76.2	1.8	85.0	60.7	70.0	15.3	22.0	10.4	3.6	90.2	53.3

Table IV
Frequency of erythema and peeling
(n = 20)

Weeks	Special Anhydrous Vehicle (Score FORMULA 1)	Vehicle + 5% Ethyl Lactate (Score FORMULA 2)	Vehicle + 2.5% Benzoyl Peroxide (Score FORMULA 3)	Vehicle + 2.5% Benzoyl Peroxide + 5% Ethyl Lactate (Score FORMULA 4)
2	1.01	1.03	3.85	3.15
4	10.1	1.06	3.91	2.98
6	1.01	1.05	3.87	3.03
8	1.00	1.08	2.93	2.88
10	1.03	1.04	2.77	2.93
12	1.00	1.01	3.01	2.75

Formula 2 versus Formula 1 = not significant (Calculated on mean × 2 weeks)
Formula 3 versus Formula 1 = $p < 0.01$ (Calculated on mean × 2 weeks)
Formula 4 versus Formula 1 = $p < 0.01$ (Calculated on mean × 2 weeks)
Formula 4 versus Formula 3 = not significant (Calculated on mean × 2 weeks)

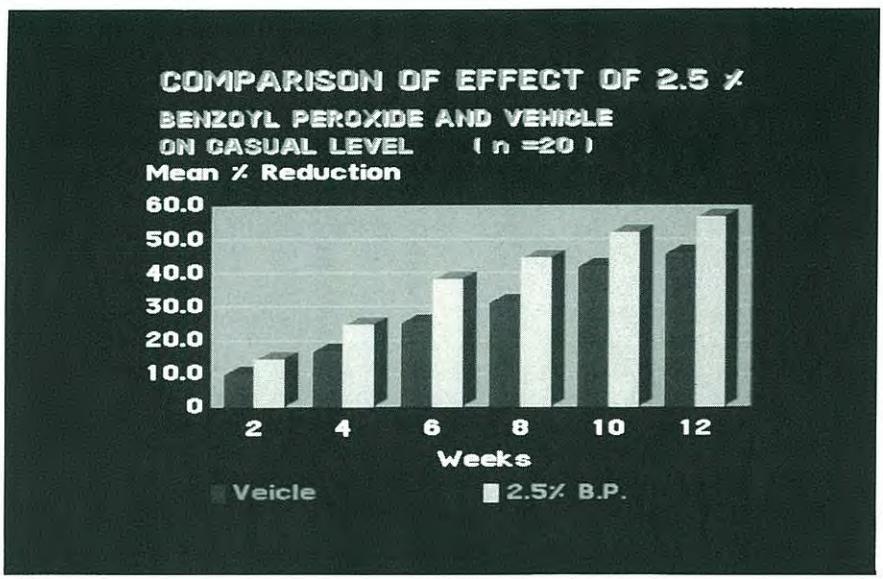

Figure 1

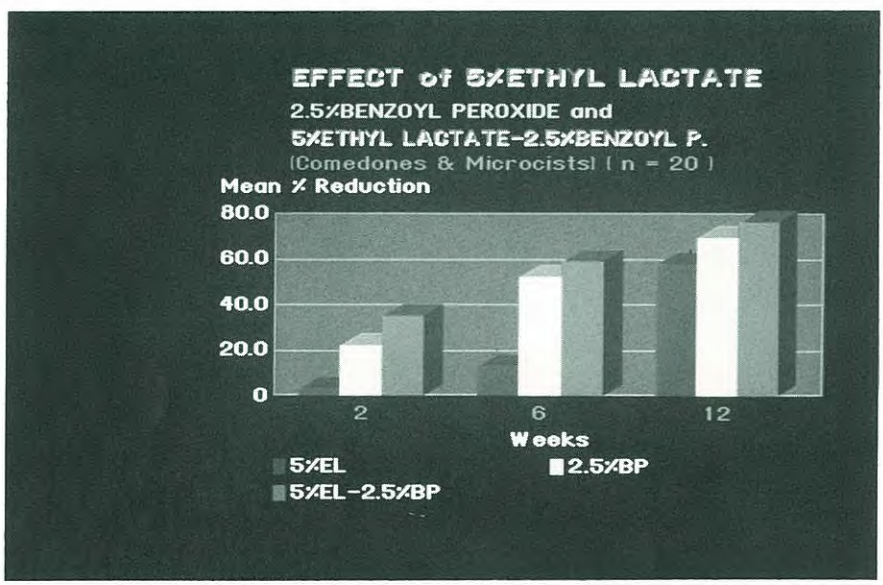

Figure 2

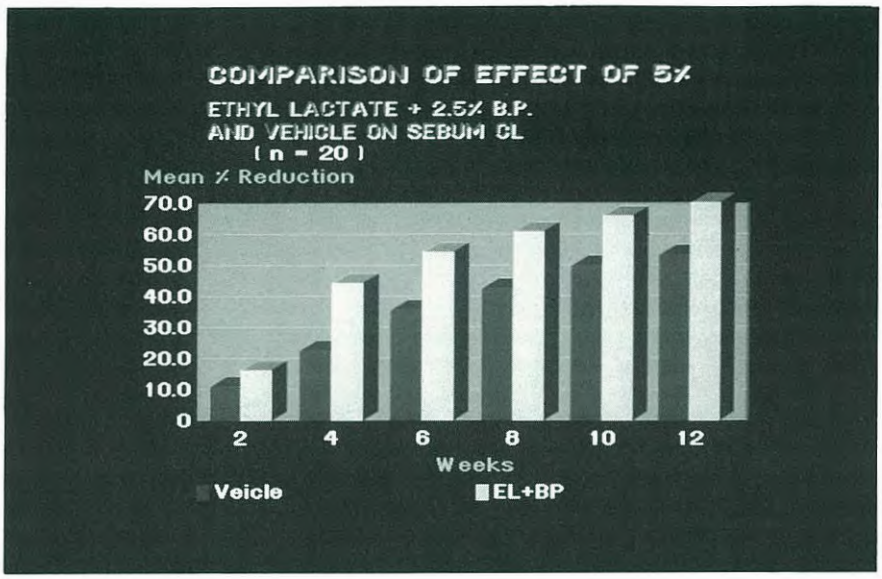

Figure 3

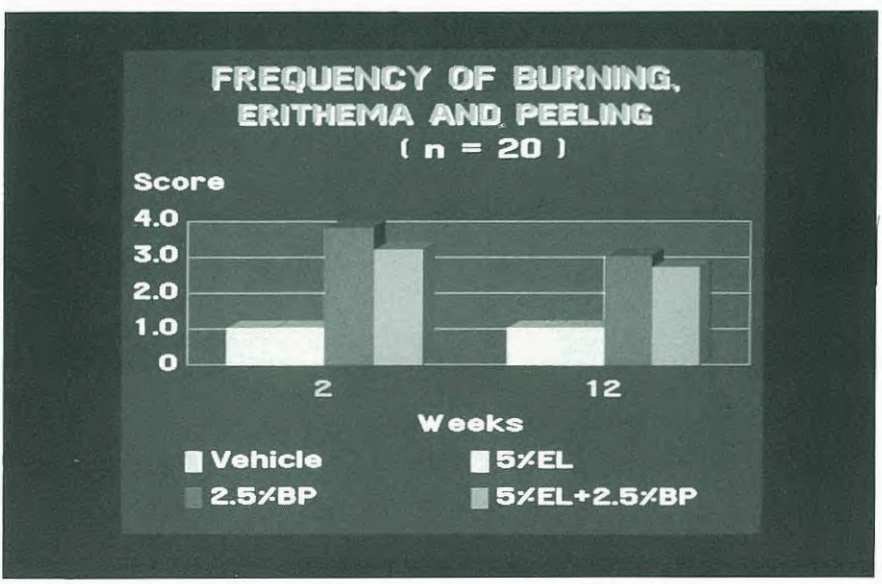

Figure 4

Evident Erythema accompanied by desquamation	5
Intense Erythema without desquamation	4
Slight Erythema without desquamation	3
Barely visible Erythema	2
No Erythema	1

The results are shown in Table IV and Figure 4.

Surface sebum

The sebum level of all the subjects under examination was determined by using a Sebumeter SM 410 (12, 13). The determination of total surface sebum was always carried out on both cheeks before evaluating the patients for the calculation of inflammatory lesions and after conditioning the subjects for 30 minutes in a room under controlled temperature and humidity (+21° C Rh 30%).
The results obtained are shown in Tables I-III and Figures 5-8.

Calculation of colonies of propionibacterium acnes

The calculation of Propionibacterium Acnes was carried out according to the method of Williamson and Kligman (14). Before taking the sample, both cheeks of the subjects (10 out of 20) were cleaned by using a sterile gauze saturated with a 0.1% sterile solution of Triton X-100, fol-

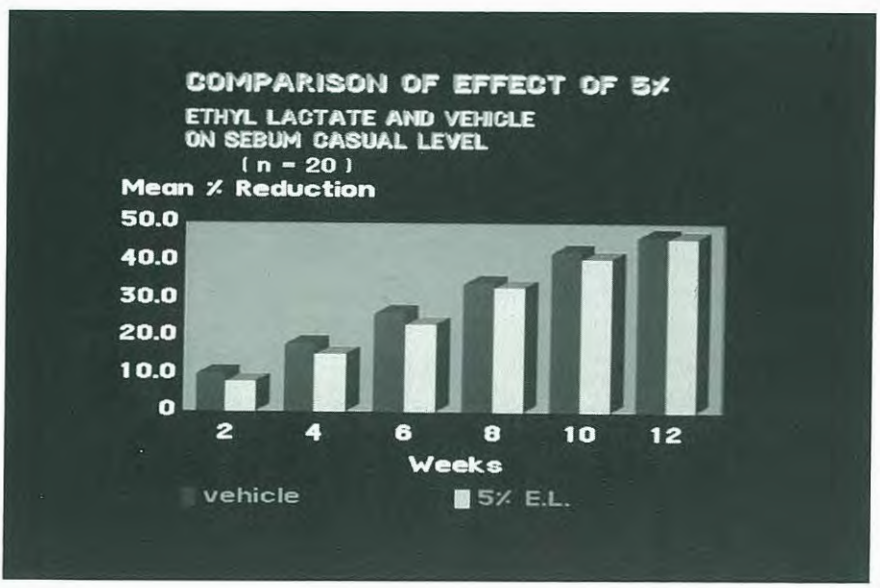

Figure 5

lowed by a further cleaning with distilled water and finally rubbed with a gauze saturated with hexane for 30 seconds. The cleansed skin was protected with a porous sterile plastic gauze, so as to maintain normal evaporative activity. After on hour a cylinder of sterile glass (internal area 3.8 cm^2) with hollow base was applied to the area. Into said cylinder 1 ml of a sterile solution of 0.1% Triton X-100 in a pH 7.9 phosphate buffer was introduced.
After having cleaned the area with a teflon spatula for one minute, two successive samples of liquid were taken. The samples thus obtained were diluted a number of times with a solution of 0.05% Triton X-100, immediately set in culture with a solution of 0.1% Tween 80 and incubated anaerobically for 7 days. The colonies of Propionibacterium Acnes were determined using the method of McGinley et. al. (15).
The results obtained are shown in Table V and Figure 9.

