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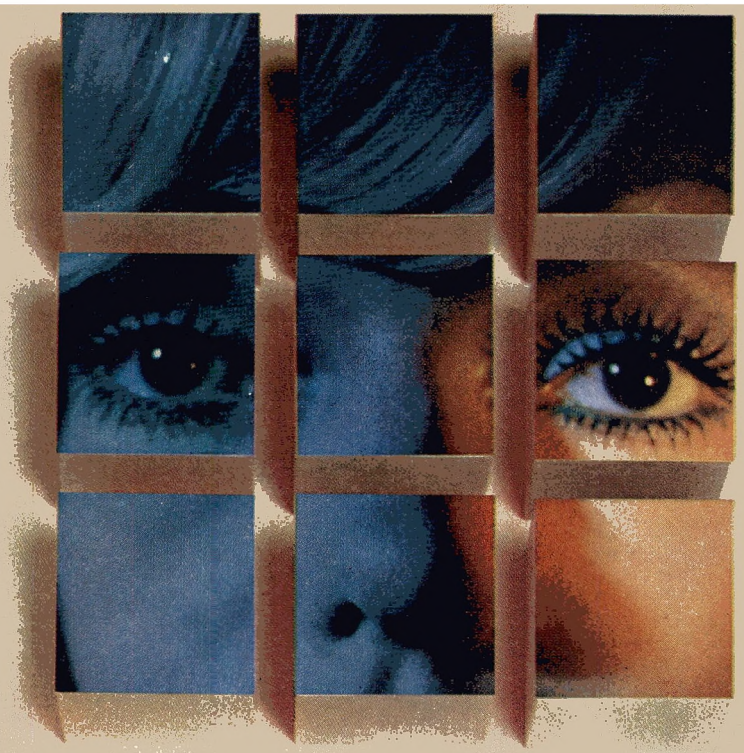
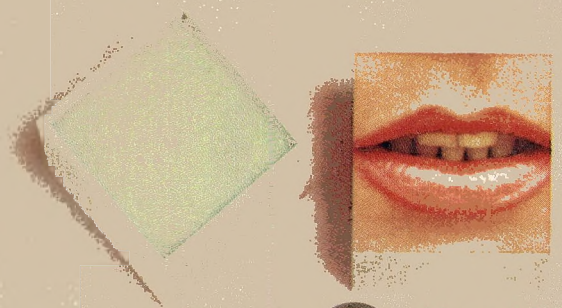
Contents





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Psycho-rheology—the relevance of rheology to consumer acceptance: JEAN V. BOYO. *Journal of the Society of Cosmetic Chemists* **27** 247–256 (1976)

Synopsis—The rheological properties commonly found in cosmetic materials are discussed. Since they are, in general, shear sensitive viscoelastic materials, the importance of using test conditions (temperature, shear rate, etc) relevant to the usage situation is discussed. In order to quantify the desirable physical properties in a product, the results of sensory and rheological tests have to be correlated. Types of panel test frequently used are briefly discussed. A method for pictorially representing texture is presented. The use of this texturegram to illustrate a range of rheological properties is demonstrated.

Sex attractants in primates: E. BARRINGTON KEVERNE. *Journal of the Society of Cosmetic Chemists* **27** 257–269 (1976)

Synopsis—The importance of olfactory communication in sexual attraction among primates is clear from our previous studies, which led to the term 'pheromone' being used to describe these olfactorily acting attractants. It is perhaps debatable whether such a term, originally defined to explain insect behaviour, can now be applied to the complex behaviour of a higher primate or man, where responses are not stereotyped. Such olfactory stimulants in these primates can produce mounting, masturbation and ejaculation, increase grooming behaviour and reduce aggression. Moreover, the ingredients in the composition of the stimulant may be added to, and produce improvements in the responsiveness of certain males. Since both the nature of the stimulants and the type of response produced can be varied, this clearly illustrates the complexity of defining an olfactory response in these higher primates.

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

ORIGINAL SCIENTIFIC PAPERS

Eye irritation tests. An assesment of the maximum delay time for remedial irrigation
R. E. Davies, B.Sc., M.I. Biol., S. R. Kynoch, B.Sc. and M. P. Liggett

SUBJECT REVIEW ARTICLES

Sex differences in odour perception
E. P. Koster, Ph.D.

Evaluation of flavours in dental creams: E. BAINES. *Journal of the Society of Cosmetic Chemists* **27** 271–287 (1976)

Synopsis—One of the most important features of a dental cream is its flavour. Consumer tests show the close relationship of overall preference with flavour attributes.

Evaluation methods can be of different types depending on which aspect of flavour is being examined. Any convenient panel can be used to determine whether two flavours are noticeably different.

Detailed flavour descriptions can be obtained using flavour profile panels. In the classic form such panels require screening and training of their members so as to render them expert.

These restrictions can be considerably relaxed, however, if the delphi technique is used to elicit an accurate consensus of opinion from the panel. Flavour descriptions can also be obtained by use of the panels of 20–30 members awarding ratings over a limited number of flavour questions. Results of such panels can be subjected to more elaborate methods of statistical analysis, such as analysis of variance and regression analysis, provided that the flavours tested conform to a carefully planned experimental design.

Panels intended to test the acceptability of flavours must be large and as representative as possible of the toothpaste using population.

The chemistry of human hair cuticle—III: The isolation and amino acid analysis of various subfractions of the cuticle obtained by pronase and trypsin digestion: J. A. SWIFT and B. BEWS. *Journal of the Society of Cosmetic Chemists* **27** 289–300 (1976)

Synopsis—The course of digestion of human hair cuticle by pronase and trypsin has been followed both gravimetrically and by the electron microscope examination of digested hair sections. This has permitted the identification of three subfractions of the hair endocuticle. The significance of the amino acid analyses is discussed.

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J. Soc. Cosmet. Chem. **27**, 247–256 (1976)

Psycho-rheology — the relevance of rheology to consumer acceptance

JEAN V. BOYD*

Presented at the Symposium on 'A Sensory Approach to Cosmetic Science' organized by the Society of Cosmetic Chemists of Great Britain at Manchester on 7–9th April 1975

Synopsis—The RHEOLOGICAL properties commonly found in COSMETIC materials are discussed. Since they are in general, SHEAR sensitive VISCOELASTIC materials, the importance of using test conditions (temperature, shear rate, etc) relevant to the usage situation is discussed. In order to quantify the desirable physical properties in a product, the results of SENSORY and rheological tests have to be correlated. Types of PANEL test frequently used are briefly discussed. A method for pictorially representing TEXTURE is presented. The use of this TEXTUREGRAM to illustrate a range of rheological properties is demonstrated.

Introduction

Cosmetics have been used by men and women the world over since earliest times. Early cosmetics consisted largely of the natural oils, sesame olive and almond, rendered fragrant with extracts of herbs and flowers. The earliest records on the use of cosmetics come from Ancient Egypt, where kings were buried surrounded by the luxuries of life. The tomb of Tutankhamun, who ruled in around 1350 BC, contained 50 alabaster vases, many of them exquisitely carved; they would have contained 400 l. of fragrant oils, had it not been stolen by thieves.

In 1770 a bill was introduced into the British Parliament (1) saying that 'All women . . . that shall impose upon, seduce, and betray into matrimony

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any of his Majesty's subjects by means of the scents, paints, cosmetic washes, artificial teeth, false hair, Spanish wool, iron stays, hoops, high-heeled shoes or bolstered hips, shall incur the penalty of the law in force against witchcraft and like misdemeanours, and that marriage, upon conviction, shall stand null and void'. A similar Act was passed in America in 1770. The author is unaware whether the Act has ever been repealed!

We can conclude that a great deal of time and expertise have been devoted to the art and science of cosmetic formulation for several hundred years. In this century many new and improved raw materials have been introduced, while many, some of them highly toxic, have disappeared. The very size of the business has rendered it imperative that it be put on a much more scientific basis than that on which it had existed for so long.

Cosmetics today consist of oils, gels, emulsions, suspensions, or a combination of the last three. Physical forms range through solids, pastes, creams, gels, lotions and liquids. They will usually contain thickening and/or stabilizing agents. Hence they show complicated rheological properties.

It is the function of the cosmetic rheologist to quantify the physical properties so that a set of numbers are available which are related to what the consumer will see and feel when he or she buys and uses the product. To be useful the numbers must relate to the conditions of use.

Types of rheological test

The physical conditions which are important in a description of a product are the stress likely to be applied, the rate at which it will be applied, and the temperature during use or storage. Every product will have a unique set of numbers describing its physical properties, and each type of product a typical set.

Shear stress–shear rate data

Practically all cosmetics have one property in common; they are shear sensitive, i.e. they break down when disturbed. This breakdown is characterized by the $\hat{\imath}$–$\dot{\gamma}$ (shear stress–shear rate) diagram. Some typical curves are shown in *Fig. 1*. *Fig. 1*(*a*) describes a simple Newtonian fluid—not likely to be encountered by many cosmetic rheologists. *Fig. 1*(*b*) describes a non-Newtonian fluid without a yield stress, also uncommon in cosmetics. *Fig. 1*(*c*) describes a fluid where the yield stress is a very important factor,

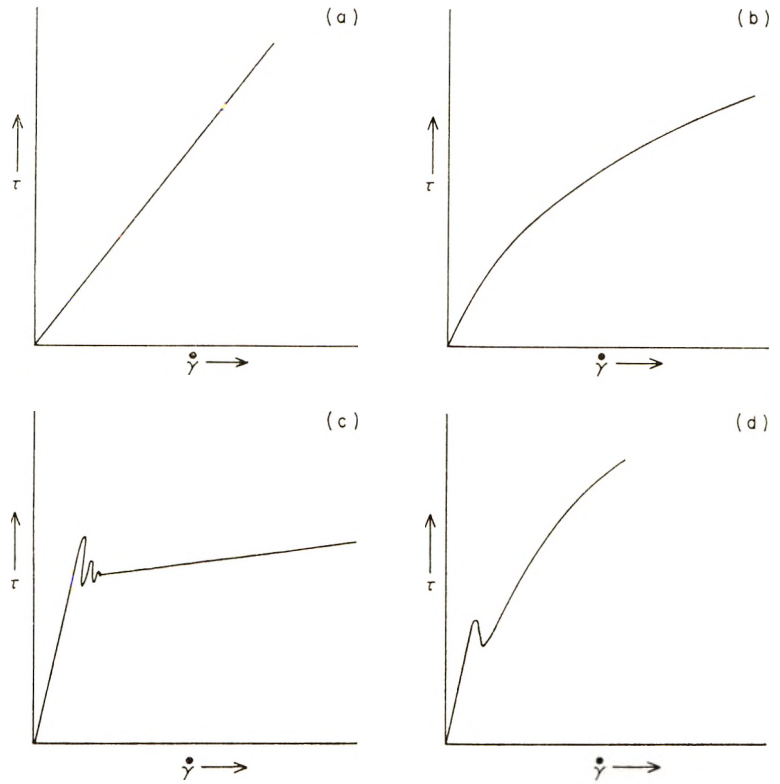

Figure 1. Typical shear stress–shear rate curves. (a) For a Newtonian liquid. (b) For a non-Newtonian liquid without a yield stress. (c) For a fluid where the yield stress is a very important factor. (d) For a fluid where the yield stress is present but not very important.

and *Fig.1(d)* a fluid where a yield stress is present but not very important.

The shear stress–shear rate diagram can be used to answer four questions, namely

How will the product appear in the container, when it may be tilted, prodded or gently shaken?

How will it stand up to transport and storage?

How will it transfer from the container to its use situation?

How will its properties change and at what rate when the product is used?

The initial impression

This is very important to the saleability of any product. A shear rate of 50–100 s^{-1} corresponds to that which the product would suffer when tilted, gently shaken or prodded. At this shear rate a measured viscosity of less than 100 cp would be easily pourable; as the viscosity increased the product would become gradually stiffer until at 1000 cp it would appear solid under these conditions. The importance of looking at the viscosity at the appropriate shear rate is obvious from *Fig. 1(c)*. Some products appear solid at low shear rates but liquid at high ones.

Storage and transport properties

Since cosmetics will often be stored for several months or even years before use, it is important that they are stable. This simply means that the physical properties chosen as being of importance to acceptability must remain within some specified limits during the expected lifetime of the product. The acceptable limits are defined by sensory evaluation. It must be ascertained by physical testing whether a product can stand up to the shearing it will receive in transport so that a product arrives on the shelf in a suitable condition. This does not apply of course to thixotropic products since they regain all their original structure when allowed to rest after shearing.

The impression on removing from the container

The yield stress has an important influence on the way in which a product will leave the container. Its position on the $\hat{\imath}$–$\dot{\gamma}$ diagram defines at what stage of usage (at what shear rate) breakdown will occur, how much force is needed (what shear stress) to initiate breakdown, and how much of the total structure is destroyed when the yield stress is exceeded. A simple example illustrates its importance. When the end is removed from a toothpaste tube the paste must not run out; it must be held in place by its yield stress. The pressure needed to exceed the yield stress must be reasonably low, otherwise either the tube would burst or the sink would be full of toothpaste! When the yield stress has been exceeded the product must still retain most of its structure, so that it will remain intact on the brush and not drip off before it can be used. Given these requirements it is possible to predict the shape of a toothpaste flow curve without knowing any of the numerical values.

Yield properties are varied by the dispersed phase concentration and the types and amounts of stabilizers used. Some gums and gelling agents give non-recoverable breakdown at low shear stresses, while clays can

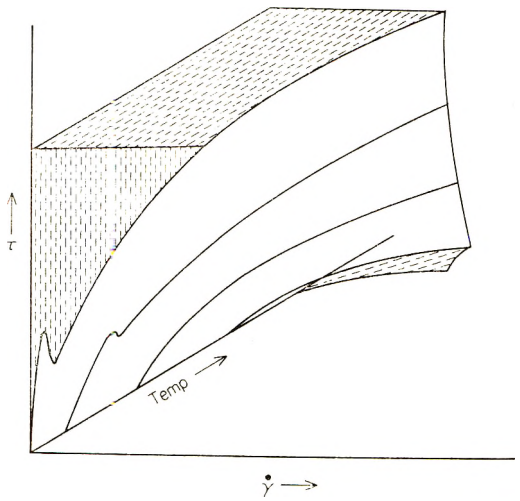

Figure 2. Representation of shear properties as a function of shear rate and temperature.

show a much more drastic but completely recoverable breakdown, giving a thixotropic product.

Properties during use

A cream, for example, is spread onto the skin at a shear rate of around 1000 s^{-1}. The degree and rate of breakdown under these conditions depend on the shear properties at this shear rate. The thermal properties also become very important, and all data must be considered as a function of both shear rate and temperature. It is possible to represent this behaviour on a 3-dimensional diagram of shear stress–shear rate-temperature (*Fig. 2*).

Elastic properties

Most cosmetics show viscoelasticity. It is a natural property of emulsions and suspensions at sufficiently high disperse phase concentrations. It is increased by the long chain molecules of the gums and other thickeners used, and by the various clays, all of which build up their own 3-dimensional network structures. The firmness of a product in the container can be described by its rigidity modulus at small strains (i.e. within the Hookean region). A product with a modulus of 10^2 nm^{-2} or less will look like an elastic liquid, e.g. some hair setting lotions. A modulus of from 10^3 to 10^5

describes a product which is moving from 'soft' to 'firm' to the touch. A modulus of 10^6 or over would indicate a hard substance not suitable for many cosmetic applications other than various types of pencils. These values provide an order-of-magnitude indication of the information to be gained from the measurement of a single physical property; in practice the perceived texture of a product results from a combination of several different properties.

