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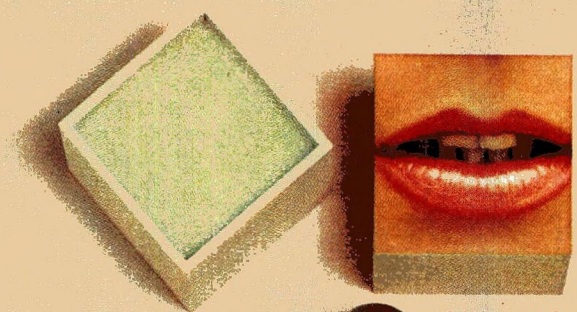

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SYNOPSES FOR CARD INDEXES

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

Exaggerated exposure in topical irritancy and sensitization testing: N. J. VAN ABBÉ, P. NICHOLAS and ELIZABETH BOON. *Journal of the Society of Cosmetic Chemists* **26** 173–187 (1975)

Synopsis—The concept of a 'safety margin' provides a convenient expression for the hazard of adverse reaction following topical administration. Exaggeration of exposure conditions in predictive testing helps to establish the safety margin, but reliability of any prediction depends on limiting the effects of exaggeration to quantitative rather than qualitative enhancement of responses. Gross exaggeration often leads to qualitative changes defying interpretation in terms of hazard during normal use.

Techniques for safety evaluation based upon causing only threshold effects and comparison of an unknown with a well-established 'control' preparation of similar type are suggested as most suitable for relatively innocuous cosmetics. Human tolerance tests would probably be ideal for the purpose but extremely time-consuming. If animal tests are used to screen for skin and eye irritancy, there should not be any need for grossly exaggerated exposure since the species mostly used approximate quite closely to man in their susceptibility to skin and eye irritants.

The prediction of sensitizing potential by exaggerating exposure is unsatisfactory owing to insufficiency of data on dose-response behaviour for mild sensitizers. Experience in normal use of a cosmetic by gradually increasing numbers of individuals would seem to be the only available way to establish sensitizing potential for cosmetic formulations, although a guinea-pig test may be useful for screening new raw materials.

Four methods for the characterization of dentifrices and other semisolids: M. BLOCK. *Journal of the Society of Cosmetic Chemists* **26** 189–204 (1975)

Synopsis—During the course of development work, the need arose for characterization and comparison of batches of dentifrices. Four methods used in other technologies are reported for their utility in the evaluation of these and other semisolids.

Force-time traces were recorded on a modified *Instron* tensiometer for a piston moving at constant speed down a full tube of toothpaste and again down the emptied one. The difference was the corrected initial force for extrusion. At the same time, a number of extruded drops were weighed. The mean weight of a drop was divided by the diameter of the orifice to obtain the tensile strength of the paste.

A polyethylene disc resting on the surface of a jar of dentifrice was withdrawn at constant speed on an *Instron* tensiometer. The force-time curve showed a characteristic maximum cohesive force. The final value obtained on separation of paste and disc was a measure of stringiness.

A notched doctor blade was used to apply 10 parallel stripes of uniform width but increasing height upon a surface. Rotation through 90° permitted sag of the semisolid, giving a stripe number characteristic of resistance to sag.

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

ORIGINAL SCIENTIFIC PAPERS

Hair breakage: the scanning electron microscope as a diagnostic tool
A. C. Brown, B.Sc., Ph.D. and J. A. Swift, B.Sc., Ph.D.

The relationship between water-borne bacteria and shampoo spoilage
S. A. Malcolm, P.Pharm., Ph.D. and R. C. S. Woodroffe, M.I.Biol.

An appraisal of human head hair as forensic evidence
J. Porter, H.N.C. and C. Fouweather, B.Sc., Ph.D.

Skin impedance and moisturization
E. J. Clar, C. P. Her and C. G. Sturelle

Analytical procedures for the determination of chlorhexidine in oral products
E. Cropper, P. Platt and N. A. Puttnam

Estimation of the general incidence of specific lanolin allergy
E. W. Clark

Cough irritation by deodorant sprays
E. M. Staal, W. Bree and P. L. C. A. Rijnbeek

The role of essential fatty acids (EFA) in the regulatory processes of Malpighian cell membranes
M. Cambrai, Ph.D.

The examination of particulate inclusions in toothpaste, by freeze-fracture, replication and transmission electron microscopy
W. B. Davies, B.Sc., A.I.M., D.A.E., M.Sc. and A. C. Macdonald, B.Sc., Ph.D.

Percutaneous absorption of Triclosan from toilet preparations: J. G. BLACK and D. HOWES. *Journal of the Society of Cosmetic Chemists* **26** 205–215 (1975)

Synopsis—The absorption of [^{3}H] Triclosan (*Irgasan*® DP300) through rat skin treated with shampoo containing 0.05% (w/v), and with aerosol deodorant containing 0.1% (w/v), has been measured. The products were applied in a manner designed to simulate consumer use, and the penetration was calculated from the amount of radioactivity excreted by the animals. From the shampoo, the penetration was 0.197 μg cm^{-2} which increased as the concentration of [^{3}H] Triclosan was increased but which was independent of duration of contact with the skin for a given concentration of [^{3}H] Triclosan. Blood levels at 48 h after treatment were proportional to concentration of applied [^{3}H] Triclosan and for 0.05% (w/v) were less than the equivalent of 0.1 μg ml^{-1}. From the aerosol deodorant the penetration was 6.85 μg ml^{-1} and the blood level reached a maximum, equivalent to 0.26 μg ml^{-1}, at 6 h after a single application.

The calculated absorption by the human is an extremely low proportion of the no-effect level in rats.

A parametric test to measure the cleaning power of toothpaste: W. B. DAVIS and D. A. REES. *Journal of the Society of Cosmetic Chemists* **26** 217–225 (1975)

Synopsis—A sensitive *in vivo* method was developed to quantify the cleaning power of dentifrices. Volunteers built up natural stain on their teeth over a period of 5 weeks by replacing their usual toothpaste with a non-abrasive paste. The estimated % area of the incisors covered by stained pellicle was observed to increase, especially towards the end of this build-up period. The natural stain was progressively removed by a series of 10 s brushing periods using an electric toothbrush and the pastes to be tested. The logarithm of the area of stain was plotted against the duration of brushing with the test paste and found to conform to a linear trace. Thus an equation for stain removal may be of the form $S = ae^{bt}$ with the coefficient b being an expression of the ability of the test paste to remove stain.

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J. Soc. Cosmet. Chem. **26**, 173–187 (1975)

Exaggerated exposure in topical irritancy and sensitization testing

N. J. VAN ABBÉ,* P. NICHOLAS† and ELIZABETH BOON*

Presented on 26–30th August 1974 in London at the IFSCC VIIIth International Congress on 'Cosmetics—Quality and Safety' organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—The concept of a 'safety margin' provides a convenient expression for the hazard of adverse reaction following topical administration. Exaggeration of exposure conditions in PREDICTIVE TESTING helps to establish the safety margin, but reliability of any prediction depends on limiting the effects of exaggeration to quantitative rather than qualitative enhancement of responses. Gross exaggeration often leads to qualitative changes defying interpretation in terms of hazard during normal use.