Determination of free fatty acids/triglycerides

The ratio FFA/TG (Free fatty acids/triglycerides) was determined using the Downing method (16).
One hour after having cleansed the area, according to the Williamson and Kligman method (14), the hollow-based glass cylinder was applied to the cheeks of the subjects (10 out of 20).

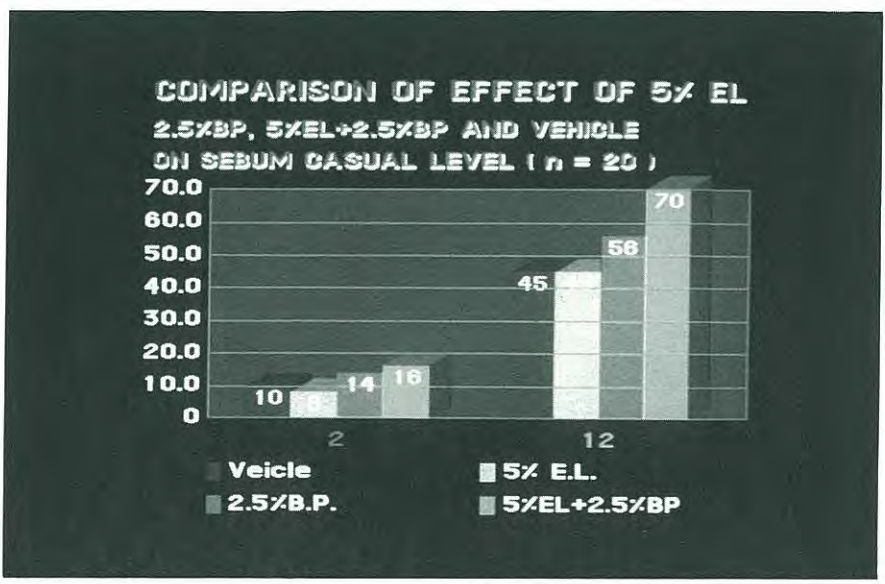

Figure 6

Into the above cylinder 2 ml of hexane were introduced, containing an internal standard of methyl nervonate. After cleansing the area with a small teflon spatula for 30 seconds, the hexane solution was filtered through a millipore filter (pore size 0.45 mm), in order to remove all the cellular debris and the bacteria, and dried under vacuum at 40° C. A TLC was then carried out to determine fatty acids and triglycerides.
The results obtained are shown in Table V and Figure 10.

Results and comments

As can be seen from Tables I-III and figures 5-8, the new vehicle alone develops a considerable antiseborrheic activity, since it reduces the sebum level by around 10% ($p<0.05$) after two weeks of therapy, arriving gradually at reductions of 40% ($p<0.01$) after three months of continous use. The sebosolving activity of this special anhydrous vehicle is further confirmed by the fact that the Tables I-III and figures 1-3 also show a total reduction of the number of comedones by about 18% ($p<0.05$) after three months use.
By controlling the activity of 5% Ethyl Lactate, of 2.5% Benzoyl Peroxide and of the mixture of both, it is evident that comedones and microcysts are reduced by 22% ($p<0.05$) and by 35% ($p<0.01$) after a treatment of only two weeks with 2.5% Benzoyl Peroxide and with the mixture of

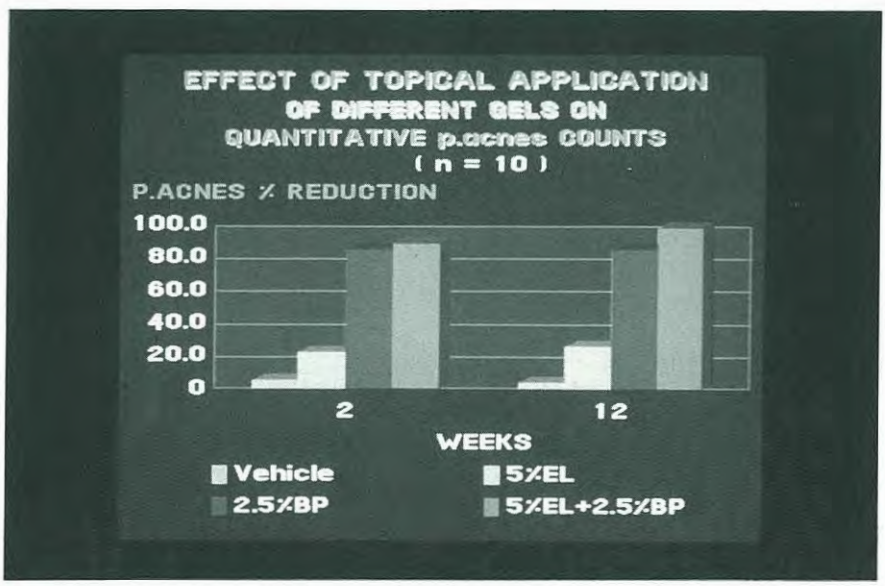

Figure 7

2.5% Benzoyl Peroxide and 5% Ethyl Lactate respectively.
On the contrary, the above lesions remain unchanged after two weeks of therapy with 5% Ethyl Lactate.
The average number of comedones and microcysts is strongly reduced even in the case of Ethyl Lactate alone, if the period of therapy is longer. In fact, after one month of therapy a reduction of about 13% ($p<0.05$) is reached, increasing to 30% after two months ($p<0.01$) and to about 60% after four months ($p<0.01$).
As regards the reduction of the number of pustules and nodules. Ethyl Lactate starts to act only after one and a half month of therapy (reduction by 10% $p<0.05$). After three months, the gradual reduction is not greater than about 30% of the starting values ($p<0.01$).
Using Benzoyl Peroxide or, even better, the mixture of Benzoyl Peroxide and Ethyl Lactate, a reduction of nodules and pustules of 30% is obtained after the first month of therapy ($p<0.05$), reaching 70% ($p<0.01$) and 80% (<0.01) respectively after three months of therapy.
The values reported in Table IV and Figure 4, indicate the irritative and keratolytic activity of both Benzoyl Peroxide and the mixture Ethyl Lactate/Benzoyl Peroxide ($p<0.01$). The vehicle alone, and the Ehyl Lactate have proved themselves to be completely free of irritative effect, even after three months of use.
Table V and Figure 9 evidence the strong bactericidal activity of both Benzoyl Peroxide and the mixture Benzoyl Peroxi-

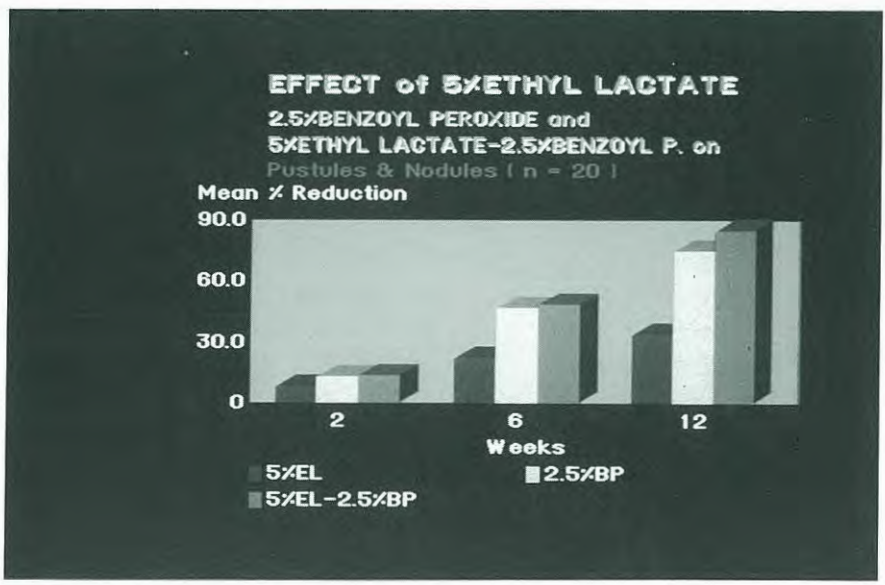

Figure 8

de/Ethyl Lactate, against the Propionibacterium acnes. These organisms are considerably reduced after only two weeks use of the products. The Ethyl Lactate alone shows only a bacteriostatic activity, whereas, combined, it appears to be capable of strengthening the effect of the Benzoyl Peroxide.

The ration FFA/TG shows (Table V and Figure 10) a reduction by about 50% of free fatty acids from the activity of both Ethyl Lactate and Benzoyl Peroxide, and from the mixture thereof ($p < 0.01$). It is known that Ethyl Lactate directly inhibits bacterial esterases, thus limiting the hydrolysis of triglycerides. Benzoyl Peroxide acts directly on the bacterial strains, thus reducing the production of esterase from Propionbacterium themselves.

From the foregoing, it plainly follows that it is possible to use Ethyl Lactate effectively in acne therapy, thus avoiding the irritative or photosensitizing effect of Benzoyl Peroxide. This is especially important in treating acne in fair skinned individuals.

Furthermore, Ethyl Lactate, in combination with a suitable vehicle, appears to be capable of enhancing the activity of Benzoyl Peroxide, thereby allowing the use of a lower concentration.

Finally, the exceptionally interesting activity of this new anhydrous vehicle, consisting of a special lipidic mixture with high volatility, is evident.

This vehicle appears able to strongly reduce the surface lipidic film while facilitating penetration of both Ethyl Lactate and Benzoyl Peroxide through the pilosebaceous ducts.

