Journal of the Society of Cosmetic Chemists

Contents

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Textbook of Dermatology

Edited by

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F.J.G. EBLING D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this edition is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

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Thomas Butterworth A.B. M.D. MED.SC.M. *Second Edition*, 1972. 104 pages. Lippincott, £1.50

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M.H. Samitz M.D. M.SC.(Med.) and Alan S. Dana Jr M.D. 1972. 210 pages, 132 colour illustrations. Lippincott, £14.00

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SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 127 × 76 mm index cards for reference without mutilating the pages of the Journal.

The chemistry of human hair cuticle—II: The isolation and amino acid analysis of the cell membranes and A-layer: J. A. SWIFT and B. BEWS. *Journal of the Society of Cosmetic Chemists* **25** 355–366 (1974)

Synopsis—Mixtures of papain and dithiothreitol have been used to effect the separation of the A-layer and cell membrane complex and the cell membrane complex alone from human hair cuticle. The course of these enzymatic digestions has been followed gravimetrically and by the electron microscope examination of digested hair sections, and it is evident that the components mentioned are isolated cleanly. The amino acid compositions of the cell membrane complex and of the A-layer (obtained by difference) are quite different from the composition of the whole cuticle. The significance of the analyses is discussed.

Use of a laboratory model to evaluate the factors influencing the performance of depilatories: T. J. ELLIOT. *Journal of the Society of Cosmetic Chemists* **25** 367–377 (1974)

Synopsis—The use of laboratory models to evaluate the performance of cosmetic and toiletry products is full of pitfalls, and subjective assessments nearly always give more accurate results.

However, the investigation of the comparatively large number of variables which influence the speed of action of depilatories cannot conveniently be carried out on a subjective assessment basis and requires the use of a suitable laboratory model.

The particular model developed (referred to as a depilometer) was designed to simulate practical use conditions as closely as possible and it enabled the more important formulation variables to be studied quickly. In-use tests on final depilatory formulations gave good correlation with the depilometer findings.

Sex hormones and skin: F. J. EBLING. *Journal of the Society of Cosmetic Chemists* **25** 381–395 (1974)

Synopsis—As well as its more obvious anatomical and physiological functions the skin plays an important part in social communication by vision, touch and smell. The epidermal surface, the activity of the glands and the distribution of the hair are particular features of skin which are concerned with sexual communication, and it is thus not surprising that they are profoundly influenced by hormones. Steroids have been widely used in efforts to improve skin texture; the effect of oestrogens is equivocal, but androgens certainly stimulate epidermal cell division. The sebaceous glands are unequivocally stimulated by androgens and inhibited both by oestrogens and anti-androgenic steroids, though their modes of action are not identical. Pituitary factors appear to be necessary, at least in the rat, for the response of the sebaceous glands to testosterone, and it seems possible they may act upon the conversion of the steroid to its active metabolites. Human body, axillary and pubic hair is similarly androgen dependent. So sebum secretion, the growth of sexual hair, and hirsutism may be inter-related by their link with steroid metabolism within the skin. Is it possible that one function, or at least one by-product, of cutaneous androgen metabolism is the manufacture of pheromones or odours?

The following papers have been accepted for publication in the *Journal*:

ORIGINAL SCIENTIFIC PAPERS

Development of a skin cream designed to reduce dry and flaky skin
J. D. Middleton, B.A.

The thin layer chromatographic detection and determination of an imidazolidinyl urea antimicrobial preservative
D. S. Ryder, L.R.I.C.

Studies of the factors controlling the action of hair sprays—III: The influence of particle velocity and diameter on the capture of particles by arrays of hair fibres
R. W. Rance, Ph.D.

A survey of the microbiological contamination in cosmetics and toiletries in the U.K. 1971
B. Jarvis, B.Sc., Ph.D., D.C.C., F.I.Biol., F.I.F.S.T., A. J. Reynolds, B.Sc., M.I.Biol., A. C. Rhodes, B.Tech., M.Sc., M.I.Biol. and M. Armstrong, B.Sc., M.Sc.

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The chemistry of human hair cuticle– II: The isolation and amino acid analysis of the cell membranes and A-layer

J. A. SWIFT and B. BEWS*

Synopsis—Mixtures of papain and dithiothreitol have been used to effect the separation of the A-LAYER and CELL MEMBRANE COMPLEX and the cell membrane complex alone from human HAIR CUTICLE. The course of these ENZYMATIC DIGESTIONS has been followed gravimetrically and by the ELECTRON MICROSCOPE examination of digested hair sections, and it is evident that the components mentioned are isolated cleanly. The AMINO ACID compositions of the cell membrane complex and of the A-layer (obtained by difference) are quite different from the composition of the whole cuticle. The significance of the analyses is discussed.

INTRODUCTION

The previous paper of this series (1) described a method for the physical isolation of cuticle from human hair and emphasized the lamellar substructure of each cuticle cell sheet. Our interest is now in the further separation of these laminae for chemical analysis. Various chemical procedures have been described for isolating different fractions from keratin fibres (cf. reference 2). Many of these lead to uncertainties about the significance of the subsequent chemical analysis and most do not allow the

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accurate specification of the morphological origin of the isolated fractions. Enzymic digestion procedures, by virtue of their high chemical specificity, are likely to lead to much cleaner fractionation and indeed Bradbury and Ley (3) have recently used pronase to dissolve wool endocuticular components selectively. Using mixtures of papain and dithiothreitol we have shown (4), by the electron microscope examination of digested hair sections, that a distinct layer remains undissolved in the vicinity of the cuticle cell membranes. The present paper is concerned with the further study of this digestion procedure which has enabled us to separate from isolated human hair cuticle the cell membrane complex and A-layer.

METHODS AND MATERIALS

The isolation of human hair cuticle

This material was prepared by shaking root-end pieces of brown Caucasian hair in water according to procedures described in the previous paper of this series (1). Cuticle fragments obtained after 2 h shaking (i.e. 5% by weight of the original hair) were separated and dried *in vacuo* over phosphorus pentoxide.

Digestion of cuticle fragments with papain/dithiothreitol

Preliminary gravimetric experiments, in which isolated cuticle was digested by dispersion in a solution containing 1 mg/ml papain (crude powder type 2, Sigma) and 2 mg/ml dithiothreitol (Calbiochem) in 0.1M phosphate buffer at pH 6.7, showed that 92% of the cuticle was dissolved after 18 h at 65°C. In addition, from observations in the transmission electron microscope of thin transverse hair sections digested under similar conditions, it was evident that some components were almost completely dissolved in as little as 15 min at 65°C. The rate of digestion could be reduced by working at 50°C and this lower temperature was used in all subsequent experiments. The course of the digestion was studied by using the following procedure. Weighed samples of cuticle (30 mg) were triturated with phosphate buffer (0.1M, pH 6.7) to form a uniform suspension. A solution of papain (2.5 mg) and dithiothreitol (10 mg) in buffer (2 ml) was then added and the suspensions incubated in a water bath at 50°C. After appropriate time intervals, samples were withdrawn and chilled to quench the enzyme reaction. The undigested cuticle residues were recovered by

centrifugation, washed twice with distilled water, dried *in vacuo* over phosphorus pentoxide and weighed.

Examination of morphological sites of digestion of human hair cuticle by papain/dithiothreitol

For this work, root-ends of brown Caucasian hair were embedded with Spurr's resin (5) in Beem capsules (LKB Produkter). It is noteworthy that these resins are unlikely to diffuse into the fibres, but nevertheless bonding of the resin with the fibre surface is sufficiently strong to provide the rigid support of the hair for subsequent thin sectioning. Transverse sections of the hair approximately 60 nm thick were cut on a diamond knife (Du Pont de Nemours) at a Porter-Blum MT-2 ultramicrotome (Sorvall) and collected on 100 mesh gold electron microscope grids (Polaron) previously covered with a thin collodion/carbon support film. Papain/dithiothreitol reagent, of the same composition as that used in the gravimetric experiments, was prepared and filtered immediately before use through 0.45 μm Millipore discs. The grids were immersed in the reagent at 50°C, and after various times of digestion, rinsed briefly in a solution containing dithiothreitol and buffer, then in two changes of distilled water each for 5 min and dried. Some of the grids were 'shadowed' by the oblique vacuum evaporation of carbon/ platinum on to the upper side of the grids (subtending an angle of 30° with the grid surfaces). The shadowing technique did not permit the discrimination of structure within the cell membrane complex on subsequent examination in the electron microscope. The remainder of the grids were therefore stained with various heavy metal compounds according to the following scheme, designed to yield maximum structural information: grids were immersed at room temperature successively in 2% aqueous osmium tetroxide for 2 h, distilled water for 1 h, saturated aqueous uranyl acetate for 2 h, distilled water for 1 h, Reynold's lead citrate (6) for 10 min, 0·02 N sodium hydroxide for 10 s, distilled water for 1 h and then dried.

The various digested hair sections, shadowed or stained, were examined in a JEM 7 transmission electron microscope at 80 kV and using a 20 μm diameter objective aperture. In the case of the shadowed sections it is already known (7) that the surfaces of undigested hair sections are not flat and that the irregularities are related to the underlying morphological structure of the sections. On the other hand the present digestions so cleanly removed some of the structures of the hair cuticle that no confusion arose with the original irregularities of the hair section surfaces.

Amino acid analyses of isolated fractions

Insoluble material remaining after digestion of hair cuticle with papain/ dithiothreitol reagent for 45 and 90 min was centrifuged at 30 000 *g*, the supernatant removed and the residue washed with three changes of distilled water, centrifuging after each wash. Material obtained by exhaustive digestion for a total of 3 days with two changes of reagent, was treated similarly. The residues were dried *in vacuo* over phosphorus pentoxide and their amino acid compositions determined by conventional autoanalysis.