Techniques for safety evaluation based upon causing only threshold effects and comparison of an unknown with a well-established 'control' preparation of similar type are suggested as most suitable for relatively innocuous cosmetics. Human tolerance tests would probably be ideal for the purpose but extremely time-consuming. If animal tests are used to screen for skin and EYE IRRITANCY, there should not be any need for grossly exaggerated exposure since the species mostly used approximate quite closely to man in their susceptibility to skin and eye irritants.

The prediction of sensitizing potential by exaggerating exposure is unsatisfactory owing to insufficiency of data on DOSE-RESPONSE behaviour for mild SENSITIZERS. Experience in normal use of a cosmetic by gradually increasing numbers of individuals would seem to be the only available way to establish SENSITIZING POTENTIAL for cosmetic formulations, although a GUINEA-PIG TEST may be useful for screening new raw materials.

Introduction

The possible hazard of adverse reaction in response to topical administration needs to be assessed by means of suitable predictive tests. The extent of such a hazard is conveniently expressed in terms of the margin

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between probable exposure of the skin or mucous membranes during normal use and the level of exposure which would produce an adverse reaction. In order to err on the side of safety, broad margins of safety are sought by exaggerating the levels and conditions of exposure in the test procedures. However, injury may result from grossly excessive direct contact of many tissues with even the most innocuous environmental chemicals and so a rational approach to exaggeration is essential.

The degree of chemical insult that the skin or mucous membranes might be expected to tolerate is somewhat problematical. The extensive testing carried out on the safety of ingested materials, such as food additives, offers little guidance as such investigations are principally aimed at demonstrating toxic effects after systemic absorption. The aspect of safety evaluation for ingested substances corresponding to topical administration is the direct effect, if any, of the test materials on the gastro-intestinal lining. Severe irritation of the lining would indeed be observed under conditions of gross exposure to many universally-accepted food materials and especially condiments such as vinegar and mustard. In other words, although there should be a wide margin of tolerance once a test material has been diluted in the body fluids following absorption, a narrower margin is to be expected in the case of a tissue in direct contact with the test material.

Whilst acknowledging that there are likely to be considerable differences between direct exposure and exposure after absorption, it might be instructive to consider the postulates on which the safety evaluation of food additives is based. A hundredfold safety factor (1) is commonly quoted; this may be deemed to offer a tenfold allowance for the greater susceptibility to systemic toxicants of man compared to laboratory animals, together with a further tenfold allowance for variation in susceptibility between individuals. In terms of systemic toxicity, such a tenfold allowance for inter-species differences in metabolic transformation and excretion seems reasonable. Effects on the skin and mucous membranes, however, are not primarily dependent on species-specific metabolic pathways. Indeed, the skin of those mammals most often used for irritancy testing approximates quite closely to human skin in its susceptibility to irritation or even shows greater sensitivity to some irritants (2, 3).

The epidermal horny layer of the skin is an important barrier to the absorption of foreign chemicals (4) and provides the first line of defence against irritants. Thickness of the horny layer varies across the human skin and in some regions it is thinner than the horny layer of other mammals. However, human epidermis overall is much thicker (5) than in most

mammals, including the rabbit, rat and mouse; this probably explains why applied substances do not easily penetrate human skin (6) and why, on the whole, it is no more susceptible to irritants than is the skin of these species. When irritancy tests are carried out on animal skin, it would therefore be irrational to allow a tenfold margin for interspecies differences.

The possible irritancy of cosmetic materials in contact with tissues of the eye is usually studied by instillation into the conjunctival sac of the rabbit eye. On the basis of wide experience of such tests, Davies (7) suggested that the rabbit eye was decidedly more sensitive to irritants than the human eye. Thus no allowance for interspecies differences seems necessary for extrapolating rabbit eye test results in terms of hazard to man.

A major factor involved in selecting the appropriate levels of exaggeration in irritancy testing is that a quantitative enhancement of responses may help to establish a meaningful safety margin, whereas a qualitative change in the type of response could well render the findings incapable of interpretation; qualitatively atypical responses might well result from gross exaggeration of exposure levels in tests for skin and eye irritancy (*Fig. 1*). To ensure that testing procedures give the information required for safety assessment, a critical re-appraisal of current methods is needed.

Test methods

In the study of systemic toxicity, suitably exaggerated dose-levels are administered to laboratory animals in the diet, by gavage or by injection. Exposure of the skin or mucous membrane to substantially exaggerated quantities of test material is seldom practical in the study of irritancy. Direct contact within a circumscribed area is essential; an exaggerated quantity applied to a larger area will not necessarily intensify the response.

Exposure to a raw material may often be exaggerated by applying concentrated solutions, but this would not be feasible for complete formulations. Even with raw materials, unrealistic effects may occur, for example, owing to hypertonicity or grossly abnormal hydrogen ion concentration; such exaggeration could well produce effects totally irrelevant to the hazards of ordinary use, by producing qualitative rather than quantitative differences in response.

An alternative way of exaggerating topical exposure is to lengthen the time of contact compared with normal use or to make multiple applications. This is helpful if it influences the response quantitatively without provoking

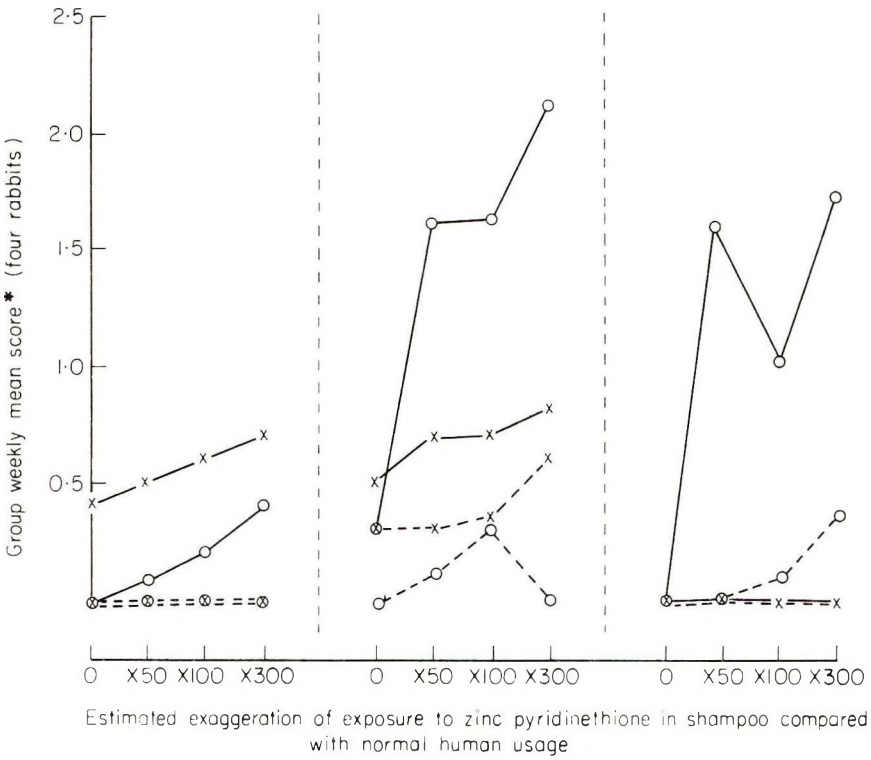

Figure 1. Comparison of skin irritancy produced by repeated shampooing (broken lines) and occlusive patch testing (solid lines). Breakage of the skin under occlusion suggests a qualitative change indicating excessive exaggeration. X, Erythema; O, breakage. *Scored according to Draize, J. H. (1959). Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.

a qualitatively different skin reaction. An example of a qualitatively altered response sometimes occurs when multiple exposures to a moderate irritant lead to an enhanced 'fatigue' response (8); this would be irrelevant in the study of short-term hazards. Thus fatigue may be pertinent to the safety of a face cream for daily use but not to a hair-waving lotion used only two or three times per year. Exaggeration by means of multiple applications should therefore be reserved for testing substances intended for frequently repeated topical use.