The pictorial representation of texture

Rheological properties of general importance for cosmetics are viscosity, rigidity and yield behaviour, and their dependence on shear rate, age and temperature. Of less general importance but nevertheless very important to individual products are such properties as greasiness, tensile strength, stickiness (or the lack of it), brittleness and surface tension. It would be very convenient to be able to represent all these properties on one texturegram. However, it would have so many dimensions as to be impossible to understand, even if it could be drawn.

Using just three variables it is possible to represent a wide range of textures on one diagram. *Fig. 2* shows how the properties during use of creams can be displayed on a $\hat{\imath}$–$\dot{\gamma}$–T diagram. Three other useful variables are viscosity, elastic modulus and the degree of quickly recoverable strain. The latter property eliminates thixotropic products from the display. Consider a material with a modulus of 5×10^5 nm^{-2} and a viscosity of 10^4 cp, something like the values to be expected for a slightly under-ripe banana. If it had a quickly recoverable strain of 50% it could be compressed to half its original thickness, and regain its former shape as soon as the stress was removed. This product would be described as rubbery to the touch. If it could only recover from a strain of 2%, breaking up if this strain were exceeded, it would be described as brittle. Similarly, a product with a low elastic modulus, say 10^2 nm^{-2}, medium viscosity, say 10^2 cp, and a fairly high recoverable strain, say 50%, would feel slimy to the touch. These three textures are plotted on the texturegram in *Fig. 3*. It must be remembered that when specifying viscosities and elastic moduli, the conditions of measurement must correspond to those in the application being considered. Various other textures can be represented on this diagram, for example hard, firm, soft and brittle solids, pourable, non-pourable and elastic liquids. Some of these are shown in *Fig. 4*.

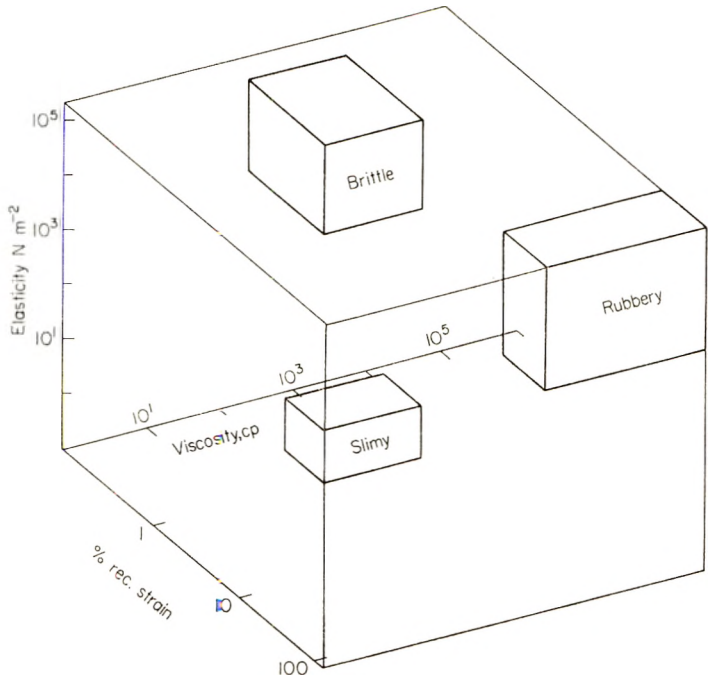

Figure 3. The pictorial representation of texture using a viscosity, elasticity, per cent of quickly recoverable strain diagram.

Specification of desirable rheological properties

Having set up methods for describing the texture of a product by means of a set of numbers, their optimum values must be established, and the allowable deviations from the optimum which do not diminish the product's acceptibility. Sensory evaluation of the products and correlation of these results with the results of physical tests is the route to this goal.

Setting up a panel

In selecting a panel there are two choices as to the type of personnel to be used; they may be non-expert, typical consumers, or a trained, expert group. The panel chosen will depend on availability, time and cost considerations, but also on the use to be made of the data. The objectives are, the following,

(i) To establish that a panel of judges can assess the physical properties to be measured.

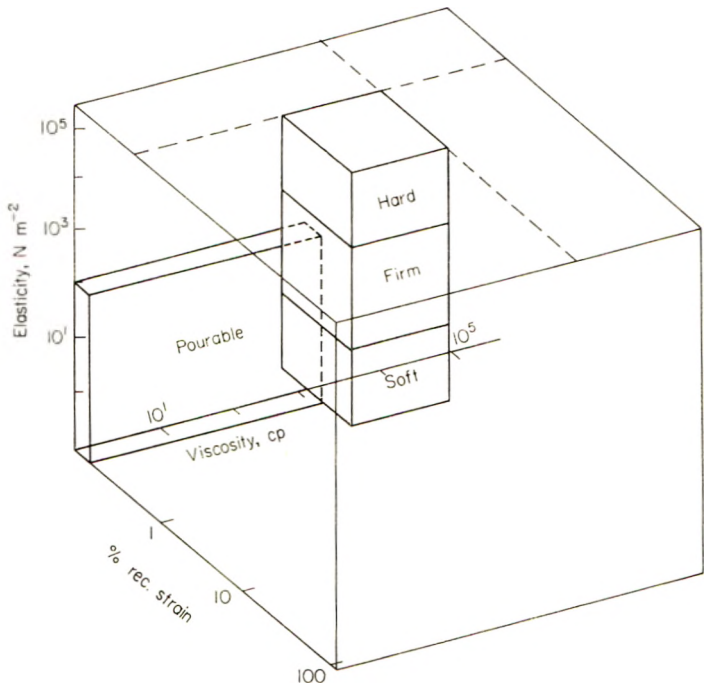

Figure 4. The pictorial representation of hard, firm, soft and pourable materials.

(ii) Having completed (i) above, to use the panel to determine the optimum for each physical property, and the acceptable limits of variation for each one.

It sounds very simple. In fact it is a little more difficult than it sounds, because of the large variation in sensory judgments between people. It is never possible to obtain an absolute answer from a sensory test. The best that can be achieved is a result which is 'statistically significant', at the 5%, 1%, or possibly even 0.1% confidence level, depending on the number of people used on the panel, and how consistent they are in their judgments.

It should be obvious from the preceding paragraph that a well-trained panel, carefully selected for their sensitivity, reproducibility and interest in participation will take much longer to set up but provide much more valuable information, and at a greater rate, once established.

Types of panel test

The organization of panel tests, and the coding and presentation of

samples is described in (2). Types of test and their usefulness are summarized below.

The triangle test

Subjects are given three coded samples, one of which is different from the other two. They have to select the odd one out. This type of test is useful to establish a panel's sensitivity, or thresholds, and to define a range of physically measurable differences in a product which cannot be perceived by the panel.

Scoring methods

Several different ways exist for scoring, or ranking samples for a particular property, e.g.

Scoring several samples together on a subdivided scale, with standard samples provided for reference.

As a ratio of two samples, a trial sample against a reference standard.

By 'paired comparison' tests, establishing which of a pair is greater in any chosen property, but not assigning any magnitude to the difference.

Scoring directly on a familiar scale without reference to standards except from time to time. This may be useful for a well-trained panel only.

Whichever methods are used, it is always necessary to analyse the results statistically. Analysis of variance (3) establishes whether there are significant differences either between the products or between the panellists, and how great the significance is. Scaling methods have been used very extensively by Szczesniak (4) to establish correlations between food textural properties and instrumental measurements. Some good correlations have been reported. Other people have carried out similar work (5). The techniques which have been applied to foods are equally applicable to cosmetics. Only the rheological properties of importance will be changed.

Conclusions

Cosmetics show complicated rheological properties, but their textures can be described, in the various usage situations, by the the correct choice of a few important properties. These properties, as measured instrumentally,

can be correlated with sensory evaluation of the same properties, thus enabling optimum properties to be specified along with acceptable departures from the optimum. Having established these quantities, an invariable standard is then available for use in new product design and as a quality control tool.

(*Received: 10th February 1975*)

REFERENCES

(1) *The art of perfumery*. G. V. Septimus Piesse (1879).
(2) *Principles of sensory evaluation of food*, chapter 6. Amerine, Pangborn and Roessler (1965) (Academic Press, New York).
(3) *Facts from figures*, chapter 19. M. J. Moroney (1951) (Penguin Books, London).
(4) Szczesniak, A. L. Correlations between objective and sensory texture measurements. *Food Technol.* **22** 981 (1968).
(5) Henry, W. F. *et al.* Texture of semi-solid foods: sensory and physical correlates. *J. Food Sci.* **36** 155 (1971).

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Sex attractants in primates

ERIC BARRINGTON KEVERNE*

Presented at the Symposium on 'A Sensory Approach to Cosmetic Science' organized by the Society of Cosmetic Chemists of Great Britain at Manchester on 7–9th April 1975

Synopsis—The importance of OLFACTORY communication in SEXUAL ATTRACTION among primates is clear from our previous studies, which led to the term 'PHEROMONE' being used to describe these olfactorily acting ATTRACTANTS. It is perhaps debatable whether such a term, originally defined to explain insect behaviour, can now be applied to the complex behaviour of a higher PRIMATE or man, where responses are not stereotyped. Such olfactory stimulants in these primates can produce mounting, masturbation and ejaculation, increase grooming behaviour and reduce aggression. Moreover, the ingredients in the composition of the stimulant may be added to, and produce improvements in the responsiveness of certain males. Since both the nature of the stimulants and the type of response produced can be varied, this clearly illustrates the complexity of defining an olfactory response in these higher primates.

Among the primates, communication of female sexual attractiveness appears to involve olfactory mechanisms, and a number of field and laboratory studies have observed sniffing of the female's genital region prior to copulatory behaviour. This is particularly true of the macaques where Carpenter (4) first observed that the vaginal overflow of the rhesus monkey possessed a characteristic odour which he thought might provide additional stimuli attracting males to females. Jay (7) perceived a strong smelling vaginal discharge in toque macaques, *Macaca sinica*, and observed males to examine the genitalia of females in the group each day. Bonnet macaques, *Macaca radiata*, have rarely been seen to present for copulation unless solicitated by the male (14). This involved flipping the tail aside, olfactory examination of the genitalia, and on occasions, insertion of the

* Department of Anatomy, University of Cambridge.

finger into the vagina followed by smelling and tasting of the secretion (13). In the pigtail macaque, *Macaca nemestrina*, the male displayed the Flehmen posture following olfactory inspection of the female's genitalia (6). In the stumptail macaque, *Macaca arctoides*, sniffing, fingering and licking of the perineal region occurs following female presentation (2) and prior to copulation (3). Although olfactory cues from the urine of receptive females are not ruled out, it would appear from these observations that in the macaques, communication of sexual status is by way of vaginal secretions.

To test this proposition use was made of operant conditioning techniques, in which male rhesus monkeys were required to press a lever in order to raise a partition which physically separated them from a female partner, but through which they could both see and smell the partner. Males had to work with some dedication, pressing the lever 250 times to gain access to the female. They regularly responded for ovariectomized partners treated with oestrogen, but rarely performed when faced with untreated ovariectomized females (9). Temporarily depriving these males of their sense of smell did not markedly affect either their pressing for or behaviour with the oestrogen-treated females; these females were presumably remembered as being attractive because of previously rewarding sexual experiences with them. However, the temporarily anosmic male failed to respond for unfamiliar ovariectomized females after these females were administered oestrogen, and shown to be sexually stimulating to normal males.

When the olfactorily deprived males had their sense of smell restored they readily began pressing for access to these females. That is to say, anosmia did not impair males' sexual arousal and sexual activity with familiar oestrogenized females, but anosmic males were not able to detect the onset of attractiveness which oestrogen promoted in their unfamiliar partners. These results were consistent with the hypothesis that oestrogenized female rhesus monkeys produced substances which stimulated the sexual interest of their partners via the olfactory sense.

The preoccupation of males with the female's genital region suggested this might be an obvious place at which to start looking for male sex attractants. We therefore studied the effects on male behaviour of transferring vaginal secretions from oestrogenized 'donor' monkeys to ovariectomized, unattractive 'recipient' partners. Application of vaginal secretions to the sexual skin area of recipients, which were themselves quite unreceptive to males, nevertheless resulted in a marked stimulation of the male partner's sexual activity (10). Oestrogen-primed vaginal secretions have

now been applied to the sexual skin area of ovariectomized recipient rhesus monkeys on 249 separate occasions, and a 1 h behaviour test observed on each occasion in an acoustically isolated testing booth housed behind a one-way screen. These tests involved 11 pairs of animals and the secretions significantly increased the male partners' sexual behaviour above pre-treatment periods when only control substances were applied (*Fig. 1*). In

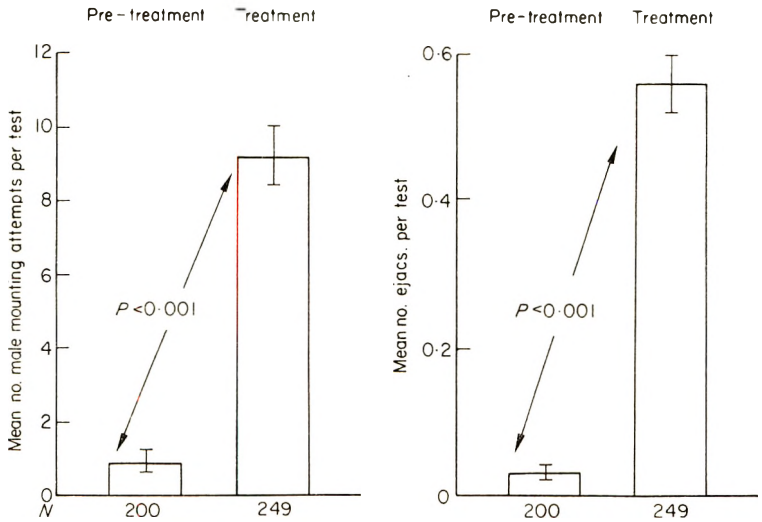

Figure 1. Effects of applying vaginal secretions from oestrogenized 'donor' females to the sexual skin area of ovariectomized 'recipient' on the sexual stimulation of males. Data for 11 pairs involving five males, five females and five donors.

the pre-treatment period eight ejaculations were recorded in 200 tests and these increased to 139 during 249 tests when vaginal secretions were smeared on the sexual skin of unreceptive recipients ($C^2 = 96.04$, P 0.001). Of even greater significance was the increase in male mounting attempts from 174 during the pre-treatment period to 2292 during applications to the same female partners. This high number of mounting attempts with few ejaculations was an indication of the unreceptive condition of the ovariectomized recipients, and clearly demonstrates the males' increased sexual interest in these pheromone treated females.

To determine the chemical nature of the substances in vaginal secretions responsible for these powerful behavioural effects, extraction and fractionation procedures were used in conjunction with behavioural assay methods.

The early stages of this procedure involved the use of ether extracts of secretions, collected by lavage with water from oestrogen-treated donor females (8). Ovariectomized rhesus monkeys were again the recipients for these extracts and the very low levels of sexual activity during the pre-treatment period was in marked contrast to the high levels seen during the applications of ether extracts. A gas chromatographic comparison of ether extracts of the vaginal secretion from ovariectomized untreated females indicated that the amounts of volatile components were absent or low, while oestrogen treatment stimulated production of volatile components and improved the sex attractant properties of vaginal secretions (11). Identification of these volatile components was obtained by preparative gas chromatography and mass spectrometry (5). The resultant mass spectra were compared with authentic samples, and established the identification of the first five peaks as acetic, proprionic, isobutyric, butyric and isovaleric acids. A mixture of authentic acids was made up to match their concentration in a pool of vaginal washings, and a small sample of this mixture when tested for behavioural activity was demonstrated to possess sex attractant properties (*Fig. 2*).