<div align="center">RESULTS AND DISCUSSION</div>

Course of digestion of isolated cuticle with papain/dithiothreitol

A plot of the amount of residue remaining against time of digestion of isolated cuticle is shown in *Fig. 1* (A). From this curve it appears that at least two components are dissolving at different rates. That this is the case is revealed by the logarithmic plot of *Fig. 1* (B). The undissolved residue at long times of digestion is approximately 7% by weight of the original cuticle and by extrapolation of the log. plot to zero time it would appear that the fast dissolving components represent about 82% and the slow dissolving component about 11% by weight of the cuticle.
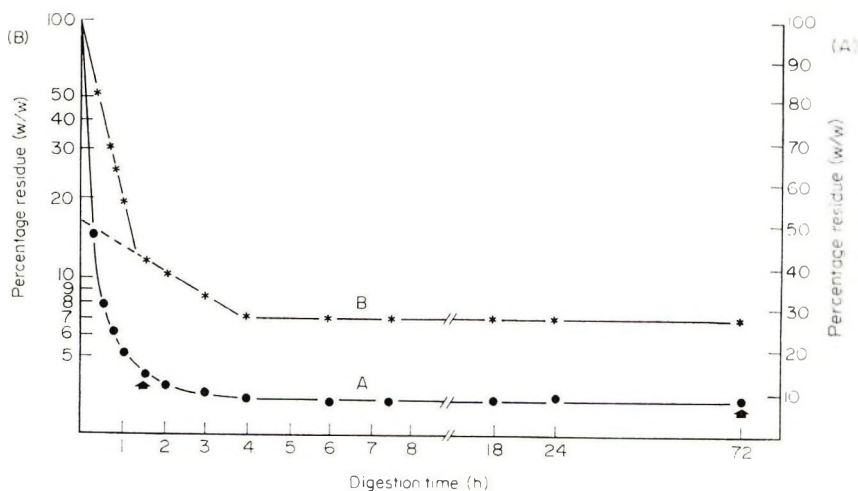
Figure 1. Graph showing the gravimetric course of digestion of human hair cuticle with papain and dithiothreitol. Curve A, axis A show the linear plot and curve B, axis B the corresponding logarithmic plot. Each point shown represents the mean of three separate determinations.

The morphological progress of digestion in the hair cuticle

Examination of digested hair sections in the transmission electron microscope revealed significant progression in the dissolution of the various structural components of the cuticle. In addition, since we believe that the accessibility to enzyme attack of the sectioned hair cuticle and of the physically isolated fragments are probably similar, some correlation could be made between the removal of cuticle components observed in the electron microscope and the various stages of digestion revealed by the foregoing gravimetric results.

After digestion of the sections for only 15 min there was almost complete removal of the endocuticle and there was some loss of material from the exocuticle (*Fig. 2*). (It is pertinent to note that *Figs 2 and 3*, which are electron micrographs of 'shadowed sections', have been prepared from intermediate negatives so that shadow regions are dark. In achieving this normal consistency for shadows, regions of increasing electron opacity are depicted by increasing brightness in the photographs.) Even at 15 min digestion it is striking that a distinct layer approximately 100 nm wide associated with the cuticle cell boundaries exhibits an electron opacity similar to that of the sectioned epoxy resin, indicating that this component is unaffected by the digestion. After digestion for 45 min virtually all the exocuticle was removed leaving the cell boundary material still apparently unaffected (*Fig. 3*). At this stage it was necessary to examine metal-stained rather than shadowed sections to reveal the detailed substructure of the cell boundary layer. It was found that this consisted of the A-layer to which was attached, on the outer-facing aspect of the hair, the complete cuticle cell membrane complex (*Fig. 4*). With further increasing time of digestion the width and general opacity of the A-layer slowly diminished. For periods of digestion exceeding 24 h the A-layer had more or less disappeared still leaving a residue associated with the cell boundaries. Close examination of this revealed that the complete cell membrane complex was still present and bounded on either side by a layer approximately 80 nm thick of stainable material (*Fig. 5*). It is noteworthy that at this stage it was difficult to discern the laminated substructure of the membrane complex but this was probably because disorientation of the residue on the electron microscope grid occurs so that the membrane laminae are no longer parallel to the electron beam of the microscope. On the other hand, by examining the point where adjacent cuticle cells overlap to give a type of T-junction of cell boundaries, the initial orientation of the membranes was maintained

and the laminated substructure of the cell membrane complex could be seen (*Fig. 5*).

From the present results it is clear that the initial fast-dissolving component observed in the gravimetric studies consists of endocuticle and exocuticle, that the slowly-dissolving component consists of A-layer and that the final insoluble residue consists mainly of cuticle cell membrane material. Indeed there is good correspondence between the percentage areas occupied by the endocuticle + exocuticle + inner layer, the A-layer and cell membrane complex in hair cuticle sections (82–85%, 10–12% and 5–6% respectively) and the foregoing gravimetric determinations for what are apparently the same components (82%, 11% and 7% respectively). Little mention has been made of the fate of the cuticle inner-layer in the present work but it is assumed because of its similarity in structure and cystine content (1) that it behaves in a manner analogous to that of the exocuticle.

The progress of digestion leading to the isolation in one case of A-layer and cell membrane complex and in the other of the cell membrane complex alone is summarized in the schematic diagram of *Fig. 6*.
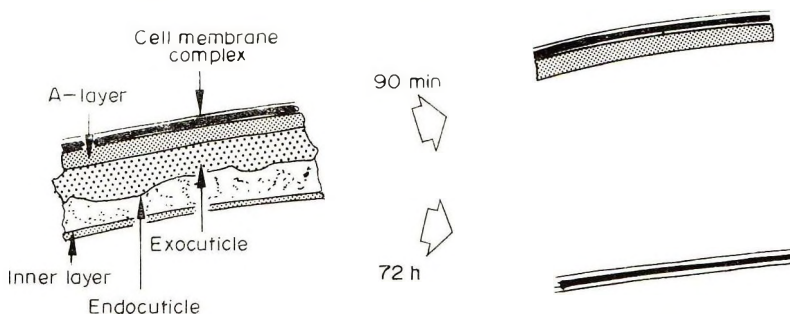
Figure 6. Schematic diagram illustrating the course of digestion of human hair cuticle with papain and dithiothreitol and leading to the separation of two major morphological components of the cuticle.

Amino acid analysis

The first column of Table I contains the amino acid analysis for isolated cuticle and columns 2, 3 and 4, the analyses for the papain/dithiothreitol-insoluble fractions of the cuticle obtained after digestion for 45 min, 90 min and 3 days respectively. The analysis in column 5 was obtained by calculating the differences in amino acid composition between the '90-minute' and '3-day' residues.
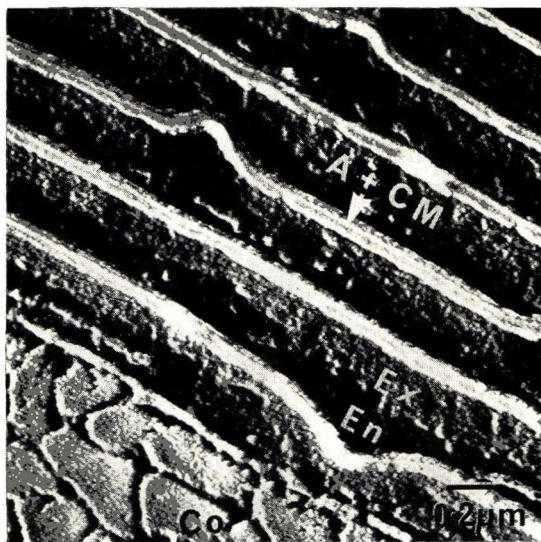
Figure 2. Transverse section of human hair cuticle digested for 15 min and then shadowed. Co, cortex; En, endocuticle; Ex, exocuticle, A+CM, A-layer+cell membrane complex.
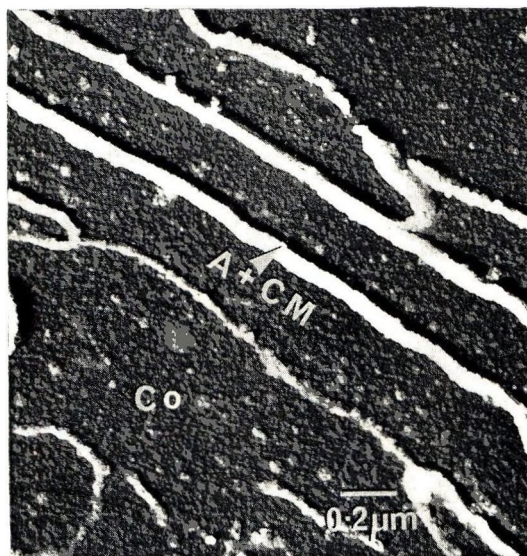
Figure 3. As Fig. 2 but digestion for 45 min.
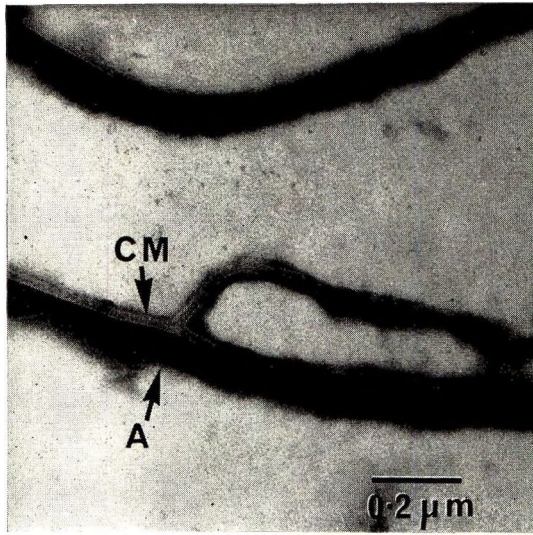
Figure 4. Transverse section of human hair cuticle digested for 45 min and stained with heavy metals. A, A-layer; CM, cell membrane complex.
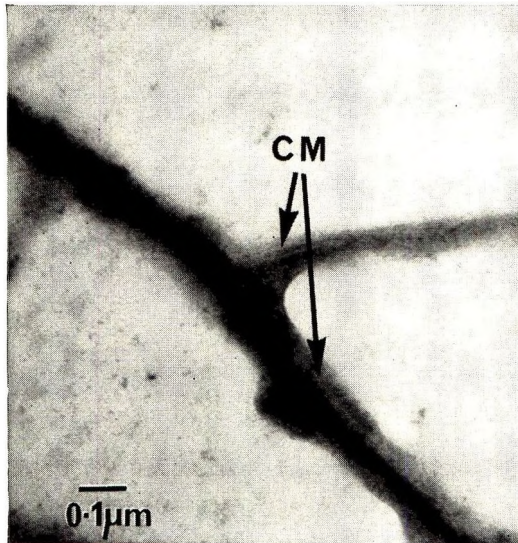
Figure 5. As Fig. 4 but digestion for 24 h.

comparison, are other published analyses for wool cuticle and various membrane fractions obtained from wool.

