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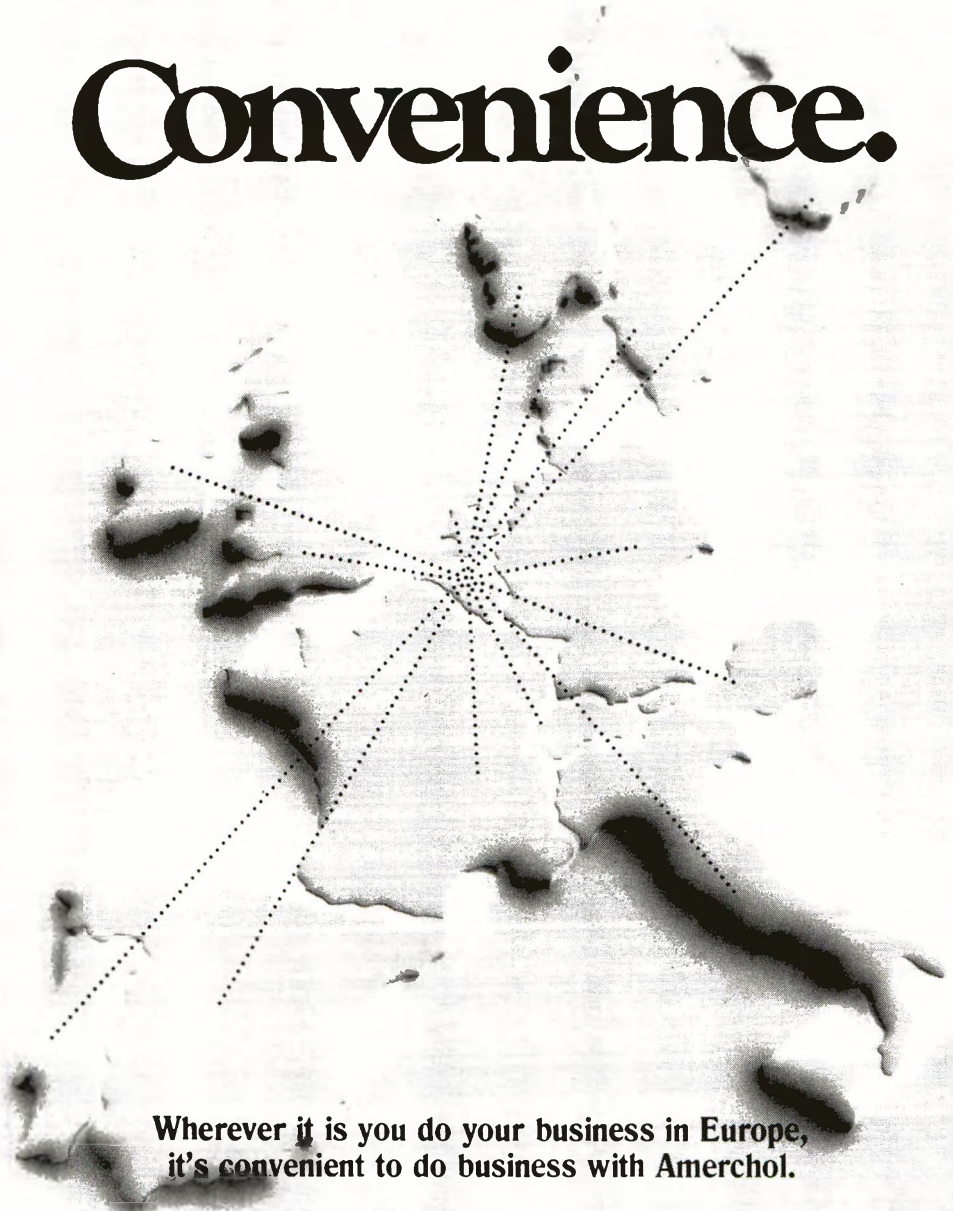

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The following synopses can be cut out and mounted on 127 × 76 mm index cards for reference without mutilating the pages of the Journal.

Substantivity of sunscreens: a study on the interaction of four alkyl 4-aminobenzoates with keratin: F. BOTTARI, E. NANNIPIERI, M. F. SAETTONE, M. F. SERAFINI and D. VITALE. *Journal of the Society of Cosmetic Chemists* **29** 353–363 (1978)

Synopsis—The interaction of four alkyl *p*-aminobenzoates with human intact keratin (HIK), human delipidised keratin (HDK) and animal (wool) keratin (AK) was investigated at 37°C in water and iu a saturated high-molecular weight hydrocarbon (pristane). The study had the purpose of assessing on a quantitative basis the skin substantivity of molecules of possible use as sunscreens. In all cases linear sorption isotherms, indicative of a constant partition of the esters between substrates and solution were obtained: the slopes of the isotherms, K, constitute a measure of the relative 'affinity' of a solute for a given substrate. The study of the time-course sorption of ethyl *p*-aminobenzoate by the three substrates in either solvent evidenced a profound influence on sorption of hydration of HIK. A second parameter characterising the interaction, $Sk = K\ Ss$ (where Ss is the solubility of the interacting substance in the solvent) was also assessed and related to the maximum binding capacity of each substrate for the esters. The influence of solvent, molecular weight and solubility of interacting substance, structure of substrate, etc., on K and Sk are discussed. Possible effects of ad- and absorption by horny layer constituents on skin penetration are also discussed.

Applanation tonometry in the assessment of eye irritation: R. M. WALTON and R. HEYWOOD. *Journal of the Society of Cosmetic Chemists* **29** 365–368 (1978)

Synopsis—The measurement of intraocular pressure has been suggested for the objective assessment of eye irritation. Applanation tonometry is the method of choice. An evaluation of this method has been made in the rabbit using a calibrated Perkins tonometer. Results are presented following the irritation produced by instillation of sodium lauryl sulphate.

Factor analysis in the evaluation of cosmetic products: ERIC BAINES. *Journal of the Society of Cosmetic Chemists* **29** 369–384 (1978)

Synopsis—In the evaluation of most cosmetic products many attributes must be investigated. These attributes are not necessarily independent and product performance can be described more simply in terms of a small number of underlying factors which adequately account for all the measured attributes. Factor analysis provides a mathematical method by which these factors can be identified and their contributions towards each of the attributes calculated. The mathematical basis of factor analysis is explained and the methods available for the extraction of factors described. Once derived the factor matrix can be rotated so that a minimum number of factors is required to specify each attribute. Factor analysis is applied to the results of a series of salon tests carried out on normal, dry and greasy hair shampoos. The seventeen attributes for which the shampoos were rated are reduced to just two orthogonal factors. After rotation these factors are found to correspond to (i) rate of foam build up, and (ii) stability of the first lather. Hair properties are found to be better described by the original foam build up

factor and a second, oblique factor corresponding to ease of rinsing. Factor loadings for the shampoos are calculated and the products mapped into the factor space. Performance is found to be affected most by the type of hair on which the test was carried out. In a second example twelve attributes of toothpaste flavours are reduced to three orthogonal factors corresponding to (i) bitterness–sweetness, (ii) lasting freshness, and (iii) flavour strength–warming. All other attributes can be satisfactorily explained in terms of these three factors.

***In vivo* screening of anti-caries agents:** K. TOMLINSON. *Journal of the Society of Cosmetic Chemists* **29** 385–397 (1978)

Synopsis—A single human mouth was used to investigate the mechanism of caries, or to screen potential prophylactic agents. Iceland Spar was used as a model substrate to predict effects on human dentine. The experiments suggest that real carious attack was reproduced in a human subject.

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Substantivity of sunscreens: a study on the interaction of four alkyl 4-aminobenzoates with keratin

Presented, October 21, 1977, 6th ADRITELF Symposium, Firenze, Italy

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Received 20 *January* 1978

Synopsis

The interaction of four **alkyl-p-aminobenzoates** with **human intact keratin** (HIK), **human delipidised keratin** (HDK) and **animal (wool) keratin** (AK) was investigated at 37°C in water and in a saturated high-molecular weight hydrocarbon (pristane). The study had the purpose of assessing on a quantitative basis the **skin substantivity** of molecules of possible use as sunscreens. In all cases linear **sorption isotherms**, indicative of a constant partition of the esters between substrates and solution were obtained: the slopes of the isotherms, *K*, constitute a measure of the relative 'affinity' of a solute for a given substrate. The study of the time-course sorption of **ethyl** ***p*-aminobenzoate** by the three substrates in either solvent evidenced a profound influence on sorption of hydration of HIK. A second parameter characterising the interaction, $Sk = K\,Ss$ (where *Ss* is the solubility of the interacting substance in the solvent) was also assessed and related to the maximum binding capacity of each substrate for the esters. The influence of solvent, molecular weight and solubility of interacting substance, structure of substrate, etc., on *K* and *Sk* are discussed. Possible effects of ad- and absorption by horny layer constituents on skin penetration are also discussed.

INTRODUCTION

Several cosmetic researchers have recently focused their attention on the loss of efficacy of sunscreens, due to removal from the skin by perspiration and/or swimming. Willis and Kligman (1) classified sunscreens into 'external' and 'reservoir' types, depending on the capacity to diffuse into the horny layer, thus resisting removal. Diffusive properties alone, however, are considered insufficient to guarantee skin-fastness unless accompanied by subtantivity of the molecule, i.e. the capacity to adhere or combine with a keratinised substrate (2). Accordingly, the research in this field is currently oriented towards the development of substantive sunscreens, such as e.g. compounds incorporating in their molecule a sulphonium or a quaternary ammonium ion intended to be bound by negative sites on the epidermal proteins, as well as a uv-absorbing moiety (3).

Whether substantivity may confer detrimental or beneficial properties to a product is a question still open to discussion. An excessive substantivity might result in irreversible binding to constituents of living tissues, thus introducing a distinct risk of toxicity (4).

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On the other hand, if substantivity is accepted as arising from adsorption of the molecule on active sites of the skin barrier, a high substantivity might induce a delayed percutaneous absorption, resulting possibly in a reduced systemic toxicity. Possible effects of keratin binding on the non-steady state processes governing skin permeability have been suggested by Flynn and Roseman (5), in consideration of their results with synthetic membranes.

It should be further pointed out that, although the term 'substantivity' has become of common use, its physico-chemical meaning is still far from being defined precisely (4). The studies hitherto reported of affinity of sunscreens for human skin *in vivo* (1, 6) or animal skin *in vitro* (7), although of practical utility, have shed little light on this point. Thus, it would appear that further investigation on the physico-chemical principles governing the interaction of selected molecules with constituents of the horny layer might be useful either for clarifying the substantivity concept, or for establishing general notions and procedures leading to a more rational formulation of sunscreen compositions.

In the present work, the interaction of four *p*-aminobenzoates (methyl, ethyl, *n*-propyl and *iso*-propyl) with human (skin) and animal (wool) keratin was investigated. The esters were chosen on account of their wide use as effective and safe sunscreens (8). One of them (ethyl *p*-aminobenzoate, benzocaine) is also a well known local anaesthetic. Animal keratin was tested to evaluate its potential usefulness as a substitute for human keratin in binding studies. In order to investigate possible solvent effects, the interaction was carried out both in water and in a high-molecular weight saturated hydrocarbon (pristane). Kinetic data on the interaction of benzocaine with the substrates in both solvents were also collected.

EXPERIMENTAL

MATERIALS

Methyl (Fluka AG, Buchs, SG, Switzerland) and ethyl (E. Merck, Darmstadt, W. Germany) *p*-aminobenzoate were recrystallised from benzene–petroleum ether (m.p., 111–113°C and 88–90°C, respectively). Chloroform (Rudipont–Industria Chimica, Torino, Italy), analytical reagent grade, was used as received. Cosmetic grade 2,6,10,14-tetramethylpentadecane (Croda Italiana Srl, Mortara, Italy) (pristane) was distilled under reduced pressure prior to use; the density of the distillate was 0·771 at 37°C (pycnometer). *iso*-Propyl *p*-aminobenzoate was prepared by refluxing the alcohol and the acid in the presence of gaseous HCl; the product, crystallised twice from benzene–petroleum ether, melted at 85–86° C [Lit. (9) 85–86°C]. The *n*-propyl ester of *p*-aminobenzoic acid, prepared and crystallised in an analogous way, melted at 72–74°C [Lit. (9) 73–74° C]. Defatted animal (wool) keratin (Fluka AG, Buchs, SG, Switzerland) was sieved before use, utilising the fraction that passed through a 70 mesh sieve but was retained by a 100 mesh sieve. The product, dried for 4 days over concentrated H_2SO_4 gave the following analytical results: N, 14·19%; S, 3·10%. The human keratin was in the form of excised human callous tissue, which had not been treated with chemical agents prior to removal. This keratin was pulverised using a liquid air-cooled mill (IKA WERK type A 105 mill, Staufen, W. Germany): after pulverisation the material was sieved as indicated for the animal keratin, and was stored over H_2SO_4 before use. The analytical results of the dried keratin were N, 16·56%; S, 0·40%. Human keratin was delipidised by stirring a suspension of the pulverised material (40 g) in 1500 ml of a 2:1 mixture of chloroform and

methanol for 6 h at room temperature. The delipidised material (31·5 g), dried over H_2SO_4, gave the following analysis: N, 16·13%; S, 0·92%. The weight loss (21·2%) can be attributed either to dehydration and to the loss of total lipids as well as of polypeptides and aminoacids (10).

SPECTROPHOTOMETRIC ANALYSES

The pristane solutions of the esters were appropriately diluted with chloroform (1 : 50–1 : 20 v/v), then were assayed (Zeiss PMQ II Spectrophotometer) at 279 nm against a suitable blank. Water solutions were assayed at 286 nm.

SOLUBILITY DETERMINATIONS

The water solubility of the esters at 37°C was determined as follows. A glass flask containing water (10 ml) together with excess solid ester was kept for 120 h in a thermostatted (37±0·1°C) shaker water bath. A sample was then filtered under isothermal conditions using a glass syringe fitted with a Millipore (Millipore S.p.A., Milano, Italy) filter holder and filter (HA type), and was assayed after suitable dilution. A similar procedure was followed for determining the solubility of the esters in pristane. Solubility data are reported in *Table I*.