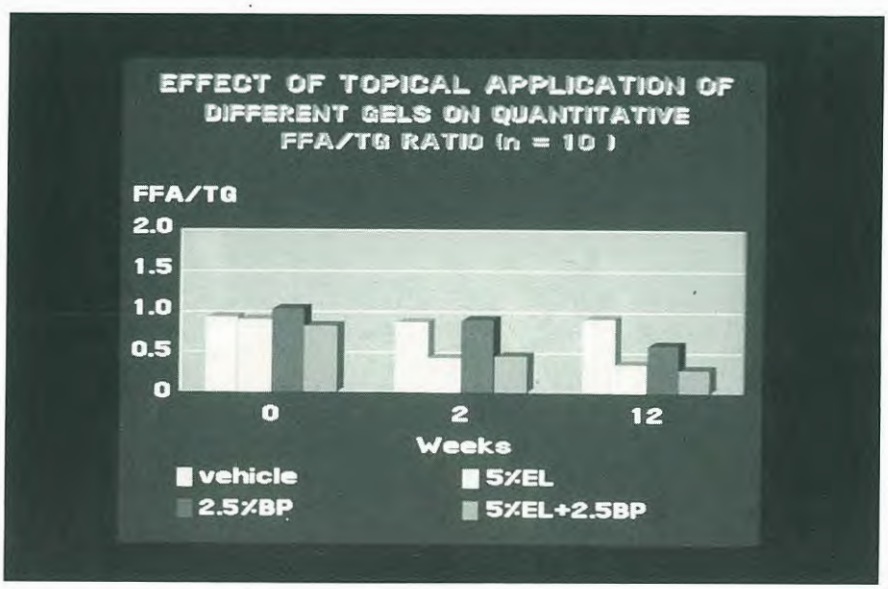

Figure 9

REFERENCES

1. **Swanbeck G. (1972)** «A new principle for the treatment of acne» *Acta Dermatovenerologica* (Stockholm), **52**, 406.
2. **Cunliffe W.J., Cotteril J.A. (1975)** «The Acnes - Clinical Festures, Phatogenesis and Treatment» In: Major Problems of Dermatology, Vol. 6, **261**. W.B. Saunders Co. Ltd., London.
3. **Grosshans E., Fourtanier A., Guidbaud B. (1978)** «Evaluation clinique d'untraitement local de l'acne juvenile par le lactate d'ethyle» *Annales de Dermatologie et Venerelogie* (Paris), **105**, 833.
4. **Prottey C., George D., Black J.G., Vickers C.F.H. (1983)** «Ethyl lactate as a treatment for acne» *British Journal of Dermatology*, **108**, 228.
5. **Prottey C., George D., Leech R.W., Black J.G., Howes D., Vickers C.F.H. (1984)** «The mode of action of ethyl lactate as a treatment for acne» *British Journal of Dermatology*, **110**, 475.
6. **Pace W.E. (1965)** «A benzoyl peroxide-sulfur cream for acne vulgaris» *Can. Med. Assoc. J.* **93**, 252.
7. **Danto J.L., Maddin W.S., Steward W.D. et al. (1966)** «A controlled trial of benzoyl peroxide and precipitated sulfur cream in acne vulgaris» *Appl. Ther.*, **8**, 624.
8. **Belknap B.S. (1979)** «Treatment of acne with 5 percent bezoyl peroxide gel or 0.05 percent of retinoic acid cream» *Cutis* **23**, 856.
9. **Smith E.B., Padilla R.S., Maccabi J.M. et al. (1980)** «Benzoyl peroxide lotion (20 percent) in acne» *Cutis*, **25**, 90.
10. **Leyden J., McGinley K., Mills O.H.D., Kligman A. (1980)** «Topical antibiotics and topical antimicrobial agents in acne therapy» *Acta Dermato-venerologica* (Stockholm), Suppl. **89**, 75.
11. **Mills O.H.D., Kligman A., Pochi P., Comite H.** «Comparing 2.5%, 5% and 10% benzoyl peroxide on inflammatory acne vulgaris».
12. **Schrader K. (1974)** «A new procedure for the determination of skin-surface fat» Dragoco Report, **8**, 171.
13. **Muti P., Celantano E., Panico S., Berrino E. (1987)** «Measurement of cutaneous sebum; reproducibility at different cleansing conditions» *Journal of Applied Cosmetology* **5**, 131.
14. **Williamson P., Kligman A.M. (1965)** «A new method for the quantitative invenstigation of cutaneous bacteria» *Journal of Investigate Dermatology*, **45**, 498.
15. **McGinley K., Webster G., Leyden J. (1965)** «Regional variations in cutaneous propionibacteria» *Journal of Applied Env. Micro.*, **45**, 998.
16. **Downing D.T. (1968)** «Photodensitometry in the thin-layer chromatographic analysis of neutral lipids» *Journal Chromatogr.* **38**, 91.

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Uterotrophic effect on rats by cutaneous administration of oestrogens

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Key words: Oestrogens, Cosmetics, Uterotrophic effect

Synopsis

Method based on uterine weight increase in immature female rats was devised to better establish the range of inactive-active concentration of several oestrogen alcoholic solutions topically administered. Standardized experimental condition were carefully the same already published by the Authors. New results presented here, in comparison with those previously obtained, allow to arrange the investigated estrogenic substances according to their decrasing uterotrophic effect as follows (being the inactive concentration indicated for each oestrogen the former number and the minimal active concentration the later): ethinyloestradiol (0.075 μg/ml - 0.125μg/ml); diethylstilboestrol (0.25 μg/ml - 0.50 μg/ml); 17 β-oestradiol (0.50 μg/ml - 0.75 μg/ml); oestradiol-3-benzoate and oestradiol-17-valerate (0.75 μg/ml - 1.0 μg/ml); oestradiol (2.0 μg/ml - 3.0 μg/ml); oestrone (4.0 μg/ml - 5.0 μg/ml).
Those definitive data confirm the realibility of the adopted method and its possibilities of application in order to detect eventual intentional presence of estrogens both in placental extracts and in cosmetic products.

Riassunto

Il metodo si basa sull'aumento del peso uterino nelle femmine immature di ratto ed è stato elaborato allo scopo di meglio stabilire la varietà di concetrazione attiva-inattiva delle diverse soluzioni alcoliche di estrogeni somministrate localmente. Si sono mantenute le stesse condizioni sperimentali tipo già rese note dagli Autori. I nuovi risultati qui presentati, a confronto con quelli precedentemente conseguiti, consentono di classificare le sostanze estrogeniche oggetto della ricerca secondo il decrescente effetto uterotrofico come segue (la concentrazione inattiva viene indicata per ciascun estrogeno dal primo numero, e la concentrazione minima attiva dal secondo): etinilestradiolo (0.075μg/ml - 0.125 μg/ml); dietilstilbestrolo (0.25 μg/ml - 0.50 μg/ml); 17 β-estradiolo (0.50 μg/ml - 0.75 μg/ml); estradiolo-3-benzoato ed estradiolo-17-valerato (0.75 μg/ml - 1.0 μg/ml); estriolo (2.0 μg/ml - 3.0 μg/ml); estrone (4.0 μg/ml- 5.0 μg/ml).
Tali dati conclusivi confermano l'affidabilità del metodo adottato e le sue possibilità di applicazione nell'individuazione di eventuali presenze intenzionali di estrogeni negli estratti di placenta e nei cosmetici.

Résumé
Pour mieux déterminer la gamme de la concentration - active ou inactive - de plusieurs solutions alcooliques d'oestrogénes qui viennent appliquées localement, on a employé une méthode basée sur le poids utérin des petits rats femelles. On a respecté attentivement les conditions expérimentales qui avaient été déjà appliquées et publiquées par d'autres chercheurs. Par rapport aux résultats précedents, ce que nous avons obtenu dans ce cas c'est une méthode qui nous permet de classer, par ordre décroissant, les effets utéotrophiques des oestrogènes que nous avons examinés. Les premières chiffres indiquent, pour chaque oestrogène, la concentration inactive tandis que les autres chifres indiquent la concentration active minimale: éthynyloestradiol (0,075 μg/ml - 0,125 μg/ml); diéthylstilboestrol (0,25 μg/ml - 0,50 μg/ml); 17 β-oestradiol (0,50 μg/ml - 0,75 μg/ml; oestradiol-3-benzoate et oestradiol-17-valerate (0,75 μg/ml - 1,0 μg/ml); oestriol (2,0 μg/ml - 3,0 μg/ml); oestrone (4,0 μg/ml - 5,0 μg/ml). Ces données définitives, confirment, tant la fiabilité de la méthode ici préesentée, que ses possibilités d'application pour détecter une éventuelle présence intentionnelle d'oestrogènes dans les extraits placentaires et/ou dans les cosmétiques.

Synopse
Eine auf dem Zuwachs des Uteringewichts in jungen weiblichen Ratte gegründete Methode wurde verfolgt, um eine tiefgreifende Bewertung zu ermöglichen, was die tätig-und-untätige Konzentration verschiedener topisch eingegebenen östrogen und Alkoholösungen betrifft. Die standardisierte Forschungsbedingungen waren die selben die von den Forschern veröffentlicht wurden. Die neuen hier gegebenen Ergebnisse ermöglichen (im Vergueich zu den schon erlangenen Daten) die folgende Klassifizierung der ausprobierten östrogenstoffe nach ihrer abnehmenden uterotrophischen Auswirkung (die erste Nummer zeigh die untätige Konzentration jedes östrogenstoffes und die zweite die am wenigsten tätige Konzentration): Ethinyloestradiol (0,0,75 μg/ml - 0,125 μg/ml); Diethylstilboestrol (0,25 μg/ml - 0,50 μg/ml); 17 β-Oestradiol (0,50 μg/ml - 0,75 μg/ml); Oestradiol-3-Benzoat und Oestradiol-17-Valerat (0,75 μg/ml - 1,0 μg/ml); Oestriol (2,0 μg/ml - 3,0 μg/ml); Oestron (4,0 μg/ml - 5,0 μg/ml).
Diese Daten bestätigen die Glaubwürdigkeit dieser Methode und ihre Anwendungsmöglichkeiten, um die eventuelle absichtliche Anwesenheit von östrogenstoffen in Plazentaextrakten und kosmetischen Produkten zu entdecken.

Resumen
El procedimiento se basa sobre el incremento del peso uterino en ratas hembras y se ha ejecutado para establecer mejor la gama de concentraciòn activa-inactiva de varias soluciones alcohòlicas de estròqenos de administraciòn tòpica. Las condiciones experimentales standard fueron precisamente las mismas que se publicaron antes por los Autores. Los nuevos resultados que se presentan aquí, en comparaciòn a los que se consiguieron previamente, permiten relacionar las substancias estrogénas investigadas con su efecto uterotrófico decreciente de la manera siguiente (siendo la concentración, inactiva para cada estrógeno indicada con el primer número y la concentración minima activa con el segundo número): etinilestradiolo (0,075 μg/ml 0,125 μg/ml); dietilstilbestrolo (0,25 μg/ml - 0,5 μg/ml); 17 β-estradiolo (0,5 μg/ml - 0,75 μg/ml); estradiolo-3-benzoato y estradiolo-17-valerato (0,75 μg/ml - 1 μg/ml); estriolo (2 μg/ml - 3 μg/ml); estrón (4 μg/ml - 5 μg/ml). Esos datos conclusivos confirman la fiabilidad del procedimiento que se ha adoptado y sus posibilidades de aplicación para detectar la posible presencia intencional de estrógenos tanto en extractos de placenta como en cosméticos.

Introduction

In a previous publication the Authors (Salvatore et al., 1987) investigated the detection of uterotrophic effect induced in immature female rats topically treated both with natural and synthetic oestrogens. The existence of a dose-response relationship, particularly in the case of 17 β-oestradiol (see Figure 1), was demonstrated. Moreover, the minimal dose capable of causing uterotrophic effect or the inactive dose under precise standardized experimental conditions were established only for a few of the oestrogens under examination.

Thus the aim of the present work is to further explore the range of inactive-active concentrations of alcoholic solutions of oestradiol-17-valerate, oestradiol-3-benzoate, oestrone, oestriol and ethinyloestradiol, for which the previous data was inadeguate.