			Amino acid composition (residues/1000)						Membrane fractions from wool-Ref. 8, 9		
	Cuticle	'45 min residue' *	'90 min residue' †	CV	Membrane fraction †	CV	'A-LAYER' (Calculated)	Wool-Cuticle (ref. 3)	Performic acid ammonia residue	Performic acid urea residue	Epicuticle
Aspartic	32.4	32.5	68.4	13.7	71.9	11.0	67.5	34.6	57.9	50.2	58.4
Glutamic	103.9	96.0	93.2	5.6	77.8	4.7	95.6	86.7	104.3	102.5	106.6
Threonine	46.0	36.9	41.0	8.2	45.4	9.5	40.1	44.4	58.9	55.7	35.9
Serine	160.5	168.3	96.1	11.9	88.1	9.7	97.2	143.4	99.9	100.8	136.3
Proline	105.2	111.6	60.3	12.3	49.2	12.3	62.2	105.1	70.1	71.3	58.0
Glycine	88.4	138.4	125.1	2.9	141.8	9.8	121.8	81.7	139.0	144.3	153.3
Alanine	54.0	54.5	58.6	8.6	50.5	13.8	59.9	57.8	69.0	60.9	46.1
Valine	72.7	69.9	72.6	5.9	74.0	8.0	72.2	75.1	46.6	51.6	57.2
Isoleucine	22.2	14.5	35.3	18.4	46.6	10.2	33.2	26.7	26.5	24.6	25.2
Leucine	22.6	31.6	61.7	16.6	82.2	8.0	57.7	61.2	50.8	46.7	54.6
½ Cystine+Cysteic acid	193.8	117.0	99.5	10.7	99.2	5.9	99.8	156.3	116.0	144.3	119.1
Methionine	3.8	0.2	1.0	34.0	1.8	45.9	0.87	3.4	0	0	0.03
Tyrosine	21.0	17.2	38.4	12.8	31.1	11.9	39.6	28.3	0	0	20.7
Phenylalanine	9.1	4.2	28.0	13.8	15.2	4.5	30.2	16.9	15.3	15.2	18.5
Histidine	5.2	4.8	10.5	17.9	16.7	5.7	8.9	8.1	12.9	13.7	10.3
Lysine	34.4	57.0	59.8	12.0	78.0	7.1	56.6	27.4	90.0	76.9	48.3
Arginine	25.1	37.8	51.7	13.9	32.8	21.5	55.0	42.9	42.6	41.2	42.7
% total amino acid	86.8	65.4	70.7		24.1		—	93	77	86	78
% by weight of cuticle	100	27.0	13.0		5.5		11-12		—	—	—

CV, Coefficient of variation expressed as percentage.
*, Single analysis.
†, Mean triplicate analyses.

Membranes

As discussed previously, the insoluble material remaining after digestion for 3 days consists mainly of cell membrane complex and contains approximately 39% lipid material. The amino acid analysis for this fraction probably represents that for the membrane-associated proteins and it is interesting in that it differs considerably from that for intact cuticle. The last three columns of Table I show the amino acid compositions of membrane fractions obtained from wool by Bradbury and co-workers (8, 9) while column 6 shows the composition of wool cuticle (3). In each case the membrane fractions show substantially higher concentrations of aspartic acid, glycine and lysine, and lower concentrations of serine, proline and cystine than the corresponding cuticle samples. The cuticular membrane fraction from human hair also contains higher proportions of isoleucine, leucine and the aromatic amino acids than does intact cuticle. Closer comparison of the human hair and wool membrane fractions is not possible since those from wool are prepared from whole wool rather than isolated cuticle.

The presence of high concentrations of basic amino acids in the cuticle membrane complex is consistent with the intense staining which is seen under the transmission electron microscope, in the intermembranous cement (δ-band) and the thin layers bounding the complex in hair sections stained with dodecatungstphosphoric acid (1). This heteropolyacid exists in solution as a trivalent anion and is generally believed to bind readily to the basic groups of proteins (10). Electron histochemical staining of hair sections for cystine (7, 11) indicates that this amino acid is absent from the cuticle cell membrane complex. Since some cystine is present in our membrane fraction, at least part of the fraction is probably derived from the protein on either side of the lamellated membrane complex proper and indeed this is consistent with our present electron microscope observations (*Fig. 5*).

The main advantage of the present method for preparing cell membrane fractions from human hair compared with the previously used oxidative procedures (8) is that the destruction of sensitive amino acids such as tyrosine and methionine is minimized. Furthermore the lipid components of the membranes are well preserved so that further study of these materials is possible.

The A-layer

The fractions obtained after digestion for 45 and 90 min evidently

contain mainly A-layer and cell membrane complex, with the 45-min fraction containing a small amount of undissolved exocuticle and the 90-min fraction having had some A-layer removed (cf. *Fig. 1*). As in the case of the membrane fraction, the accuracy of the amino acid analyses is limited by the presence of non-proteinaceous components. A more serious limiting factor is the difficulty of obtaining a well-characterized sample of the A-layer and cell membrane complex free from other contaminants such as exocuticle but not having lost any of the A-layer. Since digestion of the A-layer is essentially complete in less than 7 h, small errors in sampling time or inefficiency of quenching the enzyme reaction result in substantial variations in composition. These difficulties are reflected in the coefficients of variation shown in the third column of Table I. In spite of these limitations the calculated differences in amino acid composition between the '90-minute' and '3-day' fractions shown in the fifth column of Table I, approximate to the composition of the A-layer, and show major differences from the composition of the intact cuticle. The A-layer thus contains higher concentrations of aspartic acid, basic and aromatic amino acids and lower concentrations of serine, proline and cystine than the whole cuticle. The low content of sulphur-containing material in the A-layer/membrane residues was confirmed semiquantitatively by X-ray microanalysis where the sulphur peak/background ratio for the '90 minute residue' was substantially lower than that obtained from intact cuticle or from exocuticle isolated by pronase digestion according to the methods of Bradbury and Ley (3).

The A-layer is probably a complex mixture of different types of proteins. Analysis of residues remaining after various times of digestion between 45 min and 3 h showed that the relative concentration of aromatic amino acids increased as digestion proceeded, as indicated in Table II. No such trends were shown by the other amino acids.

Table II

Residue as % by weight of cuticle	27	14.3	13.3	12.4	9.8
Moles/1000 tyrosine	17.2	18.4	33.7	41.9	48.9
Moles/1000 phenylalanine	4.25	11.6	23.5	30.9	40.7

These results indicate the presence of a component rich in aromatic amino acids and more resistant to proteolytic enzymes than the rest of the A-layer, though whether or not this component forms a discrete lamella close to the membrane is not yet clear.

The low cystine content of the A-layer is surprising and contrary to electron histochemical evidence (7, 11). It is possible that a cystine-rich component has been removed specifically from the A-layer during the enzyme digestion but this is considered unlikely since our electron microscope observations indicate that the overall structural integrity and electron opacity of this layer is maintained for the analysed specimens. A further possibility is that the electron histochemical methods are not as specific in their staining of cystine or cysteine residues as has been claimed. Using an organomercurial method, Dobb, Murray and Sikorski (11) have shown a near-stoichiometric uptake of mercury by cysteine in reduced wool. On the other hand the uptake of mercury in such minor components as the A-layer may not be necessarily related to the presence of cysteine, for other amino acids or non-proteinaceous material could be involved in the reaction. In this respect it is interesting to note that Levy (12) has demonstrated reaction between an organomercurial halide and the amino groups of insulin. Indeed this reaction may explain the existence of the thin layer stained by mercury adjacent to the cuticle cell membranes of wool and described by Dobb *et al.* (11). Such a reaction would be consistent with our observations of high lysine concentrations in the membrane-associated fractions and the staining of a thin layer either side of the cuticle cell membranes complex by dodecatungstophosphoric acid. With respect to the silver-methenemine method for the electron histochemical demonstration of cystine, anomalous staining of the A-layer of human hair cuticle has been described already (7). The silver globules used in the latter method as a criterion for the presence of cystine (7, 13) have a physical appearance which is quite different in the A-layer than in the other components of the hair, perhaps indicating that the histochemical reaction is modified by groups other than cystine. In the light of these discrepancies in the electron histochemical demonstrations of cystine in the A-layer, we believe that our analyses showing the low cystine content of this component are realistic.

The chemistry of papain/dithiothreitol digestion

One striking feature of the present work is the rapidity with which human hair cuticle dissolves in mixtures of papain and dithiothreitol compared with papain alone (usually negligible) or mixtures of papain and sodium bisulphite (55% dissolves after 8 h rising to 70% after 28 h). One of the main reasons for this is undoubtedly the high percentage reduction of the keratin cystine by dithiothreitol (14, 15). Whereas complete reduction of

cystine in keratins by thiols is difficult to achieve because the reaction is determined by the law of mass action, dithiothreitol reduction readily splits 85% of the disulphide bonds in wool under mild conditions with only a small excess of dithiothreitol (14, 15) owing to the formation of a stable cyclic disulphide (4,5-dihydroxy-1,2-dithiane). After reduction of the disulphide bonds the proteins, previously resistant to attack by proteolytic enzymes, are rapidly digested under mild conditions. Borenfreund, Fitt and Berdich (17) have used combinations of trypsin and reducing agents including 2-mercaptoethanol and 2,3-dimercaptopropanol to degrade 'proteolytic enzyme resistant, keratin-like' components of sperm cells and similarly Pfau and McCrea (18) have used pronase and 2-mercaptoethanol to release DNA from vaccinia virus. Preliminary experiments by us have shown that a combination of pronase and dithiothreitol rapidly digests human hair cuticle (80% being dissolved in $1\frac{1}{2}$ h at 37°C pH 8.0 and over 91% within 16 h) indicating that pronase may prove to be a valuable alternative to the use of papain at 50°C.