Table I. Solubility values of the esters, in water and in pristane at 37°C, and $K\,Ss = Sk$ (mmol g^{-1}) values obtained from the sorption data using *Eq.* 2

	Solubility (mmol/100 ml)		H_2O			Pristane		
Ester	H_2O	Pristane	HIK	HDK	AK	HIK	HDK	AK
Methyl	1·669	0·367	0·353	0·242	0·576	0·147	0·143	0·433
Ethyl	0·939	0·968	0·318	0·208	0·509	0·162	0·161	0·448
n-Propyl	0·374	1·665	0·192	0·133	0·358	0·185	0·152	0·457
iso-Propyl	0·458	1·636	0·184	0·153	0·328	0·162	0·180	0·394

DETERMINATION OF SORPTION ISOTHERMS

The concentration ranges used for the equilibrium sorption experiments were the following: (ester, solvent, concentration in mg/100 ml) methyl ester, H_2O, 20–200, pristane, 10–50; ethyl ester, H_2O, 10–120, pristane, 10–130; *n*-propyl ester, H_2O, 10–50; pristane, 10–240; *iso*-propyl ester, H_2O, 10–65, pristane, 10–240. To six solutions (10 ml), covering each concentration range, contained in 25 ml glass flasks, was added 0·1 g human (intact or delipidised) keratin or 0·1 g animal keratin. A glass bead (diameter, *c.* 12 mm) was also added to ensure good mixing. Six identical solutions, without keratin, served as controls. The stoppered and sealed flasks were held for 72 h in a thermostatted (37±0·1°C) shaker bath (*c.* 100 cycles/min). Samples of the solutions were then filtered isothermally (cf. solubility determinations) and assayed spectrophotometrically. A small correction was needed to account for release of uv-absorbing substances from human (intact or delipidised) keratin to water. The correction factor was determined in separate experiments, where water keratin suspensions (containing the same amount of substrates as in the sorption experiments) were equilibrated at 37°C for 72 h. The amount of ester sorbed per unit weight of substrate (Y, mmol/g) was calculated from the concentration

difference found between the keratin-containing solutions and the corresponding control solutions using the following relationship,

$$Y = \frac{C\ V}{100\ M\ X}$$

where C is the concentration difference (mg/100 ml) found in the presence of X g of substrate, V is the volume (ml) of the solution and M is the molecular weight of the ester. Each sorption experiment was repeated at least five times; the parameters of the individual plots were calculated and averaged (cf. next section).

SORPTION KINETICS OF ETHYL *p*-AMINOBENZOATE

A solution of the ester (0·24 mmol/100 ml) in water (or pristane) was distributed, in 10 ml portions, in fifteen 25 ml flasks. To each flask was added 0·1 g of keratin (human, intact or delipidised, or animal) and a glass bead (cf. equilibrium sorption experiments). The flasks were shaken in a constant-temperature (37±0·1°C) bath: at appropriate intervals the samples were withdrawn and filtered isothermally, then were analysed as indicated in the previous sections. A correction factor, determined in separate experiments, was applied to account for release of UV absorbing substances by the substrates. The data were expressed as ester present in the solution, in percentage of the initial value, *v.* time. The final (72 h) values were in satisfactory agreement with those obtained in the equilibrium sorption experiments at corresponding concentrations.

RESULTS AND DISCUSSION

INTERACTION OF THE ESTERS WITH KERATIN

Sorption isotherms

The sorption isotherms of the four esters under investigation, in both solvents, by the three types of keratin (HIK, HDK, and AK) were in all cases linear, with the origin as intercept, and did not show a final inflection or plateau within the explored concentration range. Regression analysis allowed calculation of the parameters and statistics of all plots. The averaged ($n=5$) slopes, K, and standard errors of the slopes are reported in *Table II*. The average correlation coefficient for all experimental plots ($n=24$) was 0·964 (S.D., 0·02), and the average intercept was 0·251 (S.D., 2·914).

Table II. K (ml g^{-1}) values (and standard errors of K) for the sorption of the esters under investigation by the three substrates, in water and in pristane solution, at 37°C

	H_2O			Pristane		
Ester	HIK	HDK	AK	HIK	HDK	AK
Methyl	21·15 (1·5)	14·55 (0·95)	34·51 (1·5)	40·07 (3·1)	39·08 (2·4)	118·04 (7·6)
Ethyl	33·89 (2·2)	22·13 (0·5)	54·19 (1·6)	16·82 (1·1)	16·65 (1·05)	46·38 (1·4)
n-Propyl	51·42 (2·5)	35·54 (1·9)	95·86 (5·0)	11·14 (0·75)	9·16 (0·4)	27·47 (1·1)
iso-Propyl	40·29 (1·7)	33·50 (1·95)	71·70 (1·7)	9·90 (0·8)	11·02 (0·65)	24·12 (1·0)

Such linear plots would belong, according to the classification of Giles, MacEwan, Nakhwa and Smith (11), to the C type, which is indicative of a constant partition of a

solute between substrate and solution, analogous to the partitioning between two immiscible solvents. The linearity shows that as the solute is taken up the number of sites for adsorption remains constant, i.e. as more solute is adsorbed more active sites are formed in the substrate. A behaviour of this type can be described by the equation,

$$K = \frac{Ck}{Cs} \qquad (1)$$

where *Ck* is the concentration of the solute in the keratin substrate, *Cs* is the corresponding equilibrium concentration in the solvent, and *K*, the slope of the C type isotherm, is a factor indicative of the partitioning of the solute between substrate and solution. The *K* values for the sorption isotherms at 37°C, listed in *Table II*, were computed as a ratio of *Ck* to *Cs* expressed in mmol/g and mmol/ml, respectively.

INTERACTIONS IN AQUEOUS SOLUTION

Inspection of the *K* values reported in *Table II* shows that the distribution of the four esters between the keratinic substrates and water increases with increasing molecular weight, always in the order methyl $<$ ethyl $<$ *iso*-propyl $\leqslant$ *n*-propyl ester. Evaluation of the confidence limits for each *K* value using the *t*-distribution (d.f., 4) showed that, for each substrate, all values were significantly different from each other at the 5% probability level, with the following exceptions. For HIK, *K*-ethyl/*K-iso*-propyl and *K-n*-propyl/*K-iso*-propyl, and for HDK, *K-n*-propyl/*K-iso*-propyl were not different from each other at the stated probability level. As far as the substrates are concerned, AK shows the greatest affinity for the solutes, and is followed by HIK and HDK in decreasing order. The greater affinity for the solutes shown by AK with respect to the keratins of human origin should be related to structural differences existing between the two proteins. Indeed, AK, a 'hard' keratin, differs in its histological, physical and chemical properties from the 'soft' human epidermal keratin (12). Goddard *et al.*, (13) also found significant differences in the sorption characteristics of a cationic cellulose ether derivative by hair (a hard keratin) and mammalian stratum corneum.

The greater affinity for the esters shown by HIK with respect to HDK might be attributed to an increased state of hydration of the former substrate. Indeed, the well known effect of treatment of corneum with organic solvents is to remove all lipid materials (sterols, fatty acids, sterol esters, hydrocarbons, etc.) which have a protective effect on water-soluble substances. These are responsible for maintaining the skin in its normal state of hydration, and their loss would result in a reduced water binding capacity of the cutaneous keratin (14). An increased hydration of the keratin molecules has been found by Wurster and Dempski (15) to parallel an increased affinity for various substances. These authors assumed hydration to cause unfolding of the keratin molecules, thus exposing more binding sites.

The experiments on the time-course sorption of benzocaine by the three substrates (*Fig.* 1) provide an interesting view of the hydration phenomena of intact keratin. After *c.* 35 h of contact with the aqueous solution, the rate of sorption of the ester by HIK rises strongly, probably corresponding to full penetration of water into, and swelling of, the substrate. According to Scheuplein (16), it seems likely that the swelling and softening of the keratin filaments in water is accompanied by a partial dissolution of the cell membranes, which opens larger 'holes' through which easier diffusion may occur. This,

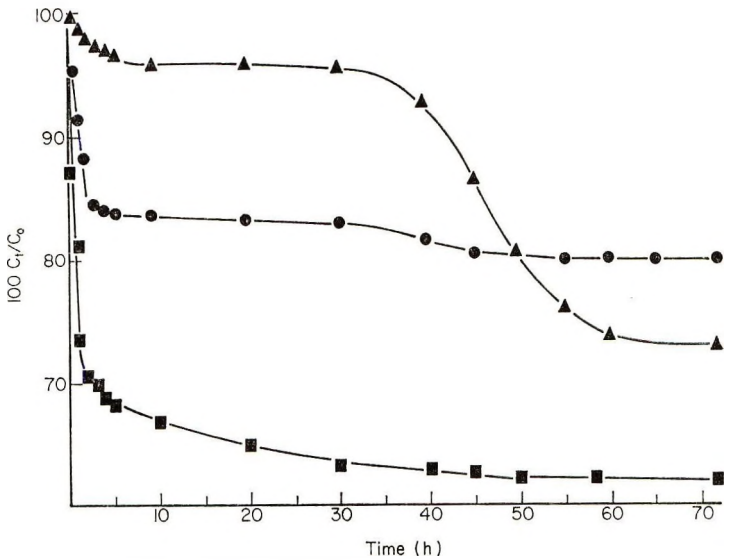

Figure 1. Sorption kinetics of ethyl *p*-aminobenzoate by the three substrates in water at 37°C. Key: ▲, HIK; ●, HDK; ■, AK.

together with the conformational changes of the protein following hydration, may account for either the increased rate and the increased extent of sorption. The human delipidised material, HDK, shows a quite different behaviour: the sorption process is 75% completed within the first 4 h of contact, and hydration effects after 35 h are barely perceptible. This evidences also the important role of total skin lipids in increasing the corneum resistance to penetration of water-soluble drugs (14). The third substrate, AK, shows also a very fast initial adsorption rate. This appears in agreement with the reported observation (17) that diffusion, e.g. of water through wool, is 100 times faster than diffusion through the stratum corneum.

Interactions in pristane solution

The K values for the sorption isotherms of the esters in pristane solution by the three substrates show that the order of affinity of the proteins for the solutes is reversed with respect to that observed in water. Indeed, at equal equilibrium concentrations of the solutes in the solvent, the concentration in the three keratinic substrates decreases, as indicated by the K values, in the order methyl > ethyl > *n*-propyl, *iso*-propyl ester. In this case, evaluation of the statistical differences among the K values, showed that, for each substrate, all values except those for the couples *n*-propyl/*iso*-propyl ester were different from each other at the 5% probability level. Of the three substrates, AK (as in the experiments in aqueous solution) shows the higher K values, indicative of a higher affinity of this substrate for the esters. In the organic solvent, only minor differences can be observed in the equilibrium sorption behaviour of HIK and HDK; both substrates show practically identical K values for the methyl and ethyl ester, and very close values for the two propyl esters. The slight influence of delipidisation on the sorption behaviour of human keratin in the presence of pristane stands in contrast with the profound influence observed in the presence of water. Hydrocarbons possess a very low affinity

for keratinised substrates (10), thus pristane would not induce modifications, resulting in a higher affinity of the substrate for the solutes, such as those induced by water in HIK. The kinetic experiments in pristane solution (*Fig.* 2), which show a qualitatively similar behaviour of the three keratins, confirm the absence of solvent–substrate interactions: equilibrium is attained slowly and steadily in all cases.

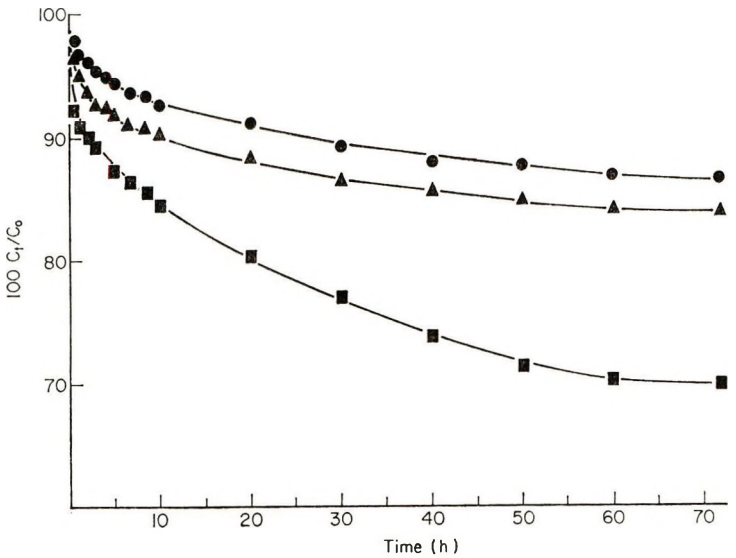

Figure 2. Sorption kinetics of ethyl *p*-aminobenzoate by the three substrates in pristane at 37°C. Key: ▲, HIK; ●, HDK; ■, AK.