Since the use of oestrogens in cosmetics, even those naturally present in placenta or in its extracts, is not permitted under Law No. 713, October 11, 1986 (recently updated by D.M. No. 530 November 24, 1987), our research is essential in arriving at a valid general screening method suitable even for the analysis of commercial samples with unknown oestrogen content.

Experimental

For each oestrogen, the following working solutions were prepared in 95° ethanol: oestradiol-17-valerate, 0.75 μg/ml and 1.0 μg/ml; oestradiol-3-benzoate, 0.75 μg/ml and and 1.0 μg/ml; oestriol 2.0 μg/ml; ethinyloestradiol, 0.07 μg/ml; oetrone, 4-5-7 μg/ml respectively; and 17 β-oestradiol 0.75 μg/ml. The last substance was used as reference to test the repro-

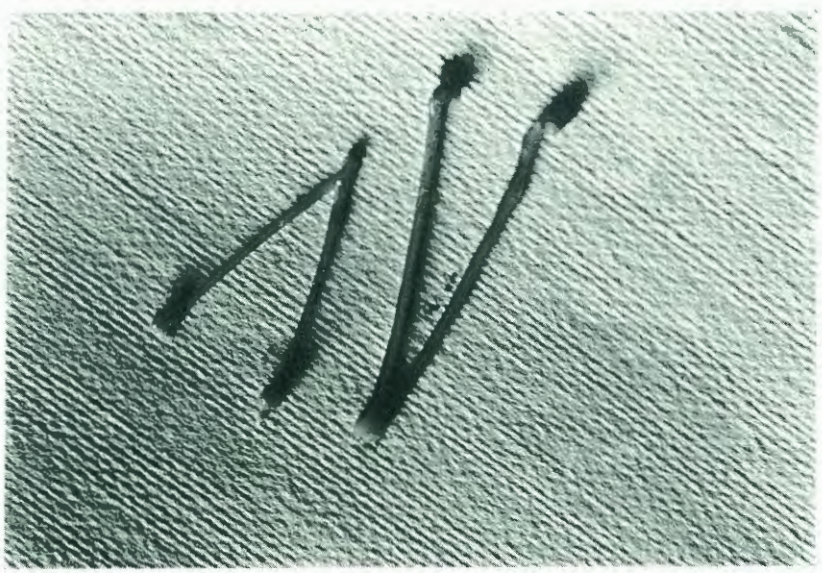


Fig. 1: Uterus of untreated immature female rat in comparison with uterus of immature female rat topically treated with 17 β-oestradiol.

ducibility of dose-response relationship previously estrablished (Salvatore et al., 1987).
The animals were chosen from a group of homogeneous size thereby reducing the possibility of error and individual variation; they were then housed in single disposable cages.
Each experiment had four animal groups (including the control group), each of which numbered 10 animals (7 animals in the case of the 0.07 μg/ml ethinyloestradiol solution). Before treatment, the backs of the rats were shaved using an electric razor (AESCULAP Favorita II CT 104; clipping height 1/20 mm) and then the animals were weighed. With each solution of a known oestrogen concentration, three topical applications were made (0.25 ml each) at 0, 8 and 24 hours. Thus, each treated rat received a total dose of 0.75 ml of alcohol solution at a known oestrogen concentration. Control animals received the same volume of 95° ethanol.

Results and discussion

Table 1 shows the experimental schema of this study.
17 β-oestradiol at a concentration level of 0,75 μg/ml was used as a reference substance. This concentration proved to be the minimal active concentration confirming the data of our previous study and showing a good reproducibility of the adopted method. A concentration of 5,0 μg/ml for oestrone represented the minimal active concentration while at 4,0 μg/ml this hormone was inactive. Oestradiol was inactive at a concentration of 2 μg/ml. On investigating synthetic oestrogens, we found that the concentration dose of 0.07 μg/ml had no effect on ethinyloestradiol. This hormonal substance proved to be the most active of the oestrogens tested to date with a minimal active concentration of 0.125 μg/ml. The 17 β-oestradiol esters showed a similar biological response since a concentration of 0.75 μg/ml resulted inactive while 1.0 μg/ml was the minimal active concentration.
In Table 2 the final results of minimal active concentrations and inactive concentrations for each oestrogen are given in their order of decreasing uterotrophic effect.
In conclusion, the data obtained demonstrated the reliability of this biological test as a screening method for detecting any deliberate addition of oestrogenic substances in commercial products of unknown oestrogen content.

Table I

Experimental design and results obtained as to the effects on rat uterine weight variations for the oestrogenes investigated. The tested concentration and the correspondent relative uterine weight for each oestrogen are reported in the first and the second line, respectively, for each assay (* $P < 0.01$).

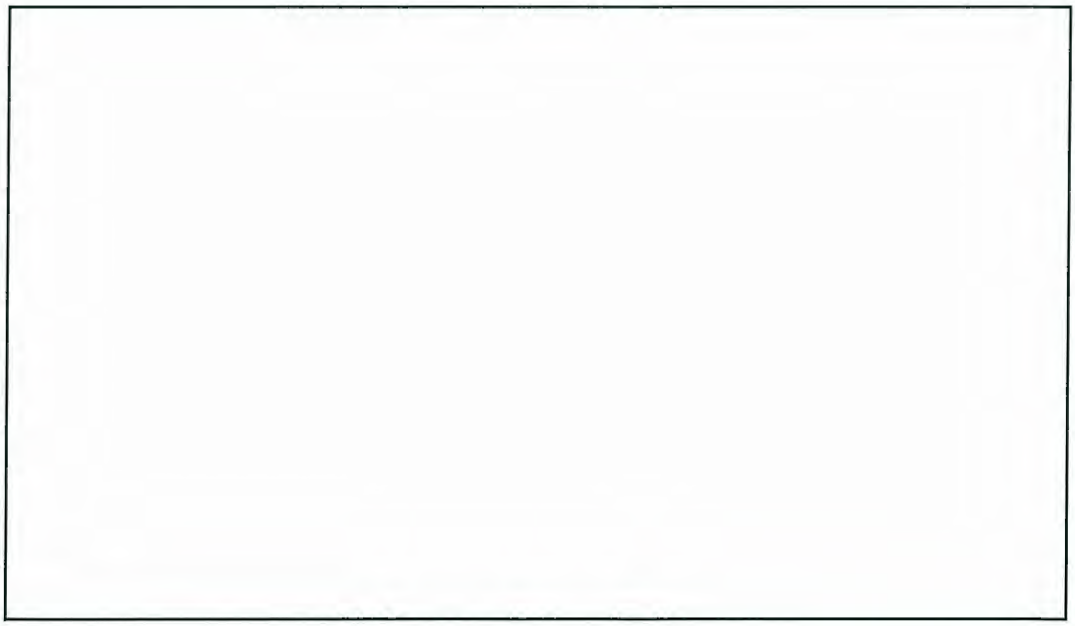
Experimental design		Treated groups							Control groups
Assay No.	Total number of rats	17 β-oestradiol	Oestradiol-17-valerate	Oestradiol-3-benzoate	Oestrone	Oestriol	Ethynyl-oestradiol	Diethyl-stilboestrol	
1	40	—	1.0μg/ml	1.0μg/ml	—	2.0μg/ml	—	—	—
	(10 per group)	—	1.69*	1.69*	—	1.38	—	—	1.33
2	47	0.75μg/ml	0.75μg/ml	0.75μg/ml	—	—	0.07μg/ml	—	—
	(10 per group)	1.74*	1.54	1.42	—	—	1.44	—	1.43
3	47 (12 per group)	—	—	—	4.0μg/ml	—	—	—	—
		—	—	—	1.66	—	—	—	1.51 (11 rats)
		—	—	—	5.0μg/ml	—	—	—	—
		—	—	—	1.96*	—	—	—	—
		—	—	—	7.0μg/ml	—	—	—	—
		—	—	—	2.56*	—	—	—	—

Table II
Definitive results of each oestrogen investigated arranged in decreasing uterotrophic effect order ($P < 0.01$)

Oestrogens	Inactive concentration (μg/ml)	Minimal concentration with uterotrophic activity (μg/ml)
Ethinyloestradiol	0.075	0.125
Diethylstilboestrol	0.25	0.5
17 β-oestradiol	0.5	0.75
Oestradiol-3-benzoate	0.75	1.0
Oestradiol-17-valerate	0.75	1.0
Oestriol	2.0	3.0
Oestrone	4.0	5.0

REFERENCES

1. **Salvatore G., De Angelis T., Moretti G., Bartolini G., Parisi G. (1987)** «Biological detection of oestrogens in cosmetics» *J. Appl. Cosmetol.* **5**, 149-160.
2. **Gazzetta Ufficiale della Repubblica Italiana (1980)** «Legge 11 ottobre 1986 n. 713 - Norme per l'attuazione delle direttive della Comunità Economica Europea sulla produzione e la vendita dei cosmetici» - Supplemento ordinario n. 101 alla G.U. n. 253 del 30 ottobre 1986.
3. **Gazzetta Ufficiale della Repubblica Italiana (1987)** «Decreto Ministeriale 24 ottobre 1987, n. 530 - Aggiornamento degli elenchi allegati alla legge 11 ottobre 1986, n. 713, sulla produzione e la vendita dei cosmetici, anche in attuazione della direttiva della Commissione delle Comunità europee n. 87/137/CEE» - Supplemento ordinario n. 3 alla G.U. n. 303 del 30 dicembre 1987.



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Computerized morphometric analysis of acne lesions

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Key words: Acne, acne evaluation, computerized analysis

Synopsis
An evaluation system of acne lesions by means of morphometric computerized analysis is presented. The method allows (even in relation to a treament): a quantitative evaluation which is effective even in the case of a high number of lesions; the evaluation of the dimensions of individual lesions and of the area they occupy even in relation to a skin area which has been chosen as sample (area %).

Riassunto
Sistema di valutazione delle lesioni da acne attraverso analisi morfometrica computerizzata. Questa metodica consente (anche in relazione al trattamento) una valutazione quantitativa efficace anche nel caso di un gran numero di lesioni, nonché la valutazione delle dimensioni delle lesioni individuali e dell'area occupata, in relazione anche alla zona di epidermide scelta a campione (area %).

Résumé
Nous illustrons une méthode d'évaluation des lésion acnéeiques basée sur une analyse morphométrique informatisée. Cette méthode nous permet d'obtenir (même lorsque associée à des soins de la peau) une évaluation quantitative que est est valable même en présence de beaucoup de lésions; en outre elle nous permet de mesurer les dimensions de chaque lésion et de la portion de tissu épitélial qu'elles occupent même en rélation avec une zone de la peau prise comme échantillon.