The effectiveness of these pheromones in stimulating sexual behaviour in the rhesus monkey does, however, vary according to social conditions: with some partners and in certain tests no sexual stimulation occurs. As more males are tested it is becoming evident that the response to pheromones varies between individuals and is also dependent in part upon the female partner with which they are paired. When the behavioural effects of fresh vaginal secretions and a synthetic mixture of their acid content were compared in the same nine pairs of animals, vaginal secretions appeared to be more effective in stimulating the male's sexual behaviour (*Fig. 3*). Although both vaginal secretions and the synthetic pheromone complex stimulated male sexual activity at significantly higher levels than in the pre-treatment tests, vaginal secretions were effective in 59% of applications compared with only 35% effectiveness of the synthetic acid mixture. Moreover, vaginal secretions stimulated 452 male mounting attempts compared with only 257 during application of synthetic pheromone to the same female partners ($t = 2.39$; $P < 0.02$).

This lower proportion of effective tests during applications of the synthetic pheromone is due to its failure to stimulate mounting behaviour in certain pairs (*Fig. 4*). The relative effectiveness of untreated vaginal secretions and the synthetic acid mixture varied with the male partners from 100% in the case of male 113 to 45% success with male 68. With males 113

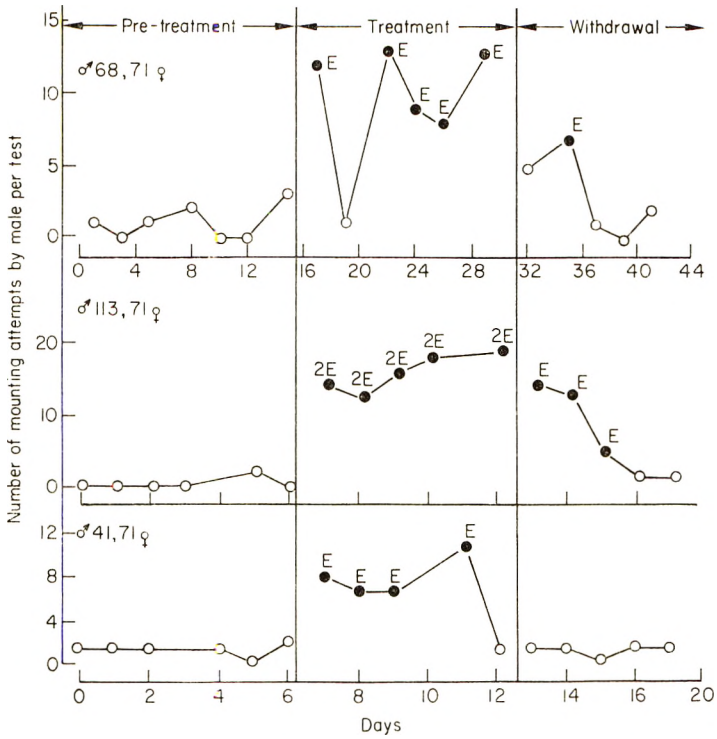

Figure 2. Sexual stimulation of male rhesus monkeys during treatment of their female partners with a synthetic mixture of aliphatic acids applied to their sexual skin area. ○, test without ejaculation; ● E, one ejaculation in the test. ● 2E, two ejaculations in the test.

and 41, acids and vaginal secretions were equally effective and this was also the case with females 71, 78 and 74, although with female 74 they were equally ineffective (*Fig. 4*). With male 67 and 68, the synthetic acid mixture was approximately half as effective as the fresh secretion. This was due to these males being paired for some of their tests with female 76 where, although secretions have stimulated sexual activity in 52% of tests, the acid mixture has always been ineffective.

Thus it can be seen that the male's response to olfactory attractants varies between individuals and is also, in part, dependent on the female partner with which they are paired. Whereas some females readily evoke a sexual response from the male, others when treated in the same manner fail to do so. Hence, the response to pheromones in these highly-evolved social primates is not stereotyped. Furthermore, it can be seen that for certain pairs, the synthetic acid mixture is not as effective in stimulating the

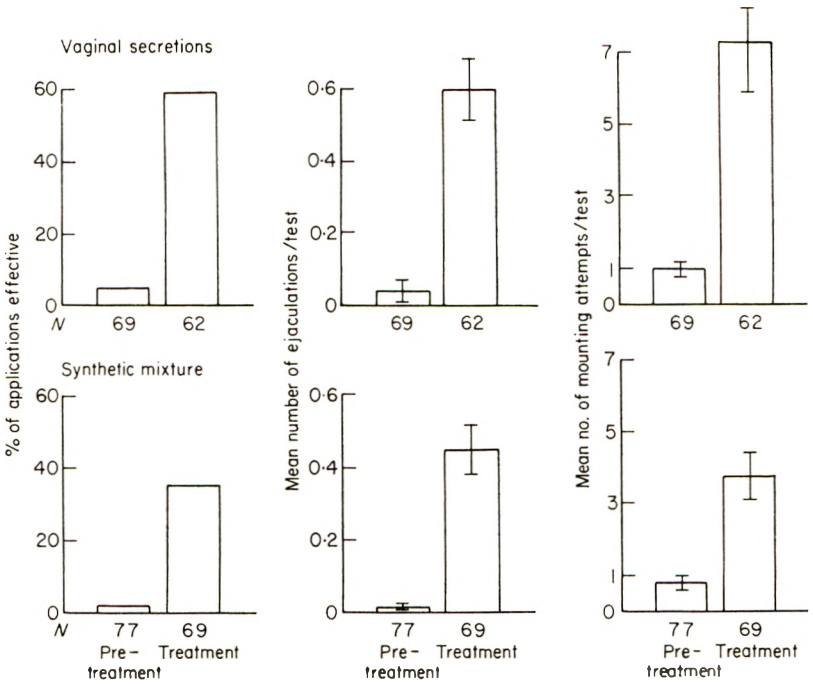

Figure 3. Comparison of the effectiveness of vaginal secretions and synthetic pheromones on the sexual behaviour of male rhesus monkeys. Treatment using both methods produced a significant response, but vaginal secretions were more effective than synthetic pheromone.

males' sexual activity as the original vaginal secretions. This could mean there is a component in untreated secretions that is lacking in the synthetic mixture.

Phenylpropanoic (PPA) and parahydroxyphenylpropanoic acids (HPPA) are both odorous compounds identified in the rhesus monkey's vaginal secretion, but are quite ineffective in stimulating male sexual activity when applied alone to the sexual skin area of an ovariectomized female partner. By addition of PPA and HPPA to the synthetic acid mixture an enhancement of the effectiveness of synthetic pheromone has been obtained (*Fig. 5*). Sexual interactions were shown by the male in more tests (65%) than when just synthetic mixture of acids alone were applied (37% of tests). The mixture containing enhancers was almost as effective as the untreated vaginal secretions (70% of tests), although the amount of sexual behaviour stimulated was not so high. Untreated vaginal secretions when applied to the sexual skin of ovariectomized females in this test series stimulated 252

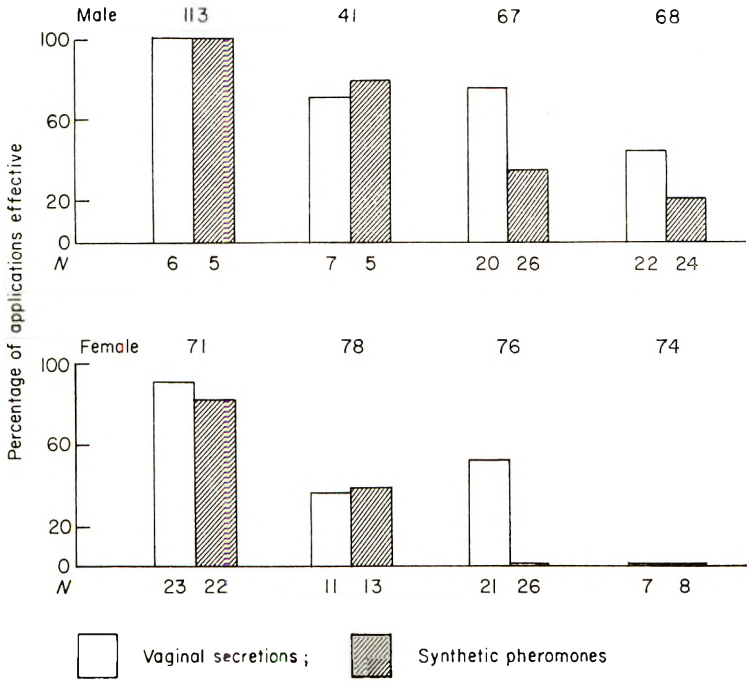

Figure 4. Variability in the response of different males to vaginal and synthetic pheromones and their relative effectiveness when applied to different female partners.

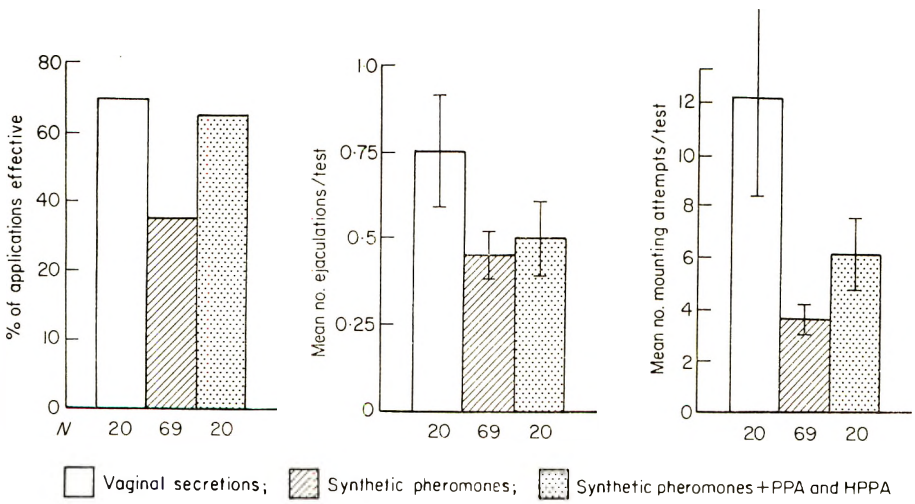

Figure 5. Improvement in the effectiveness of synthetic pheromones when phenylpropanoic and parahydroxyphenylpropanoic acids are added.

male mounting attempts and 15 ejaculations compared with 123 mounting attempts ($t = 1.6$, P ns) and 9 ejaculations ($\chi^2 = 0.82$; P ns) during 20 tests when the synthetic mixture plus enhancers was applied.

From these results there is an indication that these phenolic components have an enhancing effect on the sexual stimulating properties of the synthetic pheromones. Nevertheless, it remains true that in some pairs the simple acid mixture appears to be completely effective. It is my opinion that the odour of the oestrus vaginal secretion is the true attractant and the acid mixture, mimicking the most odorous of the components, can in certain cases act as sufficient stimulus for some males. Others require additional volatile components such as PPA and HPPA, while still others require the whole untreated oestrous vaginal secretion. Hence, the odour cue is in itself complex.

Moreover, the source of the odour cue does not appear to be either glandular or an exudate through the vaginal wall, but microbial action plays an important part in producing the odours in the vaginal secretions, since aliphatic acid concentrations increase during incubation of the vaginal lavage, while autoclaving or the addition of penicillin prevents production of these fatty acids (12). It seems probable, therefore, that the production of these acidic pheromones depends upon the bacteria of the vagina, and that the ovarian hormones exert their influence on acid production in the intact animal by determining the availability of nutrients in the form of cornified cells and mucus.

An additional complication is to be found in the plasticity in the behavioural response of the male to the odour cue. It has already been shown that this can be modified by a partner preference, but perhaps of even more interest is the different behaviours which these odour cues can sitmulate. The variability of the male's behavioural response to the same odour cue with different female partners is shown in *Fig. 6*. With the pairs 67, 76; 41, 71; and 68, 71, oestrogen primed vaginal secretions markedly sitmulated male sexual activity. With the pairs 67, 78; 41, 79; 68, 78, an increase was produced in the social responsiveness of the male and he was prepared to groom his female partner for longer, although no sitmulation of sexual activity occurred. With the pairs 67, 74; 41, 79; 68, 78, no stimulation of sexual activity occurred during the treatment with pheromone, but a marked reduction was observed in each male's aggressive behaviour towards his partner. It could be argued that we are dealing with more than one odour cue, and the vaginal secretion contains a grooming stimulant, and an aggression reducing pheromone, in addition to the sex attractant. My own feeling is that the coding for the behavioural response is not restricted to

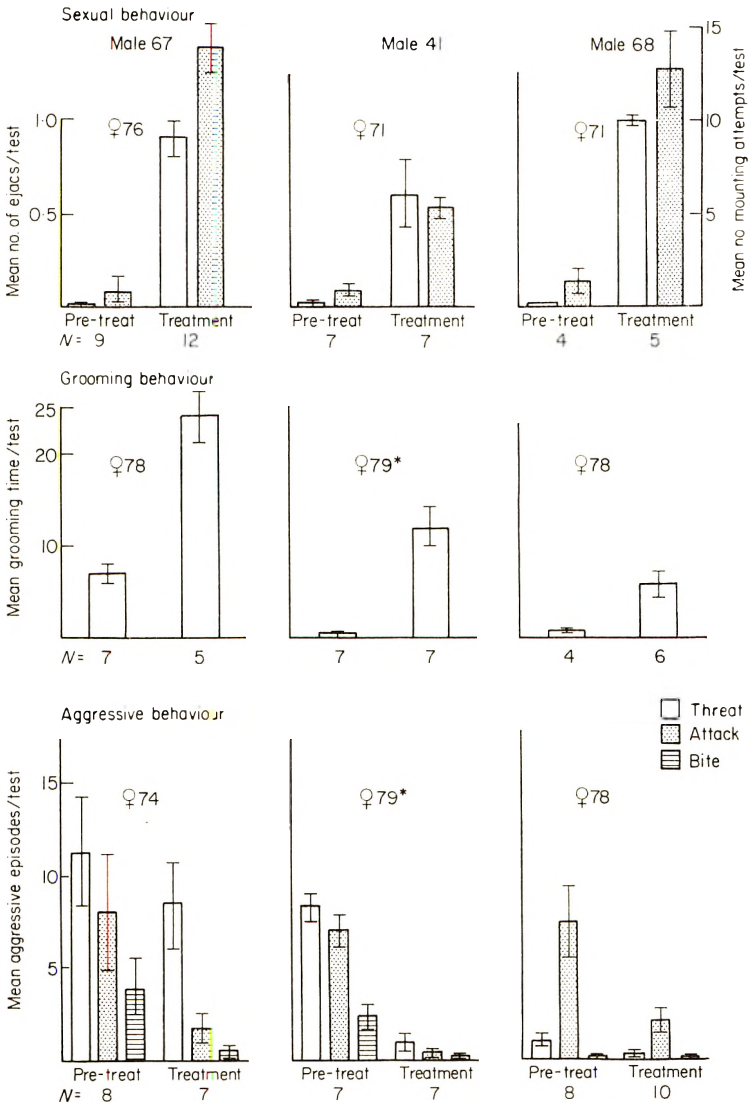

Figure 6. Increases in sexual behaviour or grooming behaviour or the reduction of aggression by applications of pheromone but depending on the female partner. Those pairs marked with an asterisk had ether extracts of vaginal secretions applied.

the olfactory cue, but is integrated in higher areas of the neocortex. This opinion is reinforced by the variability in the male's response both to ether extracts of secretions (*Fig. 6*, those pairs marked with an asterisk), and in some cases to the synthetic acid mixture itself, which clearly rules out different odour cues.