MAXIMUM BINDING CAPACITY OF THE SUBSTRATES

While the *K* values provide an indication of the relative affinity of the substrates for the esters, they do not yield information on the maximum binding capacity of the substrates for the solutes. A knowledge of the latter parameter might be useful for assessing the limiting reservoir capacity of the horny layer for a penetrating substance (18). According to *Eq.* 1, in a system at equilibrium the concentration of the solute in the substrate, *Ck*, will increase with increasing concentration of solute in the solvent (*Cs*). For a given temperature *Cs* cannot surpass a limiting value, represented by the saturation of the solution (*Ss*). To this limiting value corresponds a maximum value of *Ck*, *Sk*, indicative of the 'saturation' of the substrate. For the distribution law expressed by *Eq.* 1 to be valid also at saturation concentrations, it is essential that no deviations from ideality occur in the solutions at concentrations approaching saturation. Assuming ideality,

$$K = \frac{Ck}{Cs} = \frac{Sk}{Ss} \qquad (2)$$

where *Sk* and *Ss* are the saturation concentrations of the solute in the substrate and in the solvent, respectively. Since $Sk = K\,Ss$, *Eq.* 2 may allow an estimation of *Sk*, once known *K* and *Ss*. The *K Ss* values for all solutes and substrates of the present study are reported in *Table I.* In the same Table also appear the solubility data of the esters in water and in pristane at 37°C. It must be remembered that the *K Ss* values are only

indicative of the maximum binding capacity of a substrate for an ester, i.e. of the real *Sk* values. Indeed, although the equilibrium sorption experiments were carried out in most cases up to relatively high (*c.* 80% of saturation) *Cs* values, no proof was sought of linearity of the isotherms (i.e. of constancy of *K*) at solute equilibrium concentrations approaching saturation. Also, no proof existed of ideality of the solutions, e.g. of the absence of solute–solute interactions which might result in modification of the conditions on which *Eq.* 2 is based. Benzocaine in water, however, has been reported not to show association phenomena up to 80% of saturation, and to show only a slight tendency to self-interaction at saturation concentration (5), and the other esters studied, in view of their structural similarity with benzocaine, should behave similarly. Inspection of *Table I* shows that, in aqueous solution, the methyl and the ethyl ester have similar *K Ss* values for each substrate. A similar behaviour is observed for the two propyl esters, whose *K Ss* values are not greatly different the one from the other. For each keratin, the *K Ss* values are greater for the couple of esters of lower molecular weight, while the opposite occurs, as said before, for the *K* values, that increase with increasing molecular weight of the solute. If the existence of a relationship between the *K Ss* values and the maximum binding capacity of the substrates, *Sk*, is admitted, the differences observed between the two groups (methyl–ethyl, and *n*-propyl–*iso*-propyl) of esters might be indicative of an important role of the molecular dimensions in the binding mechanism of these compounds by keratin in aqueous solution. Although no significant differences could be detected by the present procedure among the components of each group, it is possible that differences might be detected using finer experimental techniques. A decrease of the maximum binding capacity of human stratum corneum with increasing molecular weight of a homologous series of normal primary alcohols can be detected by a simple elaboration of data reported by Scheuplein (16). Indeed, by multiplying the *Km* values (stratum corneum/water partition coefficients of the alcohols, corresponding to the present *K* values) given by the above author, by the water solubility values (*Ss*) of the compounds, reported elsewhere in the literature, *Sk* values are obtained that decrease *c.* six times from *n*-butanol to *n*-heptanol. As shown in *Table I*, the *K Ss* values of the four esters determined from data in pristane solution differed only slightly, for each substrate, the one from the other. Thus, if ideal behaviour is assumed for the solutions also in this case, each keratin would appear capable of binding the four esters to the same extent, irrespective of their molecular dimensions. A behaviour of this type might be indicative of a binding mechanism where interactions of identical functional groups with active sites on the substrate, rather than molecular dimensions, play a predominant role.

The substrates in all cases (water or pristane) behaved similarly, their apparent maximal binding capacity for each ester decreasing in the order AK $>$ HIK $\geqslant$ HDK. The possible reasons for this behaviour may be the same as those indicated when discussing the relative affinities (*K* values) of the substrates for the solutes, in the different solvents.

CONCLUSIONS

The present results appear to indicate that, in the experimental system under investigation, the sunscreen–keratin interaction may be characterised by two parameters. One of these, *K*, describes the distribution of the solute between substrate and solvent, and may be considered as indicative of the relative 'affinity' of a substrate for a solute in a given solvent. The other parameter, *Sk*, is indicative (assuming ideal behaviour of the

solution and linearity of the sorption isotherms up to saturation) of the maximum binding capacity and/or of the dissolving power of the substrates, and appears to be solvent dependent. Both factors may play a role in determining the efficacy of a sunscreen; a high K value indicates a greater affinity of the agent for the substrate, than for the vehicle, while a high Sk value may correspond to an elevated reservoir capacity of the horny layer for a sunscreen presented to the skin by a vehicle, where the sunscreen is contained at a concentration near or above saturation, as frequently is the case of cosmetic sunscreen preparations. Both K and Sk may thus concur in representing the substantivity of a compound, dissolved in a given vehicle, for the skin.

In the series of esters examined, for each substrate the K values in both solvents increased with decreasing solubility in the solvent, while the Sk values were practically identical in pristane, and appeared to show a dependency on molecular size in water. The above data stress the importance of selecting the most appropriate vehicle for a selected sunscreen if higher concentrations in the horny layer are desired. If, for example, equimolar (unsaturated) solutions of the present esters in either solvent are considered, the propyl esters in water and the methyl ester in pristane will show the highest skin affinity, while the methyl ester in water and the propyl esters in pristane, that possess less favourable K values, will prove less satisfactory (cf. *Table II*). If, on the other hand, we consider saturated solutions of the esters, the methyl and ethyl ester in water (or in an aqueous phase) will produce the highest skin concentrations with respect to any other ester in either solvent (cf. *Table I*).

The kinetic experiments have also shown that the vehicle may affect the interaction by acting on the substrate; the data do indeed indicate that hydration of the keratin may influence both the K and the $K\,Ss$ value. Although in the present equilibrium sorption experiments the hydration of the intact human keratin greatly exceeded the physiological values, it cannot be excluded that vehicle-induced hydration effects, capable of influencing the interaction, may occur also *in vivo*. Thus, a vehicle capable of modifying the state of hydration of the skin might affect the substantivity of a compound. The keratin of animal origin (wool), in spite of its structural differences, gave K and $K\,Ss$ values for the four esters in the same rank order, although of different magnitude, as the human material. Thus, the use of an animal, instead of human, substrate for studies on the relative affinity of sunscreens appears possible, at least when the human keratin is not easily accessible.

Finally it should be pointed out that the slope of the sorption isotherms, K, previously defined, on the basis of *Eq.* 1, as a parameter relative to the partitioning of a solute between substrate (considered as a homogeneous phase) and solution, is best characterised by the relationship,

$$K = \frac{Cks}{Cs} + \frac{Ckb}{Cs} \tag{3}$$

where Cks indicates the concentration of solute *dissolved* (absorbed) in the substrate, and Ckb the concentration of solute *bound* (adsorbed) to active sites on the substrate. Indeed, if one considers human epidermal keratin in its state of normal hydration, a sorbed substance will be partly dissolved in the aqueous phase surrounding the keratin filaments, and in the lipid matrix, and partly adsorbed on active sites on the protein. An individual assessment of the factors contributing to K, $Cks/Cs = Kp$ and $Ckb/Cs = K'$, might be useful for a more precise evaluation of the process of percutaneous absorption of sunscreens, as well as of other medicinal substances applied to the skin.

Indeed, the mathematical treatment of the permeation of synthetic membranes containing 'active' fillers (that can be roughly assimilated to the stratum corneum) indicates that, in the standard zero-order permeation process, the steady-state flux is influenced, among other factors, by the coefficient $Kp = Cks/Cs$, i.e. by the diffusant's partition coefficient between the continuous phase of the membrane, where diffusion takes place, and the solvent (5). The other coefficient, $K' = Ckb/Cs$, which describes the distribution of the solute between the 'active' sites and the solvent, influences only the lag time, which is roughly the time necessary for establishment of steady state conditions in the system. In other words, a strong affinity of the solute for 'active' sites of a membrane, though not affecting the flux of permeant once established, the steady state conditions, might cause a delayed establishment of these conditions. Unfortunately, in the case of the skin a separate assessment of the above coefficients appears practically impossible, particularly in view of the complexity of Kp, in the composition of which occur multiple partitions determined by the heterogeneous structure of the horny layer's 'continuous' phase. There is also both theoretical and experimental evidence (5) that in complex membranes (in series, porous, or containing dispersed phases) Kp, besides K', may influence the lag time. Although the possibility of evaluating Kp and K', and their individual contributions to the time lag appears difficult in practice, the assessment of the time lag by *in vitro* permeation studies on isolated horny layer is indeed possible. The knowledge of the above parameter, besides K and Sk, might bring a further contribution to the characterisation of the substantivity of a compound. A high lag time might correspond to a delayed percutaneous absorption and this would reduce the potential risk of systemic toxicity, since the applied agent, after fulfilling its protective role, would in time be removed from the skin (by bathing, perspiration, etc.) before reaching the circulation in significant amounts.

It is hoped that, notwithstanding the evident complexity of the stated problems, further research now in progress along these lines may make a contribution to a fuller clarification of the substantivity concept.

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Applanation tonometry in the assessment of eye irritation

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Synopsis

The measurement of **intraocular pressure** has been suggested for the objective assessment of **eye irritation**. **Applanation tonometry** is the method of choice. An evaluation of this method has been made in the rabbit using a **calibrated Perkins tonometer**. Results are presented following the irritation produced by instillation of **sodium lauryl sulphate**.

INTRODUCTION

It is a common finding that changed intraocular pressure is one of the earliest indications that a compound is producing eye irritation (1). The application of applanation tonometry in ophthalmic toxicology has been discussed by Ballantyne, Gazzard and Swanston (2). In a recent review on objectivity in the assessment of eye irritation, Heywood and James (3) considered the eye test system to be subject to such wide variation that it was unlikely that precise measurements of eye irritation would be possible. Clinical appraisal, supported by corneal thickness measurements and intraocular pressure measurements, is regarded as probably being the best that can be achieved. To date there is little published data to support the use of applanation tonometry as a measure of ocular irritation; this paper reports the potential of the technique in experiments in rabbits using sodium lauryl sulphate as the irritant.

MATERIALS AND METHODS

CALIBRATION OF THE TONOMETER

A Perkins hand-held tonometer (Clement Clarke Instruments) was used. The instrument was calibrated by cannulating the anterior chamber of the eyes of six anaesthetised rabbits to a heparinised saline manometer and an electronic transducer. Tonometer readings were taken when both the manometer and transducer gave similar readings. In addition, the instrument was calibrated in five animals in which the eyes had been irritated with 0·1 ml 10% sodium lauryl sulphate (specially pure, BDH Chemicals) so that the cornea was thickened between 25% and 55% (mean of 37%). The tonometer scale readings in grams with 95% confidence limits at varying reservoir pressures are given in *Table I* and from this data the calibration curves are drawn in *Fig.* 1.

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Table I. Calibration data

Reservoir pressure (mmHg)	Normal cornea 95% confidence limits (g)	Thickened cornea 95% confidence limits (g)
10	0·16 ± 0·38	0·1 ± 0·50
20	1·1 ± 0·37	1·0 ± 0·49
30	2·0 ± 0·38	1·8 ± 0·49
40	2·9 ± 0·39	2·6 ± 0·49
50	— —	3·3 ± 0·50

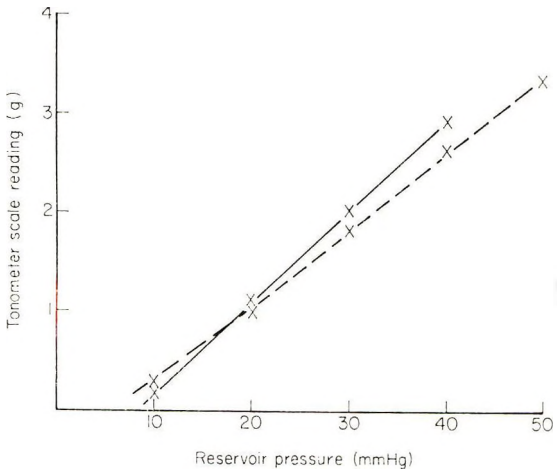

Figure 1. Calibration curves; × —— × normal cornea, × – – – × thickened cornea.

EXPERIMENTAL PROCEDURE

Six adult female New Zealand White rabbits (mean weight 2·74 kg) were used for the experiment. 0·1 ml of a 10% solution of sodium lauryl sulphate was used as the irritant. After ½, 1, 2, 4, 6 and 24 h, a clinical assessment, corneal thickness measurement, and applanation tonometry were performed. The clinical assessment was based on the degree of swelling, redness and discharge; the corneal thickness measurement followed the technique of Mishima and Hedbys (4), using the Haag-Streit depth measurement attachment with a slit lamp, and a calibrated Perkins hand-held tonometer was used for the applanation tonometry.

The intraocular pressures were measured using the calibrated tonometer with the animals restrained in stocks with the head gently held in its normal position. Prior to measurement the cornea was anaesthetised using one drop of 0·5% proparacaine hydrochloride. To visualise the applanation rings, a small drop of evaporated milk (Carnation) was applied to the tonometer prism. Three readings were taken from each eye. After each reading the eye was thoroughly irrigated with normal saline.

RESULTS

There was a rapid rise in intraocular pressure with the highest values reached 30 min after instillation of the irritant (*Table II*). Although the results slowly returned towards

baseline values, intraocular pressures which were significantly different at the 5% level of probability were recorded after 24 h. The untreated eyes showed a rise in intraocular pressure which was significantly raised above baseline values after 2 h.

Corneal thickness could be correlated to the increase in intraocular pressure. The corneas showed maximal response after 2 h, but the swelling persisted throughout the experimental period. The results of the corneal thickness measurement are consistent with those recorded by Heywood (5) with 10% sodium lauryl sulphate. The clinical assessment of damage showed a maximal response after 4 h, at which time the corneas were dull and there was evidence of iritis.

Table II. Effects of 0·1 ml 10% sodium lauryl sulphate on rabbit eyes

Time after treatment (h)	Intraocular pressure		Corneal thickness of treated eye		Clinical assessment of treated eye		
	Treated eye	Untreated eye	(mm)	Percentage increase	Redness	Swelling	Discharge
0	19·33 ± 1·47	19·17 ± 1·63	0·34 ± 0·08		0	0	0
½	32·17 ± 2·11*	18·75 ± 1·29	—	—	2	2	2
1	30·42 ± 3·14*	20·58 ± 1·74	0·48 ± 0·04	41	2	2	1
2	29·41 ± 1·49*	26·00 ± 0·58†	0·50 ± 0·04	47	2	2	1·5
4	26·80 ± 3·14*	22·25 ± 3·50	0·48 ± 0·05	41	2	3	2
6	23·58 ± 4·92†	19·42 ± 1·98	0·45 ± 0·05	32	2	2	1
24	23·25 ± 4·36†	20·33 ± 3·55	0·46 ± 0·05	36	1	1	1

* Difference from baseline significant at $P < 0·001$.
† Difference from baseline significant at $P < 0·05$.

DISCUSSION

The application of an irritant to the conjunctival sac of the rabbit initiates a variety of responses, but invariably induces some change in corneal thickness which must be taken into account when calibrating the tonometer. The technique of applanation tonometry, although basically simple, requires a skilled operator. In any technique requiring alignment procedures, inter-operator differences, usually in the region of 3–4 mmHg, have to be accepted. A particularly difficult aspect of this problem is the blurring of the inner margins of the applanation rings; the use of minimal amounts of evaporated milk, with brighter illumination, helps to reduce the problem. Even so, spuriously high or low results occasionally occur during the measuring procedure and these must be discarded from the (normal) readings.

The intraocular pressure rose more quickly and returned to near normality sooner than the other factors measured. The contralateral eye showed a sympathetic rise in intraocular pressure which was significantly above baseline levels after 2 h.