Another method of exaggerating exposure for irritancy testing is to apply the test material to damaged skin, which is more readily penetrated by irritants than intact skin (9). Damage may be artificially induced by abrasion, adhesive tape stripping or chemical pre-treatment. Direct effects

due to contact with the underlying tissues, however, may prove misleading if extrapolated in terms of normal skin with an intact horny layer. Certainly it is helpful to know what will happen when a product is applied to damaged skin, but simple quantitative relationships to irritancy for normal skin cannot thus be established and the distinction needs to be recognized.

Chemical pre-treatment of the skin, for example, by applying formaldehyde or sodium lauryl sulphate, may not produce grossly visible damage but will in many cases enhance penetration. Usually the degree of enhancement cannot be quantified in terms of relative irritancy to normal skin and the predictive value of a provocative test using chemical pre-treatment is therefore questionable. Furthermore, the intensity of adverse effects may be too severe to regard as reasonably justifiable for either animal or human studies.

Since the various methods used for exaggerating exposure by inflicting damage to the skin in one form or another so often produce difficulty in interpretation, a rational conclusion is that such damage should never exceed the minimum necessary to ensure a detectable response; discrimination between test materials in terms of skin irritancy may even be improved by limiting the overall response, e.g. by testing after dilution of the product (*Table I*).

Techniques involving grossly exaggerated exposure have led to serious problems of interpretation not only in skin irritancy testing but also in studies of eye irritancy. For example, it has long been customary to instil a

Table I. Improved discrimination between irritancy of shampoos applied to rabbit skin at 10% dilution

	Irritancy* after				
	5 h		24 h		
Type of shampoo	Neat	10%	Neat	10%	
Baby—based on amphoteric detergents	7	1.1	4	0	Erythema
	1.5	1	2	0	Oedema
Normal—based on anionic detergents	7	1.5	15	1.5	Erythema
	8.5	1	14	0.5	Oedema
Medicated—based on 0.5% Zn pyridinethione and anionic detergents	10.5	6	17	7.5	Erythema
	6	1	15.5	7	Oedema

* Scores according to Draize (Group means for six rabbits).

fixed quantity of undiluted test material into the conjunctival sac of the rabbit eye without, as well as with, a subsequent rinse (10). The effects produced by instillation of undiluted material without rinsing may be qualitatively, as well as quantitatively, different from the results likely to occur during normal use of the material. Consequently, products are rarely if ever deemed unfit for human use because of severe eye irritation when tested under these grossly exaggerated conditions in rabbits. A safety evaluation test procedure giving rise to severe effects which are commonly and quite properly ignored, obviously has little predictive value and no justification in terms of the suffering caused to the experimental animals. A more meaningful way of designing studies concerned with eye irritancy, as well as skin irritancy, is to employ test conditions resulting in threshold or minimal irritation and to include a material with known irritancy in the study to serve as a control. Whenever possible, the control material should be closely similar in chemical structure and mode of use to the test material.

Gaunt and Harper (11) reported a procedure whereby shampoo diluted to 10% concentration was instilled into the rabbit eye with no subsequent rinse. This technique avoids grossly unrealistic exposure but it would still be expected to give some enhancement of irritant effects by eliminating the rinsing procedure. As the authors acknowledged and we have confirmed, their technique has the apparent disadvantage of militating against the recognition of any tendency to corneal or iridial injury (*Table II*), but it may nevertheless have better predictive value than a test in which shampoo is instilled at 100% concentration. Improvements might be made by varying the shampoo concentration to some extent or by giving duplicate instillations (12).

Table II. Rabbit eye test findings showing effect of dilution of shampoos with water. Results at 10% dilution apparently gave better prediction of human response, no corneal or iridial injury having been notified as consumer complaints

	100% shampoo (no rinse)			10% shampoo (no rinse)		
	Cornea	Iris	Conjunctiva	Cornea	Iris	Conjunctiva
Non-medicated liquid shampoo	0	0	68	0	0	4
Non-medicated cream shampoo	35	10	108	0	0	12
Medicated cream shampoo	25	10	90	0	0	62

Scores according to Draize; each figure gives sum of scores for three rabbits after 1, 2, 3, 4 and 7 days.

The questionable features of the more usual form of rabbit eye test, apply particularly to its use in the evaluation of detergent ingredients and shampoo formulations. For cosmetic products, however, normally used away from a washbasin and without rinsing, the instillation of undiluted product may be more meaningful and seldom leads to gross injury in the rabbit eye.

A particular difficulty in using laboratory animals is the choice of species and testing site for products such as dentifrices coming in contact with mucous membranes during normal use. The hamster cheek pouch has sometimes been used for predictive testing although the presence of a cornified epithelial lining of the buccal mucosa in this species may limit the sensitivity of the test. In view of the ease of handling of these animals and the relatively large area of tissue available for examination and biopsy, this seems nevertheless to be the test of choice at the present time; possible lack of sensitivity may be overcome by reasonable exaggeration of product concentration and duration of contact with the cheek pouch.

The need to limit irritant effects in the course of tests on human volunteers is obvious, to avoid causing harm and to ensure continuing availability of willing panellists. It is also highly desirable to maintain and, if possible, to improve standards in the humanitarian treatment of laboratory animals and close attention should be given to the design of suitable procedures both for human and animal testing. The tests should preferably not, however, be designed in such a way that they mostly lead to purely negative findings, since these are as hard to interpret as grossly abnormal positive results; this is an additional reason for favouring a threshold irritancy approach.

Designs for skin irritancy studies based on threshold irritation have been put forward by Kligman and Wooding (13). These authors suggested that findings should be recorded in terms of ID50 (*concentration* to yield threshold irritant effect in 50% of test subjects) or IT50 (*time* of exposure for threshold irritation in 50% of subjects). Choice between the two methods of expressing results would depend on the feasibility of testing diluted product and the level of irritancy of the test material. Whereas the ID50 or IT50 principle may be suitable for evaluating new raw materials, formulated cosmetics will often prove altogether too bland for such an approach and are more readily tested by direct comparison with an appropriately-chosen control product.

Patch testing

Skin irritancy is usually investigated by means of a patch test procedure (14); frequently this involves the application of test material to the skin with relatively prolonged occlusion under an impermeable or semi-permeable dressing (*Table III*). Occlusion itself will produce minimal damage of the skin and a 'closed' patch test therefore embodies some degree of exaggerated exposure.

Table III. Occlusivity of patch test materials expressed as drying time of hydrated $CoCl_2$ paper on glass backing, exposed at 19.5±1°, 49.5±1.5% RH, beneath the patches, for pink → blue colour change

Material	Mean drying time (min)	n=	SD
Nil	20	7	6.3
Gauze (only)	45	6	7.8
Gauze + blenderm-backed lint square	65	6	8.4
Micropore + lint square	80	6	6.3
Micropore (only)	155	7	6.3
Micropore + blenderm-backed lint square	200	7	33.0
Dermicel + blenderm-backed lint square	230	6	21.0
Dermicel (only)	390	7	8.4
Blenderm (only)	1145	7	43.0

Micropore: supplied by 3M Co, London.
Dermicel: supplied by Johnson & Johnson Ltd, Slough.
Blenderm: supplied by 3M Co, London.