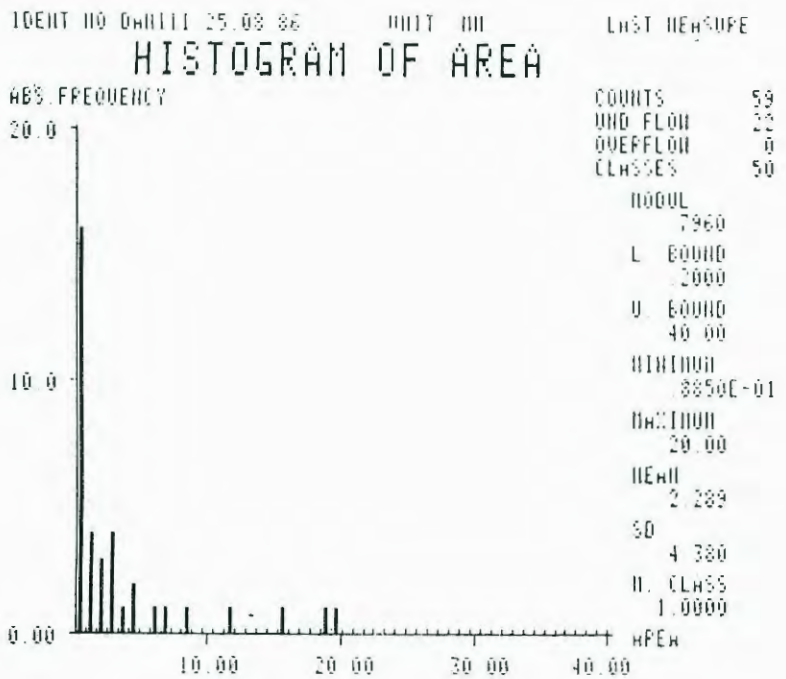
Synopse
Hier wird ein Forschungssystem für Aknebeschändigungen gezeigt, das sich auf eine morphometrischen Analyse durch Datenverarbeitungsmaschinen stützt. Diese Methode ermöglicht (auch in Zusammenhang mit einer Therapie) eine grössenmässige Bewertung durchzuführen, die auch im Falle mehrerer Beschädigungen wirkungsvoll sein kann, und dann auszuwerten wie hoch die individuellen Beschädigungen und ihre Ausdehnung auf die Haut im Zusammenenhang mit der Hautfläche, die als Muster genommen wurde, sind (Hautsflächerprozentsatz).

Resumen
Se presenta aqui un sistema para la evaluación de las lesiones de acné por medio de un anális morfométrico computarizado. Ese procedimiento permite (también con referencia al tratamiento) una evaluacíon cuantitativa eficaz también en caso de un gran número de lesiones y la evaluacíon del tamaño de lesiones individuales y de la área que ocupan con referencia también a la área de epidérmis que se ha elegido como muestra (area %).

Introduction

The course of acne and its response to therapy is usually evaluated by means of clinical observation and measurement. Clinical evaluation can be accomplished either by specific lesion counting or global evaluation: in both cases the judgement is subjective and therefore it does not allow exact quantification.
The evaluation of individual lesions requires that specific lesions in specific sites be counted so that minimum and maximum numbers be established: too few lesions would make it impossible to judge improvement, too many would make counting difficult (8).

Kligman and Plewig (2) suggested a method to simplify counting by means of grade assignment (ex. grade 1 = less than 10 comedones, grade 2 = 10-25 comedones, etc.). Comparison among groups with the same acne-grade is easier (ex. in relation to treatment) but lesions must be partly counted anyway. There are some advantages in limiting the number of lesions to be counted by using masks with gaps on correctly pre-determined face areas.
In *global evaluation*, individual lesions are not counted and the grading is based on some numerical scale, such as the Pillsbury 4-grade scale (6) or the Cook. 9-grade scale (1) which requires compa-

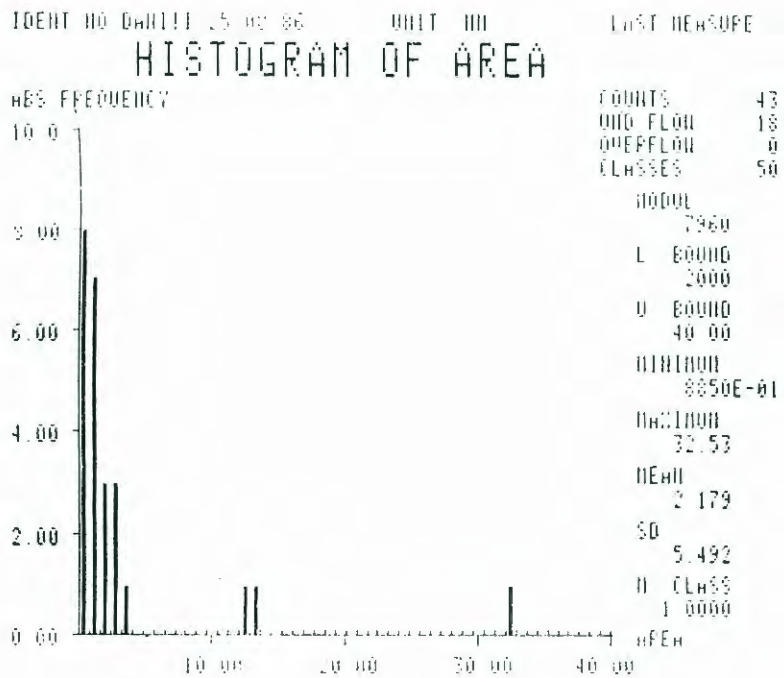


Graph 1: Pre-treatment.

ring the patient to a set of 5 standard photographic reproductions of acne. This method, adapted to office use (9), has been recently employed (3) evaluate to the effectiveness of two different antibiotic treatments (7).
Even if the global evaluation method is easier than counting specific lesions, it is less precise. In both cases the margins of error after successive visits or different observations could not be reduced sufficiently. Since 1985 (4, 5) we have been checking the possibility of using computerized morphometric analysis in evaluation of acne lesions.

Material and methods

35 mm color slides of skin areas containing acne lesions were taken through macro objective Pentax 50 mm, f 1:4 lens. These photographs were then scanned by means of a high geometric linearity TV-camera ($d < 0,3\%$ over the whole recording field of 512×512 pixels^2) connected to a computerized image analyzer Kontron Zeiss IBAS 2.
A suitable program, maintained the same for all the subjects was utilized. With this program the image was interactively elaborated by electronic filters and densito-



Graph. 2: Post-treatment.

metrically analyzed over 256 grey levels, in order to obtain a binary image suitable to the quantitative evaluation of the most significant morphometric parameters. After standardization of the primary subject image (directly taken from the color transparency), on a scale over 256 levels, the image itself was submitted to interactive elaboration in order to define the areas to analyze quantitatively. A measuring frame was placed over the image so as to de-limit a homogeneous area which avoids surfaces with different lightings. Amplitude and coordinates were memorized in the program, for a successive and exact application on the same skin area after treament. In this image were accurately analyzed the frequency of distribution of the various grey levels, by defining inside the 0-256 range a threshold for the grey interval corresponding to the examined lesions.

If the illuminating conditions of the examined areas of the subject were the same before and after a treament we chose the same grey interval values, otherwise it was necessary to modify that value in order to obtain the best possible correspondance between the electronic image and the original color transparency.

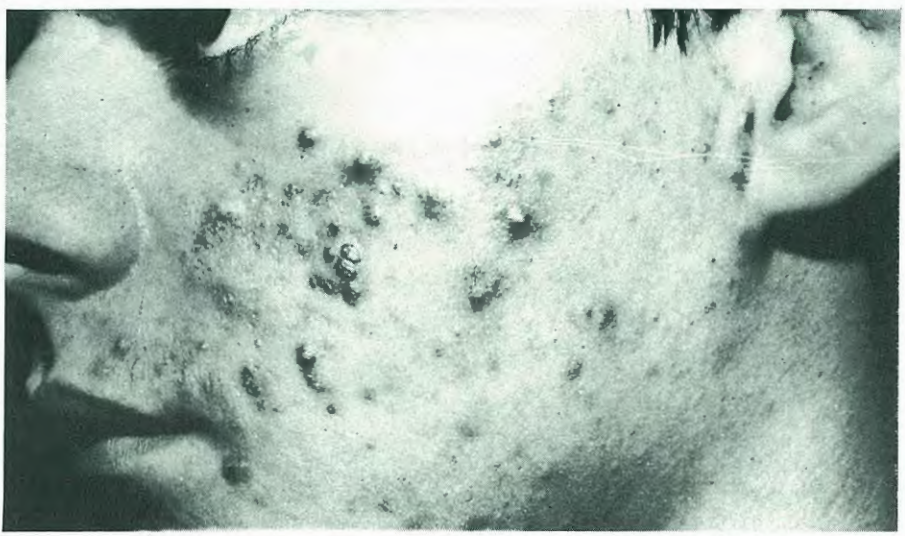


Fig. 1: Pre-treatment (black and white from color transparencies).

In this way it was possible to descriminate the lesions inside the measuring frame. The grey levels of such an interval were converted to white (leve 255), obtaining a binary image suitable to quantitative evaluation of lesions acne. This evaluation was automatically performed after identification with pseudocolours.
Morphometric analysis of acne lesions in a patient before and after a treatment (systemic antibiotic therapy) was performed.

Results

All examples of the evaluation system are presented. Two color transparencies (before and after treatment; photo 1, 2) are submitted to TV-recording connected to the computerized image analyzer. After the normalization of the primary subject image an electronic measuring frame is superimposed (photo 3, 4) and the enlargement index is calculated.
The images are densitometrically analyzed and a binary image is obtained (photo 5, 6).
Evaluation of lesions is automatically performed:

1) a quantitative evaluation: before treatment the lesions are 59 and after treatment 43;

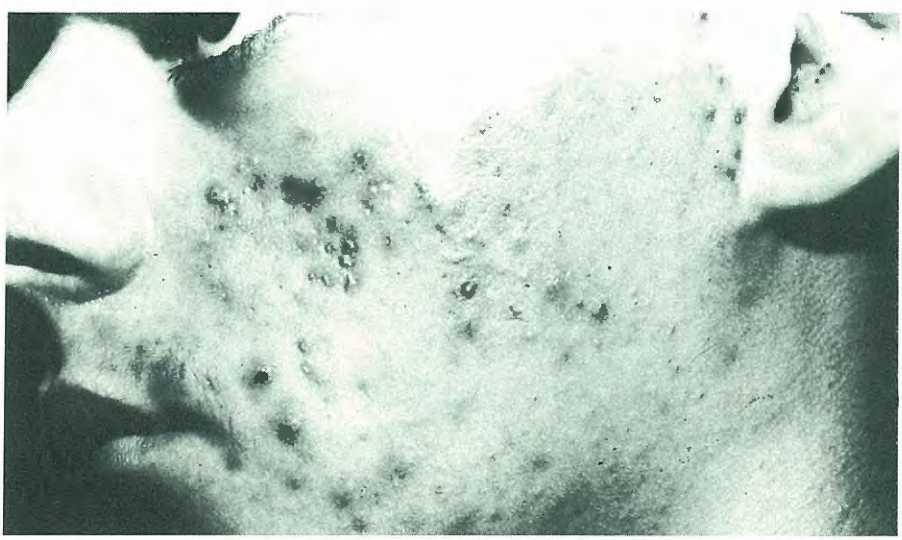


Fig. 2: Post-treatment.