If the measurement of intraocular pressure is introduced into eye irritation assessment, it must be remembered that the number of variables will be increased. For measurements to be made, local anaesthesia has to be produced, a second material (in our case Carnation evaporated milk) has to be introduced into the eye to visualise the applanation rings, and after the procedure has been carried out the eye has to be thoroughly irrigated. All these variables could influence the long term effects of the primary irritant.

The technique might have value in the assessment of local eye irritation. Much more experience, however, is required before it can be regarded as a sensitive test of ocular toxicity.

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Factor analysis in the evaluation of cosmetic products

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Synopsis

In the **evaluation** of most cosmetic products many attributes must be investigated. These attributes are not necessarily independent and product performance can be described more simply in terms of a small number of underlying factors which adequately account for all the measured attributes. **Factor analysis** provides a mathematical method by which these factors can be identified and their contributions towards each of the attributes calculated. The mathematical basis of factor analysis is explained and the methods available for the extraction of factors described. Once derived the factor matrix can be rotated so that a minimum number of factors is required to specify each attribute. Factor analysis is applied to the results of a series of salon tests carried out on normal, dry and greasy hair **shampoos**. The seventeen attributes for which the shampoos were rated are reduced to just two orthogonal factors. After rotation these factors are found to correspond to (i) rate of **foam** build up, and (ii) stability of the first **lather**. Hair properties are found to be better described by the original foam build up factor and a second, oblique factor corresponding to ease of rinsing. Factor loadings for the shampoos are calculated and the products mapped into the factor space. Performance is found to be affected most by the type of **hair** on which the test was carried out. In a second example twelve attributes of **toothpaste** flavours are reduced to three orthogonal factors corresponding to (i) bitterness–sweetness, (ii) lasting freshness, and (iii) flavour strength–warming. All other attributes can be satisfactorily explained in terms of these three factors.

INTRODUCTION

In the evaluation of cosmetic products no instrumental methods are available to measure most of the attributes of interest and recourse must be had to panel testing. This can lead to difficulty in the interpretation of results when scores are obtained for many questions where the relationships between the attributes for which questions were asked are not known and where there is no knowledge as to the relative importances of the various attributes.

Attributes can be grouped or clustered to some extent by using common sense or rules of thumb developed over many years' experience. There exists, however, a mathematical method by means of which the data can be reduced to order. The relative importance of questions can be determined and the many questions asked reduced to a few underlying, basic factors, in terms of which all of the results on all of the questions can be explained. This technique is known as factor analysis.

First attempts at such an analysis were made by Spearman (1) in his search for a means by which intelligence could be measured objectively. He successfully demonstrated the existence of a common factor underlying all of the many different measures of intellectual attainment. The method was further refined by Thurstone (2), who abandoned the

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notion of a single general factor and, instead, described intelligence in terms of a series of overlapping group factors. Later workers, such as Catell (3), applied the technique to the investigation of personality traits with considerable success.

With the advent of computers the extremely heavy load of calculation entailed no longer posed problems and the original approximate methods of extracting factors have become statistically respectable and less approximate. The method is now used in market research and sociology and can be applied very conveniently to the type of product evaluation where the multiplicity of attributes to be investigated calls for simplification.

The results of factor analysis can be used to determine the relationships among attributes and so give a framework within which the mode of action of the product can be better understood. Product performance can be compared in terms of a small number of factors, thus avoiding the complications which arise from comparison on the basis of many measured attributes.

MATHEMATICAL BACKGROUND

Factor analysis is based on mathematical manipulation of the correlation matrix of the variates under investigation, in this case the attributes for which scores have been obtained by means of a panel test. Because of this basis the method is suitable for analysis of variates which can be measured only imprecisely.

The basic model can be expressed algebraically by *Eq.* 1. The correlation coefficients between all the pairs of the p attributes give a square $p \times p$ matrix. This matrix can be expressed as the product of a $p \times k$ factor matrix and its $k \times p$ transpose.

The residual matrix is added to bring the values on the diagonal of this product up to 1.

Eq. 1

$$\Sigma = \Lambda\Lambda' + \Psi$$

Correlation matrix	Factor matrix	Transpose of factor matrix	Residual matrix

$$\begin{Bmatrix} R_{11} & \cdots & R_{1p} \\ R_{21} & & \vdots \\ \vdots & & \vdots \\ \vdots & & \vdots \\ R_{p1} & \cdots & R_{pp} \end{Bmatrix} = \begin{Bmatrix} L_{11} & \cdots & L_{1k} \\ \vdots & & \vdots \\ \vdots & & \vdots \\ \vdots & & \vdots \\ L_{p1} & \cdots & L_{pk} \end{Bmatrix} \times \begin{Bmatrix} L_{11} & \cdots & L_{p1} \\ \vdots & & \vdots \\ \vdots & & \vdots \\ \vdots & & \vdots \\ L_{1k} & \cdots & L_{pk} \end{Bmatrix} + \begin{Bmatrix} \Psi_{11} & & & 0 \\ & \Psi_{22} & & \\ & & \ddots & \\ 0 & & & \Psi_{pp} \end{Bmatrix}$$

$$(p - k)^2 > p + k$$

The number of factors k can be considerably less than the number of attributes. In fact the p attributes have been reduced to a much smaller number of k underlying factors.

Several methods exist for the estimation of Λ, the factor matrix, the best known being Principal Factor Analysis and the associated approximate method, Centroid Analysis. Details of these can be found in several textbooks on multivariate analysis (4, 5, 6). Computer programs are available for carrying out these calculations.

The number of factors to be extracted can be determined in several ways, but subject to the constraint that

$$(p - k)^2 \text{ be greater than } p + k$$

where p is the number of variates and k is the number of factors. For $p = 17$, $k \leqslant 11$. Probably the simplest method of determining the number of factors needed is to check whether the product of the two highest loadings on the last factor to be extracted yields a correlation coefficient which is significantly different from zero.

The rest of this paper will be devoted to two examples which show how factor analysis can be applied to the evaluation of cosmetic products.

FACTOR ANALYSIS OF A SERIES OF SHAMPOO PANEL TESTS

The data for the first analysis were obtained from a series of half-head tests carried out on pairs of shampoos. Two of the tests compared shampoos for normal hair, one compared dry hair shampoos and one, shampoos for greasy hair. One normal hair shampoo appeared in both of the normal tests.

Procedure consisted of two latherings during which scores were awarded for various attributes of the foam and for ease of rinsing. After the second rinse comb drag and hair tangling were rated and after drying scores were given for comb drag, fly, softness, gloss and body. For simplicity the mean scores on each attribute over all thirty subjects on the panel have been used for the analysis.

It would have been possible to use scores from the individual subjects, but the amount of computation would have been appreciably greater. *Table I* shows the scores for all seventeen attributes of the eight shampoos tested. These scores have been converted to standardised form using the equation

$$z = \frac{x - \bar{x}}{s}$$

where z is the standardised score, x is the original score for the particular attribute and shampoo, $\bar{x}$ is the mean of the original scores for a particular attribute over all shampoos and s is the standard deviation of the original scores for a particular attribute over all shampoos tested.

The reason that standardised scores are shown is that they will be needed in this form later when the factor loadings of the shampoos themselves are calculated.

The seventeen attributes are listed in the table. It can be seen that they are presented in a crudely clustered form with the first lather questions grouped together, likewise the second lather questions, the wet hair properties and also the dry hair attributes. Factor analysis will show whether these apparently logical groupings have any validity.

Table I. Standardised scores for shampoos

		Normal		Normal		Dry		Greasy	
		A1	B	A2	C	D	E	F	G
1	First foam build up	0·27	−0·14	1·09	1·50	−0·45	−0·09	−1·68	−0·58
2	First amount of foam	0·43	−1·19	0·64	1·96	−0·55	0·00	−0·89	−0·26
3	First foam texture	−0·95	−0·68	1·11	1·68	−0·05	0·21	−1·21	−0·26
4	First foam stability	−1·65	−1·38	0·23	0·23	1·15	1·04	0·08	0·15
5	First ease of rinsing	0·83	0·91	0·73	0·76	−1·48	−1·47	−0·18	−0·08
6	Second foam build up	0·69	0·32	0·95	0·91	0·12	0·14	−1·52	−1·54
7	Second amount of foam	0·07	−0·21	0·54	1·13	0·68	0·68	−1·70	−1·25
8	Second foam texture	−0·30	−0·34	0·81	0·85	0·81	0·95	−1·35	−1·43
9	Second foam stability	−0·81	−0·81	0·44	0·44	1·36	1·25	−0·97	−0·97
10	Second ease of rinsing	0·88	1·11	0·80	0·75	−1·23	−1·32	−0·56	−0·46
11	Wet hair comb drag	−0·81	−0·58	−0·99	−0·96	0·35	0·21	1·31	1·50
12	Wet hair tangling	−0·66	−0·65	−0·99	−0·99	0·27	0·17	1·32	1·53
13	Dry hair comb drag	−0·96	−0·68	−1·13	−0·89	0·89	1·23	0·51	0·99
14	Fly	−1·26	−1·27	−0·59	−0·43	0·81	0·78	0·78	1·19
15	Softness	0·10	−0·10	1·47	1·40	−0·04	−0·62	−1·26	−0·95
16	Gloss	0·18	0·35	1·50	1·29	−0·74	−0·68	−0·70	−1·19
17	Body	0·61	0·30	1·18	1·39	−0·74	−0·81	−1·08	−0·91

The purposes of factor analysis in this particular case are to reduce the unwieldy number of seventeen attributes to a more manageable number of factors on which the shampoos can be compared, and to determine, if possible, the relationships among the seventeen attributes so as to better understand how the various wet and dry properties of the hair are produced by the shampoo.

EXTRACTION OF THE FACTORS

The original scores from the tests were processed using a computer program written in BASIC to extract factors by the Centroid Method. Sufficient factors were considered to have been obtained when the conditions of the test described above were met and the product of the highest loadings on the last factor was found to be not significantly different from zero. The last factor was then dropped and only the earlier factors used.

In addition to the factor matrix the computer also printed out the matrix of the correlation coefficients between all pairs of attributes. The correlation matrix appears as *Table II* and the factor matrix as *Table III*.

The seventeen attributes have been reduced to a mere two factors and in all but very few cases the communality for each attribute is satisfactorily high with most being greater than 0·9. This means that at least 90% of the variation of most of the attributes is accounted for by the two factors. The communality is the sum of the squares of the factor loadings for any one attribute.

None of the lather attributes is loaded entirely on one factor although first and second foam build up lie heavily on factor I and first and second foam stability are heavily loaded on to factor II.

Table II. Correlation matrix for shampoo attributes

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	First build up	1·000	0·835	0·827	−0·072	0·458	0·853	0·798	0·686	0·358	0·571	−0·850	−0·850	−0·675	−0·562	0·932	0·842	0·900
2	First amount	0·835	1·000	0·799	0·113	0·316	0·568	0·614	0·511	0·303	0·331	−0·558	−0·542	−0·465	−0·241	0·744	0·641	0·711
3	First texture	0·827	0·799	1·000	0·448	0·097	0·567	0·724	0·715	0·595	0·162	−0·500	−0·523	−0·291	−0·065	0·799	0·640	0·611
4	First stability	−0·072	0·113	0·448	1·000	−0·781	−0·172	0·244	0·415	0·750	−0·781	0·370	0·334	0·643	0·787	−0·042	−0·273	−0·389
5	First rinsing	0·458	0·316	0·097	−0·781	1·000	0·321	−0·070	−0·223	−0·624	0·970	−0·543	−0·518	−0·886	−0·789	0·484	0·693	0·750
6	Second build up	0·853	0·568	0·567	−0·172	0·321	1·000	0·886	0·814	0·470	0·518	−0·967	−0·971	−0·683	−0·717	0·854	0·792	0·820
7	Second amount	0·798	0·614	0·724	0·244	−0·070	0·886	1·000	0·961	0·783	0·127	−0·754	−0·773	−0·315	−0·336	0·753	0·560	0·586
8	Second texture	0·686	0·511	0·715	0·415	−0·223	0·814	0·961	1·000	0·889	−0·028	−0·658	−0·685	−0·195	−0·198	0·687	0·506	0·463
9	Second stability	0·358	0·303	0·595	0·750	−0·624	0·470	0·783	0·889	1·000	−0·469	−0·245	−0·280	0·249	0·255	0·366	0·109	0·041
10	Second rinsing	0·571	0·331	0·162	−0·781	0·970	0·518	0·127	−0·028	−0·469	1·000	−0·711	−0·692	−0·949	−0·903	0·601	0·782	0·839
11	Wet drag	−0·850	−0·558	−0·500	0·370	−0·543	−0·967	−0·754	−0·658	−0·245	−0·711	1·000	0·998	0·836	0·854	−0·860	−0·882	−0·910
12	Wet tangling	−0·850	−0·542	−0·523	0·334	−0·518	−0·971	−0·773	−0·685	−0·280	−0·692	0·998	1·000	0·818	0·838	−0·866	−0·883	−0·901
13	Dry drag	−0·675	−0·465	−0·291	0·643	−0·886	−0·683	−0·315	−0·195	0·249	−0·949	0·836	0·818	1·000	0·911	−0·759	−0·899	−0·926
14	Fly	−0·562	−0·241	−0·065	0·787	−0·789	−0·717	−0·336	−0·198	0·255	−0·903	0·854	0·838	0·911	1·000	−0·584	−0·743	−0·801
15	Softness	0·932	0·744	0·799	−0·042	0·484	0·854	0·753	0·687	0·366	0·601	−0·860	−0·866	−0·759	−0·584	1·000	0·922	0·923
16	Gloss	0·842	0·641	0·640	−0·273	0·693	0·792	0·560	0·506	0·109	0·782	−0·882	−0·883	−0·899	−0·743	0·922	1·000	0·962
17	Body	0·900	0·711	0·611	−0·389	0·750	0·820	0·586	0·463	0·041	0·839	−0·910	−0·901	−0·926	−0·801	0·923	0·962	1·000

Table III. Unrotated factor scores for shampoos

	Attribute	Factor I	Factor II	Communality
1	First foam build up	0·93	−0·23	0·91
2	First foam amount	0·68	−0·29	0·54
3	First foam texture	0·63	−0·57	0·72
4	First foam stability	−0·27	−0·87	0·83
5	First ease of rinsing	0·58	0·75	0·90
6	Second foam build up	0·94	−0·20	0·92
7	Second foam amount	0·76	−0·62	0·96
8	Second foam texture	0·65	−0·71	0·92
9	Second foam stability	0·24	−0·90	0·87
10	Second ease of rinsing	0·73	0·68	0·99
11	Wet hair comb drag	−0·98	−0·06	0·96
12	Wet hair tangling	−0·98	−0·03	0·96
13	Dry hair comb drag	−0·85	−0·51	0·98
14	Fly	−0·78	−0·57	0·93
15	Softness	0·94	−0·18	0·91
16	Gloss	0·93	0·13	0·88
17	Body	0·97	0·18	0·97

Wet comb drag and tangling are composed mainly of factor I, in the negative direction.