In the hands of an experienced clinician, the occlusive patch test is a valuable technique for diagnosing the causal agents of pre-existing irritation and sensitization. Using occlusive testing in a prophetic manner, however, involves different considerations. Some preparations (antiperspirants, for example) are normally used under conditions tantamount to occlusion; relevance of an occlusive patch test is then obvious. For many other products, e.g. face creams and shampoos, occlusive patch tests may be somewhat less realistic. However, an occlusive or partially occlusive prophetic patch test is probably the best available procedure for predicting the effects of long-term exposure by means of a relatively short-duration test, taking into account the fact that many toiletries and cosmetics are used repeatedly over long periods of time (*Tables IV and V*). Data showing quantitative results for occlusive *vs* non-occlusive exposure in 21-day human patch tests (2) indicate that occlusion gives a substantial enhancement of effect

for many irritants. Skin irritancy testing carried out under 'open' patch test conditions (e.g. with the applied material under a loosely-woven gauze covering) might therefore be preferable in order to avoid too many 'false positive' results. However, since toiletries and cosmetics are invariably formulated to give minimal skin reaction, 'open' patch testing in practice would nearly always yield wholly negative results which would be hard to interpret. 'Closed' patch tests resulting in threshold irritation, preferably including controls with known irritant potential, allow decisions on the acceptability of a cosmetic ingredient or product to be reached with greater confidence.

Another important consideration in patch testing concerns the form and amount of test material applied. When a volatile solvent is present in the formulation, this should obviously be permitted to evaporate before a 'closed' patch is applied to the skin; if this precaution is not taken, irritant effects due to the solvent will tend to give 'false positive' responses in the sense that solvent evaporation during normal use would avoid any irritation from this source. A further complication is that some toiletry products are

Table IV. Detection of moderate increases in skin irritation, using partially occlusive human patch test (Uttley, M. and Van Abbé, N. J. *J. Soc. Cosmet. Chem.* **24,** 217 (1973))

Product	Irritancy score
Nil (blank lint patch)	6.6*
Lotion base	6.6
Base + DHA (aged)	8.4
Base + DHA (aged) + fresh DHA**	9.3
Base (fresh) + fresh DHA (10%)	10.9*

DHA = Dihydroxyacetone.
* Difference significant at 1% level (Wilcoxon).
** Adjusted to 10% concentration.

Table V. Correlation between partially-occlusive human patch test (Uttley and Van Abbé) and consumer reports

	Irritancy score	Interpretation of score	Consumer reports
Moisturizing cream	6.3*	Non-irritant	No irritation
Cheek gloss no. 1	11.0+	Slight irritant	No irritation
Cheek gloss no. 2	12.6*	Slight irritant	No irritation
Cheek gloss no. 3	18.1	Moderate → severe irritant	Irritation

* Significant at $< 1\%$ level (Wilcoxon).
+ Significant at $< 2\%$ level (Wilcoxon).

rinsed off the skin during normal use soon after application. In these instances, loading a patch with the undiluted product and leaving it in contact with the skin at full concentration for many hours is highly unrealistic and may well prove intolerable for the volunteers.

For a shampoo, a more informative method of patch testing is to apply the product to the patches at a dilution of 10–20% with water. When a physiologically-active constituent occurs in the formula (e.g. an antimicrobial agent) an alternative procedure is to load the patch with a quantity based on an amount per unit area of skin equivalent to the residue left on the scalp after shampooing, as determined by assay.

Where the assay of actual residues presents serious difficulty, open patch tests may be carried out by actually shampooing a small test area of skin daily for several days; experience suggests that this procedure may even have better predictive value than a closed patch test based on estimates of residual quantities after rinsing.

There should thus be no insuperable difficulty in showing an adequate safety margin for the topical administration of cosmetics and toiletries by reasonable exaggeration of the exposure conditions in tests for irritancy. Since, for the reasons already stated, the present authors contend that no allowance is usually necessary for interspecies variation, the choice between using human volunteers or laboratory animals does not appear to have great significance from the investigator's standpoint. If, however, the irritant potential of the test material really is unknown, initial screening is certainly best carried out with laboratory animals. Moreover, if effects on damaged skin are being examined, an animal screening test is obviously desirable before extending the study to human skin, as a reasonable safeguard for the volunteers. Despite the similarity in responses to irritants generally shown, if interspecies differences are discernible when results for animal and human irritancy tests are compared, greater reliance should obviously be placed on the human data.

To avoid uncertainty in extrapolation from animal responses to man whilst minimizing the risk of serious harm to volunteers, human studies may sometimes take the form of tolerance tests. The degree of exposure (with respect to amount of test material applied, its concentration or the duration of contact) is gradually increased until a threshold response occurs. The predictive value of such a test should be satisfactory provided that precautions are taken to avoid fatigue by careful choice of site of application or interval between applications. Human eye irritancy testing should generally follow this type of cautious approach (12). The drawback to more

widespread use of the human tolerance test, however, is its time-consuming nature and the difficulty of recruiting volunteers.

Sensitizing potential

Sensitization is an important type of possible adverse reaction to toiletries and cosmetics; it usually involves the risk of causing an allergic contact dermatitis and needs to be considered separately from irritancy. Allergens may sensitize occasional individuals at concentrations which are without any significant effect on the majority of the population; this feature of allergenicity causes great difficulty in predictive testing. Some dermatologists nevertheless take the view that exposing a group of randomly-chosen subjects under test conditions to an exaggerated concentration of a suspected allergen increases the chance of recognizing its sensitizing potential. If so, what degree of exaggeration is appropriate?

Marzulli and Maibach (15) recently reported a detailed investigation of sensitizing properties using exaggerated concentrations of test materials. For example, with sorbic acid tested at 20% concentration, one of the 33 subjects they tested gave a positive reaction. The crucial question is what proportion of users, if any, would be sensitized at a typical use-concentration of about 0.1–0.2%. Clearly this is unanswerable without knowing the shape of the dose-response curve for an allergen of the same or a similar type, extending right down to normal use-level concentrations of the test substance. These authors did, in fact, conduct tests at several concentration levels with a number of compounds but the proportion of subjects with positive responses did not show a dose-response pattern justifying any broad conclusions; a graded dose-response relationship may perhaps be inferred on theoretical grounds but even the extensive work carried out by Marzulli and Maibach (15) was evidently insufficient to demonstrate it clearly.

Some toxicologists concerned with toiletries and cosmetics prefer to conduct tests for sensitizing potential at normal use-concentration of the test materials, using human volunteers. If such tests involve multiple exposures under occlusive patches, the resulting minor degree of skin damage should marginally increase the likelihood of a positive response. However, since it is well known that toiletries and cosmetics in general sensitize less than, say, 1 in 10 000 users, the predictive value of any use-concentration test carried out with only a few hundred volunteers must be exceedingly

small. Selection of atopic subjects for test panels is sometimes considered to improve predictive value but the evidence to indicate that atopics show enhanced susceptibility to topical allergens in general is questionable (16).