The activation of papain is dependent upon the existence of cysteinyl residues at the prosthetic site (16) so that the presence of dithiothreitol will give maximum yield of the groups and thereby maximum proteolytic activity.

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Use of a laboratory model to evaluate the factors influencing the performance of depilatories

T. J. ELLIOT*

Presented in part on the 14th November 1973 in Nottingham, at the Symposium on 'Evaluation of Product Performance', organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—The use of laboratory MODELS to evaluate the performance of COSMETIC and TOILETRY products is full of pitfalls, and subjective assessments nearly always give more accurate results.

However, the investigation of the comparatively large number of variables which influence the speed of action of DEPILATORIES cannot conveniently be carried out on a subjective assessment basis and requires the use of a suitable laboratory model.

The particular model developed (referred to as a DEPILOMETER) was designed to simulate practical use conditions as closely as possible and it enabled the more important formulation variables to be studied quickly. In-use tests on final depilatory formulations gave good correlation with the DEPILOMETER findings.

INTRODUCTION

The main problem of testing depilatories with a users panel on a day-to-day basis is the practical difficulty of the necessary time required to re-grow the hair in between tests.

Another problem is the known variability of hair coarseness between individuals so that in extreme cases this can be a more significant factor in speed of depilation than changes in formulation.

There have been several attempts in the past to develop an *in vitro* test for measuring the effectiveness of depilatories and these have mostly been

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based on measuring the breaking time for mechanically stressed hair in contact with the depilatory preparations.

Yablonsky and Williams (1) criticized these methods and developed their own test based upon the measurement of time of maximum hair swelling of individual hairs suspended in the depilatory preparations.

The essential criteria for our own laboratory test model was that the the instrument should simulate as closely as possible the conditions under which the depilatory would normally be used and the important factors in this context were seen as follows: (a) use of leg hair and under-arm hair; (b) application of product for predetermined time without use of mechanical force; (c) use of moderate force after this time to produce breakage (simulating the use of a spatula).

PROCEDURE

The development of the instrument or 'depilometer' was handed over to Beecham Special Services laboratory, and I am indebted to Mr J. D. Cheshire and Mr H. Ashmore for developing this piece of equipment.

Mode of action

Ten similar hairs are tested together. After the application of the depilatory product to the hairs, a suitable time interval elapses before a mechanical force (load) is applied for 10 s at each successive minute until the hairs break. The procedure is automatically timed, requiring attention only to fit new hairs and apply the depilatory. The key observation made is the number of minutes taken to break the hairs.

Apparatus and operations

The instrument is in two parts—the load applicator and the control box and timer—joined by a cable.

(1) *Load applicator*. The mechanism consists of a load bar which falls transversely across the mounted hair sample.

The load bar is mounted on the plunger assembly which consists of a circular table for carrying ring weights, an axial support rod which slides freely in a sleeve and a guide rod to prevent rotation. The axial support normally rests on a platform which drops away when the solenoid is energized.
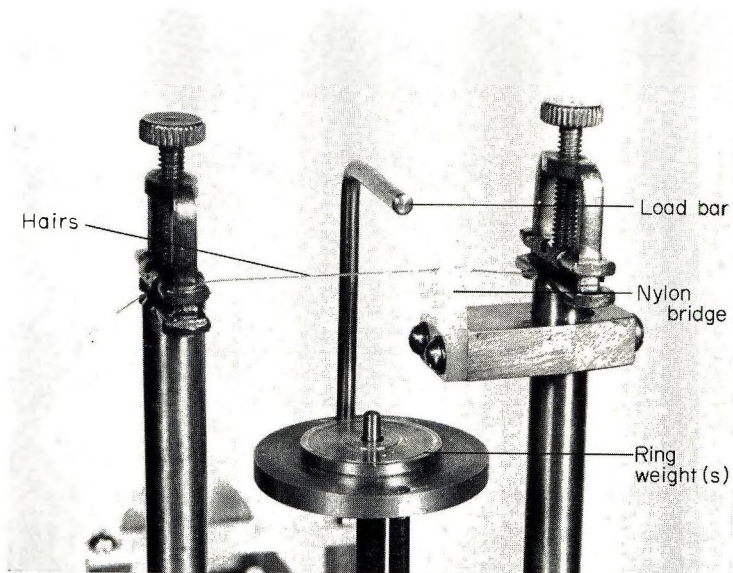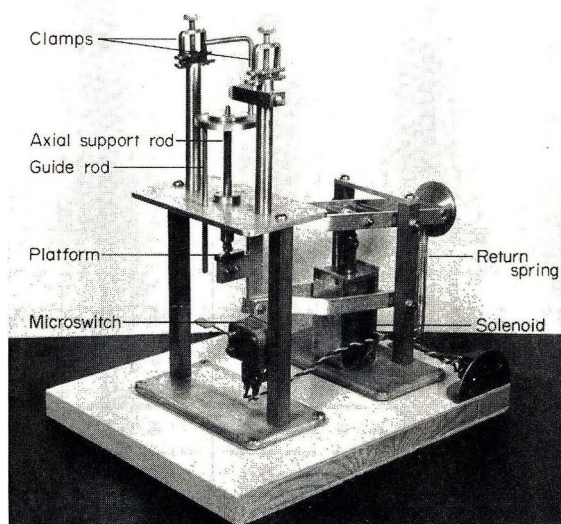
Figure 1. Hairs in position on load applicator.

Figure 2. Load applicator.
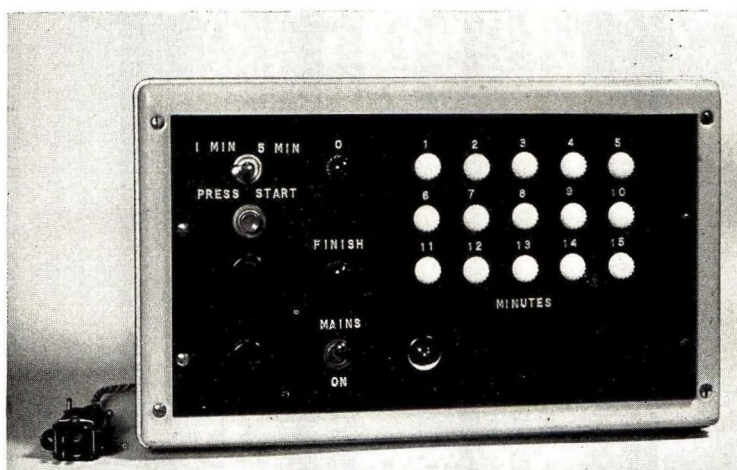
Figure 3. Control box and timer.

Hairs are laid with minimal tension between the two rubber-lined clamps, and the depilatory preparation is applied where the hair rests on the nylon bridge.

At the programmed time intervals after depilatory application, the solenoid is energized rapidly, removing the platform from the plunger assembly which drops onto the hair under its own weight and rests there for the load duration. When the solenoid is de-energized, the return spring lifts the load off the hair. When the hair breaks under load, the guide rod actuates the micro-switch and arrests the timing indicator.

(2) *Control box and timer*. Immediately after application of the depilatory preparation, a START button is depressed to zero the timer and start the test sequence. A cam driven by a synchronous motor causes a stepping relay to move forward at regular intervals, indicating the time elapsed and applying a short duration loading to the hair via the solenoid.

An initial delay to the application of hair loading may be introduced if required.

Test conditions

Simple alterations to the timing mechanism enable the intervals between and duration of hair loading to be changed. Similarly, alterations in the load applicator can be made to enable different loads to be applied. We have adopted the following conditions for all testing:

Number of hairs	10
Length of hairs between clamps	55 mm
Length of hair treated	3–5 mm
Width of nylon bridge	3 mm
Diameter of load bar	3 mm
Distance between load bar and nylon bridge	8 mm
Height of load bar above hair	13 mm
Total weight of plunger assembly	50 g
Time of first load application	1–5 min
Frequency of load application	1 per min
Duration of load application	5 s

Although we would have preferred to use axillary hair and leg hair for the tests, we found that this was not easily available in the quantities we required, so of necessity we had to use standard untreated European head hair.

However, we carried out tests to evaluate the differences in time of depilation between samples of head, leg and axillary hair, using a controlled depilatory application.

Men (10 subjects)

Depilation time (Meter)	Leg hair	Axillary hair	Head hair
Range of times	6–10	7–12	10–15
Average time	7	9.5	12.5

Women (10 subjects)

Depilation time (Meter)	Leg hair	Axillary hair	Head hair
Range of times	4–7	6–13	8–15
Average time	6	10	10

These results show that, although there was considerable variation in depilation times between individuals, in general leg hair is easier to depilate than axillary hair (which is confirmed by practical experience); and that head hair is similar to axillary hair in depilation time.

Formulation factors

The factors which we were interested in evaluating with regard to their effect on speed of depilation were as follows.

Nature of thio-compound.
Choice of neutralizing base.
Concentration of thio-compound.
Effect of pH.

The thio-compounds selected were as follows: (a) thioglycerol, (b) thioglycollic acid, (c) thiomalic acid, (d) 2-mercapto propionic acid, (e) 3-mercapto propionic acid, (f) thiodiglycol, (g) 2-mercapto ethanol.

The neutralizing bases were as follows: (a) lithium hydroxide, (b) sodium hydroxide, (c) potassium hydroxide, (d) calcium hydroxide, (e) strontium hydroxide, (f) barium hydroxide.

In order to measure the speed of depilation of the various combinations of thio-compounds and neutralizing bases, a basic cream depilatory formula was used:

Cetyl alcohol	6.00 % w/w
Lanbritol Wax	10.00 % w/w
Thio-compound	5.00 % w/w
Neutralizing base	q.s.
Water to	100

In most cases this formula gave a satisfactory cream, but even where separation of the product took place, for the purposes of the test it was not considered that this had any significance.