2) a quantitative evaluation of each lesion area. The lesions are distributed according to their extension in 25 predetermined classes. If elements are below 0,2 mm^2 they are eliminated for calculation and graphs: a verification has shown that below said value a number of misleading elements can be included, such as scales, debris and ostia. Before treatment the average area is mm^2 2.289 (graph 1). After treatment the average area is mm^2 2.179 (graph 2).
3) An evaluation of the skin area covered by lesions in relation to the measuring frame area (area %) that before treatment is 12.14% and after treatment is 8.445%.

Discussion and conclusions

The evaluation methods of acne lesions and of their regression, in relation to treatment, which have been presented up to now allow, both in vivo or in photographic reproductions, the simple counting of a number of lesions, which is generally limited, and whose global evaluation is insufficiently precise.
The method presented here has allowed (in the same pre- and post-treatment sample area): a quantitative evaluation, effective even with a high number of lesions, a quantitative evaluation of individual lesion areas, and an evaluation of the lesions as a percentage of a chosen skin area. It is a method that minimizes subjectivity in the evaluation of the smallest differential quantitative modifications of selected parameters, induced by a therapy, in a single subject. Nonetheless there are some drawbacks: it is impossible to automatically distinguish elementary lesion types and the interactive determination of a densitometric threshold is required.

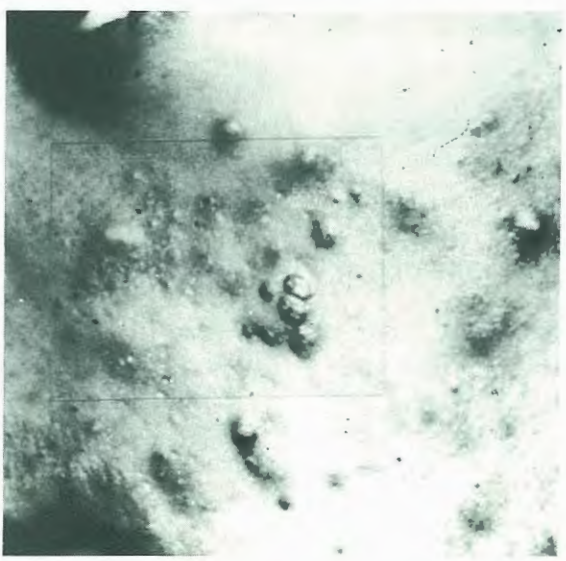


Fig. 3: Pre-treatment: «normalized electronic image with a superimposed measuring frame (electronic window).

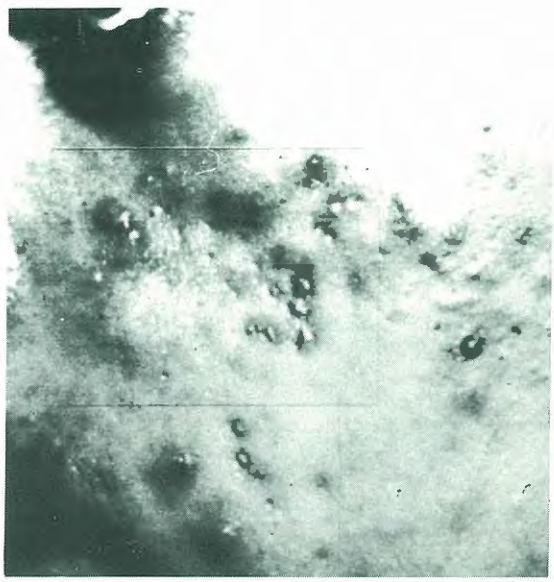


Fig. 4: Post-treatment, «normalized» image with the same measuring frame.

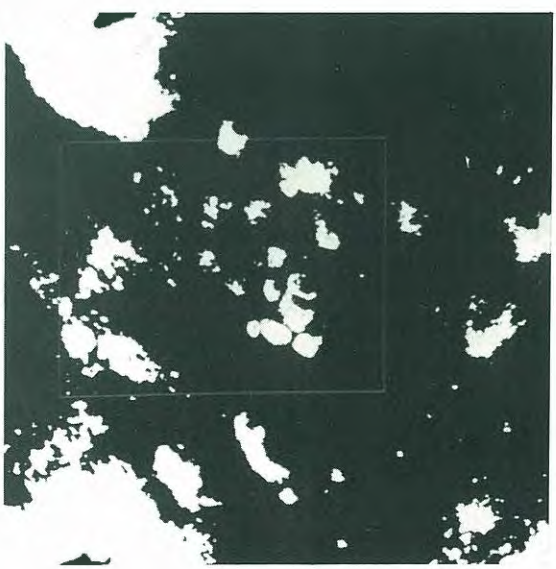


Fig. 5: Pre-treatment: interactive determination of densitometric threshold (segmentation).

Fig. 6: Post-treament.

REFERENCES

1. **Cook C.H., Center R.L., Michaels S.E. (1979)** «An acne grading method using photographic standards» *Arch. Derm.* **115**, 571-575.
2. **Kligman A.M., Plewig G. (1975)** «Classification of acne» *Cutis* **16**, 520-522.
3. **Nacht S. (1986)** «Efficacy of Anti-Acne Drugs and Formulations» *Cosmetic & Toiletries* **101**, 47-55.
4. **Pelfini C. (1985)** «Analisi morfometrica computerizzata di lesioni acneiche» *Comm. at.* 62th Nat. Congr. SIDEV, Rome, 12-15/6/1985.
5. **Pelfini C. (1986)** «La valutazione della regressione delle lesioni acneiche» *Comm. at: 25th Congr. ADOI, Grado, 17-20/10/1986.*
6. **Pillsbury D.M., Shelley W.B., Kligman A.M. (1968)** «Dermatology» *WB Saunders Co. Philadelphia*, 810-811.
7. **Samuelson J.S. (1985)** «An accurate photographic method for grading acne: initial use in a double-blind clinical comparison of minocycline and tetracycline» *J. Amer. Acad. Derm.* **12**: 461-467.
8. **Strauss J.S. (1982)** «Principles of efficacy evaluation of acne products» In: Kligman A.M., Leyden J.J. (eds.) Safety and Efficacy of Topical Drugs and Cosmetics. Grune & Stratton, New York, 389-400.
9. **Wilson R.G. (1980)** «Office application of a new acne grading system» *Cutis* **25**, 62-64.

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Enhancement of normal hair growth by topical treatment

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Key words: Hair growth, Topical treatment, Vasodilators, Vasoactive drugs, Hair density, Trichosaccharides

Synopsis
The effect on hair growth a hair lotion containing trichosaccarides[R] and vasodilators was assessed on 13 healthy volunteers in an open experiment. On side of the scalp was treated, the other was used as a control. In a first study the right side was treated; in a second one, 9 months later, the left side was treated. After 30 days of treatment, on the treated side a significant ($p < 0.001$) increase in the number of hair shafts per cm^2 of scalp area was found together with a higher hair lenght ($p < 0.001$).

Riassunto
Durante un esperimento aperto si è valutato su un campione di 13 volontari sani l'effetto di una lozione per capelli contenente tricosaccaridi e vasodilatatori sulla crescita dei capelli. È stato trattato un lato del cuoio capelluto, usando l'altro come riferimento. In un primo studio è stato trattato il lato destro, e in un secondo, 9 mesi dopo, il sinistro. Dopo 30 giorni di trattamento, sulla parte trattata si è riscontrato un aumento significativo ($p < 0.001$) del numero di capelli per cmq, nonché un allungamento ($p < 0.001$) dei capelli.

Résumé
L'effet d'une lotion qui contient des trichosaccharides et des vaso-dilatateurs, a été testé sur 13 volontaires sains. On a traité une partie seulement du cuir chevelu des sujets, à fin de pouvoir établir une comparaison entre les deux parties. Le test a été divisé en deux phases: la première fois on a soumis au test la partie gauche, après deux mois on a traité la partie droite du cuir chevelu. Aprés 30 jours de traitement, la partie traitée a révélé une augmentation importante du nombre ($p < 0,001$) et de la longeur ($p < 0,001$) des cheveux pour chaque cm^2 du cuir chevelu.

Synopse
Die Auswirkung eines aus Trichosaccaryd und gefässerweiternden Stoffen zusammengesetzen Haarwassers wurde im Lauf eines offenen Experiments auf 13 gesunde Menschen analysiert, die sich freiwillig solchen Experimenten unerziehen. Nur eine Seite der Kopfhaut wurde mit diesen Haarwasser behandelt, und die andere wurde als Kontrollmuster benutzt.
Zuerst wurde die rechte Seite und dann (nach 9 Monaten) die linke Seite behandelt. Nach 30 Tagen Behandlung konnte man einen bedeutenden Haarzuwachs ($p < 0{,}001$) und Haarlänge ($p < 0{,}001$) bemerken, was die behandelte Hauftläche anbelangte.

Resumen
Se ha valorado el efecto que una loción para el pelo con tricosácarides y vasodilatadores ha ejercitado sobre el crecimiento del pelo de 13 voluntarios sanos durante un experimento abierto. Se ha tratado un lado del cuero cabelludo y se ha utilizado como control el otro lado. Durante un primer estudio se ha tratado el lado derecho, y en un segundo, 9 meses después, se ha tratado el lado izquierdo. Después de 30 dias de tratamiento, sobre el lado que se había tratado se ha encontrado un incremento significativo ($p(0{,}001)$ del número de pelos por cmq junto a un alargamiento del pelo ($p < 0{,}001$).

Introduction

For decades hair lotions have been claimed to promote hair growth, but without any scientific evidence. The main reason for that was the lack of reliable methods to assess hair growth and hair density of the scalp. The trichogram technique (1, 2) based on the study of the bulbs of pulled hairs was an important advance and allowed interesting progress in the understanding of pathological hair conditions. But its use is limited, being this technique mostly qualitative. Furthermore, there are strong variations in «hair formula» depending on scalp areas and seasons (3). A more quantitative, though still uneasily practicable, method is the phototrichogram described by Saitoh (4) which allows the measurement of hair density and hair growth through a macrophotography.
Using the latter technique, we have measured the influence of a topical lotion on hair growth in normal people.

Material and methods

13 healthy volunteers, aged 16-30 years, without any sign of male pattern alopecia or scalp disease were selected for the experiment.
The tested lotion* was a mixture in an base of nicomethanol tartrate and ethyl nicotinate, both well-known vasodilators, and mucopoly saccharides extracted from mammalian gut (trichosaccarides^R+) (Table I). It was applied once every other day by gently spreading on the scalp without any rubbing, for at least one month. In a first study, the hair lotion was applied only on the right side of the scalp (late winter 1984). In a second study only the left side was treated (Autumn 1985). In each experiment, the untreated side served as a control. Each study involved 10 volunteers but 7 people participated in both studies. The statistical study was made using the analysis of variance and the paired t test comparisons, for each study separately, as an interaction was found between treatment and side effect.
Hair growth parameters were assessed as follows: two symmetrical areas, of about 4 cm^3, selected on each side of the scalp, were shaved and photographed four days later with a Polaroid CU5 close-up hand camera. After enlargement of the picture, hair shafts were counted on an area of 1 cm^2 using a network (see photograph). Hair length was measured before, during (at regular intervals), and at the end of treatment, using magnified photographs according to the Saitoh's method.