Bearing in mind the objections to grossly exaggerated exposure testing and recognizing that sensitization testing at normal use-concentration often yields negative results that cannot be interpreted or which may be unreliable, there are certain attractions in devising a test procedure to enhance responses to use-concentrations and to ensure that positive results are obtained even with moderate or mild sensitizers. Kligman (17) in proposing his 'maximization' test, offered a procedure giving 24/24 positive responses to *p*-phenylenediamine even though he was still unable to detect some known mild sensitizers such as lanolin. The value of this type of test for cosmetic evaluation does not therefore yet seem to have been established.

At the present time, despite the energetic attempts by a number of highly-skilled investigators, there is clearly no satisfactory way of predicting the sensitizing potential of cosmetics and toiletries by means of human volunteer studies; nevertheless, such studies carry a definite risk of sensitizing volunteers, possibly for some years (18), and their justification is therefore doubtful. As an alternative, a reliable test for sensitizing potential using laboratory animals would obviously be helpful.

Whereas the response of some other mammals closely resembles the human response to irritants, there are marked interspecies differences in allergic responses. Suitability of the guinea-pig for sensitization testing has been validated to some extent (19) but it would be unwise to expect guinea-pig studies to eliminate any but the most potent sensitizers. Thus, although it is reasonably practical to test for the sensitizing potential of raw materials by conducting challenge tests at elevated concentrations using animals or man, no comparable procedure is yet available for studying complete formulations likely to display no more than mild sensitizing ability. Rather than applying maximizing procedures of dubious predictive value, it is probably better to allow a product to be used normally by gradually increasing numbers of individuals. This view takes for granted a prior scrutiny of the raw materials to eliminate any potent known sensitizers and an adequate scheme for monitoring adverse reactions if they are reported by users of the product.

Conclusion

Unreasonable criteria for assessing the potential hazards of topical administration do not necessarily help to protect the consumer. Thus, although animal feeding tests on a proposed food colour may well show that the maximum no-untoward-effect level is several hundred times greater than the expected human intake, even the most harmless materials applied to the skin with such exaggeration are likely to prove injurious. Frazer (1) claimed that the acceptable usage level of a substance—he was referring specifically to food additives, though others have applied his concept more widely—should be regarded as one-hundredth of the level required to produce significant modification of structure or function in not more than 50% of a group of test animals. Further, at a dose-level equal to one-tenth of the ED50, no significant changes of any kind should occur. Such margins, however, could not be applied generally to substances coming in contact with the skin or mucous membranes. For example, none of the synthetic anionic surfactants would be acceptable if a shampoo had to be formulated with no more than one-hundredth of the detergent concentration giving threshold irritation in a closed patch test. Nevertheless, present-day shampoos are used almost universally with minimal known adverse effect; a different basis for judging acceptability is therefore needed and one of the possible approaches might be to seek a tenfold margin in relation to experimental findings to allow for individual differences in susceptibility. As already shown, no allowance for interspecies differences need usually be made in irritancy testing. A tenfold safety criterion on these lines may prove quite helpful for the safety evaluation of raw materials but it will seldom be a technically feasible criterion for use in testing formulated products. Direct comparison of a newly-formulated product in a threshold irritancy test with other formulations of similar type, whose effects during normal use are known, will be more appropriate. Such a comparison will certainly give practical guidance on probable safety-in-use. A study on human volunteers will clearly be the most reliable and ideally the study should take the form of a comparison with a known 'safe' and a known 'unsafe' material of similar type (i.e. with 'negative' and 'positive' controls). Comparison with a 'positive' control (e.g. a known irritant) might facilitate quantitative expression of the findings, if a human tolerance test is carried out. In circumstances where laboratory animal studies are judged to be required, it will be equally desirable to conduct these as threshold irritancy tests to forecast the onset of hazard to man in normal use.

Reasonably exaggerated exposure in cosmetic safety evaluation may theoretically be achieved by designing 'in use' tests. Human volunteers use the test material for a few weeks with more frequent applications than would normally be made and subject to repeated examination for adverse effects. Such a procedure may involve a risk that the investigator will be unable to control the amount and frequency of application effectively and that comparisons with suitable controls may be difficult to arrange. Unless the conditions of testing prove suitable for achieving threshold responses, interpretation may depend on negative findings which will limit the reliability of the study. 'In use' testing warrants serious consideration, however, as an alternative to the highly empirical, grossly exaggerated procedures currently favoured by some investigators.

ACKNOWLEDGMENT

The authors are indebted to Mr J. Brown for the data given in *Table III.*

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Four methods for the characterization of dentifrices and other semisolids

M. BLOCK*

Presented on 12–14th November 1973 at Nottingham at the Symposium on 'Evaluation of Product Performance' organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—During the course of development work, the need arose for characterization and comparison of batches of DENTIFRICES. Four methods used in other technologies are reported for their utility in the evaluation of these and other SEMISOLIDS.

Force-time traces were recorded on a modified *Instron* TENSIOMETER for a piston moving at constant speed down a full tube of toothpaste and again down the emptied one. The difference was the corrected initial FORCE for EXTRUSION. At the same time, a number of extruded drops were weighed. The mean weight of a drop was divided by the diameter of the orifice to obtain the TENSILE STRENGTH of the paste.

A polyethylene disc resting on the surface of a jar of dentifrice was withdrawn at constant speed on an *Instron* tensiometer. The force-time curve showed a characteristic maximum cohesive force. The final value obtained on separation of paste and disc was a measure of STRINGINESS.

A notched doctor blade was used to apply 10 parallel stripes of uniform width but increasing height upon a surface. Rotation through 90° permitted sag of the semisolid, giving a stripe number characteristic of RESISTANCE to SAG.

INTRODUCTION

Numerous semisolids are in household use to-day, ranging from medicinal ointments, skin creams and dentifrices in the bathroom, to sandwich spreads, mayonnaise, whipped cream and jellies in the kitchen,

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and to putty, polishes, grease and paint in the workroom. Thermoplastics, ceramics, glass and other materials appear rigid at room temperature but are processed in the semisolid state. The measurement and control of rheological properties of such materials presents great difficulties. Historically, each craft and later each industry has tried to solve its particular rheological problems in isolation, so that to-day a vast amount of knowledge is available but dispersed among numerous specialized sectors of technology.

During the course of product development on dentifrices, the need arose for characterization and comparison of batches of pastes. Four methods were transferred from other technologies and are reported for their potential utility in the evaluation of semisolids. The first two methods utilize the principle of extrusion, the next measures values related to cohesion and adhesion and the fourth ascribes a number to the property of sag.

Earlier work on extrusion

A variety of simple rheometers has been used to measure the extrusion of butter (1), fats (2–4), molten lead and wax (5), clay pastes (6), mastics (7) and foods (8). Prentice (9, 10) and later Wiedermann (11) found a good correlation between the ease of extrusion of fats and their spreadability. Further measurements on the extrusion of foods through simple devices have been made (12–16). The force for extrusion of ointments and pastes from tubes and bottles has also been measured (17–20). The ductility of petrolatum has been measured by extruding a column vertically downwards, permitting it to break under its own weight and measuring the length of the residual cone adhering to the orifice (21). Food texture has been assessed by back extrusion through the concentric annulus between a cup and a metal plunger (22–24).

Experimental

Materials

A number of viscous liquids, suspensions, gels and pastes were prepared for this study.