All determinations were carried out in duplicate at room temperature at 25°C. The depilation times shown below are the mean of two readings.

RESULTS

Type of thio compound and neutralizing base
Thioglycerol.

Neutralizing base	% w/w of commercial material	pH	Depilation time (min)
Lithium hydroxide	2.0	11.6	6.0
Sodium hydroxide	1.9	11.2	4.5
Potassium hydroxide	2.6	11.5	6.5
Calcium hydroxide	1.7	11.9	6.0
Strontium hydroxide	6.2	11.0	8.0
Barium hydroxide	7.3	11.0	8.0

Thioglycollic acid.

Neutralizing base	% w/w of commercial material	pH	Depilation time (min)
Lithium hydroxide	4.6	11.8	5.0
Sodium hydroxide	4.5	11.4	5.5
Potassium hydroxide	6.1	11.7	7.0
Calcium hydroxide	4.0	12.1	7.0
Strontium hydroxide	14.5	12.0	7.5
Barium hydroxide	17.2	12.0	13.0

Thiomalic acid.

Neutralizing base	% w/w of commercial material	pH	Depilation time (min)
Lithium hydroxide	4.2	11.6	> 15.0
Barium hydroxide	15.8	12.1	> 15.0

2-mercaptopropionic acid.

Neutralizing base	% w/w of commercial material	pH	Depilation time (min)
Lithium hydroxide	4.0	11.8	8.0
Sodium hydroxide	3.9	11.3	7.5
Potassium hydroxide	5.3	11.4	14.0
Calcium hydroxide	3.5	12.2	13.5
Strontium hydroxide	12.5	11.0	13.0
Barium hydroxide	14.9	11.2	10.0

3-mercaptopropionic acid.

Neutralizing base	% w/w of commercial material	pH	Depilation time (min)
Lithium hydroxide	4.0	11.5	6.0
Sodium hydroxide	3.9	11.9	7.0
Potassium hydroxide	5.3	11.5	8.0
Calcium hydroxide	3.5	11.3	14.0
Strontium hydroxide	12.5	12.7	6.5
Barium hydroxide	14.9	12.7	6.5

Thiodiglycol.

Neutralizing base	% w/w of commercial material	pH	Depilation time (min)
Lithium hydroxide	3·1	11.5	> 15.0
Sodium hydroxide	2.9	11.0	> 15.0
Potassium hydroxide	3.6	12.2	12.5
Calcium hydroxide	2.7	12.2	> 15.0
Strontium hydroxide	9.7	12.6	> 15.0
Barium hydroxide	11.5	12.2	> 15.5

2-mercapto ethanol.

Neutralizing base	% w/w of commercial material	pH	Depilation time (min)
Lithium hydroxide	2.7	11.6	3.5
Sodium hydroxide	2.6	11.2	4.0
Potassium hydroxide	3.2	11.1	4.0
Calcium hydroxide	2.4	11.8	5.0
Strontium hydroxide	6.4	11.9	6.0
Barium hydroxide	10.2	11.6	5.0

[Note: No attempt was made to bring these products to a constant pH but
only to approximately the right range of pH.]

Conclusions. Of the two factors investigated, the most significant
one influencing the speed of depilation is the choice of thio compound. In
order of effectiveness based on depilation times averaged for all the neutra-
lizing bases the results show the following.

Thio-compound	Average depilation time (min)
2-mercapto ethanol	4.0
Thioglycerol	6.5
Thioglycollic acid	7.5
3-mercapto propionic acid	8.0
2-mercapto propionic acid	11.0
Thiodiglycol	15.0
Thiomalic acid	15.0

The influence of neutralizing base is not so significant as the choice
of thio compound, but there is a relationship between speed of depilation
and choice of base as follows.

Base	Average depilation time (min)
Sodium	5.7
Lithium	5.7
Potassium	6.5
Barium	6.5
Calcium	7.1
Strontium	8.3

In general monovalent bases would seem to give faster acting depilation
than di-valent bases.

Based upon these experiments, and on considerations of odour, it was
decided to eliminate at this stage certain thio compounds as follows.

Thiomalic acid—slow acting, very bad odour.

Thiodiglycol—slow acting, poor odour.

2-mercapto propionic acid—slow acting, poor odour.

3-mercapto propionic acid—moderate speed action, good odour.

The choice for retention of thioglycerol and thioglycollic acid was obvious as they were both fast-acting and with reasonable odour 2-mercapto ethanol was also retained because it gave the fastest acting depilation, although it has a very poor odour.

From the monovalent bases, sodium hydroxide was selected as the best overall; and from the divalent bases calcium hydroxide was selected.

Effect of pH

Using the basic depilatory cream formula previously described, depilatory creams were made up containing 5% w/w of each of the following thio compounds: (a) 2-mercapto ethanol; (b) thioglycerol; (c) thioglycollic acid. Each cream was neutralized to a range of pH values with (a) sodium hydroxide, and (b) calcium hydroxide; the depilation times were determined for each formulation.

2-mercapto ethanol.

Neutralizing base	pH	Depilation time (min)
Sodium hydroxide	9	14.0
Sodium hydroxide	10	5.0
Sodium hydroxide	11	< 3.0
Calcium hydroxide	9	8.0
Calcium hydroxide	10	3.0
Calcium hydroxide	11	3.0
Calcium hydroxide	12	6.0

Thioglycerol.

Neutralizing base	pH	Depilation time (min)
Sodium hydroxide	9	> 15.0
Sodium hydroxide	10	7.0
Sodium hydroxide	11	5.0
Calcium hydroxide	9	10.0
Calcium hydroxide	10	10.0
Calcium hydroxide	11	8.0
Calcium hydroxide	12	6.0

Thioglycollic acid.

Neutralizing base	pH	Depilation time (min)
Sodium hydroxide	9	>15.0
Sodium hydroxide	10	12.0
Sodium hydroxide	11	5.0
Calcium hydroxide	9	>15.0
Calcium hydroxide	10	15.0
Calcium hydroxide	11	11.0
Calcium hydroxide	12	7.0

Conclusions. Depilation time is decreased as pH is increased as would be expected. In practical terms a pH of 9 would be too low to give a reasonably fast-acting depilatory.

In the range of pH of 10–12 the choice of thio compound and neutralizing base can have more effect on speed of depilation than the pH.

Effect of concentration of thio compound

Using the basic depilatory cream formula previously described, depilatory creams were made up containing (a) 2.5% w/w, (b) 5.0% w/w and (c) 7.5% w/w of each of the following thio compounds: (a) 2-mercapto ethanol; (b) thioglycerol; (c) thioglycollic acid. Each cream was neutralized to a pH of 11 with (a) sodium hydroxide and (b) calcium hydroxide, and the depilation times were determined for each formulation.

2-mercapto-ethanol.

Neutralizing base	% w/w of thio compound	Depilation time (min)
Sodium hydroxide	2.5	8.0
Sodium hydroxide	5.0	<3.0
Sodium hydroxide	7.5	<3.0
Calcium hydroxide	2.5	8.0
Calcium hydroxide	5.0	3.0
Calcium hydroxide	7.5	4.0

Thioglycerol.

Neutralizing base	% w/w of thio compound	Depilation time (min)
Sodium hydroxide	2.5	14.0
Sodium hydroxide	5.0	5.0
Sodium hydroxide	7.5	5.0
Calcium hydroxide	2.5	10.0
Calcium hydroxide	5.0	8.0
Calcium hydroxide	7.5	6.0

Thioglycollic acid.

Neutralizing base	% w/w of thio compound	Depilation time (min)
Sodium hydroxide	2.5	10.0
Sodium hydroxide	5.0	5.0
Sodium hydroxide	7.5	6.0
Calcium hydroxide	2.5	12.0
Calcium hydroxide	5.0	11.0
Calcium hydroxide	7.5	15.0

Conclusions. Whilst an increase in concentration from 2.5% to 5.0% with all three thio compounds gives a decrease in depilation times, a further increase to 7.5% does not have any further effect (probably due to solubility limits having been reached).

An optimum concentration of thio compound of approximately 5% would seem to be indicated for practical purposes.

DISCUSSION

This series of results enabled us to formulate a range of practical depilatories which could then be compared with competitive products to give a measure of relative performance.

Although speed of depilation is probably the most important factor in the development of a commercial depilatory, other characteristics (particularly potential irritation) have to be considered.

When skin irritation studies were carried out using the most rapid acting depilatories, it was found in general that speed of action and depilation were inversely proportional.

However, the factors which were most significant for speed of depilation did not necessarily have the same order of importance for irritation. Using the results obtained from the Depilometer studies together with the results of irritation studies a number of practical depilatory creams were formulated which represented different degrees of compromise between speed of action and irritation.

User tests with these depilatory creams then enabled the best overall compromise to be selected.

(Received: 6th September 1973)

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SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN

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Society of Cosmetic Chemists
of Great Britain
1974 Medal Lecture

Professor J. Ebling was the recipient of the 1974 Medal of the Society of Cosmetic Chemists of Great Britain. The Society's Medal was presented to Professor Ebling at a meeting held at the Royal Society of Arts on Thursday, 7th March 1974. In making the presentation, the President of the Society, Mr G. A. C. Pitt, expressed the great pleasure it gave the Society to honour one of its own members for the first time, in this way. Professor Ebling, who holds the chair of Zoology in the University of Sheffield, was a regular contributor to the scientific activities of the Society as well as being a major contributor to the science of dermatology. His lecture, 'Sex Hormones and the Skin', was then delivered to the meeting, and Miss Anne Young, the Vice-President of the Society, proposed a vote of thanks.

Professor F. J. G. Ebling (left) receiving the Silver Medal
from the Society's President, Mr G. A. C. Pitt (right)

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SYMPOSIUM

COSMETIC SCIENCE AND

HUMAN SENSES

Papers are invited for a Symposium dealing with Cosmetic Science and the Human Senses which is due to be held on

7 – 9 April, 1975.