For dried hair, softness, gloss and body are strongly, positively loaded on factor I.

ROTATION OF THE FACTOR MATRIX

Factor matrices obtained in this way are not unique and merely correspond to one of an infinite number of possible solutions. These other solutions result from there being an infinite number of positions into which the factor axes can be rotated, although the relative positions of the variates or attributes in the factor space remain unaltered. It is customary, therefore, to carry out a further rotation of the original factor matrix so as to minimise the number of factors needed to describe each of the attributes. This can be carried out manually when only a few factors have been isolated, but becomes difficult for more than three factors. The second example will be rotated in this way.

Objective methods of rotating the factors are available. The Quartimax method was the first of these developed and works on the principle of maximising the variance of the loadings within the rows of the factor matrix (7). The result of this is to maximise the number of high and low factor loadings and to minimise the number of intermediate loadings. It has, however, the disadvantage of tending to produce only one major factor when it is generally preferable to distribute the variates as evenly as possible over all of the factors isolated.

The most commonly used method is Varimax rotation which maximises variance within the columns of the factor matrix (5). In this way the undesirable features of the Quartimax method are avoided and the factors are rotated to give mainly high or low loadings on all of the factor axes. The Varimax method is usually supplied as an extra feature of factor analysis computer programs.

A Varimax rotation was therefore carried out, again using a computer program written in BASIC.

The rotated factor matrix is shown in *Table IV*. It can be seen that the communalities of the attributes remain unaltered except for minor variations due to rounding error. The rotated factors are now called A and B.

Table IV. Rotated factors for shampoos

	Attribute	Factor A	Factor B	Communality
1	First foam build up	0·96	0·03	0·92
2	First foam amount	0·73	−0·10	0·54
3	First foam texture	0·76	−0·38	0·72
4	First foam stability	−0·03	−0·91	0·83
5	First ease of rinsing	0·36	0·88	0·90
6	Second foam build up	0·96	0·06	0·92
7	Second foam amount	0·90	−0·39	0·96
8	Second foam texture	0·82	−0·51	0·93
9	Second foam stability	0·47	−0·80	0·86
10	Second ease of rinsing	0·52	0·85	0·99
11	Wet hair comb drag	−0·93	−0·32	0·97
12	Wet hair tangling	−0·93	−0·29	0·95
13	Dry hair comb drag	−0·68	−0·72	0·98
14	Fly	−0·60	−0·76	0·94
15	Softness	0·95	0·08	0·91
16	Gloss	0·86	0·38	0·88
17	Body	0·88	0·44	0·97

Positions of the attributes in the two-dimensional factor space are shown in *Fig.* 1. The original factor I passed through the wet hair attributes at its negative end and

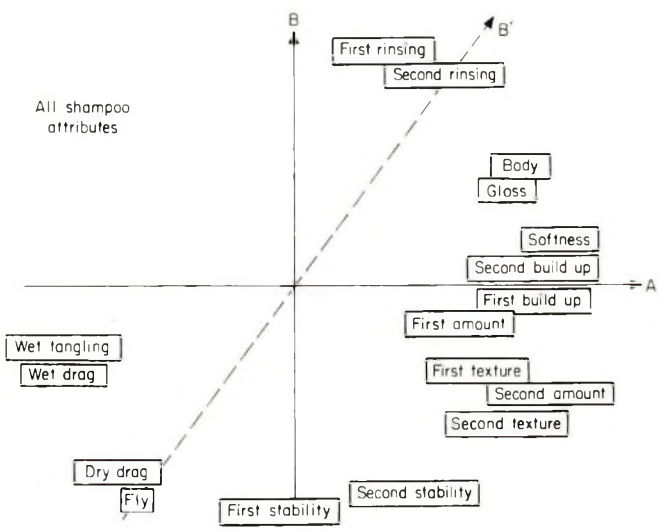

Figure 1. Factor projections for all shampoo attributes.

through softness, gloss and body at its positive extreme. Factor II was perpendicular to this and passed between the first and second lather foam stabilities.

Factor A passes through first and second lather build up and Factor B through first foam stability at its negative extremity. The factors can be identified on this basis, Factor A being named Foam Build Up and Factor B Foam Stability, although this refers specifically to first foam stability. This may be important because it is a measure of stability in the presence of soil. There is a pleasing symmetry in the relationship of the other foam attributes to these two factors. Most of the attributes fall within the quadrant lying between the Foam Build Up and the first Foam Stability axes. First foam amount depends almost entirely on rate of build up. First and second foam textures and second foam amount depend almost equally on rate of build up and first foam stability. Second foam stability depends strongly on first foam stability but also has some small dependence on rate of build up. As would be expected both first and second ease of rinsing have a strong negative dependence on stability and, rather less expectedly, a small positive dependence on rate of build up.

When the hair condition attributes are examined they are seen to fall into three major clusters. These are (11) wet hair comb drag and (12) wet hair tangling, with (15) softness, (16) gloss and (17) body negatively correlated with the first cluster, and (13) dry hair comb drag and (14) fly.

When the positions of these clusters in the factor space are examined it can be seen not only that Foam Build Up remains an important factor for the conditioning attributes, but that a second, oblique factor can be drawn to pass through second ease of rinsing and fly and dry hair comb drag and that this factor appears to be of greater relevance than first foam stability. This oblique axis has been added to the figure and called B′.

It is tempting to take the Foam Build Up factor as corresponding to the detergent and cleaning action of the shampoo and the Ease of Rinsing factor as a measure of efficiency of removal of both soil and detergent once cleaning has been accomplished. Using this interpretation the relationships of the conditioning attribute clusters to these two basic properties of the shampoo become immediately apparent.

Dry hair drag and fly have a very strong negative dependence on ease of rinsing and are virtually independent of foam build up. Incomplete removal of soil, or more likely, detergent from the hair increases comb drag, produces static and increases the amount of fly.

Softness and wet hair comb drag and tangling depend almost entirely on the cleaning action of the shampoo. More efficient cleaning produces greater softness and reduces the undesirable wet hair properties. Gloss and body, however, appear to depend almost equally on cleaning and efficiency of detergent or soil removal (ease of rinsing).

The first objective of the factor analysis has been accomplished. The 17 attributes have been reduced to two orthogonal factors, (A) rate of lather build up for first and second lathers, and (B) first lather stability, i.e. stability in the presence of soil.

Hair properties are best described by factor A and an oblique factor B′ corresponding to ease of rinsing for first and second lathers.

A systematic account of the relationships among the seventeen attributes has been achieved and this account is consistent with previous knowledge of shampoo performance. It should be pointed out, however, that the relationships described above are merely a set of hypotheses which can be tested in further experiments and either confirmed or rejected.

SHAMPOO FACTOR LOADINGS

Once the factor matrix has been extracted and rotated it is possible to calculate factor loadings for the products that were tested and to determine the positions within the factor space of these products. Two methods are available for doing this (8). In both methods a transformation matrix is constructed which, when multiplied by the vector of standardised attribute scores for a given product, gives the factor loading for that product.

Factor loadings for the shampoos are shown in *Table V* and positions of the shampoos in the factor space are plotted in *Fig.* 2.

Table V. Estimated factor loadings for shampoos

	Factor	
Shampoo	A	B
Normal		
A1	0·77	1·18
B	0·53	1·22
A2	1·23	1·14
C	1·29	0·90
Dry		
D	−0·62	−1·41
E	−0·67	−1·56
Greasy		
F	−1·33	−0·60
G	−1·30	−0·74

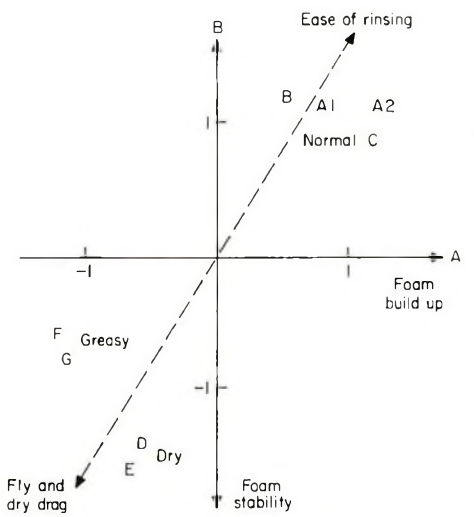

Figure 2. Positions of the shampoos in factor space.

The normal hair shampoos performed best with C being the best of all. Shampoos A1 and A2 were, in fact, the same shampoo tested on two different panels. The loadings on Factor B are unchanged over the two tests, although there is a slight improvement in foam build up for A2.

The dry hair shampoos performed relatively badly giving poor rinsing and increased dry hair comb drag and fly. This may not, however, be a reflection on the performance of the shampoos, but merely a result of their being tested on a panel of dry haired subjects. If this is the case then the shampoos fell far short of the ideal of making dry hair behave as if it were normal during and after shampooing.

The greasy hair shampoos performed poorly on foaming and in producing the desirable properties of body, softness and gloss. There was a corresponding increase in the undesirable wet hair properties. Again this may merely indicate that tests were carried out on a panel of subjects with greasy hair and that the shampoos failed to overcome the characteristic behaviour of that type of hair.

If it is desired to test for significant differences between shampoos on the factors that are isolated, it becomes necessary to calculate factor loadings for each individual subject. This gives enough replications for one of the standard significance tests to be applied. In the interests of rigour it is better in this case to carry out factor analysis using the individual scores as the data.

FACTOR ANALYSIS OF A TOOTHPASTE FLAVOUR PANEL

The results of a panel carried out on fifteen toothpaste flavours provide the data for the second example of factor analysis. Twelve semi-expert panel members brushed their teeth with the test toothpastes and awarded scores for eleven flavour attributes and for overall flavour preference. The method used was the same as that described in an earlier paper (9), although a smaller panel than usual was used. Because the panel members were all experienced in this type of testing and all but the preference question were attributes that could be rated objectively, the smaller number of respondents was considered adequate.

The attributes rated are listed in *Table VI* and the object of the factor analysis was to identify some simple underlying structure among these attributes. In this particular analysis factor rotation was carried out manually so as to illustrate the principles on which such rotation is based.

EXTRACTION OF THE FACTORS

Mean scores for all of the attributes of all the toothpastes were analysed using the Centroid program referred to above. *Table VI* shows the matrix of the correlation coefficients between all pairs of attributes. The factor matrix is given in *Table VII*.

Three factors were extracted. Although the highest loadings on Factor III do not quite meet the requirements stated above for retention of the factor, there were several attributes with fairly high loadings and the factor was retained. Communalities for all but two of the attributes were satisfactorily high.

ROTATION OF THE FACTORS

The first step in this process is to identify what Thurstone (2) called simple structure. This arises when a large number of the attributes have zero loadings on all but one or two of the rotated factors. The original factor matrix is examined for evidence of this by simply plotting the positions of the attributes in the factor space, when only two factors have been isolated, or by plotting the projections of the attribute vectors on an arbitrary plane when three factors have been obtained. For more than three factors the process becomes much more difficult to visualise.

Table VI. Correlation matrix for toothpaste flavour attributes

		1	2	3	4	5	6	7	8	9	10	11	12
1	Flavour strength	1·000	0·840	0·718	−0·208	−0·806	0·391	0·824	0·397	0·462	0·346	0·160	−0·670
2	Tingling	0·840	1·000	0·649	−0·297	−0·794	0·622	0·909	0·489	0·468	0·416	0·461	−0·478
3	Warming	0·718	0·649	1·000	0·019	−0·601	0·208	0·557	0·163	0·425	0·208	0·139	−0·445
4	Sweetness	−0·208	−0·297	0·019	1·000	0·522	−0·401	−0·428	−0·783	0·037	−0·769	−0·293	0·540
5	Mildness	−0·806	−0·794	−0·601	0·522	1·000	−0·300	−0·864	−0·625	−0·389	−0·640	−0·103	0·818
6	Freshness	0·391	0·622	0·208	−0·401	−0·300	1·000	0·445	0·322	0·403	0·250	0·851	0·056
7	Sharpness	0·824	0·909	0·557	−0·428	−0·864	0·445	1·000	0·631	0·313	0·601	0·287	−0·700
8	Bitterness	0·397	0·489	0·163	−0·783	−0·625	0·322	0·631	1·000	0·121	0·959	0·326	−0·655
9	Lasting flavour	0·462	0·468	0·425	0·037	−0·389	0·403	0·313	0·121	1·000	0·052	0·476	0·000
10	Lasting bitterness	0·346	0·416	0·208	−0·769	−0·640	0·250	0·601	0·959	0·052	1·000	0·233	−0·705
11	Lasting freshness	0·160	0·461	0·139	−0·293	−0·103	0·851	0·287	0·326	0·476	0·233	1·000	0·254
12	Flavour preference	−0·670	−0·478	−0·445	0·540	0·818	0·056	−0·700	−0·655	0·000	−0·705	0·254	1·000

Table VII. Unrotated factor matrix for toothpaste flavours

	Attribute	Factor I	Factor II	Factor III	Communality
1	Flavour strength	0·83	0·14	−0·46	0·92
2	Tingling	0·88	−0·15	−0·29	0·88
3	Warming	0·57	0·13	−0·54	0·63
4	Sweetness	−0·61	−0·13	−0·65	0·81
5	Mildness	−0·88	−0·31	0·16	0·90
6	Freshness	0·62	−0·67	0·21	0·88
7	Sharpness	0·90	0·14	−0·16	0·86
8	Bitterness	0·74	0·23	0·52	0·87
9	Lasting flavour	0·44	−0·45	−0·36	0·53
10	Lasting bitterness	0·69	0·34	0·53	0·87
11	Lasting freshness	0·47	−0·77	0·26	0·88
12	Flavour preference	−0·68	−0·73	−0·01	0·99

Figure 3 shows the attribute vectors extended to cut the plane defined by factor I = 1 so as to give the projections of the attributes onto that plane.