It is most important that this lack of a stereotyped response is emphasized in this highly evolved social primate, particularly if consideration is to be given to the human situation and a search for human pheromones started. These aliphatic acids are present in human vaginal secretions (12) and the human male can distinguish variance in these odours between the phases of the menstrual cycle of the rhesus monkey. This does not, however, imply any causal relationship between these odour cues and the sexual behaviour of the human male. If we are to consider the complexity and plasticity in the response of the male rhesus monkey, then the further social and cultural evolution of man may make the search for an olfactory aphrodisiac with sexual releasing properties a fruitless task. Indeed, it may be argued that stimulation or provocation by female odours could be disruptive to our social order, and perhaps this is why we take such pains to disguise our body odours. This is not to say that these odours play no part in human sexual behaviour, but to give them significance at the level of sex attractants underestimates the complexity of human behaviour. What then might be the effect of these odour cues in human behaviour? *Fig. 7* shows data which has been extracted from a number of experiments which I feel has some bearing on the kind of level these odour cues might be seen to act on the human. Following withdrawal of the sex attractant from the female partner, males usually lose sexual interest in the female and make no further mounts, but occasionally (*Fig. 7(a)* and *(b)*) males maintain their sexual arousal and, paradoxically, show increased mounting and thrusting prior to a loss of sexual interest. Moreover, if the oestrogenized female donor is given progesterone, her vaginal secretions lose their sexual stimulating properties when applied to the recipient. However, prior to the male's loss of sexual interest there is a marked increase in the male's mounting and thrusting (*Fig. 7(c)*). Since the only change in all these experiments is the odour of the female partner, I interpret this increased mounting behaviour as an increase in male sexual performance to compensate for the odour deficit of the female partner; that is, an increase in tactile input compensating for decreases in another sensory cue, namely olfactory. Similarly, following reversal of anosmia there may be an increase in male ejaculations with no marked increase in mounting (*Fig. 7(d)*). Here the introduction of

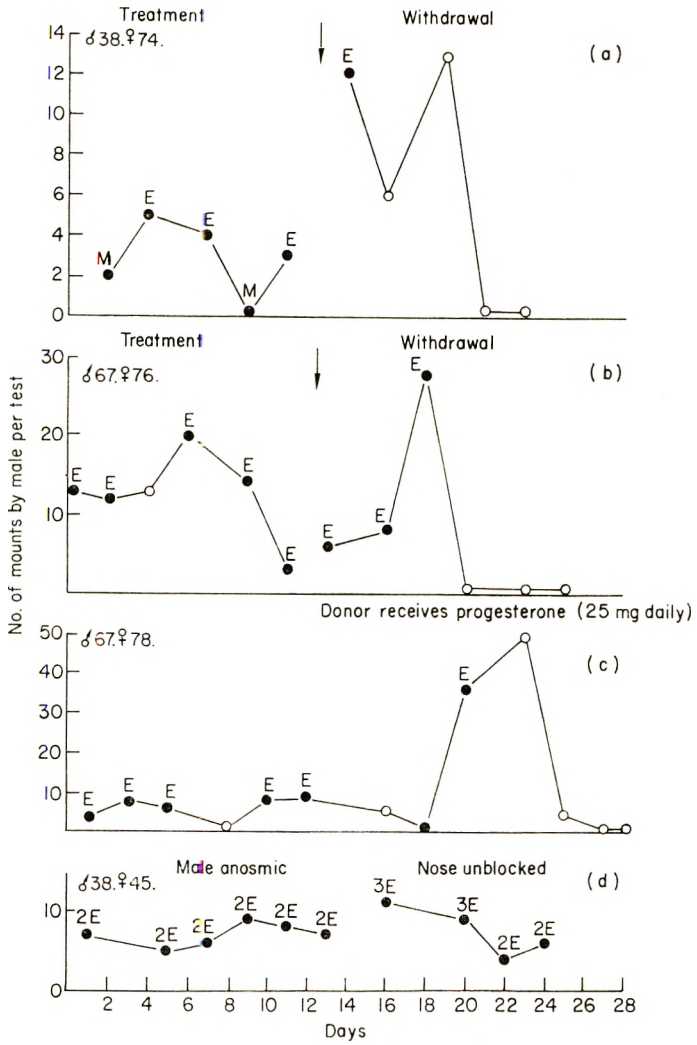

Figure 7. Changes in the sexual performance (mounting patterns) of male rhesus monkeys following odour changes of their female partner.

the olfactory sense with an attractive female initially improves the sexual performance of the male, and briefly increases his ejaculatory score.

It would appear from these results that at least two separate neural pathways are involved in the integration of olfactory cues. It is possible that sexual atrraction involving releaser pheromones is brought about via olfactory connections to the pyriform cortex and other cortical regions

before passing on to the limbic system via the amygdala and medial forebrain bundle to the hypothalamus. This I envisage as serving a filtering function, with the cortex analogous to a computer incorporating certain 'go' and 'no go' programmes. As we have already seen, the rhesus monkey's behaviour incorporates a number of 'no go' programmes, as for example the modifying effects of partner preferences, past experiences with certain females, and the presence of other males, etc. If we consider the human with infinitely complex behaviour patterns involving traditions, rituals, religions and past experiences, there are many variables which might constitute 'no go' programmes, making it pointless to look for any overt behavioural response. If, on the other hand, we consider sexual performance (as in *Fig. 7* for the rhesus monkeys) it is possible that a more direct neural input to the hypothalamus is involved, possibly monosynaptic or probably involving only a few synapses. This I see as being analogous to tactile input as in genital stimulation (1), with the hypothalamus serving as an area of integration, while the threshold for response is modulated by hormonal status.

In conclusion, olfactory cues are of importance in the sexual behaviour of the male rhesus monkey but the behavioural response is far from stereotyped. This presents obvious problems when considering an attractant role for such odours in the human where social and cultural determinants of behaviour are even more complex. I would like to suggest that an alternative approach for the human might be in considering a role for these odours in sexual performance. This could be analysed objectively, and is less likely to be influenced by cultural variables.

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REFERENCES

(1) Barraclough, C. A. and Cross, B. A. Unit activity in the hypothalamus of the cyclic female rat: effect of genital stimuli and progesterone. *J. Endocrinol.* **26** 339 (1963).

(2) Bertrand, M. The behavioural repertoire of the stump-tailed macaque. *Bibliotheca Primat.* **111** (1969).

(3) Blurton-Jones, N. G. and Trollope, J. Social behaviour of stump-tailed macaques in captivity. *Primates* **9** 365 (1968).

(4) Carpenter, C. R. Sexual behaviour of free ranging rhesus monkeys (Macaca mulatta) II Periodicity of oestrous, homosexual, antoerotic and non-conformist behaviour. *J. Comp. Psychol.* **33** 143 (1942).

(5) Curtis, R. F., Ballantine, J. A., Kerverne, E. B., Bonsall, R. W. and Michael, R. P. Identification of primate sexual pheromones and the properties of synthetic attractants. *Nature* **232** 396 (1971).

(6) van Hoof, J. A. R. Facial expressions in higher primates. *Symp. Zool. Soc., Lond.* **8** 97 (1962).

(7) Jay, P. Field studies in *Behaviour of non-human primates*, 525–592. Eds A. M. Schrier, H. F. Harlow and F. Stollnitz (1965) (Academic Press, New York).
(8) Keverne, E. B. and Michael, R. P. Sex-attractant properties of ether extracts of vaginal secretions from rhesus monkeys. *J. Endocrinol.* **51** 313 (1971).
(9) Michael, R. P. and Keverne, E. B. Pheromones in the communication of sexual status in primates. *Nature* **218** 746 (1968).
(10) Michael, R. P. and Keverne, E. B. Primate sex-pheromones of vaginal origin. *Nature* **215** 84 (1970).
(11) Michael, R. P., Keverne, E. B. and Bonsall, R. W. Pheromones: Isolation of male sex-attractants from a female primate. *Science* **172** 964 (1971).
(12) Michael, R. P., Zumpe, D., Keverne, E. B. and Bonsall, R. W. Neuroendocrine factors in the control of primate behaviour. *Rec. Prog. Horm. Res.* **28** 665 (1972).
(13) Rahaman, H. and Parthasarathy, M. D. (1969) Studies on the sexual behaviour of Bonnet monkeys. *Primates* **10** 149.
(14) Simonds, P. E. The Bonnet macaque in South India in *Primate behaviour*, 175–196 (ed. by I. de Vore) (1965) (Holt, Rhinehart and Winston).

J. Soc. Cosmet. Chem., **27**, 271–287 (1976)

Evaluation of flavours in dental creams

ERIC BAINES*

Presented at the Symposium on 'A Sensory Approach to Cosmetic Science' organized by the Society of Cosmetic Chemists of Great Britain at Manchester on 7–9th April 1975

Synopsis—One of the most important features of a DENTAL CREAM is its FLAVOUR. CONSUMER TESTS show the close relationship of overall preference with flavour attributes.

Evaluation methods can be of different types depending on which aspect of flavour is being examined. Any convenient PANEL can be used to determine whether two flavours are noticeably different.

Detailed flavour descriptions can be obtained using flavour profile panels. In the classic form such panels require screening and training of their members so as to render them expert.

These restrictions can be considerably relaxed, however, if the DELPHI TECHNIQUE is used to elicit an accurate consensus of opinion from the panel. Flavour descriptions can also be obtained by the use of panels of 20–30 members awarding ratings over a limited number of flavour questions. Results of such panels can be subjected to more elaborate methods of statistical analysis, such as analysis of variance and regression analysis, provided that the flavours tested conform to a carefully planned experimental design.

Panels intended to test the acceptability of flavours must be large and as representative as possible of the toothpaste using population.

Introduction

Flavour is one of the most important attributes of a toothpaste, it is what is noticed during brushing and immediately afterwards. Although consistency and texture are also important, they tend to be standardized

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over most toothpastes on the market, and flavour contributes most to sensation in the mouth. Analysis of consumer test data confirms this. *Fig. 1* is a two-dimensional view of the three-dimensional factor space produced by carrying out a factor analysis on a set of consumer test results. The view is that looking into the corner formed by the origin and the three factor axes.

Factor analysis is a mathematical method whereby the 10 questions asked in the consumer test have been reduced to a smaller number of underlying, basic factors so that each question can be represented by a point in the factor space (1). This enables a toothpaste to be described in terms of three numbers, each corresponding to one of the factors, rather than by the 10 numbers resulting from the original 10 questions. The closer two questions fall in this space the more highly correlated they are. In *Fig. 1* it can be clearly seen that 'overall preference' and 'flavour in use' fall

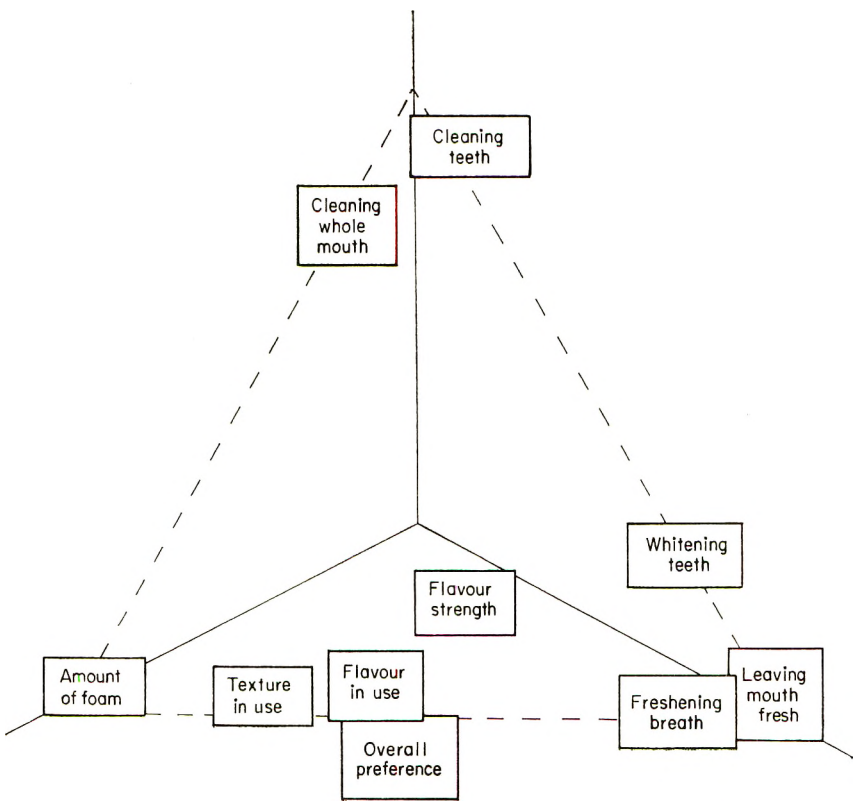

Figure 1

very close together with 'texture in use' and 'flavour strength' being next closest to 'overall preference'.

Surprisingly the range of flavours used by toothpaste manufacturers has been narrow and tended to be conservative, being largely confined to peppermint, spearmint and combinations of these two. There is recent evidence, however, that more exotic flavours can be viable and hold a respectable market share in this country and the USA. The spice flavour of a well-known clear gel is a good example of this. A breakdown of the UK toothpaste market by flavour type for major products is shown in *Fig. 2*. The dominance of mint flavours is obvious.

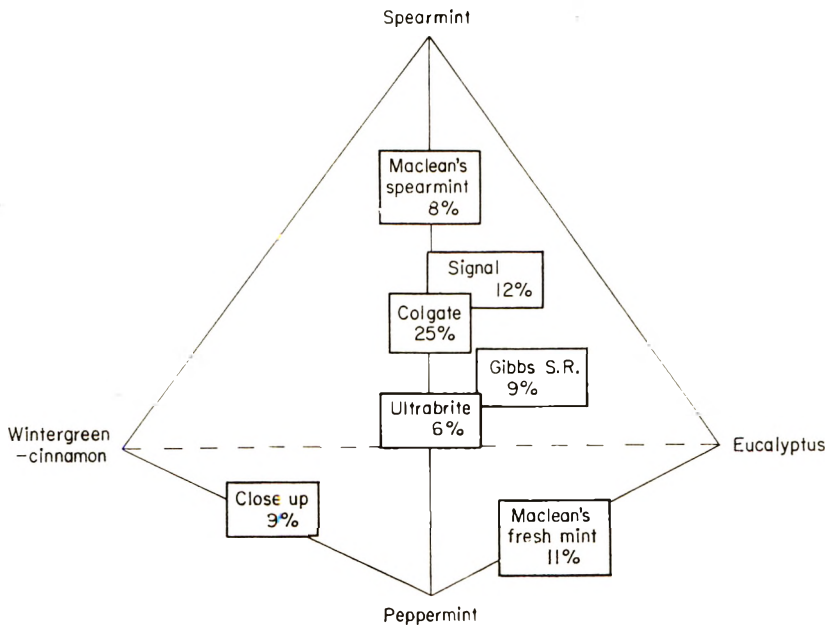

Figure 2

PHYSIOLOGICAL BACKGROUND

The sensation of flavour is made up of more than just the four basic tastes, sweet, sour, bitter and salty, which are registered by the taste buds

on the tongue. Smell plays an important part, as the more volatile flavour components are picked up on the olfactory mucosa of the inner nose. Tactile nerve endings in the mouth also register such sensations as burning, cooling and drying (2).

Taste buds occur mainly on the tip, the sides and rear of the upper surface of the tongue. They are contained in protuberances of the skin or papillae. Each bud is made up of from two to twelve taste cells which are arranged like the staves of a barrel. At the tip of each cell is a cilium which protrudes into a pore in the skin leading into the mouth. Electric impulses are carried from the taste cells to the brain via nerve fibres (3).

Taste is a chemical sense. Sourness is produced by hydrogen ions, the more acid a substance, the greater its sour taste. As a consequence of this buffers can be successfully used to reduce sourness. Saltiness is produced by cations in the order, from greatest to least, NH_4, K, Ca, Na, Li, Mg and by anions, in similar order, SO_4, Cl, Br, I, HCO_3, NO_3. Sugars, some amino acids, saccharine, cyclamates and some lead salts are sweet. Bitterness is associated with many different types of chemical compound, the most bitter being the alkaloids such as caffeine, quinine, etc. Many of the compounds listed above do not give the sensation of a single taste, but rather produce a mixture of tastes, for example sodium bicarbonate is both salty and bitter and saccharine is not only extremely sweet, it has a distinctly bitter aftertaste (4).