Results

1 - Hair density

On treated areas, the mean hair densities

Table I
Formula of the tested hair lotion

Trichosaccarides^R	50 mg
Nicomethanol tartrate	14 mg
Sodium pantothenate	7 mg
Ethyl nicotinate	5.6 mg
Biotin	0.175 mg
Hydroalcoholic base 7.1^c ad 7 ml	

(Table II and III) had increased by day 34 as compared to day 4, while on control areas, hair densities had clearly decreased in both studies. The analysis of variance on the whole data showed a significant difference due to the treatment ($F=18.5$ 1-18 df-$p<0.001$) but a weak interaction between side and treatment effect ($F=6.2$ 1-18 df-$p<0.05$). Consequently, a paired t. test was made on each study separately. Due to the large range of data obtained, the result of the first study was not statistically significant. The number of subjects displaying and increase in hair density by more than 2% on the treated side was 14/20 as compared to 3/20 on the control side ($p<0.01$, chi 2 test).

2 - Hair length after 30 day's growth

By the 30th day following shaving (Table IV), the mean length of hair was clearly (1st study) or barely (2nd study) higher in the treated side. Under paired t test comparison, the difference between treated and not treated sides highly significant ($p<0.001$).

In the first study (where the treatment had been maintained up to 68 days), a significant increase in hair length was found on the treated side on days 4 and 30, but not on day 64. In the second study, a greater hair length was found by days 20 and 30 but this was not statistically significant. There were only intraindividual differences, hair length being at a time significantly higher on treated si-

Table II
Number of hair shafts per cm² (mean and standard deviation) **in 10 normal subjects**

	1st study		2nd study	
	Day 4	Day 34	Day 4	Day 34
Treated side	255 ±54	266 (+4.3%) ±55	205 ±37	209 (+2.0%) ±39
Control side	235 ±36	226 (—3,8%) ±54	216 ±50	193 (—10.6%) ±36
Total difference	+8.1%		+12.6%	
Paired t test	(NS)		($p<0.001$)	

Table III
Number of hair shafts per cm². Difference between D 34 (end of treatment) **and D 4** (before treatment)

	1st study	2nd study
Treated side	10.5 ± 23.4	3.7 ± 15.8
Control side	—8.6 ± 38.0	—23.3 ± 20.9
Treated minus control side	19.1 ± 46.7 (NS)	27.0 ± 16.4 ($p<0.001$)

des in 6 subjects and on the control side in one subject (Table V).

Discussion

In the experiment acceptable variation coefficients were obtained both for mean hair density (15.3% to 23.9%) and hair growth (7.6% to 16.7%). As expected control sides demonstrated spontaneous trends with time. The reduction in hair density was statistically significant and occurred mostly during the second study, thus partly explaining the lower increase in hair shaft number as compared to the first study. Conversely, the rate of hair growth was significantly higher on both sides in the second study than in the first one.

Despite these physiological variations, statistically significant increases in hair density and hair growth ranging from 8.1% to 12.6% for hair density and from 2.3% to 20.1% for growth rate, were observed on treated sides. It is unlikely that a mechanical effect on hair growth has occurred as the lotion was applied only every other day and without rubbing. The used formula comprised known vasodilators which have long been used for promoting hair growth. Their effectiveness has never been quantitatively assessed, although they have been commonly used, sometimes successfully, in alopecia areata.

Other ingredients, such a mucoplysaccharides, may also have an effect as their increase in the dermis has been shown to be associated with hair growth (5, 6). Penetration studies should be performed together with further experiments to substantiate this point.

Nevertheless, the above experiment demonstrates that the Saitoh's method is sensitive enough to allow the identification of changes in hair growth parameters even in normal people and to assess the stimulating effect of a lotion applied every other day only.

Acknowledgement: The authors wish to thank Deglaude Laboratories for providing the lotion, and Mrs Roche for typing the manuscript.

Table IV
Hair lenght (mm) on day 30 (mean and standard deviation)

	1st study	2nd study
Treated side	12.3 ± 1.5	13.5 ± 1.9
Control side	10.2 ± 1.7	13.2 ± 1.0
Treated minus control side	2.1 ± 1.1 (+20.1%) ($p < 0.001$)	0.3 ± 1.1 (+2.3%) (NS)

Table V
Statistical analysis of mean hair length difference (t test), between treated and control sides, at days 20 and 30 of treatment

Subject number	Day	t	p	treated (T) versus control (C) side
1	D20	1.42	NS	
	D30	1.39	NS	
2	D20	2.27	<0.05	T<C
	D30	1.04	NS	
3	D20	4.42	<0.001	T>C
	D30	0.03	NS	
4	D 20	3.14	<0.01	T>C
	D30	0.61	NS	
5	D20	7.45	<0.001	T>C
	D30	0.57	NS	
6	D20	0.81	NS	
	D30	0.54	NS	
7	D20	2.29	<0.05	T>C
	D30	0.59	NS	
8	D20	1.65	NS	
	D30	7.23	<0.001	T>C
9	D20	2.59	<0.05	T>C
	D30	0.24	NS	
D20	1.57	NS		
	D30	0.74	NS	

REFERENCES

1. **Van Scott E.J. (1958)** Response of hair roots to chemical and physical influence. In «The biology of hair growth» *Academic Press*, New York, 441-449.
2. **Barman J.M., Astore, Pecoraro (1965)** «The Normal trichogram of the adult», *J. Invest. Dermatol.*, **44**: 233-236.
3. **Barman J.M., Pecoraro, Astore (1964)**, Method, technic and computations in the study of the trophic state of the human scalp hair. *J. Invest. Dermatol.* **42**: 421-425.
4. **Saitoh M., Uzuka, Sakamoto (1970)** «Human hair cycle» *J. Invest. Dermatol.*, **54**: 65-81.
5. **Moretti G., Cipriani (1967)** «Correlation of tissue mucopolysaccharides with the hair cycle» *J. Invest. Dermatol.*, **48:** 498-503.
6. **Elling H. (1986)** «Penetration of mucopolysaccharides into the skin of diverse animal species» *Drug Res*, **36:** 1525-1527.

Lead-time

We need a lot of this! There are many of you who have wonderful ideas for meetings. There are ideas for location, for theme, for time of year. There are ideas for subjects you would like to hear discussed and for subjects upon which you would like to speak.
Of course, some of the ideas will not be wonderful. Nevertheless, they are there — you do have them. What I would now like you to do is share them. Once I have the ideas, we can explore using them. There is a remote chance that they might fit into this year's plan. Possibly into next year's. Probably they can become the plan for a few years hence.
That is an explanation of «lead-time». In order to have your ideas utilized, I must have them well in advance. For us to place a meeting in a location of choice may require up to five years advance planning and reservations in peak seasons. A program with depth in a single area of interest requires twelve to eighteen months of preparation. And so on - and on.
The simple fact is, we want all of our programs to be excellent in site, topic, scope and presentation. We need your help in the form of ideas and comments. We need the help with a lot of «lead-time».
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So, please send me your ideas. And let me have some lead-time.

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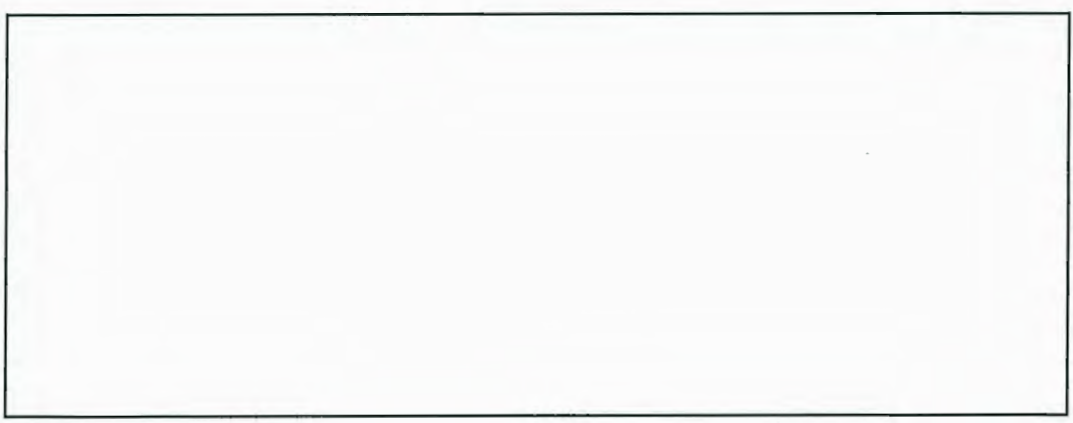

Book Review

J. Appl. Cosmetol. **6**, 52 (January-March 1988)

DISEASES OF THE ORAL MUCOSA, by Fulvio Allegra and Pier Umberto Gennari, 256 pages, 492 photograph and designs, 620 analytic index references, 26×30.7 cm, book cover in cialux closth with a plastic, in color, book cover. Ciba Geigy Ed., 1987
ISBN 88-7645-054-8

The first edition of a book on diseases of the oral mucosa has recently been published by Ciba Geigy. The authors are Fulvio Allegra, M.D., Chairman of the Dept. of dermatology and Pier Umberto Gennari, M.D., Chairman of the Dept. of Dentistry, Professors of Medicine and Dentistry, University of Parma.

The book is not only dedicated to Doctors, Dentists, Dermatology, Venereology and Dentistry residents, but is also of great interest to plastic surgeons, maxillofacial surgeons, and some chapters, to the Ear, Nose and Throat Specialist. All these specialists, although practicing professions in which highly specialized technical skills are required, find the basis for these skills in a detailed knowledge of anatomy, physiology, pathophysiology, clinical examination and the development and embryology of the mucosa and structures of the oral cavity. The authors gave precedence to visual images, contributing not only static images, but also images demonstrating the evolution of the lesions, frequently accompanied by tables and charts. Another merit of the book is the presentation of many designs by Dr. Frank Netter, the master artist of medical illustration.

The book has 14 chapters, presented in the following order: macroscopic anatomy, histology and ultrastructure of the oral cavity, physiology, clinical examination, the development and embryology of the mucosa and the structures of the oral cavity with references to dysembryogenetic diseases, genetic diseases and two chapters, subdivided into two sections, cover paradontal anatomy and deep and marginal (gingivitis) paradontopathy, illustrating their anatomic, functional and pathological aspects. The next chapter is an extensive look at infectious diseases of the oral cavity, with particular attention to mycotic, viral and sexually transmitted diseases, and syphilis is covered in-depthly. AIDS, and it's oral manifestations, are excellently treated, given the importance of the disease, and of recognizing it's clinical manifestations in the mouth. The book doesn't forget hypersensitivity immune, endocrine and nutritional diseases, vitamin deficiencies and traumatic lesions of the oral cavity.