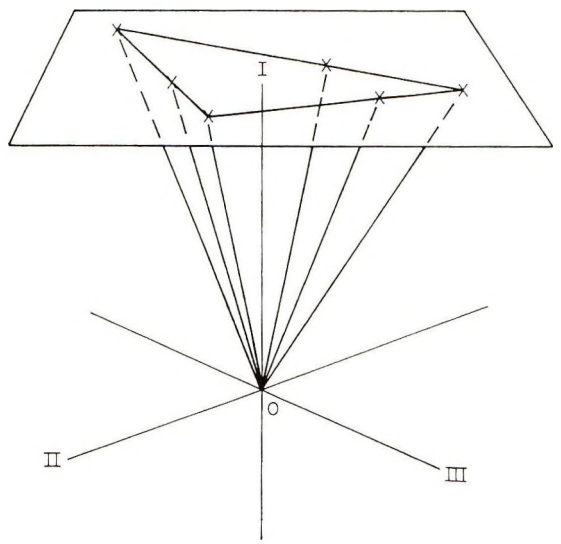

Figure 3. Three-dimensional factor projections. Plane I = 1.

For the toothpaste flavours the projections of the attribute vectors can be plotted by calculating the ratios II/I and III/I for each attribute and plotting one against the other. Simple structure is revealed in the projections by the presence of a triangular configuration of the attributes. The factors giving this simple structure are defined by the vertices of the triangle.

Attributes having loadings on two of the factors, but not on the third, lie along the sides joining the vertices corresponding to the factors.

The flavour attribute projections are shown in *Fig.* 4. Inspection of the figure shows that there are three clusters of attributes which could each identify a factor giving the hoped for simple structure. There is also some indication that most other attributes tend to lie along the edges of the triangle obtained by locating one vertex within each cluster. The approximate position of the triangle is indicated in the figure. The clusters are (1): (4) sweetness, (8) bitterness, and (10) lasting bitterness; (2): (1) flavour strength, and (3) warming; and (3) (6) freshness, and (11) lasting freshness.

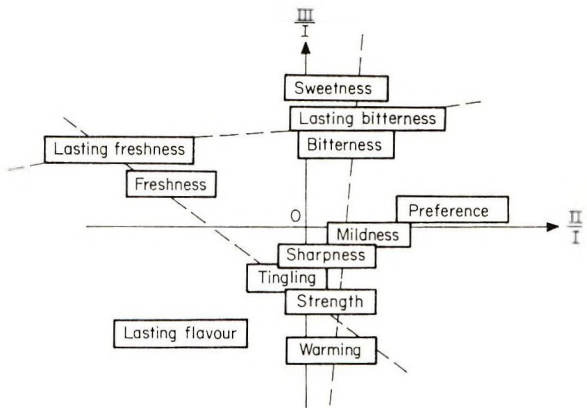

Figure 4. Flavour factor projections.

The appearance of a sweetness–bitterness factor in the simple structure is encouraging as these attributes correspond to two of the recognised basic tastes.

Factors revealed in the search for simple structure are not necessarily orthogonal, i.e. at right angles to each other and independent. Although such oblique factors can be handled, they introduce complications and will be avoided in this analysis if possible. Consequently the correlation matrix of the flavour attributes was searched for coefficients, between members of the different clusters, which were approximately zero and which would indicate independence of the attributes.

Lasting bitterness, warming and lasting freshness were found to satisfy this requirement, the correlations between all pairs from these three being less than 0·25. The original factor matrix was then rotated so that new factors were obtained which passed through or very close to the three approximately orthogonal attributes.

Rotation was carried out in three steps. Loadings on Factors I and III were plotted against each other and the angle of rotation required to locate lasting bitterness on one factor and warming on the other was determined. This rotation is illustrated in *Fig.* 5. Flavour attributes are identified by the numbers listed in *Tables VI* and *VII*.

The factor matrix was multiplied by the rotation matrix

$$\begin{Bmatrix} \cos\theta & 0 & -\sin\theta \\ 0 & 1 & 0 \\ \sin\theta & 0 & \cos\theta \end{Bmatrix}$$

where θ is the angle of rotation. The new factors were labelled A1 and C1. In the second step A1 was plotted against II so as to locate lasting bitterness on one axis and lasting

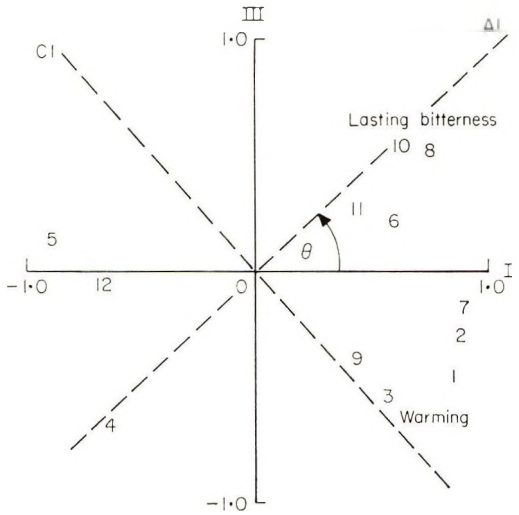

Figure 5. Rotation of factors I and III.

freshness on the other. A new rotation matrix was constructed and the factor matrix multiplied by it to give the new factors A2 and B1.

The final step consisted of reversing the signs of B1 and C1, an operation equivalent to rotation through 180°, to give the final factors B2 and C2.

Table VIII gives the final rotated factor matrix. The communalities have again stayed constant except for small changes due to rounding error. Although the three marker attributes do not fall exactly on the three factors, A, B and C, they lie very close to them.

Table VIII. Rotated factor matrix for toothpaste flavours

	Attribute	Factor A	Factor B	Factor C	Communality
1	Flavour strength	0·33	0·02	0·90	0·92
2	Tingling	0·34	0·35	0·81	0·89
3	Warming	0·11	−0·09	0·78	0·63
4	Sweetness	−0·85	−0·30	0·07	0·82
5	Mildness	−0·62	0·02	−0·71	0·89
6	Freshness	0·22	0·87	0·26	0·87
7	Sharpness	0·56	0·14	0·72	0·85
8	Bitterness	0·90	0·22	0·11	0·87
9	Lasting flavour	−0·14	0·44	0·56	0·53
10	Lasting bitterness	0·93	0·11	0·07	0·88
11	Lasting freshness	0·10	0·92	0·12	0·87
12	Flavour preference	−0·79	0·41	−0·45	0·99

Positions of the twelve attributes in the factor space are shown in diagram form in *Fig.* 6. The view is isometric, with factor A lying in the plane of the paper and factors B and C coming obliquely out of the plane. Dotted lines connect those attributes not lying on or near a factor with the nearest axis.

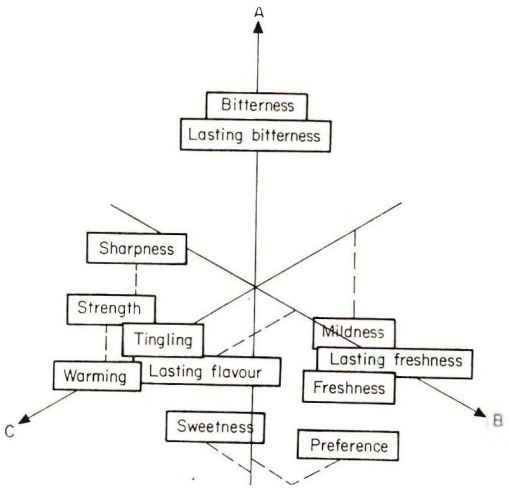

Figure 6. Flavour factor space.

Factor A clearly corresponds to bitterness and lasting bitterness with its negative extremity approximating to sweetness, although sweetness itself is displaced slightly towards the warming–flavour strength axis.

Factor B can be identified with lasting freshness and Factor C with warming–flavour strength.

All of the other attributes can be described in terms of their dependence on these three factors. Tingling is closely associated with warming–flavour strength, but has small components from both bitterness and lasting freshness.

Sharpness is equally dependent on bitterness and warming–flavour strength. Mildness is the exact opposite of this being equally associated with sweetness and whatever may be the negative form of warming–flavour strength.

Lasting flavour has equal contributions from warming–flavour strength and lasting freshness.

Flavour preference was loaded heavily on sweetness with a small contribution from lasting freshness and a slight negative component of warming–flavour strength. As stated above the results for flavour preference must be treated with caution as the panel was far from representative and small in numbers. It would appear that this particular panel had a sweet tooth.

A satisfactory structure for the toothpaste flavour attributes has been found and all have been described plausibly in terms of three factors which approximate to (i) bitterness–sweetness, (ii) lasting freshness, and (iii) flavour strength–warming.

The objective of the analysis has been achieved. If so desired it is now possible to continue the process and calculate factor loadings for each flavour, as was done for the shampoos, and to compare the performances of the flavours on the three factors.

CONCLUSION

Factor analysis has been applied to panel evaluations of two types of product and has drastically reduced the numbers of parameters required to describe product performance in both cases. In addition useful insights have been gained into the relationships among the various attributes measured.

There are many other products to which this method of analysis can be extended and its usefulness is not confined to the results of panel evaluations alone. Any battery of tests can be analysed in this way and the results can provide a fruitful source of ideas and hypotheses on how a product achieves its effect and how its performance can be improved.

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In vivo screening of anti-caries agents

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Synopsis

A single human mouth was used to investigate the mechanism of **caries**, or to screen potential prophylactic agents. Iceland Spar was used as a model substrate to predict effects on human **dentine**. The experiments suggest that real carious attack was reproduced in a human subject.

INTRODUCTION

It is now well accepted that the major cause of dental caries is the presence of fermentable sugars in the diet. This view was first expressed by Miller in the 1890s and time has confirmed his views (1). The World Health Organization has endorsed the coincidence of dental caries with a high intake of fermentable sugar.

The mechanism of the process is also well accepted; bacteria present in the mouth release enzymes which degrade sugars to acids which attack and dissolve the enamel of teeth. This is an oversimplified statement of the chain of reactions which lead to tooth decay, but it is worth restating if only to emphasise that the mechanism is a chain in which the breaking of any one link destroys the whole chain.

The mechanism immediately invites suggestions for agents which interfere with the various steps and which hopefully would prevent the development of caries. Numbers of potential prophylactic agents have been proposed. Unfortunately there is no simple means of checking the effect of such agents. *In vitro* experiments do not correspond to the dynamic environment of the human mouth, and the results of animal experiments are not necessarily applicable to humans. The only means of testing potential prophylactic agents is by clinical trial. Such trials normally last several years and are very expensive to administer. Companies who normally finance such trials are increasingly reluctant to underwrite this expense without some guarantee of success.

It would be desirable, therefore, to have a small scale screening test which would reproduce human caries in a real situation. This paper will suggest that this could possibly be done in a one man clinical trial.

THE ORAL CELL

The subject (the author) had three teeth missing in his lower left jaw ($|\overline{4, 5, 6}$). A hollow cell constructed of white gold was fitted into this space (*Figs.* 1 and 2). The cell was perforated to permit the flow of saliva through the cell and was fitted with a trap door to enable samples to be put into the cell. The cell was worn without discomfort, day and night for

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long periods. It was removed night and morning for external cleaning. Every 2 or 3 days, when samples in the cell were to be examined, the interior of the cell was cleaned.

Samples were removed from the cell for examination at intervals of 1–2 days to a week. They were washed with distilled water, dried with filter paper and kept in a desiccator over calcium chloride for a fixed period.

Since caries represents the actual loss of tooth substance it seemed logical to follow the progress of caries by weight loss of the samples. If the technique was followed precisely there was no difficulty in obtaining reproducible and meaningful results.

The salivary pH of the subject, determined by holding the electrode of a pH meter in the left buccal pouch, varied between 5·8 and 6·8 when on a normal diet. The most convenient way of establishing and maintaining a lower pH was to suck tablets of a proprietary mint confection. These were found to contain over 90% sucrose, and a routine was established whereby one tablet was sucked every hour of the working day. This produced a salivary pH value of about 5·3.

In order to investigate the validity of the system, initial experiments were done using crystals of Iceland Spar (pure calcium carbonate) as a model, representing tooth substance. Such crystals are, of course, readily attacked by acids. Crystals of suitable size were therefore placed in the oral cell and the weight loss was checked from time to time.

EXPERIMENTS WITH CALCITE

HIGH SUCROSE DIET

The cell containing the crystal was worn continuously for a period of 89 days with the following programme: 42 days, normal diet; 13 days, high sucrose diet (with regular use of mint confections); and 34 days, normal diet.

The crystal was removed from the cell at approximately weekly intervals and was washed, dried and weighed. The results are in *Table I* and are shown graphically in *Fig. 3*.

Table I. Effect of high sucrose diet

Duration (days)	Conditions	Weight loss rate (μg/day)
42	Normal diet	565
13	High sucrose diet	1190
34	Normal diet	437

It will be seen that even under 'normal' diet conditions there is some acid attack on the crystal, but the rate of attack is doubled by the high sucrose diet.

Further screening experiments were therefore conducted without the high sucrose diet and were planned to investigate conditions which might interfere with the chain of reactions leading to the development of acid in the mouth and to carious attack. These conditions were: the effect of reducing the bacterial population of the mouth; the effect of inhibiting the enzymes responsible for the glycolytic breakdown of sugars; and the effect of fluorides in protecting the substrate.