Most mucilages consisted of a dispersion in 70% sorbitol syrup of gums and other hydrocolloids, such as Carbopol *940*, a carboxy-vinyl polymer

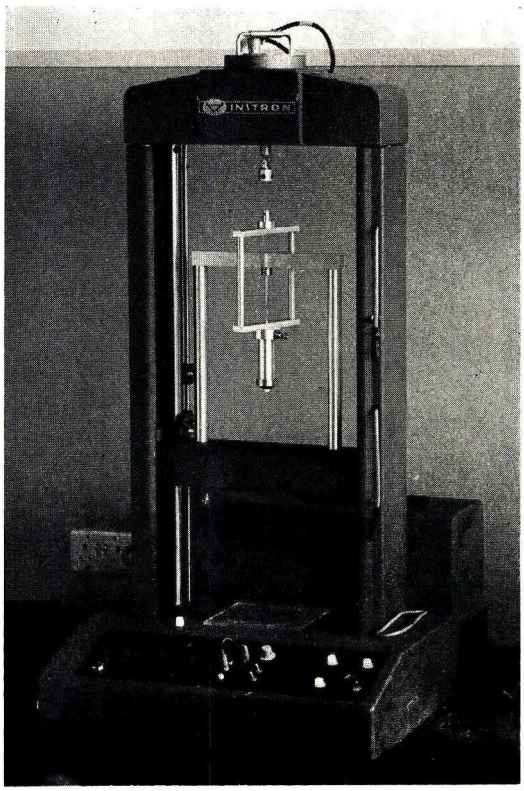

Figure 1. *Instron* tensiometer with Mk I extrusion rheometer.

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which was neutralized with sodium hydroxide. Another consisted of a 20% suspension of a synthetic silica thickener in sorbitol.

Dentifrice 1 contained 14% of an abrasive, dentifrice 3 contained 32% of a mixture of abrasives, Seven different hydrocolloids were used at the stated concentrations, with corresponding minor changes in water content, in order to achieve acceptable consistency and different rheological properties (*Table I*).

Methods of rheological characterization

Initial force of extrusion (corrected)

Vasic and deMan's method (16) was adapted for use on an *Instron* tensiometer.

Extrusion rheometer attachment. Figs. 1–3 illustrate the apparatus, which consisted of a motor crosshead rigidly joined to the *Instron* crosshead. A stainless steel rod fitted to an aluminium piston was attached to the motor crosshead. The piston was fitted at first to a metal tube bearing a brass cap,

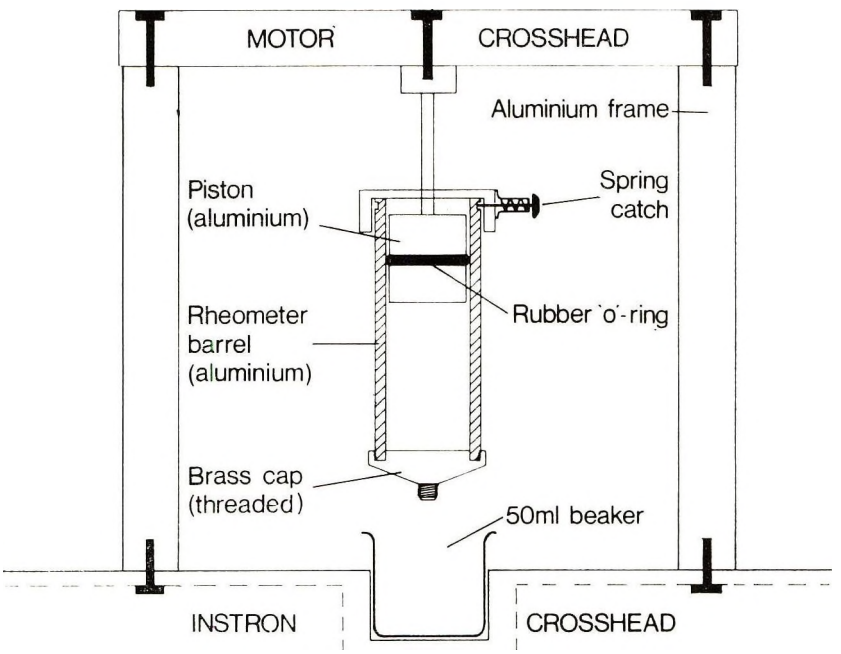

Figure 2. Mk I rheometer barrel.

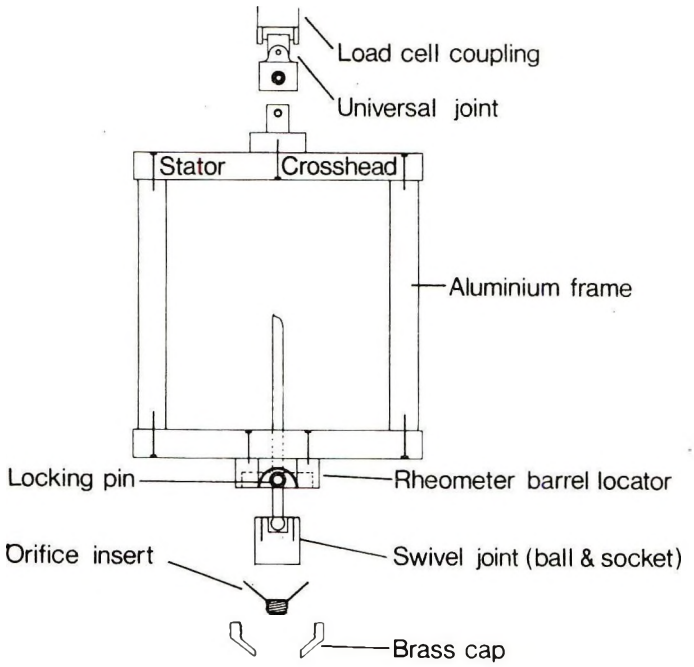

Figure 3. *Instron* crosshead assembly.

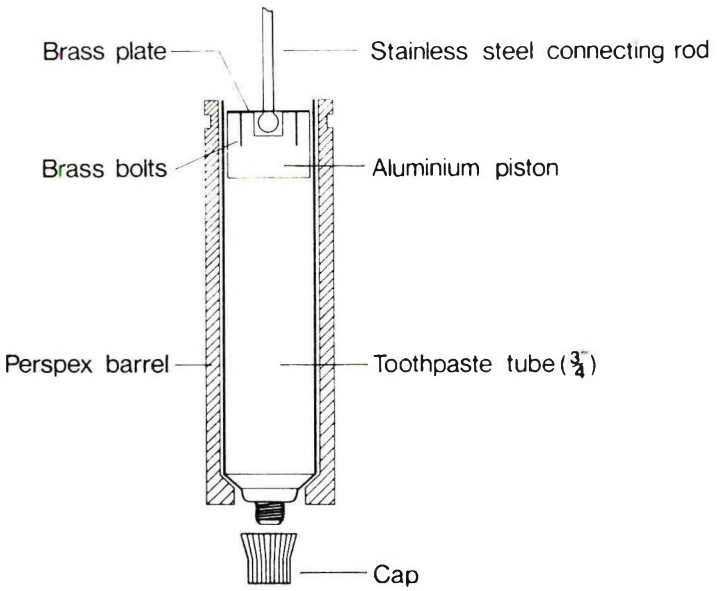

Figure 4. Mk II rheometer barrel.

which held the orifice insert, *Fig. 2.* It was later found more convenient to fit the piston to a standard, inverted, uncrimped tube of dentifrice supported in a *Perspex* holder, *Fig. 4.* The latter was attached to the *Instron* crosshead, which was linked as usual by a pin to coupling of the load cell.