The last, but not least, of this series of in-depth presentations covers neoplasias of the oral mucosa, including precancerous, benign and malignant lesions. The book also provides the reader with an up-to-date and complete bibliography, as well as an analytic index.

J. Appl. Cosmetol. **6**, XIX-XXI (January-March 1988)

Recenti acquisizioni e orientamenti nella detersione della cute e dei capelli

1° Corso Europeo di Dermatologia Cosmetologica

Roma, 25-26 giugno 1988
Sede: Aula della Clinica Dermatologica
Università degli Studi di Roma

A chi ne farà richiesta il dr. P. Morganti potrà fornire tutte le delucidazioni scientifiche riguardanti il corso:
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Scopi del corso

Il corso ha lo scopo di definire tutti i problemi chimico-dermatologici connessi con la detersione fisiologica della cute e dei capelli. Verranno posti in rilievo sia i principi scientifici su cui sono fondati i diversi tipi di detergenti (saponi, syndet, latti, shampoo, ecc.) che le ragioni cliniche connesse con la loro prescrizione. Un particolare rilievo verrà dato, inoltre, alla descrizione dei meccanismi dei diversi prodotti detergenti ed agli aspetti allergologici connessi con il loro uso continuativo esterno, esteso anche a vaste superfici cutanee. Il corso è riservato ai medici dermatologi, ai chimici cosmetologi e a tutti i laureati nelle discipline affini che intendano perfezionare le loro conoscenze sui problemi clinico-cosmetologici riguardanti la detersione. Questo corso, che si avvale della collaborazione e consulenza di eminenti scienziati europei, sarà articolato in dodici lezioni teorico-pratiche tenute tutte da docenti italiani, della nazione, cioè, che organizza il corso.
Lo stesso corso verrà ripetuto nelle altre nazioni CEE.
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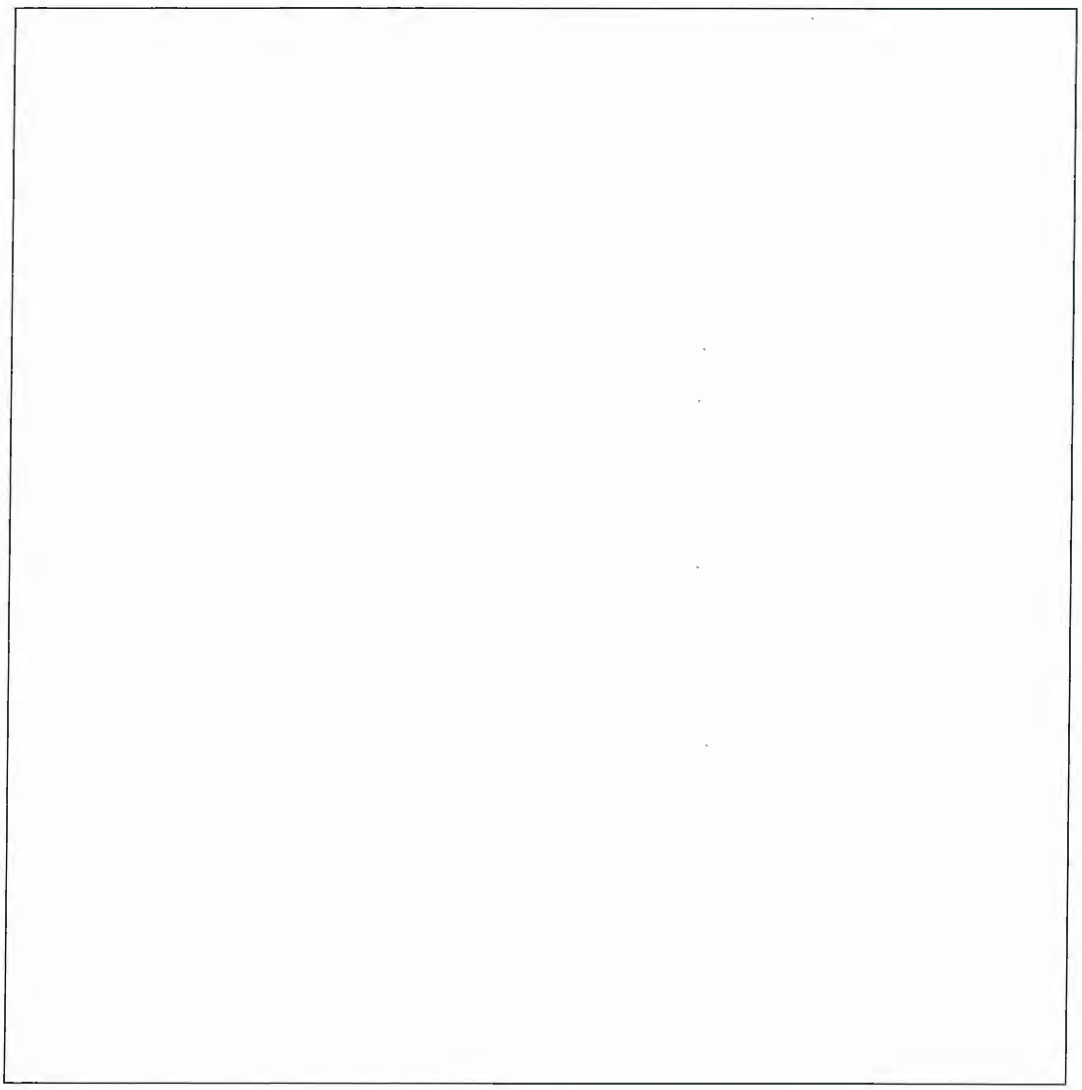
PROGRAMMA

Sabato 25 giugno

ore 8.30 - Introduzione: E. Panconesi
ore 9.00 - Struttura macromolecolare e biochimismo della cute (B. Berra)
ore 9.30 - Caratteristiche e proprietà fondamentali delle cheratine (D. Innocenzi - F. Todisco)
ore 10.00 - Struttura morfologica e macromolecolare dei capelli (S. Calvieri - S. Giustini)
ore 10.30 - Proprietà fisiche e reattività chimica dei capelli (M. Ambroso)
ore 11.00 - Break
ore 11.15 - Le basi chimiche della detergenza (E. Chiacchierini)
ore 11.45 - La detersione della cute infantile (G. Camplone - E. Angelini)
ore 12.15 - La detersione della cute secca (U. Bottoni - B. Annibali)
ore 12.45 - Problemi connessi con la detersione della cute dell'anziano (S.D. Randazzo)
ore 13.15 - Brunch
ore 15.00 - La detersione della cute seborroica (S. Pala - B. Tiberti)
ore 15.30 - La detersione fisiologica della cute alterata: problemi e prospettive (G. Panasiti - R. Clerico)
ore 16.00 - I componenti fondamentali e le forme cosmetiche degli shampoo S. Giorgini)
ore 16.30 - I preparati per il bagno: i bagni schiuma e olii da bagno nella Dermatologia Cosmetologica (P. Morganti)
ore 17.00 - Break
ore 17.15 - L'attività detergente dei saponi e dei syndet (A. Teglia - S. Bonazzi)
ore 17.45 - Chiusura dei lavori

Domenica 26 giugno

ore 8.30 - Latti e creme detergenti nella detersione del viso (M.C. Melli)
ore 9.00 - Igiene della mucosa orale e detersione dei denti (E. Benagiano)
ore 9.30 - La mucosa vaginale; i detergenti intimi: problemi di detersione (R. Forleo)
ore 10.00 - Controllo dell'attività di detergenza mediante l'uso di apparecchiature specifiche (G. Secchi)
ore 10.30 - Chiusura dei lavori



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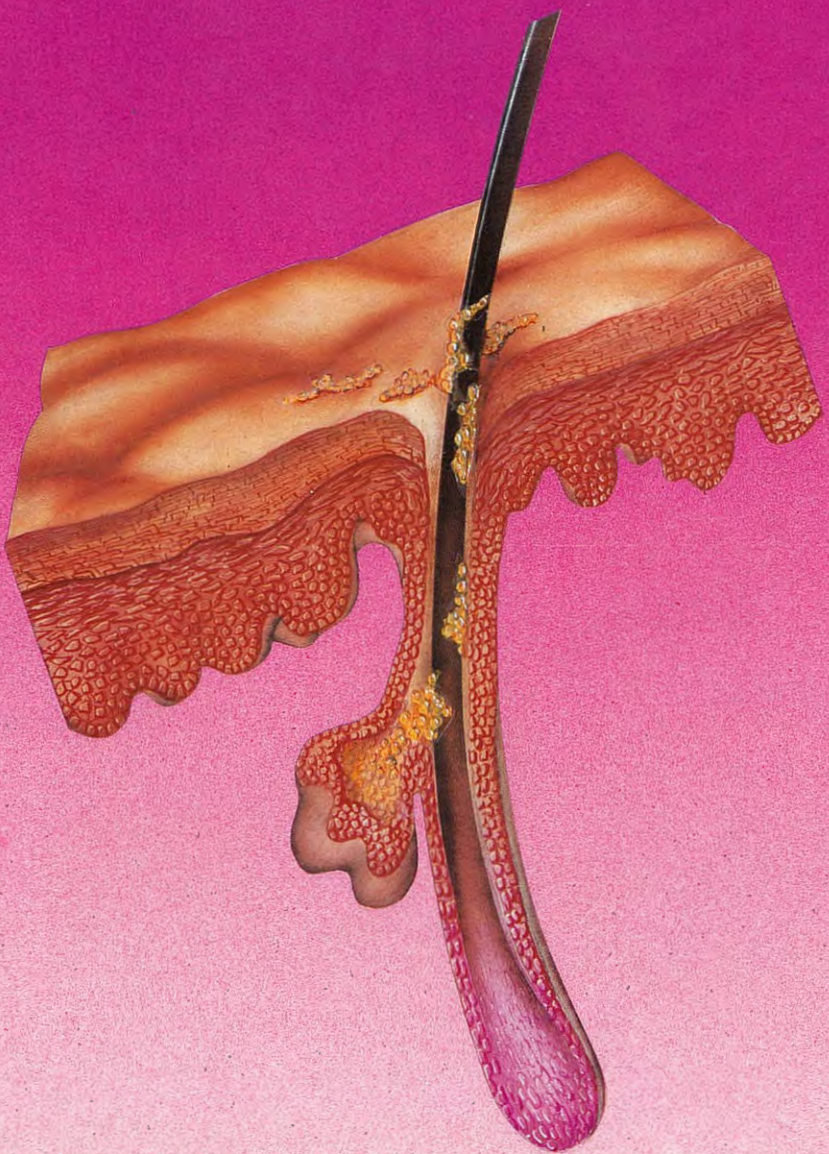

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