Bitter flavours are detected at the back of the tongue, sweet at the tip, sourness along the sides of the tongue from about midway to the back and the sensation of saltiness is detected more or less equally over the whole tongue (4).

Not only do some substances have different tastes which are experienced simultaneously, they also can taste quite differently to different people. Phenylthiourea is probably the best-known example of such a compound and is tasted by most of the population as being slightly bitter or salty. To some people, however, the taste is overwhelmingly and unbearably bitter. Sensitivity of this nature is known to be genetically determined and the high incidence of this sensitivity which the North American Indian population has in common with the peoples of East Asia is part of the evidence for the Asian origin of the American Indians (5).

For every person there are thresholds below which the four basic tastes cannot be detected. These can be determined very simply by using a series of solutions of increasing concentration of citric acid for sourness, sodium chloride for saltiness, sucrose for sweetness and quinine for bitterness and

finding that concentration at which each taste is first detected. Thresholds vary considerably over the population and typical distributions found during the screening of candidates for a flavour profile panel are shown in *Figs. 3, 4, 5* and *6*. The distribution for bitterness is fairly flat and that for sweetness highly peaked.

Adaptation and fatigue to flavours limit the number of and interval between tastings that any single panel member can be expected to carry out. Recovery from sour taste is usually very rapid because of the high solubility of hydrogen ion and the ease with which it is washed from the vicinity of the taste buds. Saltiness and sweetness present few problems as

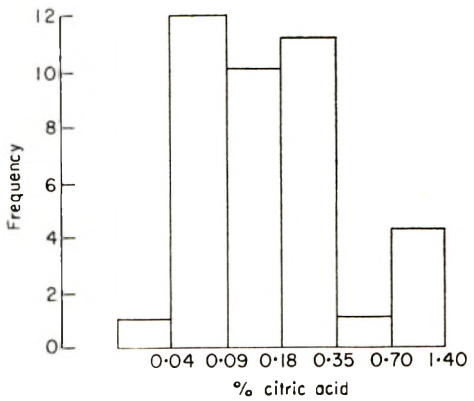

Figure 3. Sourness.

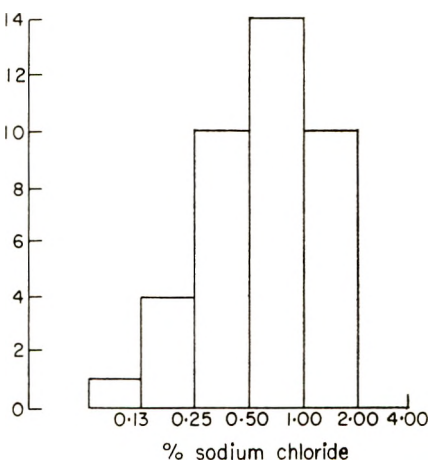

Figure 4. Saltiness.

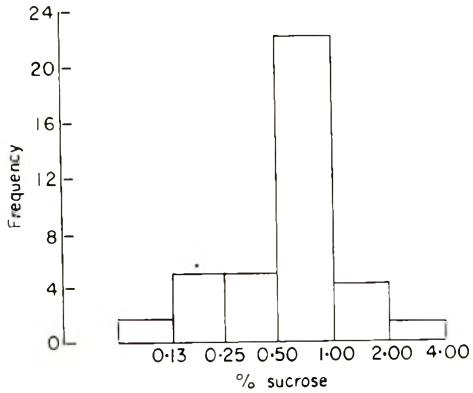

Figure 5. Sweetness.

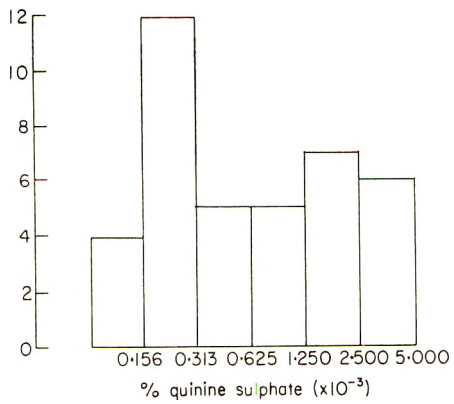

Figure 6. Bitterness.

recovery from these sensations is also fairly quick. Bitterness can linger in the mouth for a minute or more, probably because bitter compounds tend also to be substantive to the skin and disappear more slowly from the site where they are tasted (6).

The odour component of flavours presents the least problem from this point of view, recovery from odour sensation being quick. It has been reported that more than 70 odours can be evaluated in 1 h, provided that interest is maintained (7).

Tactile and temperature, not to mention pain, stimuli present the greatest obstacle to carrying out many flavour evaluations in a short time. The cooling and tingling effects of menthol considerably slow the rates at which toothpastes can be tasted.

Flavour difference tests

The simplest form of flavour evaluation is that required to demonstrate a difference between two flavours. This can arise when it is necessary to find out whether a change in some other component of a toothpaste has altered the taste of the finished product to such a degree that it can be detected by the consumer. In this case any flavour difference is likely to be small and the appearance, consistency and texture of the products to be tested should be effectively identical.

A panel of about 30 subjects is recruited. Just about anyone will do, there being no necessity for any special flavour expertise. Each panel member is presented at various times during the day with four toothpastes to taste. The test is completely blind, the creams being identified only by randomly numbered tubes and all differences in appearance and consistency eliminated as far as possible. These four creams are, in fact, two each of the cream under investigation and of a standard corresponding, say, to the regular product. They are presented to the panel in a random order previously worked out and known only to the person running the test. When all the creams have been tested each panel member is asked which numbered creams corresponded to the pairs. In the case of four creams described here, his probability of being right by accident in his pairings is exactly one-third. No matter how he has chosen the first cream there are equal chances of his choosing one from the remaining three if there is no special reason for his choosing any one rather than another. It is then a simple matter to carry out a χ^2 test on the results. For no detectable flavour difference the number of panel members pairing the creams correctly is expected to be one-third of the total taking part in the panel. The table of results will appear as follows:

	Correctly paired	Incorrectly paired	Total
Observed	m	n	N
Expected	$N/3$	$2N/3$	N

and $\chi^2 = \dfrac{(m-N/3)^2}{N/3} + \dfrac{(n-2N/3)^2}{2N/3}$ with one degree of freedom.

The value of χ^2 can be looked up in tables to determine whether or not it is significant and whether a detectable flavour difference probably exists.

In the case of toothpastes it is more realistic to insist that the panel brushes with the creams rather than tastes the cream straight from the tube. This type of test is very general in its application. It is used in the food industry and can be used without difficulty for cosmetics such as lipstick (8). All panel members should be persuaded to make a decision on the pairings in this form of testing because 'Don't Knows' cannot be treated in the analysis and are a waste of everybody's time.

If necessary the test can be repeated on two or three successive days so as to give the panel a better chance of finding a difference. The non-expert nature of the panel, the fact that it is run completely blind and the realism introduced by brushing with the creams being spread over a whole day make this test method very useful for assessing whether flavour differences which can be picked up by an expert are, in fact, likely to be perceived by the consumer.

Another version of this test uses two samples of the control cream and one of the cream under investigation. The statistical analysis can be worked out in a similar way to that described above.

Flavour profiles

If a description is sought of how a flavour is perceived by the user it is necessary to use some form of flavour profile panel (9). Members of such a panel must be expert and will have to be trained over a considerable time in recognition of flavour notes and sensations in the mouth and the accurate description of intensity and the order in which the sensations appear. Potential panel members must first be thoroughly screened for taste thresholds, as described above, and for the ability to recognize and describe a wide range of odours. As only five or six members are needed for a panel it should be possible to select two or three panels from 60 or so applicants.

Screening tests have revealed no convenient rules of thumb or short cuts that can be used in panel selection. Men and women appear to perform more or less equally and under-30's and over 30's show no significant differences in taste thresholds. Smokers, however, tend to have a higher bitterness threshold than non-smokers, but are no different for the other three tastes or for odour recognition. This may be due to some form of nicotine tolerance in the smokers. Laboratory workers have been found to be significantly better than others at identifying odours, but this appeared to be due to the screening being slanted towards odour description for which laboratory workers, not surprisingly, had a better vocabulary.

Thresholds for the four basic tastes are quite independent. Sensitivity to one particular taste does not necessarily imply sensitivity for any other taste. This effect is well known (10). As the four tastes are perceived by four totally separate systems of taste buds this is only to be expected.

Tests designed to show whether phenylthiourea was perceived as bitter or tasteless and whether sodium benzoate was tasted as sour, sweet, salty or bitter gave the results shown in *Fig. 7*. These are what would be expected of an average population and the responses to the two compounds are again completely independent. Perception of phenylthiourea as bitter did not mean that sodium benzoate would also taste bitter to the same person.

	Sodium benzoate				
	Sweet	Sour	Salt	Bitter	Total
Phenylthiourea					
Bitter	9	2	3	14	28
Tasteless	4	2	1	4	11
Total	13	4	4	18	39

Figure 7

Having chosen the panel it is then necessary to train it. Training takes the form of running practice sessions on specially prepared toothpastes consisting of unflavoured bases of various types to which flavour components are added singly and in increasingly complicated combinations. In this way the panel builds up a vocabulary of flavour notes and gains practice in describing events in the mouth in terms of these notes. Sessions are best held in the hour before lunch and panel members should refrain from drinking, eating or smoking for at least 30 min before a tasting session. Hands should be washed before a session using water only and no soap.

The session itself is composed of two parts. Aroma is evaluated first and flavour second. In both parts the odours, flavours and other sensations are identified and described as accurately as possible. An intensity rating from the scale

0	1	2	3	4
Not present	Just noticeable	Slight	Moderate	Strong

is then awarded to each sensation. The order in which the sensations occurred is recorded and finally, aftertaste and after effects are noted.

In the first session with any flavour a considerable amount of time will be spent on discussion within the panel to settle on an agreed nomenclature

for the observed sensations. At this point an independent supervisor for the panel is essential since forceful personalities on a panel can influence this stage considerably. With a trained panel, however, this problem can be avoided by using a version of the Delphi technique developed by the Rand Corporation (11). Each panel member carries out his evaluation in private, noting down sensations, time order and intensities on a blank form. After the first tasting the forms are collected and a questionnaire for the second tasting compiled which shows the order of sensations with their mean intensities as scored on the previous session and the ranges within which the panel rated the intensities. The panel member carries out his second tasting with all this information in front of him and modifies his evaluation in the light of this. Questionnaires for the third tasting are based on the results of the second in a similar fashion and the process repeated until the panel reaches a reasonable consensus. Three or four repetitions are usually enough to produce a satisfactorily tight range of evaluations from the panel.

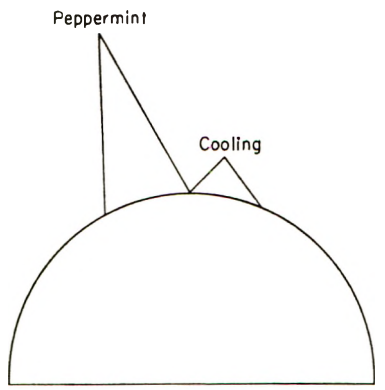

Figure 8. Odour profile.

Presentation of the results of flavour profile analysis traditionally made graphically in the form of profiles are shown in *Figs. 8* and *9*. The sensations are arranged from left to right around the circumference of a semicircle in the order in which they were recorded. Intensity is indicated by the length of sensation segment above the semicircle. An alternative form of presentation is merely to plot intensity against time and add descriptions of the sensations at appropriate points as shown in *Fig. 10*. Results can be depicted equally effectively as histograms.

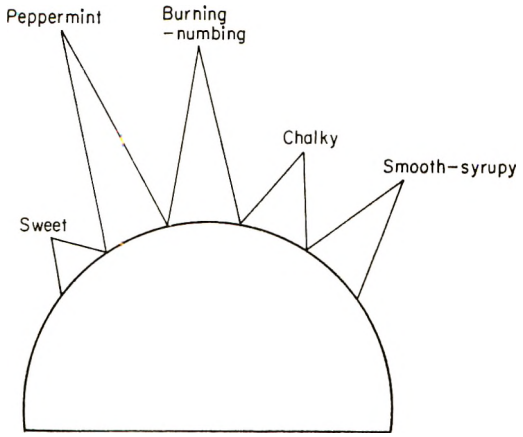

Figure 9. Flavour profile.

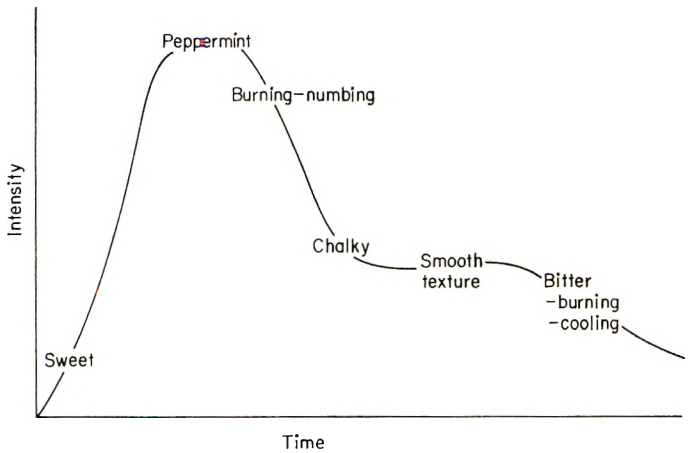

Figure 10

Despite the use of numerical intensity ratings the results of flavour profile analysis remain largely qualitative and difficult to use in even the simple case of variation in the level of one or two flavour components. A simpler form of the test is required.

Simple flavour description panels

In the simplified version a larger panel is used, consisting of about 30 members who answer specific questions about flavour sensations. The

questionnaire can be based on the results of previous flavour profile analysis of the toothpastes to be tested. The same intensity rating scale is used or some similar five-point scale, possibly of the form

0	1	2	3	4
Absent	Slight	Moderate	Appreciable	Great

The number of members on the panel is some multiple of the number of creams to be tested and preferably in the region of 30. Using random number tables allocations of test cream to individual panel members are worked out such that equal numbers of the panel use each cream on the first brushing and the process is repeated for all brushings until all panel members will brush once with each cream. The panel is then run and intensity ratings recorded. An example of a typical questionnaire is shown in *Fig. 11*.

Rating	None	Slight	Moderate	Appreciable	Very
	0	1	2	3	4
Flavour strength					
Tingling					
Warming					
Sweetness					
Freshness					
Drying					
Bitterness					
Lasting flavour					

Figure 11

Analysis of the results of this type of panel is straightforward. The panel design is that of a completely randomized block and can be subjected very easily to analysis of variance (12). This analysis is carried out separately for each question and, for the example of six creams tested by a panel of 30, will give an analysis of variance table as follows/

Source of variation	Sum of squares	Degrees of freedom	Mean square	*F*
Between toothpastes	.....	5	.....	..
Between panel members	.....	29	.....	..
Error	.....	145	.....	
Total	.....	179		

The removal of the variation due to systematic differences in rating between panel members appreciably reduces the error sum of squares and hence increases the sensitivity of the test. Comparison of the between

toothpastes mean square with the error mean square using the *F*-test will show whether any significant differences in mean ratings exist among the creams. The Duncan multiple range test can be used to identify these individual differences (13). Although the use of a limited and discrete rating scale would seem to require that statistical analysis be carried out by non-parametric methods, the classical analysis of variance is robust enough to give reliable results, particularly as the toothpaste ratings of interest are the means of 30 readings.