Experimental procedure. Tests with a stopwatch and dentifrices extruded manually from standard tubes showed an extrusion velocity of about 1.3 cm sec^{-1}. Speeds of 5 and 10 cm sec^{-1} for the *Instron* crosshead corresponded to extrusion velocities of 0.9 and 1.8 cm sec^{-1} from the tube. Inverted tubes were filled to a fixed height, stoppered and left for 24 h. They were then placed, one at a time, in the *Perspex* barrel. The screw cap and rubber stopper were removed and the piston was inserted in the inverted tube. As the crosshead began to descend and the piston began to extrude the paste, the force for extrusion, which was recorded, rose sharply; the rapidly attained maximum was taken as the 'initial force for extrusion'. A repeat run was made with the empty tube. The initial force for extrusion recorded on the emptied tube, due to friction between piston and the aluminium walls, was subtracted from the initial force for extrusion for the filled tube.

Results. Table I shows the results for the initial force for extrusion determined at two shear rates, corresponding to consumer practice when extruding dentifrice from this standard size of tube. *Table II* shows the effects of time of storage on this parameter.

For most pastes, there was an increase in the force required for extrusion with increase in velocity of extrusion, as was expected. *Table II* shows that for most pastes, the initial force required increased with the time elapsed between filling of a tube and extrusion.

The modified cellulose thickener in dentifrice 1 was the only material to show a decrease in initial force for extrusion with increasing speed. The two types of xanthan gum showed a remarkably low force for extrusion in both dentifrices, as can be seen in *Table II.* The effect persisted in a mixture with sodium carboxymethyl cellulose.

A small panel of laboratory staff confirmed that ease of extrudability of the dentifrices tested varied directly with the initial force for extrusion.

Discussion. The initial force for extrusion from a new tube of dentifrice is particularly important for the consumer, who makes a judgement on the quality of the product from this sensory cue. With some pastes, the force required varies dramatically with temperature and this could prove unsatisfactory in either summer or winter. There seems to be a range of acceptable pressures for deforming the tube and extruding the paste, outside which products are judged unfavourably. Although it is possible to alter

Table I. Initial force for extrusion (f) and tensile strength (t) at two shear rates

		Force (f)				Tensile strength (t)		
		5 cm sec^{-1}		10 cm sec^{-1}		5 cm sec^{-1}		10 cm sec^{-1}
Material	Thickener	f	Range	f	Range	t	Range	t
Dentifrice 1	0.3% modified cellulose	340	320–360	300	250–350	5.9	5.8–6.0	5.2
	0.3% SCMC A	520	490–540	570		5.3	5.2–5.4	5.7
	0.6% carragheenan A	680	550–720			18.5	18.0–19.0	
	0.8% carboxy vinyl polymer	150	100–250	170		3.5	3.0–3.7	4.4
Dentifrice 2	0.75% SCMC B	775	740–800	1050	1000–1085	7.5	7.4–7.6	9.0
	0.75% xanthan gum A	370	330–420	480	470–500	4.6	4.4–4.7	5.3
70% sorbitol	20% silica gel	640	570–700	900	600–1150	1.7	1.5–1.9	2.4
	0.5% carboxy vinyl polymer	360	300–400	560		7.0	6.9–7.1	7.4

Table II. Initial force for extrusion and tensile strength after 1 and 2 days

Material	Thickener	Initial force		Tensile strength	
		1 day	2 days	1 day	2 days
Dentifrice 2	1.2% modified cellulose	600, 400	780, 750	9.6, 10.5	11, 18
	0.9% SCMC B	670, 760	960, 1160	5.5, 5.7	
	1.0% xanthan gum B	105	295, 260, 260, 260	2.7	2.7, 2.8
	0.5% SCMC B with 0.3% xanthan gum A	440, 460	530, 590, 620	4.2, 4.2	
	1.1% carragheenan B	390, 435	620, 620	4.2, 4.2	
	1.3% carragheenan A	370, 370	450, 495	3.4, 3.5	
Dentifrice 3	1.5% SCMC B	650, 685	780, 820	6.6, 6.3	9.0, 10.3, 9.6
	1.7% xanthan gum B	190, 170		4.9, 4.9	

the pressure required by changing the diameter of nozzle on the tube, a change in formulation is generally more convenient. Of course, when different batches of toothpaste are to be compared, the force required to deform the tube need not be taken into account.

In his work on the horizontal extrusion rheometer, Prentice (8) considered the force of extrusion to consist of two parts (*Fig. 5*). The force required to push the sample of cooking fat along the cylinder depended upon the friction between metal and fat and was found to decrease linearly with length of stroke. The force required to drive the sample through the orifice was considered constant and was of major interest to this investigator.

Although Prentice (9, 10) and Hoffer (25) found a linear decrease in force with time, that is, decreasing length of stroke, as cooking fats, margarine and butter were extruded through the orifice, this was not found to be the case with dentifrices on a *FIRA-NIRD* extruder* (26). The smooth brass tubes had been filled with pastes and extruded after 24 h. A most irregular series of variations of force with time was obtained. These variations were attributed to the friction of hard particles of abrasive wedged between piston and tube.

Vasic and deMan (16) were interested in the force required to push material along the tube as well as that required for extrusion through the orifice and used the average force on one downstroke in their calculations. They repeated the run with the emptied cylinder and subtracted this force from the average.

* Gaydon Ltd, Croydon, Surrey.

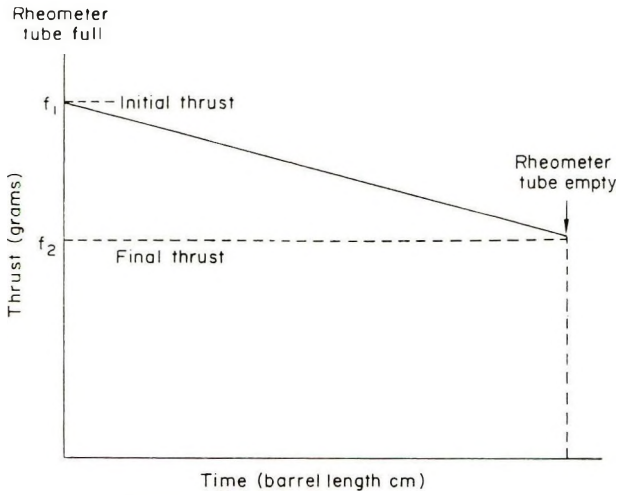

Figure 5. Force for extrusion—idealized.