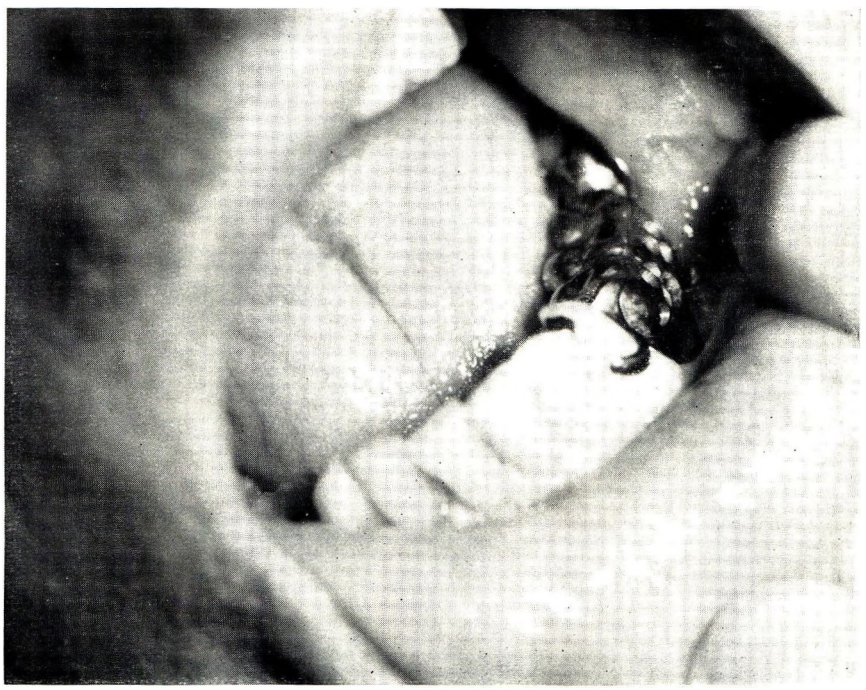

Figure 1. Oral cell in subject's mouth

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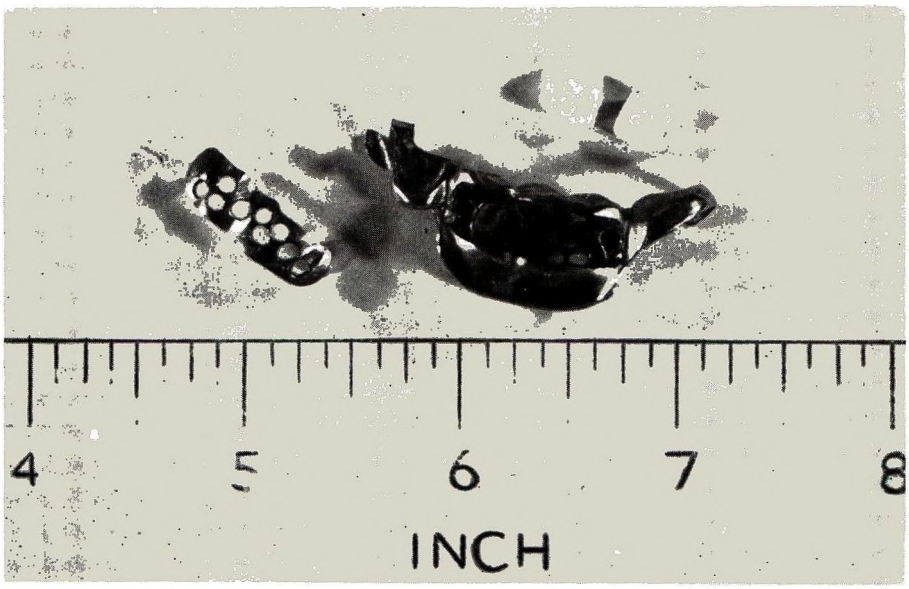

Figure 2. The oral cell

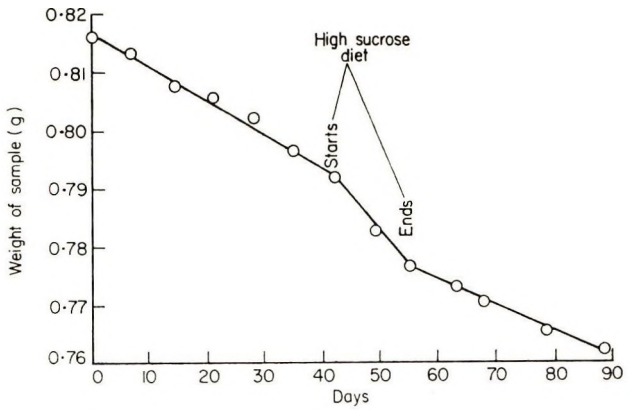

Figure 3. Loss of weight of calcite crystal with high sucrose diet.

EFFECT OF SODIUM HYPOCHLORITE

Sodium hypochlorite is a powerful and non-discriminating bactericide which might be expected to reduce the bacterial population of the mouth for a short time.

During the course of this experiment the mouth was washed out seven times a day with 100 ml of 100 ppm available chlorine sodium hypochlorite solution. The results are shown in *Table II* and *Fig. 4*.

Table II. Effect of sodium hypochlorite

Duration (days)	Conditions	Weight loss rate (μg/day)
13	Normal diet	590
14	Normal diet plus NaOCl mouthwash	170
31	Normal diet	440

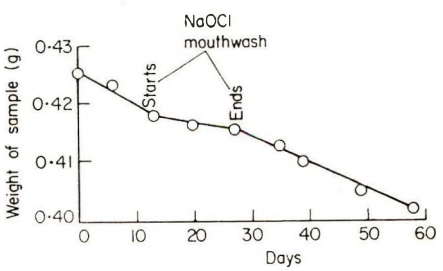

Figure 4. Inhibitory effect of NaOCl mouthwash.

Sodium hypochlorite clearly slows down the rate of acid attack on the crystal, but does not prevent it entirely. This suggests that the bacterial population of the mouth was reduced, but not eliminated. This is what might be expected from the transitory nature

of sodium hypochlorite and the rapidity with which a bacterial population can re-establish itself. Nevertheless, it does imply that a properly chosen bactericidal mouthwash could reduce the amount of acid produced by glycolysis.

EFFECT OF ENZYME INHIBITORS

Sodium N-lauroyl sarcosinate is a well known enzyme inhibitor (2) which is known to affect the glycolytic pathway by inhibiting the enzyme hexokinase. Its effect can easily be demonstrated *in vitro*.

Its effect on glycolysis in the mouth and the consequent effect on a calcite crystal was demonstrated by washing out the mouth three times a day after meals with a 1% solution of sodium N-lauroyl sarcosinate.

The results are shown in *Table III* and graphically in *Fig.* 5.

The rate of acid attack has thus been slowed down to a tenth of its former value, presumably by the inhibition of the enzyme hexokinase.

Table III. Effect of enzyme inhibitor

Duration (days)	Conditions	Weight loss rate (μg/day)
29	Normal diet	370
13	Normal diet + mouthwash	38
29	Normal diet	390

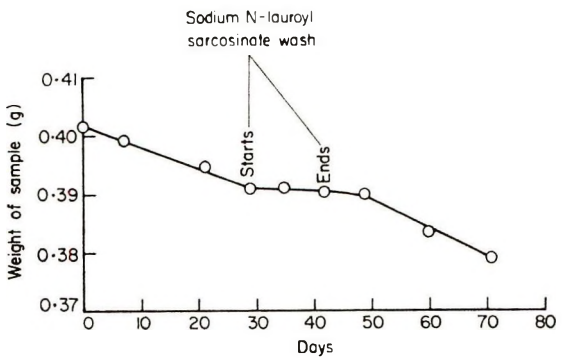

Figure 5. Inhibitory effect of SNLS.

EFFECT OF FLUORIDES

Ionic fluorides are known to affect the incidence of caries (3). The effect may be due to any or all of the following; ion exchange of F^- for OH^- in the apatite lattice may protect teeth from subsequent acid attack; low concentrations of F^- may inhibit some enzymes; low concentrations of F^- ion in the presence of Ca^{2+} and PO_4^{3-} ions may favour the precipitation of apatites on carious lesions (remineralisation).

For experiments with fluorides the high sucrose diet was used and two situations were covered; a crystal of calcium fluoride (solubility 17 ppm equivalent to 8 ppm F^-) was placed in the cell adjacent to, but not touching, the calcite crystal, and a solution of 0·1% NaF was used as a mouthwash three times/day.

Calcium carbonate is known to ion-exchange with F^- and in mechanically static conditions might be expected to cause the precipitation of CaF_2 on the crystal surface, thus inhibiting acid attack.

The results of these experiments are shown in *Table IV* and are shown graphically in *Fig. 6*.

Table IV. Effect of fluorides

Duration (days)	Conditions	Weight loss rate (μg/day)
35	Normal diet	468
21	High sucrose diet	1014
14	High sucrose diet + CaF_2 crystal	486
4	High sucrose diet + NaF mouthwash	0
32	High sucrose diet	344

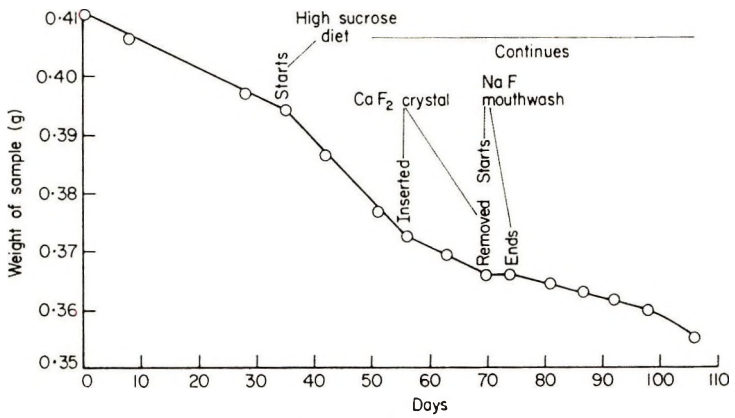

Figure 6. Inhibitory effect of fluorides.

Thus even the very low concentration of F^- ion available from the CaF_2 crystal was sufficient to halve the rate of acid attack, presumably by interference with enzyme action.

The effect of the NaF mouthwash was more dramatic and stopped acid attack completely. Even after the mouthwash was stopped the effect continued and at 32 days the rate of loss had not reverted to its expected level.

This dramatic result appeared to be due to the precipitation of CaF_2 on the surface of the calcite crystal.

SUMMARY OF PRELIMINARY RESULTS

Iceland Spar is not tooth enamel and conclusions on caries should not necessarily be drawn from the results. Nevertheless, the model system does appear to reproduce the effect of acid attack and gives some confidence in the validity of the technique.

Screening experiments with human teeth (deciduous teeth from children) showed that the rate of attack was very slow unless, either the surface layer of enamel was acid etched, or the interior enamel was exposed by drilling out the dentine.

Both these techniques appeared unnatural and it was decided to use human dentine as a substrate, thus representing the advanced stage of carious attack.

EXPERIMENTS WITH HUMAN DENTINE

Slices of human dentine from molars were prepared and used in the next series of experiments. In most cases two slices were placed in the cell so that one could, if necessary, be used as a control on the other when certain treatments were used.

The weight reproducibility of dentine slices was not as good as with Iceland Spar, particularly in the first few days of any experiment. Nevertheless, with care, reproducible and meaningful results could be obtained.

PRELIMINARY RESULTS WITH DENTINE SLICES

Two slices of dentine were placed in the cell and the weights were followed after period of normal and of high sucrose diet. The results are shown in *Table V* and graphically in *Fig. 7.*

Thus dentine would appear to be an ideal substrate material for further investigations.

Table V. Use of dentine as substrate

Duration (days)	Conditions	Weight loss rate (μg/day)
41	Normal diet	Negligible
27	High sucrose diet	1540

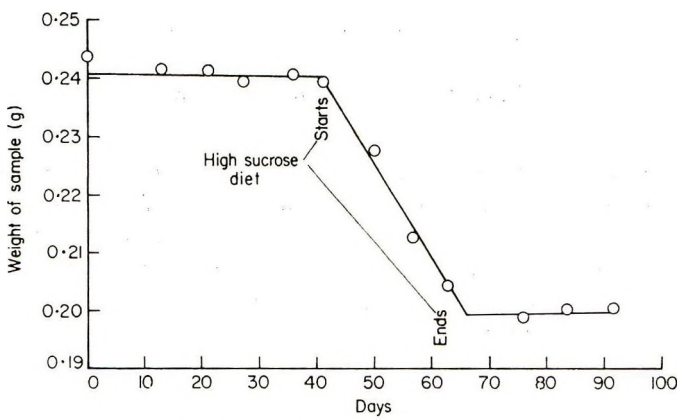

Figure 7. Loss of dentine with high sucrose diet.

EFFECT OF TOPICAL FLUORIDES ON DENTINE

Two slices of dentine were placed in the cell. Normal diet was taken for 16 days, followed by the high sucrose diet. On day 24, one piece of dentine was removed from the cell, soaked for 1 h in a 1% solution of NaF at pH 6·7, washed and replaced in the cell. The

second piece was washed in distilled water only. The results are shown in *Table VI* and graphically in *Fig. 8.*

Table VI. Effect of NaF

	Duration (days)	Conditions	Weight loss rate (μg/day)
Slice A (control)	16	Normal diet	Negligible
	32	High sucrose diet	650
	22	Normal diet	Negligible
Slice B (NaF treated)	16	Normal diet	Negligible
	8	High sucrose diet	735
	24	High sucrose diet following NaF treatment	285
	22	Normal diet	Negligible

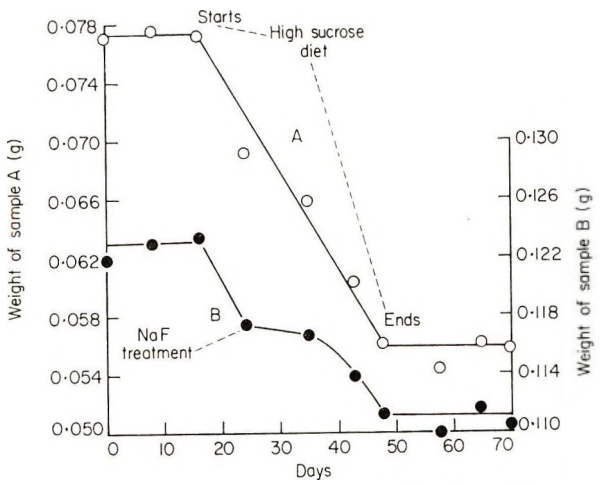

Figure 8. Inhibitory effect of NaF on weight loss of dentine.

It is clear from the graph that the NaF treatment has a marked effect in reducing the rate of loss and that this effect persists for many days.

EFFECT OF SODIUM MONOFLUOROPHOSPHATE

Sodium monofluorophosphate (MFP) is a known anti-caries agent (4) though the mechanism of its effect is not fully understood. Commercial material always contains a few per cent of NaF and so for the experiments to be described, freshly prepared Na_2FPO_3, free of F^- ions, was used.