The venue has not yet been decided but will most likely be in the North of England.

Please send titles and synopsis of papers to:-

Mr W. W. F. Scotland
c/o Society of Cosmetic Chemists of Great Britain,
56 Kingsway, London WC2, England.

Sex hormones and skin

F. J. EBLING*

The 1974 Medal Lecture by Professor F. J. Ebling, Department of Zoology, University of Sheffield, Sheffield S10 2TN, delivered before the Society of Cosmetic Chemists of Great Britain on the 7th March 1974 with G. A. C. Pitt Esq., President of the Society in the Chair.

Synopsis—As well as its more obvious ANATOMICAL and PHYSIOLOGICAL functions the SKIN plays an important part in social communication by vision, touch and smell. The epidermal surface, the activity of the glands and the distribution of the HAIR are particular features of skin which are concerned with sexual communication, and it is thus not surprising that they are profoundly influenced by HORMONES. STEROIDS have been widely used in efforts to improve skin texture; the effect of OESTROGENS is equivocal, but ANDROGENS certainly stimulate epidermal cell division. The SEBACEOUS GLANDS are unequivocally stimulated by androgens and inhibited both by oestrogens and anti-androgenic steroids, though their modes of action are not identical. PITUITARY factors appear to be necessary, at least in the rat, for the response of the sebaceous glands to TESTOSTERONE, and it seems possible they may act upon the conversion of the steroid to its active metabolites. Human body, AXILLARY and pubic hair is similarly androgen dependent. So sebum secretion, the growth of sexual hair, and hirsutism may be inter-related by their link with steroid metabolism within the skin. Is it possible that one function, or at least one by-product, of cutaneous androgen metabolism is the manufacture of pheromones or odours?

INTRODUCTION

When the human skin is viewed against its evolutionary inheritance, certain features are clear. We do not question its functions as a sensory structure or as a defensive barrier against both physical injury and chemical assault. Its role in preventing loss of precious water has been part of vertebrate history ever since some distant amphibious ancestor took the first steps to emerge from a primaeval swamp.

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The evolution of warm blood-bloodedness in the mammals was accompanied by the development of an insulating material known as hair. To adjust the pelt at intervals to seasonal changes in temperature, hair follicles undergo cycles of activity which appear to be linked to environmental changes through the endocrine system; even the human hair may retain a remnant of this moult cycle. But men were enabled to leave the forests and fan out through new fields by the development of superb mechanisms for keeping cool. Hair was lost, its insulating qualities being to some extent replaced by fat; the vascularity of skin became greatly increased, though heat loss could be reduced by shunting the blood through the deeper layers; and eccrine glands which could be brought into play for cooling developed over virtually the whole surface of the body.

The first men were almost certainly dark skinned; as the body hair became reduced, melanin pigmentation provided a protection against the damaging effects of ultraviolet radiation. But as man moved into areas where even the summer sun moved low in the sky the threat of radiation damage was replaced by another, namely the failure to synthesize vitamin D. So natural selection switched to favour pale skin. The evolutionary legacy is still reflected by the fact that Europeans tend to develop skin cancer in the tropics and West Indian immigrants to get rickets in Great Britain.

Our thinking about skin tends to be dominated by these evolutionary facts which concern our biological survival as individuals. But there is another level at which natural selection has operated, namely that of the social group. The survival of the group depends upon its coherence by communication, between the sexes, as Darwin himself realized, in the family, and throughout the troop. Although overshadowed by the sophisticated use of language, and sometimes suppressed because of its embarrassing inevitability, the role of skin remains important.

Much of the behaviour of our vertebrate ancestors was innate, that is to say it was programmed by heredity; patterns of courtship or aggression in one individual were automatically set in motion by so-called releasing mechanisms—physical structures, colours, movements, scents or sounds—in another. While mammalian and particularly human social interactions have become increasingly dependent on learned and thus much more rapidly modifiable patterns, the releasing features are still present, and more than a remnant of the innate responses still remains.

The buttocks provide a good fundamental example of a visual releaser. When a female ape is sexually receptive, they become red and swollen and she displays them to attract the male. In man they become permanently

inflated, especially in the female, to reach their greatest natural development in the steatopygia of the African Bushwoman, and their ultimate cultural exaggeration in the bustle. Desmond Morris has made the further interesting suggestion that since all the remaining sexual signals are in front of the body, the breasts have developed as a buttock mimic, thus making the human female equally attractive from either aspect.

I could discuss many more examples of structural releasers of sexual interest, such as lips, cheeks, eyes and hair, and it is hardly necessary for me to point out that all these are the targets of cosmetic chemistry in its efforts to improve on nature. I want, however, to confine myself to three features of the skin which are of sexual significance and which underlie the understanding of its hormonal connections, namely texture, smell and hair distribution.

At the beginning of this century, a work on pathology described skin as 'a tissue which is silk to the touch, the most exquisitely beautiful surface in the universe to the eye . . . more beautiful than velvet, softer and more pliable than silk, more impervious than rubber . . . '. The sensation of touch is one of the earliest of all pleasurable experiences and it becomes a powerful sexual stimulus; in the words of Havelock Ellis (1) 'Touch sensations constitute a vast gamut for the expression of affection, with at one end the note of minimum personal affection in the brief and limited touch involved by the conventional hand-shake and the conventional kiss, and at the other end the final and intimate contact in which passion finds the supreme satisfaction of its most profound desire . . . . Even the hand-shake of a sympathetic man is enough in some chaste and sensitive women to produce sexual excitement or sometimes even the orgasm'. (He was, I think, writing of an age when women's sexual feelings were severely repressed.) The erotic appeal of skin is visual as well as tactile. It looks and feels best when it is unwrinkled, smooth or even wet—facts exploited by photographers as well as by cosmeticians—and for some persons it seems that leather or even rubber works even better than the real thing.

Odour is a characteristic of the human body. In addition to the breath, Havelock Ellis (1) distinguished between the odours of the general skin, the hair and scalp, the armpit, the perineum, the mons veneris and the prepuce, and pointed out that the scents were detectable even in healthy and well-washed persons under normal conditions. Some of these regions of the body, for example the axilla and the genital areas, contain aggregations of tubular apocrine glands opening into the hair follicles and there is little doubt that these are responsible for the odorous secretion. But the general body skin

and the scalp have only holocrine sebaceous glands and the eccrine sweat glands; one cannot dismiss the thought that the former, if not the latter also, may contribute to the smell as well as to the texture of the skin.

Both the sebaceous and apocrine glands remain small during infancy and become active only after puberty. Body odours change at puberty, and characteristic odours are said to be emitted during sexual excitement by both men and women. Havelock Ellis (1) records the case of a woman who emitted a rose odour for 2 days after coitus, and mentions that in the seventeenth century there was a monk in Prague who was prepared to diagnose the chastity of women by their smell. Whatever is true of man, there is increasing recognition of the role of odours, or pheromones, as sexual or social signals in other mammals (2–4). They function not only as sex attractants, but also in the marking of territory and the establishment of social hierarchy, and they are produced by a wide variety of specialized skin glands, either apocrine, sebaceous, or mixed, which can occur in almost every region of the body. The rabbit, for example, has three pairs of apocrine glands (5), the chin and anal which are used for marking territory and appear to have similar secretions, and the inguinal which is concerned with sex attraction and produces a different chemical material (6).

The chemical structure of a few animal scents is known and some, indeed, form the classic materials of human perfumery. Such are civetone, from the civet cat, and muscone, from the male musk deer, which are macrocyclic ketones (7). The musk rat produces dihydrocivetol, a saturated ring alcohol, and the black tailed deer a lactone (8).

Facial and body hair are other features of the skin which develop at puberty, though follicles growing only a fine down have been present since birth. Whether male facial hair is sexually directed towards the female as well as serving for aggressive display to other males, I am unsure. Presumably it is commonly shaved off in many cultures, not to reduce sex appeal, but as a contribution to social harmony. And in spite of the exploitation of full frontal pubic hair to arouse sexual interest in the theatre, and a recent study in which 50% of women admitted to being excited by male body hair, I suspect that the main evolutionary significance of the pubic and axillary hairs is to act as wicks for the dissemination of odour produced by the apocrine and, probably, the sebaceous glands. Whatever the function of facial, pubic, axillary and other body hair, it is clear that it is, whether in male or female, an adult character manifested only during the reign of male type sex hormones.

It is reasonable to expect, therefore, that at least three features of the

skin—texture, glandular activity and body hair—should be profoundly susceptible to the influence of sex hormones. Steroid hormones have been widely used in cosmetics to improve skin texture for such circumstantial reasons. Is this justified? The question needs, perhaps, to be put precisely: 'Are there any effects which can be objectively measured, and can their value be discerned in a double blind trial against a placebo?' If, as only too commonly, the investigator has been asked 'What evidence is there to justify the marketing of this product?' the answer, if not invariably useless, must be somewhat less respectable!

What are the qualities of 'skin texture'? Obviously, certain features can be loosely assessed by eye or touch, but do these reflect any anatomical changes which remain measurable after the skin has been assaulted by biopsy and histology? Are there differences in dermal constitution or in epidermal cell replacement, or are there more subtle changes in the keratinized surface or its lipid mantle?

Attempts to answer these questions have yielded confusing and equivocal results. In 1949, Eller and Eller (9) reported that topical application of oestrogenic ointments to the backs of senile female subjects locally increased the size of the epidermal cells, as well as accentuating the waviness of the basal layer, which in senile skin normally lacks rete-pegs. Their results might appear reasonably informative were it not for the facts that inunction of oestrogen free ointment by itself had a similar—if less marked—effect, that the ointments were applied under occlusion, and that the effective concentrations of oestrogen must have been at least 100 times greater than any amounts normally used in hormonal cosmetics.