The method is best illustrated by taking a concrete example. A major component of a toothpaste had been altered and this had resulted in a definite change in the perceived flavour of the modified product. It was felt that the simplest way of tackling the problem would be to alter the flavour level. Five different levels of flavour in the modified product were tested against the original, giving a total of six creams. A panel of 30 was used. The only two questions to show a significant 'between creams' difference were 'bitterness' and 'flavour strength'.

If only one question had shown these differences the solution would have been straightforward. Mean ratings would have been plotted against flavour level and the match to the original product read off from this line. In the example, however, it was necessary to plot 'bitterness' ratings against 'flavour strength' ratings. *Fig. 12* shows the results of this. The points corresponding to the five flavour levels in the modified cream fall, gratifyingly, in

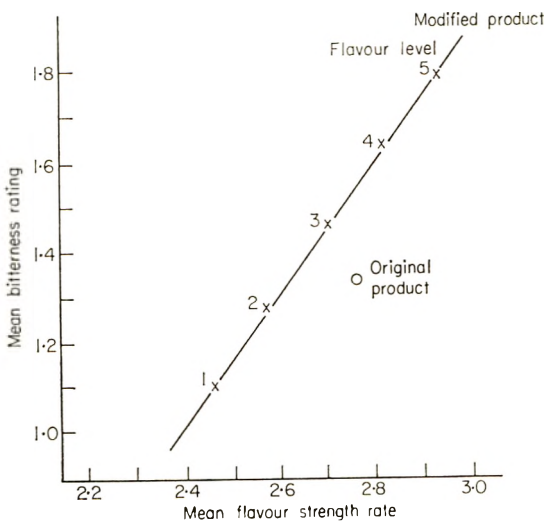

Figure 12

a straight line with the point for the original product falling somewhat below and to the right. The original product is clearly less bitter than the modified product having the same apparent flavour strength. The nearest point on the modified product line to the original point was taken as a suitable match and when that flavour level was substituted into the modified product consumer testing showed that the match had been successful.

In the example above a very simple experimental design had been used for the test creams, i.e. five levels of one variable to be compared with a standard. More complicated designs can be very easily incorporated into this type of test. For example, it may be desired to test two levels of each of three flavour components. This would mean that eight experimental creams, corresponding to all the combinations of components and levels, would have to be tested against the standard giving nine creams in all. A panel of 27 would be enough for this test and the initial analysis of variance table would have the following form:

Source of variation	Sum of squares	Degrees of freedom	Mean square	*F*
Between toothpastes	.....	8	.....	...
Between panel members	.....	26	.....	....
Error	.....	208	.....	
Total	.....	242		

In order to explore the effects of the different levels of the three flavour components the between experimental toothpastes sum of squares can be subdivided to give the analysis of variance table shown below.

Source of variation	Sum of squares	Degrees of freedom	Mean square	*F*
Between levels of component 1	.....	1	.....	....
Between levels of component 2	.....	1	.....	....
Between levels of component 3	.....	1	.....	.....
Interactions:				
Component 1 × Component 2	.....	1	.....	.....
Component 1 × Component 3	.....	1	.....	.....
Component 2 × Component 3	.....	1	.....	.....
Components 1 × 2 × 3	.....	1	.....	.....
Error (from previous table)	.....	208	.....	

This analysis is confined to the eight experimental creams, hence the total number of degrees of freedom of seven. *F*-tests will show which flavour components or combinations of components had a significant effect on the attribute analysed. The mean ratings corresponding to the significant effects can then be compared with that of the ninth cream, the

standard, using the Duncan multiple range test described above. A nearest match to the standard can easily be worked out on this basis.

Extending the method further, even more complicated experimental designs can be used for the toothpastes, such as Simplex or star designs (14). Regression analysis can be used to fit equations relating the main rating scores to all the variable flavour component levels. The fitting of such regression equations will be valid only if a significant effect between toothpastes has been shown by the initial analysis of variance. Linear programming can be used to determine the nearest match to a standard over all the relevant flavour questions although this will only work if all the regression equations are first order, i.e. correspond to straight lines, planes or hyperplanes. Curvature due to higher order terms leads to complications and the best method of choosing a match in this case is by inspection.

When large experimental designs are used it will be necessary to test a great number of creams. It will no longer be possible to use a complete randomized block as the basic design for the panel. This can, however, be overcome by the use of incomplete block or lattice designs in which each panel member need use only a few out of the many experimental creams, and the duration of the panel reduced once more to more manageable proportions (14).

It is quite possible for any member of a simplified flavour description panel to clean his teeth five or six times a day without impairing his ability to judge flavour notes and intensities. An average panel can, therefore, be completed in three or four days. Should any panellist drop out of the test it is quite permissible to estimate his missing ratings using the recognized method of calculating missing values for the particular panel design used (12).

The simplified flavour description panel is, therefore, quick and easy to run. Analysis of the results can be as complicated as desired and depends only on the experimental design chosen for the test creams.

Flavour acceptability tests

Flavour acceptability is more difficult to test than flavour description. The test method described above can, however, be adapted for this purpose by modifying the questionnaire and ensuring that panels are larger and more representative of the toothpaste-using population. Usually only one

question will need to be asked, i.e. for flavour preference, and a rating scale can be used of the following form:

0	1	2	3	4
Very poor	Poor	Average	Good	Very good

Other flavour description questions can be asked if it is wished to consider the influence of particular flavour sensations on acceptability.

Because of the obvious variability of flavour likes and dislikes over the population, panels used for acceptability testing must be large and as representative of that population as possible. Panel size should be at least 50 and local panels confined to a single factory or workplace would seem to be unsuitable because unrepresentative, although they could be used for a first rough screening of flavours for acceptability.

Experimental design can be built into the testing by using different levels of a range of flavour components. Regression analysis can again be used and linear programming or inspection used to detect flavour combinations with optimum flavour preference ratings, the object of the testing being no longer to match a standard, but to optimize flavour preference.

Conclusions

The test procedures described above are intended to enable flavour evaluation, particularly description, to be carried out in an orderly and scientific manner. Emphasis has been placed on the generation of numerical results which can be subjected to statistical analysis. The numerical prescriptions applied to flavour acceptability have been deliberately left somewhat vague. In this area the ideas for new flavours will usually come from a creative flavour specialist although panel testing will be necessary to judge the success of his efforts.

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REFERENCES

(1) Fruchter, B. *Introduction to factor analysis* (1954) (Van Nostrand, Princeton).
(2) Moncrieff, R. W. 'Gustation', Food Mfr, June, 1944, p. 203.
(3) *A.S.T.M. Publication S.T.P.* 433. 'Basic principles of sensory evaluation' (1968).
(4) Moncrieff, R. W. 'The tastes', *Perfum. Essent. Oil Rev.* (Oct 1950 to Feb. 1951).
(5) Harris, H. *Introduction to human biochemical genetics* (1955) (Cambridge, London).
(6) Wells, F. V. 'Duration of odorants on the skin'. *S.P.C. Year Book*, 107 (1966) (United Trade Press, London).

(7) Pfaffman, C. 'Symposium on food acceptance testing methodology'. *Quartermaster Food Container Inst.* 6 (Oct. 1954).
(8) Perryman, D. R. and Swartz, V. W. 'Measurement of sensory difference'. *Food Technol.* **5** 207 (1950).
(9) Cairncross, S. E. and Sjostrom, B. L. 'Flavour profiles—a new approach to flavour problems'. *Food Technol.* 308 (Aug. 1950).
(10 McBurney, D. H. 'Are there primary tastes for man?' *Chem. Senses Flavour* **1** 17 (1974).
(11) Jolson, M. A. and Rossow, G. L. 'The Delphi process in marketing decision making'. *J Market. Res.* VIII 443 (1971).
(12) Cochran, W. G. and Cox, G. M. *Experimental designs* (1950) (Wiley, New York).
(13) *A.S.T.M. Publication S.T P.* 434. 'Manual of sensory testing methods (1968).
(14) Davies, O. L. *Design and analysis of industrial experiments* (1954) (Oliver and Boyd).

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The chemistry of human hair cuticle—III: The isolation and amino acid analysis of various subfractions of the cuticle obtained by pronase and trypsin digestion

J. A. SWIFT and B. BEWS*

Synopsis—The course of digestion of human HAIR CUTICLE by PRONASE and TRYPSIN has been followed both gravimetrically and by the ELECTRON MICROSCOPE examination of digested hair sections. This has permitted the identification of three subfractions of the hair endocuticle. The significance of the AMINO ACID analyses is discussed.

Introduction

In the first two papers of this series we described a new technique for the physical isolation of cuticle from human hair (1) and the enzymatic isolation of the A-layer and cell membrane complex from the cuticle (2). Our attention has now turned to an examination of those cuticular components dissolved by the proteolytic enzymes trypsin and pronase. Although Bradbury and Ley (3) have shown already that pronase can be used for the isolation of whole endocuticle from wool, the present experiments are more comprehensive and have enabled us to

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identify and isolate for analysis new and interesting subcomponents of the endocuticle of human hair.

Methods and materials

The experimental procedures were broadly the same as those described in the previous paper (2), namely (a) a study of the gravimetric course of digestion of isolated cuticle by pronase and trypsin, (b) the electron microscope examination of human hair sections after digestion with the enzymes to establish which morphological components are dissolved, and (c) the amino acid analysis of various identifiable fractions isolated from the bulk cuticle.

The enzyme solutions used were 0.1% w/v pronase (Calbiochem grade B) and 0.1% w/v trypsin (Sigma, 2 × crystallized) in 0.1M ammonium acetate/ammonia buffer at pH 7.8 containing 10% w/v ethanol (the ethanol was used to minimize bacterial growth and also to facilitate the wetting of the samples to be treated (3)). The corresponding buffer at pH 7.8 without the added enzymes was used for washing the various insoluble fractions and hair sections. The insoluble fractions were obtained as described earlier (2) and the soluble fractions by evaporation *in vacuo* to small bulk followed by freeze drying.

The heavy metal stains used for treating the enzyme-digested hair sections were different from those used in the previous paper. They were (a) 0.1N silver nitrate to which 0.880 ammonia was carefully added until the precipitate just dissolved; grid-mounted sections were treated for 30 m at room temperature, followed by 2 × 15 m immersions in distilled water and then dried, and (b) 10% w/v dodecatungstophosphoric acid (PTA) (BDH Analar grade) in 50% w/v ethanol filtered through 0.45 μm Millipore membrane; grid-mounted sections were treated for 2 h at 40°C followed by a 20-s rinse in 50% ethanol and rapid drying of the grid on the corner of a filter paper.

Results

Course of digestion of isolated cuticle with trypsin and pronase

A graph showing the loss in weight of hair cuticle against time of digestion with trypsin and pronase is shown in *Figure 1*. The rate of digestion with trypsin was fairly rapid for the first 5 h and then the rate slowed down so that between 10 and 60 h it was about 1/12 of the initial rate. No further digestion occurred after 70 h by which time 20% of the cuticle had dissolved. It is clear that the two distinguishable rates of digestion arise through the dissolution of two separate components of the cuticle. By extrapolating the linear portion of the curve between 10 and 60 h, it was established that the fast-dissolving component constitutes

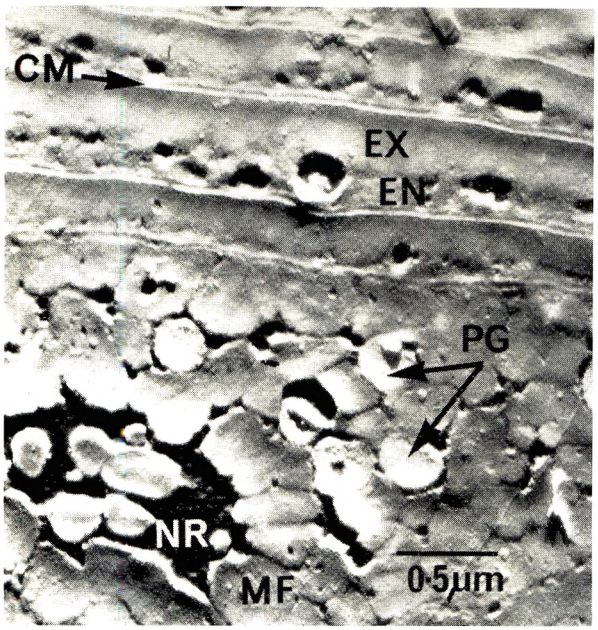

Figure 2. Transverse section of human hair digested with trypsin for 5 h and then shadowed with carbon and platinum. EX, exocuticle; EN, endocuticle; CM, cell membrane complex; NR, nuclear remnant of cortex; PG, melanin pigment granule; MF, macrofibril. NB. This photograph was prepared from an intermediate negative.

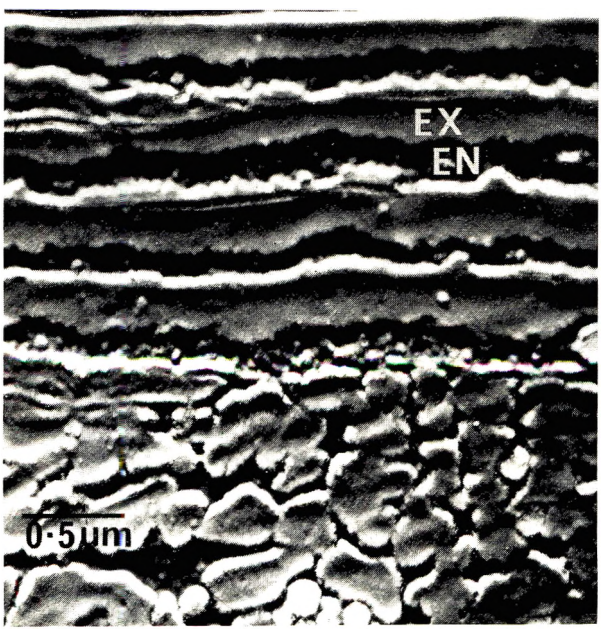

Figure 3. Transverse section of human hair digested with pronase for 30 h and then shadowed. (*Facing p.* 290)

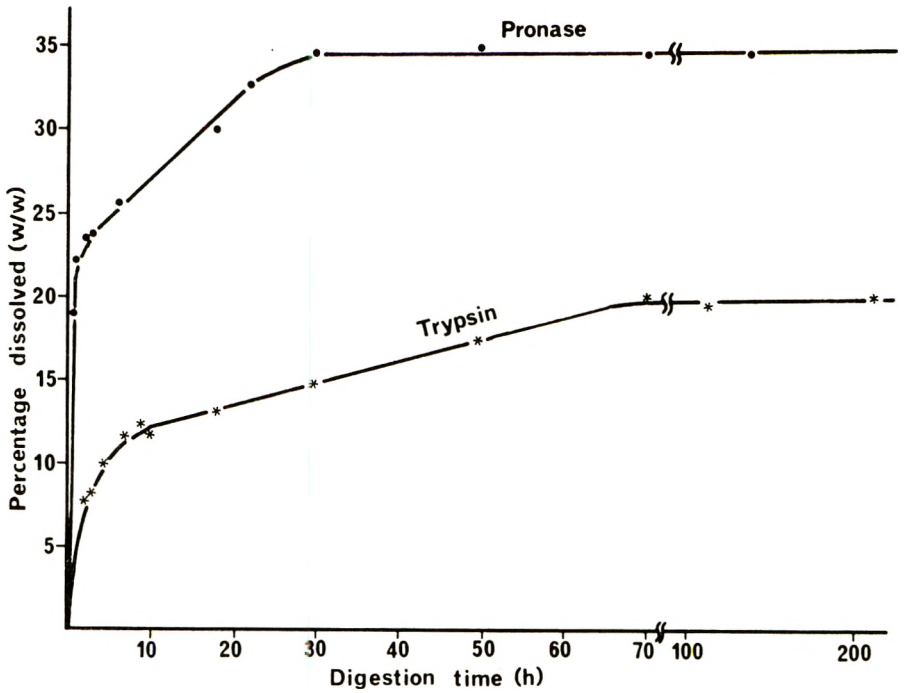

Figure 1. Graph showing the gravimetric course of digestion of human hair cuticle with trypsin and with pronase. Each point shown represents the mean of two separate determinations.

about 11% and that the slower dissolving component comprises about 9% by weight of the whole cuticle.