Two pieces of dentine were placed in the cell and when normal conditions had been established, one of them was removed, soaked in a 1% solution of Na_2FPO_3 for 1½ h at pH 6·8, washed and replaced in the cell. It was separated from the other untreated piece of dentine by a polythene screen. The results of this experiment are shown in *Table VII* and graphically in *Fig. 9.*

Table VII. Effect of MFP

	Duration (days)	Conditions	Weight loss rate (μg/day)
Slice A (Na_2FPO_3 treated)	21	Normal diet	Negligible
	14	High sucrose diet	1305
	First 7	High sucrose diet following Na_2FPO_3 treatment	240
	Next 10	High sucrose diet following Na_2FPO_3 treatment	1222
Slice B (control)	21	Normal diet	Negligible
	31	High sucrose diet	1064

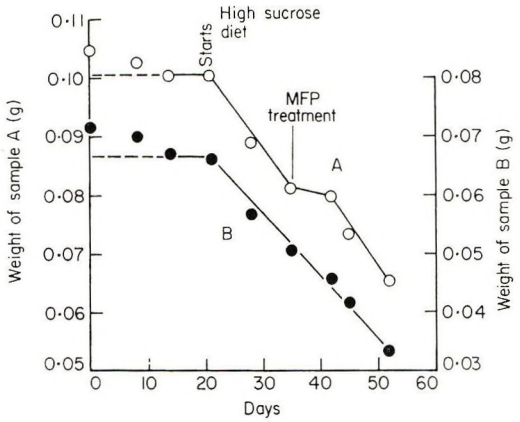

Figure 9. Inhibitory effect of MFP on weight loss of dentine.

There is clearly a halt in the rate of loss of weight of the treated sample, but after about 10 days this effect disappears. This suggests that MFP is incorporated into the apatite lattice, or is adsorbed onto the surface, two mechanisms which have been suggested to explain the action of MFP (5).

EFFECT OF ENZYME INHIBITOR

Sodium N-lauroyl sarcosinate (SNLS) was shown to be an effective enzyme inhibitor in the model system using calcite as a substrate. The experiment was, therefore, repeated with dentine.

After normal weight loss conditions had been established with a high sucrose diet, the mouth was washed out three times a day after meals with a 0·5% solution of SNLS. The effect of this treatment on two separate slices of dentine is shown in *Table VIII* and graphically in *Fig. 10.*

It is clear that SNLS effectively halts the rate of loss of dentine over the period during which it is used. The effect did not persist beyond the treatment period which is what would be expected from an enzyme inhibitor.

EFFECT OF BACTERIOSTAT

Sodium hypochlorite was shown to be effective in reducing the rate of loss of calcite in the model experiment. This would not be the antibacterial of choice for a mouthwash

Table VIII. Effect of enzyme inhibitor

Duration (days)	Conditions	Weight loss rate (μg/day) B	A
21	Normal diet	Negligible	Negligible
11	High sucrose diet	828	1168
7	High sucrose diet + SNLS mouthwash	Negligible	Negligible
11	High sucrose diet	881	1257
20	Normal diet	Negligible	Negligible

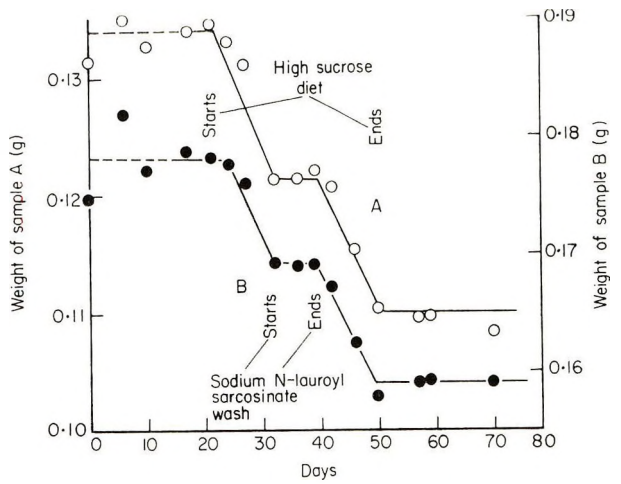

Figure 10. Inhibitory effect of SNLS on weight loss of dentine.

since it is not adsorbed onto the mucous membrane and is so readily inactivated by proteins.

For experiments with dentine a proprietary mouthwash containing 0·075% of *p*-diisobutylphenoxy ethoxyethyl dibenzyl ammonium chloride (benzethonium chloride) was chosen. This would be expected to adsorb onto mouth tissues so that its effect might persist beyond the period of its use.

Two slices of dentine were placed in the cell and after stable conditions of weight loss had been established the mouth was washed four times a day with 12 ml of the mouthwash. The results of this experiment are shown in *Table IX* and are given graphically in *Fig. 11*.

The mouthwash used clearly had a marked effect in reducing the rate of loss of dentine to less than 10% of its former level. Even after the use of the mouthwash ceased, the rate of loss did not revert immediately to its former level which suggests that the active component is adsorbed and continues to exert its effect.

EFFECT OF Ca^{2+} AND PO_4^{3-} IONS

If the mechanism of carious attack is the commonly accepted one, then saturating the plaque (in which the reaction occurs) with Ca^{2+} and PO_4^{3-} ions should prevent it.

Table IX. Effect of mouthwash

Duration (days)	Conditions	Weight loss rate (μg/day) A	B
25	Normal diet	Negligible	Negligible
7	High sucrose diet	1808	1872
17	High sucrose diet + mouthwash	117	53
11	High sucrose diet	979	1048
10	Normal diet	Negligible	Negligible

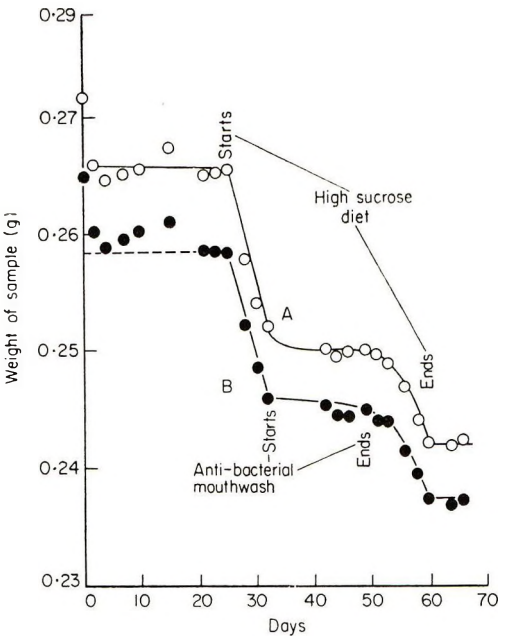

Figure 11. Inhibitory effect of bacteriostat on weight loss of dentine.

To investigate this supposition tablets were made consisting of 80% sucrose + 20% dicalcium phosphate ($CaHPO_4.2H_2O$) and these were used in place of the mint sweets normally used during one phase of the experiment.

Two slices of dentine were placed in the cell and after a normal high sucrose diet, the tablets were used. The results are shown in *Table X* and graphically in *Fig. 12.*

Table X. Effect of DCP in diet

Duration (days)	Conditions	Weight loss rate (μg/day) A	B
17	Normal diet	Negligible	Negligible
14	High sucrose diet	1139	1225
15	High sucrose + DCP diet	625	635
27	Normal diet	Negligible	Negligible

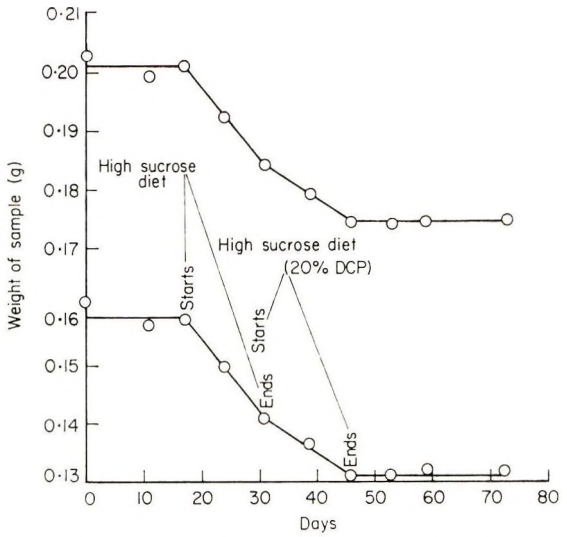

Figure 12. Effect of DCP/sucrose diet on weight loss of dentine.

The rate of loss of dentine has clearly been reduced to about 50% of its former level but it has not been prevented. The tablets used would certainly increase the salivary concentration of Ca^{2+} and PO_4^{3-} ions, but this does not necessarily mean that the plaque concentrations would also rise to the same extent and it is in the plaque that the carious reaction occurs.

EFFECT OF CALCIUM FLUORIDE

It had been noticed in previous experiments that the weight of a sample of dentine kept for long periods under normal diet conditions actually increased. This could be due to calculus production (the subject is a heavy calculus producer) or remineralisation or both.

A dual experiment was done in which dentine slices were kept under normal diet conditions for 63 days. Subsequently the high sucrose diet was used and once established, a calcium fluoride crystal was placed in the cell.

The results of these experiments are shown in *Table XI* and graphically in *Fig.* 13. Only approximate figures can be given because of the short duration of some of the steps.

The weight increase noted after 63 days quickly disappears when the high sucrose diet is started. The effect of the introduction of a CaF_2 crystal into the cell is marked by a considerable reduction in the rate of loss. This effect did not persist after removal of the

Table XI. Development of calculus

Duration (days)	Conditions	Weight loss rate (μg/day)
63	Normal diet	Weight increase
13	High sucrose diet	600–800
4	High sucrose diet + CaF_2 Crystal	140–150
6	High sucrose diet	800–900
14	Normal diet	Negligible

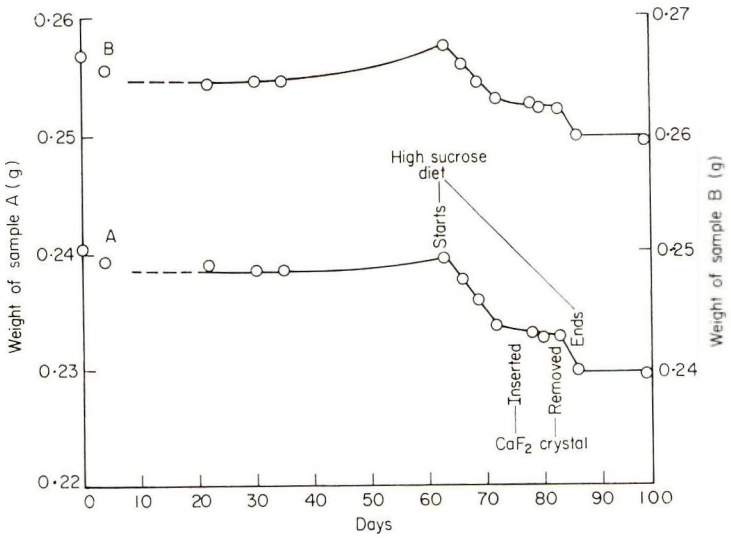

Figure 13. Calculus growth and subsequent removal by sucrose diet.

crystal, which suggests that the mechanism is that the small concentration of F^- ion (max. 8 ppm) acts as an enzyme inhibitor rather than an ion exchanger.

CONCLUSIONS

The experiments described suggest that the mouth of a single human subject can be used as a clinical test bed. This can be used to investigate the mechanism of the carious process, to check the mechanism of known anti-caries agents, and to screen new potential anti-caries agents.

The procedure is tedious and demands a certain degree of personal dedication. It is, however, very cheap compared to a full scale clinical trial which may cost up to £15,000 a year for 3–4 years and it is free of the ethical problems of conducting clinical trials with children.

Critics who speculate that the technique used does not necessarily reproduce dental caries will be reassured to hear that at the conclusion of the trial the subject had to have two natural teeth adjacent to the oral cell, filled because of real caries.

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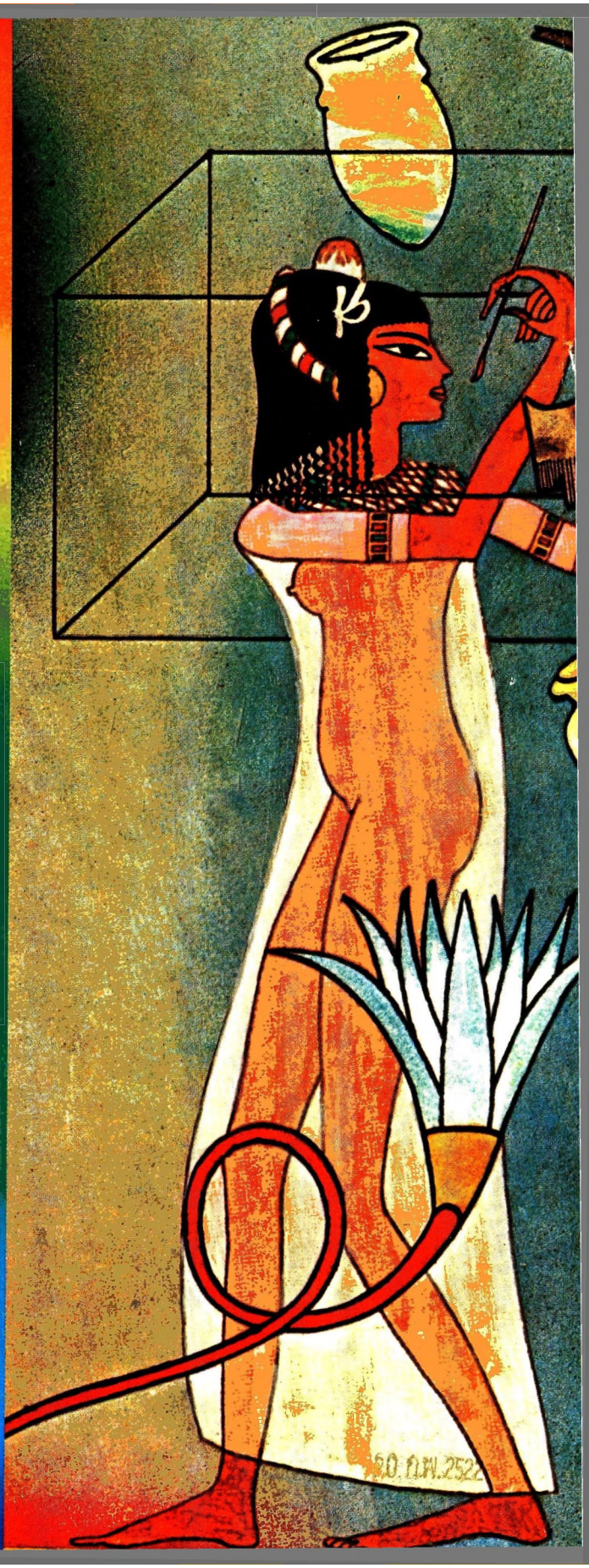

Sun Chemical Corporation

Pigments Division