The conclusions can, moreover, be contrasted with those reported in 1962 by Montagna, Formisano and Kligman (10). Three groups, each of 10 men and 10 women, aged 65 or more, were anointed daily for 6 weeks on one side of the face and on the back of one hand, with 1% testosterone propionate, 1% progesterone, or 0.5% ethynyl oestradiol, respectively. The opposite side of the face and the other hand were treated with ointment base alone. In untreated senile skin the Malpighian cells were shrivelled and vacuolated, with irregular nuclei, but testosterone or progesterone regularly restored the properties of younger skin, as well as giving it increased fullness and producing some alleviation of wrinkles. Only a few of those treated with oestrogen or the ointment base alone showed any amelioration. This effect of testosterone is not perhaps surprising, since there is general agreement that given systemically, it stimulates epidermal cell division in

experimental animals, in spite of the disputed contention that oestrogens do so (for review see 11).

Clinical trials of hormonal creams have produced equally inconclusive results. Behrman (12) put an oestrogen on one side of the face in some 30 women, using an oestrogen free cream on the other. Neither the observers nor the women themselves noticed any differences. Similarly, in a double blind trial carried out by the British Consumers' Association (13), a face cream containing oestrogen scored no better than one without hormone.

Steroids used in face creams have not been confined to oestrogens. It has, for example, been claimed that pregnenolone (14), a common precursor of progestogens, androgens and oestrogens, will produce a degree of oedema in senile skin, though it must be admitted that the cosmetic results are not dramatic (15).

When we turn to the skin glands, the experimental results are much easier to interpret. In experimental animals or in man, and whether measured histologically or by changes in sebum secretion, it is generally agreed that male hormones increase, and oestrogenic female hormones decrease the activity of the sebaceous glands (6, 16, 17). The effect of injecting testosterone propionate or oestradiol benzoate on the sebaceous glands of immature female rats was described by me in 1948 (18). Similarly, it can be shown in the rabbit that specialized glands of apocrine type are enlarged by testosterone and diminished by oestradiol (19).

Using a simple technique in which the sebum is collected from the forehead on pads of cigarette papers, Strauss and Pochi (20, 21) have clearly shown that sebaceous secretion is influenced by similar factors in man. They have established that secretion is very low until puberty, when it rises in both males and females, that oral treatment with methyl testosterone prior to puberty, though not afterwards, will greatly increase it, and that oral oestrogens will markedly suppress it in adult males.

We have studied sebum production in rats by measuring the changes in the level of hair fat (22). The animals are washed in detergent and warm water to reduce the base level, and dried with a hair dryer. A sample of hair is then clipped from one flank, and the level of fat determined gravimetrically after successive extraction with di-ethyl ether. After an interval of days, a sample from the other flank is similarly treated. That testosterone increases and oestradiol decreases secretion has been clearly demonstrated in castrated male and in spayed female rats.

The sebaceous glands are holocrine, that is to say that their secretion is formed by complete disintegration of the cells, which are replaced from

the periphery. Clearly there are two main components of secretion: the formation of new cells and the synthesis of sebum within them. It was therefore of great interest to discover whether steroid hormones act at one or both of these points. The rate of cell division was determined by mitotic counts in histological sections, the rats being injected with colchicine 5 h before they were killed, a procedure which arrests dividing cells in the metaphase. The somewhat unpredictable conclusion, confirmed a number of times, was that whereas testosterone does indeed greatly increase cell division, oestradiol has little or no effect on it, even though in low doses it markedly depresses secretion (23). By administering both steroids simultaneously, it is thus possible to produce a high rate of mitosis coupled with a low rate of secretion (24). It must, therefore, be concluded that oestradiol reduces secretion not by inhibiting cell replacement but by interfering with sebum synthesis within the cells themselves.

The opportunity for further testing of this hypothesis arose by the discovery of steroids which were anti-androgenic but not oestrogenic. It was of obvious interest to see whether such compounds would, in common with oestrogens, reduce sebum secretion in animals stimulated by testosterone. But, if the compounds are anti-androgenic as distinct from oestrogenic, they ought, unlike oestrogens, also to reduce cell division. We were able to show that this was true for several anti-androgenic steroids. In an experiment on cyproterone acetate (25), six litter-mate groups of six castrated male rates were used (*Fig. 1*). A dose of 2 mg per day of the anti-androgen significantly reduced both sebum secretion and sebaceous mitoses in rats treated with testosterone implants giving an uptake of 0.2 mg per day. A dose of oestradiol of the order of only 2 µg per day, however, caused a much greater reduction in sebum secretion, without significantly affecting mitosis. If the two suppressing steroids are acting at different points, their effects when administered together should be greater than either by itself. This is so. When 2 µg of oestradiol was added to 2 mg of cyproterone acetate, the result was a further reduction in sebum secretion. If the steroids had acted at the same point, it is inconceivable that any effect would have been produced by increasing the dose by one-thousandth. These results ought to dispose of the view that oestrogen competes with androgens in any 'anti-androgenic' sense. They are also evidence against the view that oestrogens reduce sebaceous secretion by suppressing endogenous androgen production, for that would inevitably result in reduced sebaceous mitosis.

Sebaceous secretion is also influenced by the pituitary (*Fig. 2*). Removal of the pituitary reduces sebum production in castrated rats and treatment
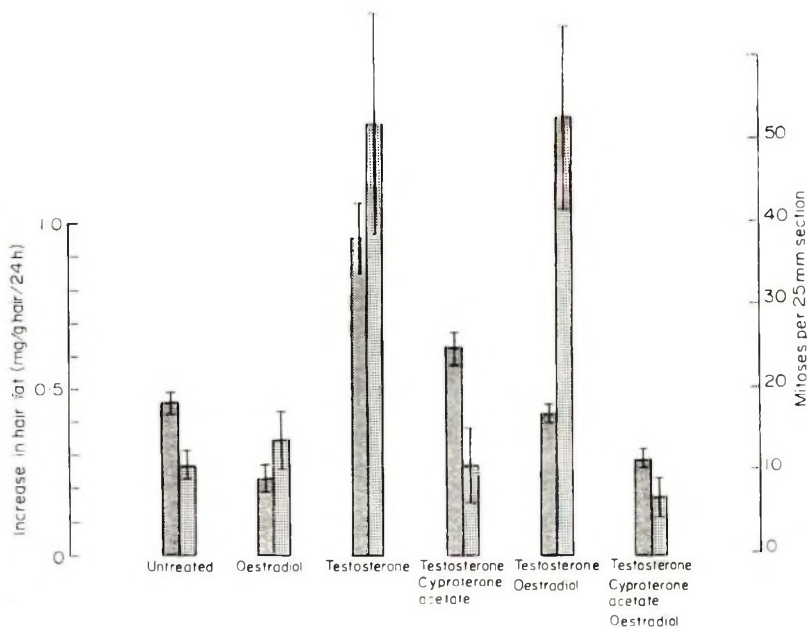
Figure 1. Independent and combined effects of oestradiol and cyproterone acetate in castrated rats treated with testosterone. Means $\pm$ SEM for six litters, each of six rats. The changes in hair fat were measured between 16 and 24 days after the start of treatment. Details of dosage are given in the text.
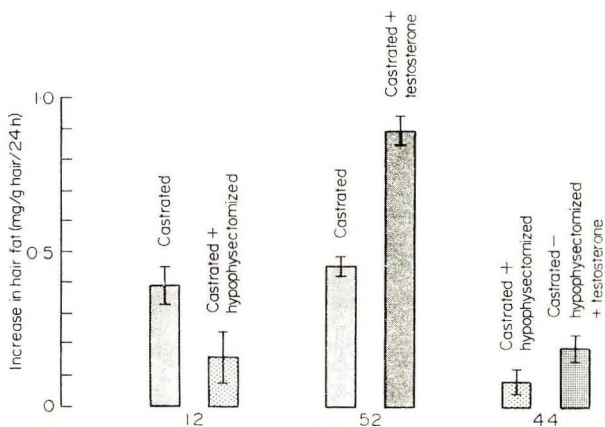
Figure 2. Effects on sebum secretion of hypophysectomy in castrated rats (left-hand group), and of testosterone in castrated rats (centre group) and in hypophysectomized-castrated rats (right-hand group). The numbers of litter-mate pairs used for the comparisons are shown below each set of histograms.

with testosterone then results in a much lower level of secretion than when the pituitary is present (26, 27). There is general agreement about this, but considerable dispute about how the facts should be explained (28). Shuster and Thody maintain that the increment actually due to testosterone is the same whether or not the pituitary is present, but that pituitary hormones have an independent effect on the skin, either directly or indirectly through other endocrine organs. They claim that MSH is the major, indeed the only, pituitary hormone acting directly on the skin (29), and they suggest that thyrotrophic hormone, acting through the thyroid gland, indirectly but independently affects the sebaceous glands (30). A full response to testosterone thus requires the additive effects of all these hormones.

I do not dispute that thyroid hormone or pituitary factors may have direct and independent effects on sebaceous secretion. The disagreement arises because I believe that irrespective of any such effects, the pituitary may actually potentiate the response to testosterone, producing a synergistic effect over and above any purely additive one. Moreover, I do not believe MSH has been established as the sole pituitary factor; growth hormone and prolactin are still candidates.

That pituitary factors play such a permissive role in the response to testosterone was indicated in experiments, performed about 20 years ago by Rothman and his co-workers (31) and by myself (32), using gland size as the criterion. The conclusion is amply borne out by hair fat measurements. During the last 7 years we have measured the effect of testosterone on sebum secretion in 52 castrated rats using matched litter mates as untreated controls, and in 44 hypophysectomized castrated rats similarly matched (*Figs 2 and 3*). The mean increment due to testosterone in the hypophysectomized animals was only 22% of that in those with intact pituitaries (27). Moreover, in somewhat smaller groups of rats, we have been able to restore the full response with preparations of growth hormone (*Fig. 3*) or prolactin (26).