In the case of the pronase treatment the rate of digestion within the first hour was very high and in that time some 22½% of cuticle dissolved. Thereafter the rate of digestion diminished abruptly to a linear portion between 2½ and 22½ h. Beyond 30 h no further digestion occurred and in all 35% of the cuticle had dissolved. The fast- and slow-dissolving components represent approximately 22½% and 12½% by weight of the cuticle respectively.

Morphological progress of digestion of cuticle with trypsin and pronase

An electron microscope examination was made of transverse hair sections treated with trypsin for 5 h and 70 h and then 'shadowed' with a mixture of carbon and platinum. After 5 h the regions corresponding to the endocuticle contained discrete holes measuring 0.05–0.1 μm in diameter (*Fig. 2*), and after 70 h larger holes were observed in the endocuticle. Often these latter holes were long and

narrow as if a discrete laminar subcomponent of the endocuticle had been dissolved. It is also pertinent to mention that after trypsin treatment for 70 h the nuclear remnants of the cortex were completely dissolved.

For the shadowed pronase-treated sections it was found that the endocuticle was peppered with holes at 2 h, and after 30 h *(Fig. 3)* the endocuticle was completely dissolved. In addition at 2 h the nuclear remnants of the cortex were removed completely and after 30 h it was clear that the 'non-keratinous' material of the intermacrofibrillar matrix had also been degraded *(Fig. 3)*.

The electron microscope examination of hair sections stained with either silver or tungsten was valuable for determining in detail which cuticular structures had been affected by the enzymes. The results were generally in accord with those obtained from the shadowed sections and in addition indicated that the inner layer, exocuticle, A-layer and cell membrane complex were unaffected by the enzyme treatments (*cf. Figs 4–7*). In the case of the extensive pronase treatments it was found that a thin network of material remained in the endocuticular regions (*Figs 6 and 7*). Since this latter material stained fairly intensely with silver (*Fig. 7*) it was assumed to be relatively rich in cystine and that it was part of the exocuticle which diffusely permeates the endocuticle. It is noteworthy that despite extensive treatments with the enzymes the intercellular cement of the cuticle (δ-band) was stained as intensely with PTA (*Figs 5 and 6*) as the sections which had not been treated with the enzymes (*cf.* Ref. 2).

Amino acid analyses

The amino acid analyses for some of the fractions isolated by trypsin and pronase digestion of whole cuticle are listed in *Table I*. The first column contains the amino acid analysis of whole human hair cuticle. The fractions listed in the next six columns of *Table I* were obtained as follows:

ENDO — Soluble fraction obtained by digestion of whole cuticle for 5 days with pronase.

EXO+A+I+M — Insoluble material remaining after pronase digestion for 5 days.

ENDO A — Soluble fraction obtained by trypsin digestion for 10 days.

ENDO A1 — Soluble fraction obtained by trypsin digestion for 9 h.

ENDO A2 — Soluble fraction obtained by taking the insoluble material remaining after trypsin treatment for 11 h and digesting it with trypsin for a further 10 days.

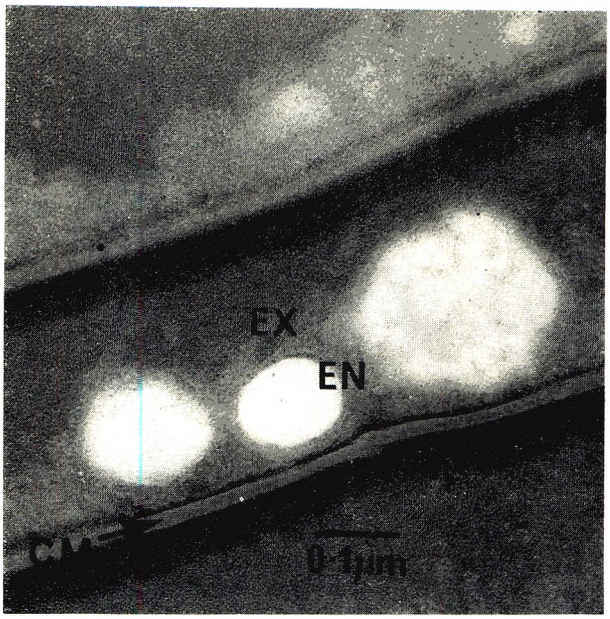

Figure 4. Transverse hair section digested with trypsin for 5 h and then stained with ammoniacal silver nitrate; A, A-layer.

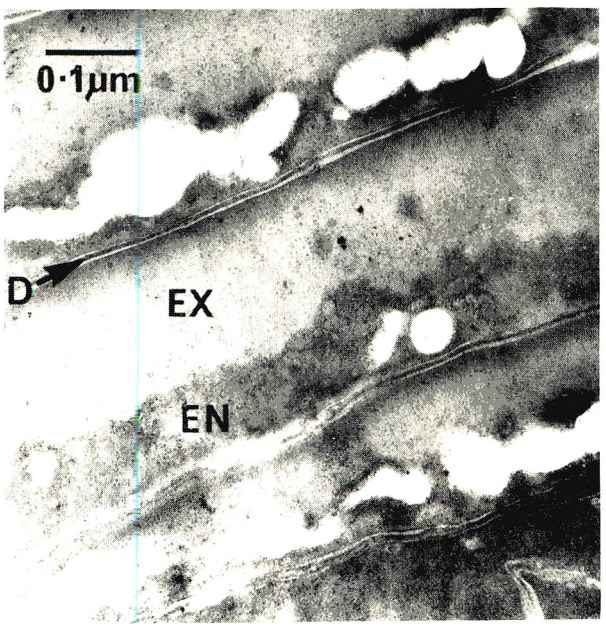

Figure 5. Transverse hair section digested with trypsin for 70 h and then stained with dodecatungstophosphoric acid (PTA). D, intercellular cement of δ-band.

(*Facing p.* 292)

Table I. Amino acid compositions of whole cuticle and various fractions obtained in the present work. Also included for comparison are other published analyses for wool cuticle. All analyses are in residues of each amino acid/1000 amino acid residues of total protein in each fraction.

	Present work							Wool analyses (Ref. 3)		
Amino acid	Whole cuticle	ENDO	EXO+A +I+M	ENDO A	ENDO A1	ENDO A2	ENDO B	Whole cuticle	ENDO	EXO
Aspartic acid	32.4	93.3	17.9	95.3	128.3	44.1	56.9	34.6	74.1	20.6
Glutamic acid	89.2	123.3	79.7	134.3	154.6	96.6	115.2	86.7	103.1	85.6
Threonine	46.1	58.7	41.9	55.0	51.7	54.2	52.0	44.4	55.4	38.9
Serine	160.7	102.9	170.0	84.3	68.2	125.5	130.7	143.4	107.0	118.5
Proline	105.4	73.0	108.0	59.7	13.6	59.3	79.1	105.1	89.2	123.6
Glycine	88.4	68.6	90.1	79.6	63.6	110.2	65.0	81.7	81.5	86.5
Alanine	54.0	59.3	45.6	80.8	71.7	73.8	65.3	57.8	66.5	64.4
Valine	72.7	64.8	71.7	69.9	57.8	69.7	75.3	75.1	74.7	81.6
Isoleucine	22.3	40.8	10.4	42.4	44.8	47.6	41.0	26.7	39.4	28.8
Leucine	44.6	86.5	26.1	90.5	101.1	68.7	78.0	61.2	93.1	45.7
½ Cystine + Cysteic Acid	180.8	56.9	250.8	18.3	6.5	37.3	94.5	156.3	31.0	199.5
Methionine	4.6	14.9	2.6	23.1	12.6	17.9	13.6	3.4	8.1	1.6
Tyrosine	21.1	33.9	16.6	32.9	40.8	34.3	30.4	28.3	35.8	19.6
Phenylalanine	11.7	23.8	7.7	31.4	40.0	15.6	20.9	16.9	38.5	11.6
Histidine	5.2	10.0	3.6	10.6	12.7	18.7	9.2	8.1	10.7	5.2
Lysine	34.4	41.2	34.6	42.9	47.0	51.6	38.1	27.4	42.1	20.6
Arginine	26.4	48.0	22.4	49.0	84.8	24.7	34.8	42.9	49.8	47.7
% Total amino acid in fraction	86.8	70.9	77.2	66.8	97.2	73.0	70.7	43.0	30.0	77.0
% of fraction in whole cuticle	100.0	34.5	65.2	21.0	10.9	10.6	14.2	100.00		

ENDO B — Soluble fraction obtained by taking the insoluble material remaining after trypsin treatments for 10 days and digesting it in pronase for 5 days.

NB. In the case of all the soluble fractions appropriate corrections were made to the amino acid analyses for the contributions due to the presence of the enzymes.

All the data listed in columns 1–7 of *Table I* were obtained from duplicate amino acid analyses of the various fractions. Although not listed, analyses were also obtained for all the corresponding insoluble fractions and of soluble material obtained by digesting with pronase for 5 days the insoluble material remaining after trypsin treatment for 15 h. It is worthy of mention that the analyses of the soluble fractions inferred by taking the appropriate differences in analysis of these additional fractions were in general agreement with the more accurate direct analyses for the corresponding fractions shown in *Table I*.

In the last three columns of *Table I* are reproduced for comparative purposes the analyses for various fractions obtained by Bradbury and Ley (3) in their work on the pronase digestion of wool cuticle.

Discussion

Since several distinct subfractions of the human hair cuticle have been identified in the present work it is convenient to subdivide most of this discussion according to the morphological origins of the subfractions.

(a) *Whole endocuticle (ENDO)*

From the electron microscope examinations of hair sections treated with pronase for 5 days it was clear that virtually all the endocuticle had dissolved and that no other cuticular components had been degraded. Only sparse material remained in the endocuticular regions and since this appeared to contain cystine at least some of it may be diffuse exocuticle. It could also contain some of the non-proteinaceous components derived from the membranous organelles of the original follicle cells, such as endoplasmic reticulum, Golgi apparatus, mitochondria etc. which are compressed into this part of the cell during the keratinization process.

It is striking that the percentage by weight of cuticle which dissolves in pronase after 5 days (i.e. 35%) is similar to the percentage of endocuticle in whole cuticle determined from a large number of measurements of the cross-sectional areas occupied by the same components in electron micrographs of transverse sections of untreated human hair (i.e. 33–35%).

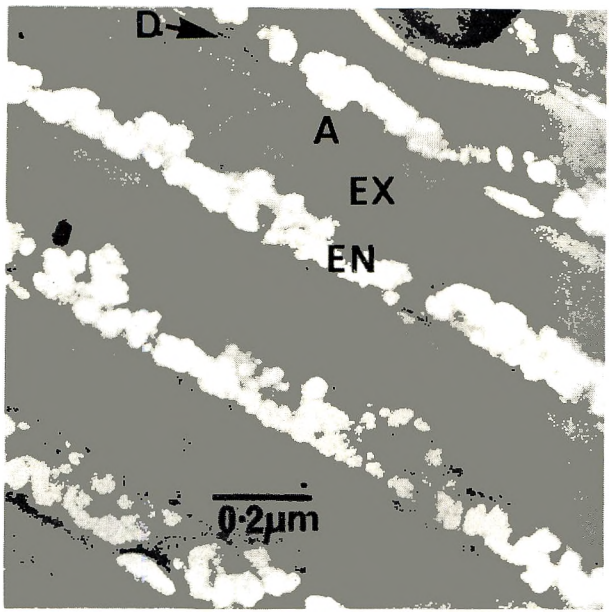

Figure 6. Transverse hair section digested with pronase for 100 h and then stained with PTA.

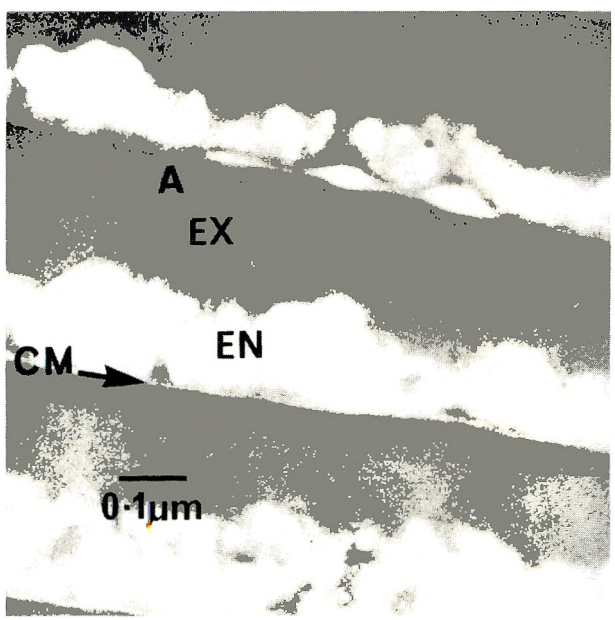

Figure 7. As Fig. 5 but stained with ammoniacal silver nitrate.

(*Facing p.* 294)

In the light of the foregoing results we consider that the amino acid analysis of the material from whole cuticle which dissolves after 5 days treatment with pronase is representative of the proteins in the whole endocuticle. This analysis is designated ENDO in *Table I*. The endocuticle evidently contains more aspartic acid, glutamic acid, threonine, alanine, isoleucine, leucine, methionine, tyrosine, phenylalanine, histidine, lysine and arginine and less serine, proline, glycine, valine and cystine + cysteic acid than whole cuticle. This is in very good agreement with the analyses made by Bradbury and Ley (3) for the corresponding components isolated from wool cuticle with pronase (*cf.* columns 8 and 9 of *Table I*). By comparing the analysis for the endocuticle not only with that of whole cuticle but also with that of the exocuticle (*cf.* column 3 and the next section of the discussion) it is evident that the major proportions of the acidic and basic amino acid residues in the cuticle are contained within the endocuticle. This is in accord with electron histochemical observations concerning the distribution of acidic and basic groups within the cuticle (1). That the amount of cystine in the endocuticle is quite small is also in agreement with electron histochemical observations (4, 5).

(b) *Exocuticle + A-layer + inner layer + cell membrane complex (EXO + A + I + M)*

We have established that treatment of whole cuticle with pronase for 5 days dissolves the major portion of the endocuticle perhaps leaving some cystine-rich exocuticular-like material and cytoplasmic lipid debris in the endocuticle region. It follows that the material remaining after pronase digestion will comprise the inner-layer, exocuticle, A-layer and cuticle cell membrane complex. Bradbury and Ley (3) have isolated a similar fraction from wool cuticle with pronase and although the exocuticle will certainly comprise the major proportion of this fraction, A-layer and membrane components will also be present.

Our analysis for the long term pronase-insoluble material is shown in column 3 of *Table I*. As would be expected, the analysis is complementary to that of the whole endocuticle in that it contains more serine, proline, glycine, cystine + cysteic acid and less aspartic acid, glutamic acid, threonine, alanine, valine, isoleucine, leucine, methionine, tyrosine, phenylanaline, histidine, and arginine than whole cuticle. There is excellent agreement between these results and those of Bradbury and Ley (3) for the comparable exocuticle-rich fraction isolated from wool cuticle (*cf.* columns 8 and 10 of *Table I*). That the exocuticle-rich fraction contains high concentrations of cystine and lower concentrations of the acidic and basic amino acids relative to the endocuticle is also consistent with electron histochemical observations.