The concept of a permissive role in the response to androgens for one or more pituitary factors may be important. Following the evidence that testosterone is metabolized in its target tissues to 5α-dihydrotestosterone, we tested the effect of this steroid on sebaceous secretion. In castrated rats the response was similar to that of testosterone, but in hypophysectomized rats it was relatively greater (33). We were similarly able to demonstrate significant responses to androstenedione (33) and to 5α-androstane-3β, 17β-diol (34), though a number of other metabolites, including 5α-androstanedione and androsterone, gave insignificant responses (*Fig. 3*). These
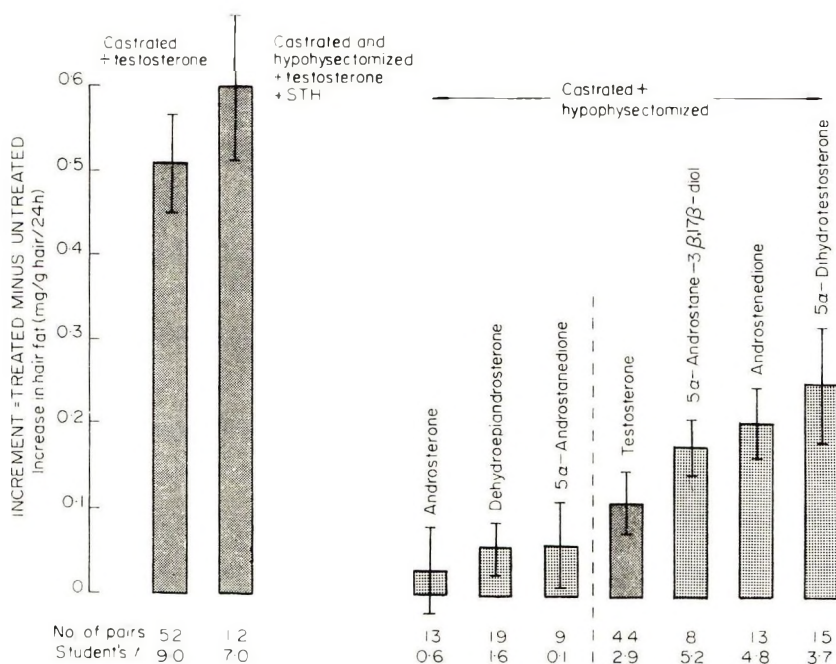
Figure 3.   Increments in sebum production produced in rats by doses of 0.2 mg/ 24 h of steroid, calculated by subtracting the increase in hair fat (expressed as mg/g hair/24 h) in a litter mate control from the increase in each treated animal STH = bovine growth hormone (Squibb).

results suggest that the response to testosterone may be dependent on its conversion to 5α-dihydrotestosterone and perhaps to the 5α-androstane-diols (*Fig. 4*) and that the conversion may, at least in the skin, be pituitary dependent. This hypothesis would be very tidy, were it not for the fact that androstenedione, also, appears fairly potent without further conversion.

The next stage in such an investigation is clearly to compare the metabolism of testosterone in the presence and absence of the pituitary. So we have attempted to identify the principal metabolites present in the skin and other target organs 1 h after injection of testosterone-4-^{14}C. In castrated rats, only about 5% of the total activity in the skin samples remained present as unchanged testosterone; about 35% was recovered as 5α-dihydrotestosterone and about 5% as androstenedione. In contrast, in two experiments each using pooled material from 25–30 hypophysectomized-castrated rats, 70 and 40% of the radioactivity, respectively, was recovered in unchanged testosterone. The evidence is consistent with the view that the
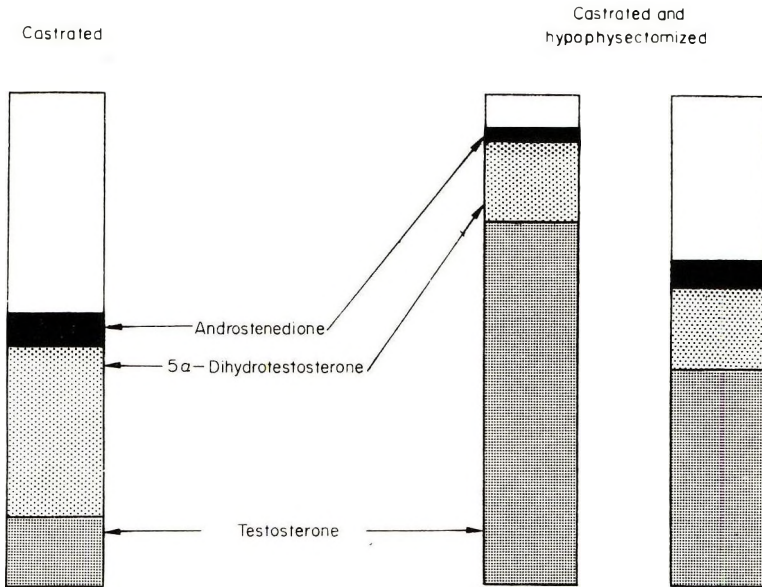
Figure 4. Recovery of identified metabolites, expressed as percentage of total recovered radioactivity in skin samples, 1 h after injection of testosterone-4-^{14}C into male rats. Each histogram represents results from a pool of 25–30 rats.

response of the skin to androgens involves their conversion to active metabolites which is pituitary mediated, though more results are needed to substantiate the hypothesis.

Finally, we come to body hair. The problem is usually not how to stimulate hair growth, but how to suppress it in women in which it occurs in culturally unacceptable amounts. While there is no doubt that the facial, axillary, pubic and body hair is all androgen dependent, growth of facial and body hair is often abnormal in women who show no apparent endocrine disturbance. Is it possible that this 'idiopathic' hirsutism is also related to peripheral androgen conversion? There is evidence that genital skin from both men and women is better able than general body skin to convert testosterone to 5α-dihydrotestosterone, and this is true for the foetus as well as the adult (35). But an even more interesting clue comes from a recent report that hirsute women excrete five times as much 5α-androstanediol as normal subjects (36). Does the growth of sexual hair perhaps depend on the conversion at site of endogenous androgens not only 5α-dihydrotestosterone but to the 5α-androstanediols?

I have travelled a fair distance in my discourse, and the time has come
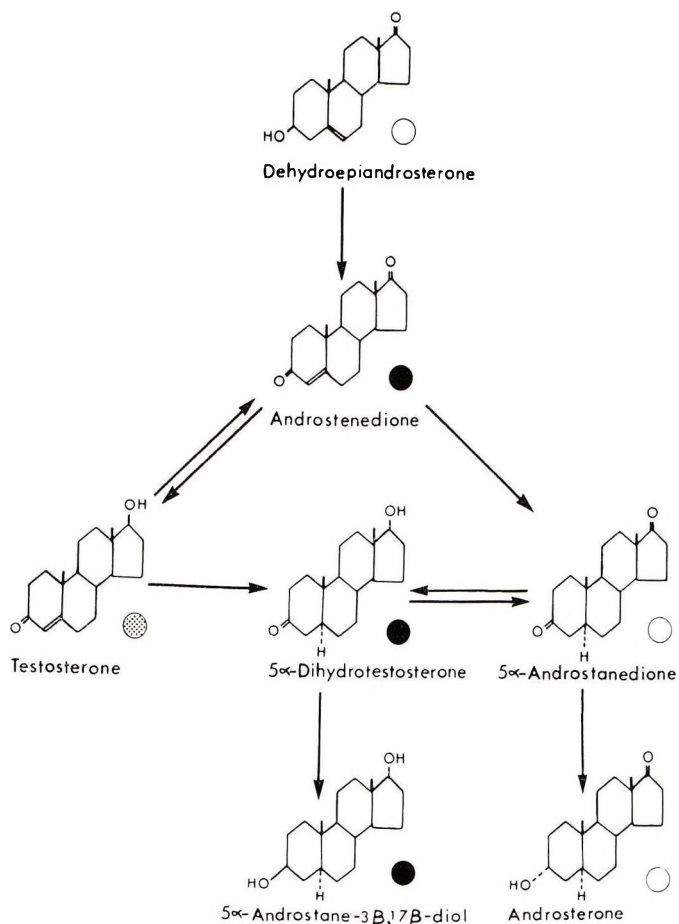
Figure 5. Possible pathways of androgen metabolism in rat skin. Solid circles indicate compounds which have been clearly shown to have significant effects on sebum production in hypophysectomized-castrated rats; open circles indicate compounds which have failed to do so; the stippled circle indicates a small effect significantly demonstrated only by the use of a large number of rats.

to summarize some of the details of the journey. I have drawn attention to those qualities of the skin, namely texture, appearance, feel and smell, which are concerned with sexual communication and discussed the underlying mechanisms especially in relation to the epidermis, the sebaceous and apocrine glands, and the body hair. In man, no less than in other animals, these structures change at puberty and are all profoundly influenced by both male and female hormones. Sebum secretion, the growth of sexual

hair and the problem of hirsutism may be interrelated by common involvement in the transformation of steroids in the skin, which is possibly, at least in relation to the sebaceous glands, mediated by pituitary hormones. Perhaps the epidermis, also, may be affected by steroids, and the application of non-active precursors was not a foolish idea, even if pregnenolone did not produce a striking effect. To add one more facet to the problem, is it possible that one function, or perhaps by-product, of cutaneous androgen metabolism could be the manufacture of pheromones? When we injected testosterone-4-^{14}C into rabbits, we were able to recover the label from the odoriferous part of the inguinal gland secretion (6). We do not know the chemical nature of the rabbit scent. But not all musk-like scents are macrocyclic ketones or lactones, some are 16-unsaturated steroids; for example, the compound 5α-androst-16-en-3β-ol has been identified in human urine as well as being responsible for the characteristic smell of the boar (37). Such a compound could, in theory, be formed from an endogenous androgen.

My story has included a modicum of firm evidence, a deal of speculation, and even a whiff of pure innuendo, for which I should need to ask no forgiveness here. Perhaps it was just a convenient way of linking the discovery, over 25 years ago, that rat sebaceous glands were influenced by hormones with our current study of steroid transformations in the skin, taking in the work on anti-androgens and the pituitary on the way. Or perhaps, at least, in hindsight, and admitting the gaps in logic, there is a connecting thread. In either case, Mr Chairman, I present it to you as the 1974 Medal Lecture. And may I add that I have been not only honoured, but more than a little overcome by your invitation to me to give it. I have been a member of the Society for 10 years, and although I cannot be described as a Cosmetic Chemist I have always felt, and feel now, that I am among friends.

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it may seem like a drop in the ocean

but it's the bitter truth