Of particular interest is the very high cystine concentration in the long term pronase-insoluble fraction in which ½ cystine occupies 1 in 4 of the amino acids of

the protein. Taking into account the fact that the cystine concentration of the cuticle cell membrane complex is quite low (2), we have calculated that the order of 1 amino acid residue in 3.7 is present as $\frac{1}{2}$ cystine in the combined exocuticle. and A-layer. In our previous paper (2) we also presented evidence for suspecting that the cystine of the A-layer may not be as high as electron histochemical observations had led us to believe. In this case it is interesting to speculate that there might therefore be as much as 1 amino acid residue in 3.3 as $\frac{1}{2}$ cystine in the proteins of exocuticle and inner-layer. Notwithstanding this latter speculation it is clear that the exocuticle, A-layer and inner-layer as a whole are remarkable for their high cystine content.

(c) *Endocuticle fraction A*

In the neck of the hair follicle those cells derived from the follicle matrix which will eventually be transformed into cuticle are compressed into a sheet-like form; at this stage the cells contain recognizable nuclei that are compressed into disc-like units within each cell. During the subsequent hardening the cystine-rich exocuticle and A-layer are laid down in laminae at one side of the cell (i.e. that side of the cell at the greater radius from the fibre axis) and the nucleus is further compressed along with other effete cell organelles into that part of the cell later identified as the endocuticle. No distinct entity corresponding to this effete cuticle nucleus can be identified under the electron microscope in metal-stained sections of untreated hair, although recently Kassenbeck (6) has reported that he has observed under the optical microscope entities corresponding to this effete nucleus in the cuticle of hair treated with *p*-toluene sulphonic acid in ethylene glycol.

About 20% by weight of human hair cuticle dissolved after long term trypsin treatment, and from electron microscope observations it was established that this material was derived from the endocuticle. Since the zones of digestion were usually sheet-like in form we therefore believe that it was the effete nucleus of the cuticle which had been dissolved. This fraction comprises about 57% by weight of the whole endocuticle and the amino acid analysis for it is shown under the title ENDO A in *Table I.* It evidently contains significantly more glycine, alanine, leucine, methionine and phenylalanine and less serine, proline and cystine + cysteic acid than the whole endocuticle.

Since the proportion by weight of cuticle which dissolves after long-term trypsin treatment (*c.* 20%) is similar to that proportion which dissolves very rapidly within the first hour of pronase treatment (*c.* 22%), we conclude that both these enzymes under their respective conditions are dissolving the same component, i.e. the endocuticle nucleus. The extreme rapidity of the pronase digestion precluded the possibility of obtaining a good corresponding sample for analysis,

but insoluble material remaining after long term trypsin digestion was almost identical in composition to that remaining after 2 h digestion with pronase.

(d) *Endocuticle subfractions A1 and A2*

From our study of the gravimetric course of digestion of whole cuticle with trypsin, two components dissolving at different rates were identified. Since we believe that long term trypsin treatment dissolves the effete cuticle nucleus, it follows that the two new fractions identified are subcomponents of this nucleus. That this is so is in agreement with the morphologic progression of trypsin digestion observed with the electron microscope. The component of the cuticle which dissolves in trypsin after 9 h and that which dissolves after treating with trypsin for a further 10 days have been designated endocuticle A1 and A2 respectively (i.e. ENDO A1 and A2). The amino acid analyses of these two subcomponents of the nucleus are shown in columns 5 and 6 of *Table I*. As would be expected the analyses of A1 and A2 are complementary with respect to the whole ENDO A fraction. In particular A1 contains significantly higher concentrations of aspartic acid, glutamic acid, leucine, tyrosine, phenylalanine and arginine and lower concentrations of serine, proline, glycine, valine and cystine + cysteic acid than A2. It is also worth pointing out that the analysis for A1 is most extreme from that of whole cuticle.

The nuclei of normal viable mammalian cells contain two major types of protein, namely the histone proteins generally characterized by their high basic amino acid content (7), and the non-histone proteins which are relatively rich in acidic amino acids (8). It is difficult to imagine what changes occur to the corresponding proteins from the nuclei of the matrix cells of the hair follicle during the keratinization process and certainly their ultimate fate following loss of the nucleic acids of these cells is not known. Perhaps the two fractions A1 and A2 originate from the histone and non-histone proteins of the cuticle cell nucleus modified during the keratinization process.

(e) *Endocuticle fraction B (ENDO B)*

From the foregoing it is clear that there is a further fraction of the endocuticle which is not digested by long-term trypsin treatments but which does dissolve in pronase. This component comprises about 15% by weight of whole cuticle and about 43% by weight of the endocuticle. The amino acid analysis for this fraction, which we have designated ENDO B, is shown in column 7 of *Table I*. It is clear from comparison with the ENDO A fraction that ENDO B contains the bulk of the cystine present in the whole endocuticle and that the concentration of aspartic acid is lower.

It follows from the foregoing proposals about the origin of ENDO A and its subfractions A1 and A2, that ENDO B probably originates from the non-nuclear cytoplasmic debris of the cuticle cell left over after the formation of the exocuticle, A- and inner-layers.

(f) *Consideration on the properties and nature of the cuticle cell membrane complex*

It has been known for some time that single cuticle and cortical cells are apparently liberated into suspension when keratin fibres are treated with solutions of various proteolytic enzymes and it has been generally assumed that this is because the cell membrane complex separating the cells has been degraded (*cf.* review by Bradbury (9)). From our critical electron microscope examinations of hair sections, treated not only with pronase and trypsin but also with papain/dithiothreitol (2), we can find no evidence whatsoever for the dissolution of the cuticle cell membrane complex. Even despite extensive enzyme treatments, the full structural integrity and pattern of heavy metal staining of the intercellular membrane glue (δ-band) is retained. We therefore believe that the liberation of cuticle cell-like units in the proteolytic enzyme treatment of bulk mammalian keratin fibres is due to digestion along the endocuticle sheet rather than splitting of the cell membrane complex. Further evidence for believing that this is so is that mild mechanical agitation is always necessary to release the cell-like units presumably by rupturing the retaining cell membrane which now only loosely holds the remaining exocuticular segment. A similar situation may also exist in relation to the liberation of cortical cells by proteolytic enzymes with digestion occurring in the intermacrofibrillar matrix, and particularly in that matrix immediately adjacent to the cortical cell membranes, as a necessary prerequisite for subsequent mild mechanical agitation to release cell-like units.

The chemical composition of the intercellular membrane cement of the cuticle (δ-band) is puzzling. Electron histochemical observations using not only phosphotungstic acid as a stain but also silver/N-acetyl homocysteine thiolactone (10) indicate that this component is rich in free amino groups. If these amino groups were either those of the lysyl groups or the end groups of proteins it is surprising that proteolytic enzymes had no effect on the δ-band. One possibility is that the protein content of the δ-band is small and that the major component is a polysaccharide rich in amino-containing sugar residues. Polysaccharides containing *vic*-diol groupings are certainly present in the cell coat around hair follicle matrix cells and in the intercellular desmosomal plaques between adjacent follicle cells (17). It is not unreasonable to suppose that the polysaccharide coat of the hair follicle cells is retained throughout the keratinization process and, in the case of the cuticle, becomes organized into the layer later identified as the δ-band. Some support for this idea also comes from the fact that we have tentatively identified

amino sugars and pentoses in a cuticle cell membrane fraction, rich in δ-band, obtained by our papain/dithiothreitol procedures (2). Also this fraction had a very low protein content.

(g) *Consideration of the properties of the cuticle in the light of the composition of the cuticle subcomponents*

The hair cuticle is divided into two main subcomponents whose chemical compositions are quite different and which can be expected to behave differently under the influence of various cosmetic treatments.

The exocuticle and A-layer laminae are oriented within each cuticle cell sheet towards the outside of the hair. Since they are highly cross-linked by cystine they will be extremely tough and resilient and fitting in their role for protecting the surface of the hair shaft from environmental exposure. On the other hand these components are likely to be relatively inelastic and susceptible to brittle fracture on bending. An indication of this brittleness to bending is that we have never observed with the scanning electron microscope cuticle scale edges bent back on themselves at the surface of virgin hair. Indeed where the edge of a cuticle cell is not adhering immediately to the underlying cell, it is usually tilted upwards by only a few degrees. Despite extensive combing either with or against the natural scale overlap the scale edges tend to break off in small pieces rather than be bent back (12). If, on the other hand, the cystine disulphide crosslinks are destroyed by oxidation (as occurs in weathered hair or to some extent on bleaching with hydrogen peroxide) or by reduction (as in thioglycollate perming) the exocuticle and A-layer become much more pliable. This is indicated by the fact that we sometimes see cuticle edges bent back through quite large angles when weathered, bleached or reduced hairs have been combed. It is also noteworthy that Makinson (13) believes that the oxidative softening of the wool cuticle is important in the anti-felting treatments of wool fabrics and it is this which eliminates the directional dependance of the coefficient of friction of the fibres.

In contrast to the exocuticle, the proteins of the endocuticle contain very little cystine but relatively high concentrations of acidic and basic amino acid residues. Electron histochemical experiments indicate the existence of fairly high concentrations of free carboxyl and amino groups in the endocuticle, but it is not possible to determine from these experiments alone what proportions of the groups are the side chains of the acidic or basic amino acid residues or the end groups of the protein chains, though we would expect the latter to be quite a small proportion. It is possible that some of the acidic and basic amino acid residues are involved in intermolecular isopeptide cross-linking (14). On the other hand since the presence of isopeptide links in proteins such as hair medulla tends to confer trypsin insolubility (15, 16) we must presume that the amount of isopeptide link present in our trypsin-soluble ENDO A components of the cuticle is quite small.

The exocuticle and endocuticle can be expected to behave quite differently in

water. The exocuticle, because of its high cross-link density, is unlikely to swell appreciably, but the relative absence of cross-links and the presence of ionic groups in the endocuticle will mean that it will absorb fairly large quantities of water (17). The resultant swelling may even be high enough to convert some of the endocuticular components into a gel. This could have interesting implications on the behaviour of the hair cuticle in the wet state and could perhaps contribute to the directional frictional properties of wet hair where there is a strong tendency towards felting.

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REFERENCES

(1) Swift, J. A. and Bews, B. The chemistry of human hair cuticle—I: A new method for physical isolation of cuticle. *J. Soc. Cosmet. Chem.* **25** 13 (1974).
(2) Swift, J. A. and Bews, B. The chemistry of human hair cuticle—II: The isolation and amino acid analysis of the cell membranes and A-layer. *J. Soc. Cosmet. Chem.* **25** 355 (1974).
(3) Bradbury, J. H. and Ley, K. F. Separation and analysis of exocuticle and endocuticle. *Aust. J. Biol. Sci.* **25** 1235 (1972).
(4) Swift, J. A. The electron histochemistry of cystine-containing proteins in thin transverse sections of human hair. *Jl R. Micros. Soc.* **88** 449 (1967).
(5) Dobb, M. G., Murray, R. and Sikorski, J. Specific labelling of thiol groups in mammalian keratin suitable for electron microscope studies. *J. Microsc.* **96** 285 (1972).
(6) Kassenbeck, K.—private communication.
(7) Stellwagen, R. H. and Cole, R. D. Chromosomal proteins. *Ann. Rev. Biochem.* **38** 951 (1969)
(8) Garrard, W. T., Pearson, W. R., Wake, S. K. and Bonner, J. Stoichiometry of chromatin proteins. *Biochem. Biophys. Res. Commun.* **58** 50 (1974).
(9) Bradbury, J. H. The structure and chemistry of keratin fibres. *Advn. Protein Chem.* **27** 111 (1973).
(10) Swift, J. A. Unpublished observations on the application to human hair of an electron histochemical procedure devised by P. A. Kendall and E. A. Barnard, *Jl R. Microsc. Soc.* **81** 203 (1963).
(11) Orwin, D. F. G. A polysaccharide-containing coat on keratinising cells of the Romney wool follicle. *Aust. J. biol. Sci.* **23** 623 (1970).
(12) Swift, J. A. and Brown, A. C. The critical determination of fine changes in the surface architecture of human hair due to cosmetic treatment. *J. Soc. Cosmet. Chem.* **23** 695 (1972).
(13) Makinson, K. R. The integration of various observations on the mechanisms of 'degradative' anti-felting treatments. *J. Textile Inst.* **64** 228 (1973).
(14) Asquith, R. S., Otterburn, M. S. and Sinclair, W. J. Isopeptide crosslinks—their occurrence and importance in protein structure. *Angew. Chem.* **13** 514 (1974).
(15) Rogers, G. E. Structural and biochemical features of the hair follicle. In: *The epidermis*, eds W. Montagna and W. C. Lobitz, 179 (1964) (Academic Press, New York).
(16) Harding, H. W. J. and Rogers, G. E. The occurrence of the ε-(γ-glutamyl)lysine crosslink in the medulla of hair and quill. *Biochem. Biophys. Acta* **257** 37 (1972).
(17) Leeder, J. D. and Watt, I. C. The stoichiometry of water sorption by proteins. *J. Coll. Interface Sci.* **48** 339 (1974).

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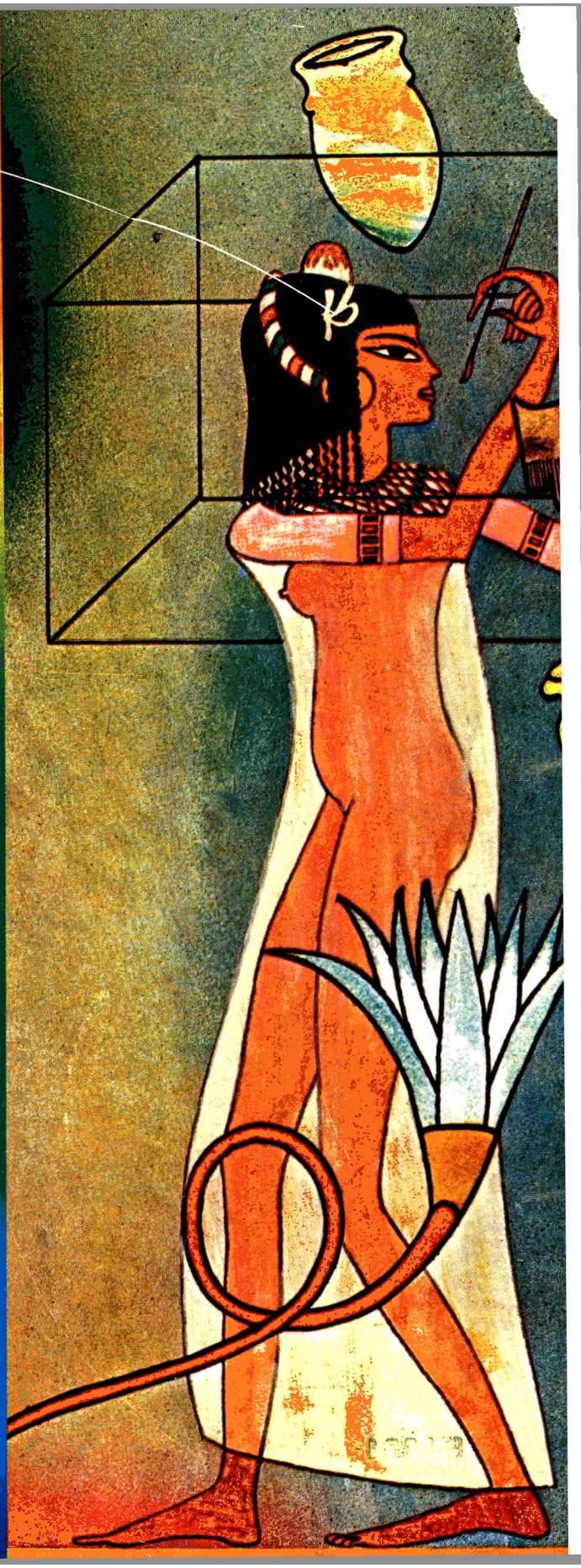

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