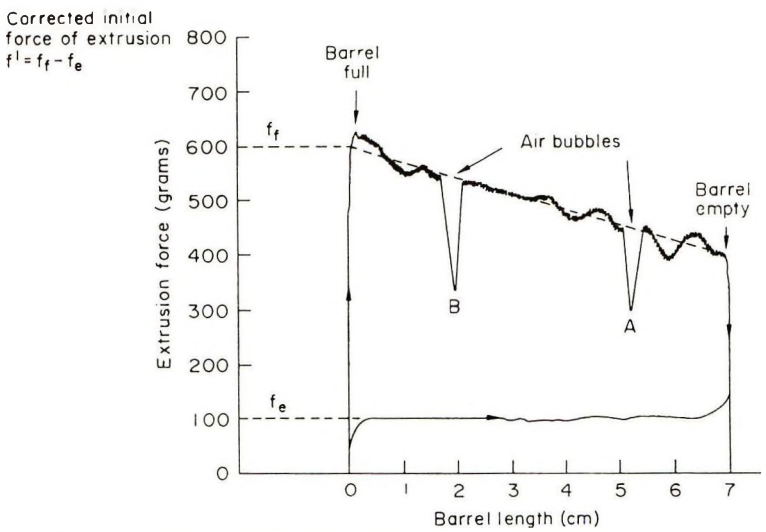

Figure 6. Force for extrusion—actual. Crosshead speed = 5 cm min^{-1}.

In this work the maximum, initial force required for extrusion was considered of greatest interest. Considerable variations were found on samples from the same batch stored for the same length of time in separate tubes. These variations were considerably reduced when the initial force for extrusion for each emptied tube was subtracted from the initial force determined on the full tube, *Fig. 6*.

Tables I and II show that despite the scatter of experimental results, the method can usefully detect differences in formulation between similar toothpastes.

Characterization by tensile strength

Ben-Arie (27) pointed out that much apparatus for the measurement of rheological properties of viscoelastic materials, such as 'gel strength', was adapted to specific uses and that the experimental values were dependent upon the specific equipment employed. Some devices measured rigidity, some plasticity and others elasticity. Moreover, there was generally no physical correlation between the results of the different methods. On the other hand, tensile strength is a clear, well-defined concept.

Ben-Arie (27) extruded Napalm gels with piston and barrel downwards through various nozzles. A number of drops extruded at constant speed were weighed. The mean weight of a drop divided by the diameter of the nozzle was the tensile strength. He established that the experimentally determined values were independent of the areas and of the area-length ratios of the nozzles used for extrusion. As the tensile strength increased linearly with velocity of extrusion, the values were determined at two convenient velocities and extrapolated to zero.

Charm (28) extruded mayonnaise and ketchup through tubes using air pressure and calculated the tensile strength in a similar way. The diameter of the tubes had to be below that critical value at which the material would flow under gravity.

For this work the tensile strength at the velocity of extrusion used for dentifrices was of interest, and Ben-Arie's method was employed.

Experimental procedure. The extrusion rheometer was used to determine the initial force for extrusion and the tensile strength of the paste in one run. The drops and pieces of extruded ribbon falling from the rheometer after extrusion were simply counted and weighed, *Fig. 7.* The mean weight of a drop was divided by the diameter of the nozzle to obtain the tensile strength.

Results. Table I shows the results for two velocities of extrusion and *Table II* the effect of increasing times of storage on a number of formulations. For most pastes there was an increase in tensile strength with increased velocity of extrusion (or driving pressure) and with increasing time of storage. Only the modified cellulose thickener in dentifrice 1 showed a decrease in tensile strength with increasing velocity of extrusion. The xanthan gums in dentifrice 2 showed a remarkably low tensile strength both 1 and 2 days after filling.

A small panel confirmed that ease of extrudability of the dentifrices tested varied inversely with the tensile strength.

Discussion. Determination of tensile strength was found to be simpler and more reproducible than determination of initial force for extrusion. The necessary equipment can be much less sophisticated than the *Instron* tensiometer and of course no recording equipment is required.

Many dentifrices increase in tensile strength with time of storage. Indeed, a low degree of thickening immediately after manufacture, permitting easy filling of tubes, followed by development of structure over a period of several weeks, has been claimed as an advantage for one type of thickener (29). In fact, shear-thinning is highly desirable in dentifrices; the paste should be easy to squeeze out of its tube to form a firm ribbon on the brush. In the mouth it should then again rapidly deform under shear. With most toothpastes, this is a thixotropic, time-dependent process. Measurement of tensile strength at two time intervals after filling, as in *Table II*, would distinguish between the slow time-dependent and other processes.

The speed of the method and the low loss of heat to the surroundings due to the *Perspex* support permit measurements to be made over a range of temperatures.

Characterization by maximum cohesive force and stringiness

Claassens (30) developed the so-called 'hesion' balance for measuring the adhesion-cohesion of butter to various materials. The vertical pull required to separate butter from a solid disc was determined. Henry and Katz (31) adapted this method for use with the *Instron* tensiometer and measured the adhesion and stringiness of starches, gums and whipped cream to solid surfaces. Their method was adopted for use on gels and dentifrices.

Experimental procedure. A polyethylene disc of 2.5 cm diameter was fixed to a spindle attached to the jaws of the calibrated *Instron* tensiometer and a jar containing the paste was raised until complete contact was made all around the disc, care being taken to avoid entrapment of air between disc and gel. A time for equilibration was allowed, according to the degree of thixotropy of the semisolid. The crosshead was then started and a force-time curve was recorded, *Figs. 8–10*. The stringiness or S-factor was measured just after the thin neck of semisolid had ruptured.

Theory. As the disc was being lifted at constant speed, the abscissa in *Fig. 10* has been plotted as distance in cm rather than time in seconds. The force-distance curve is in many ways similar to those for metals with respect to three well-defined regions:

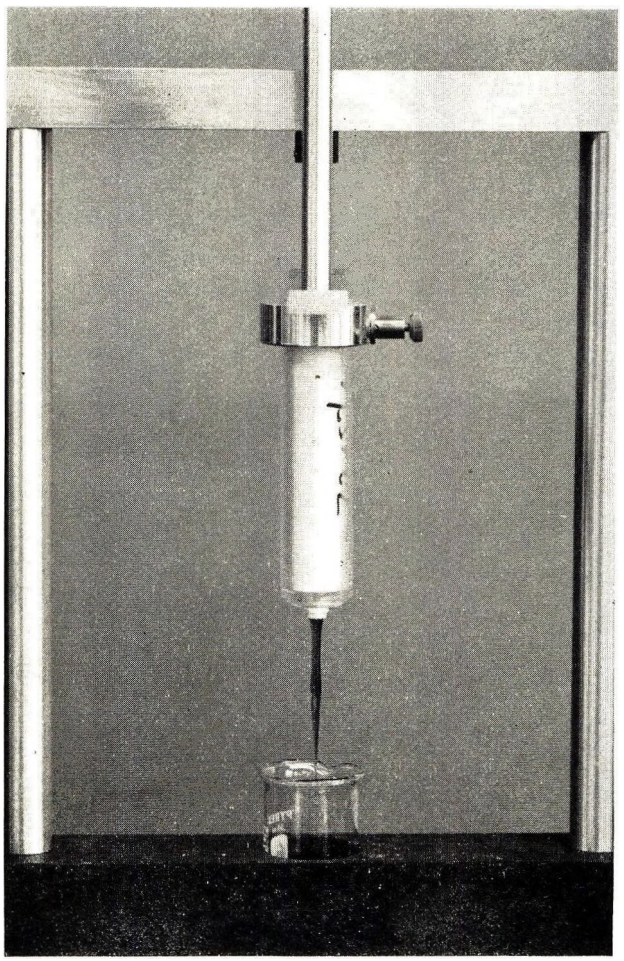

Figure 7. Mk II rheometer with toothpaste tube insert.

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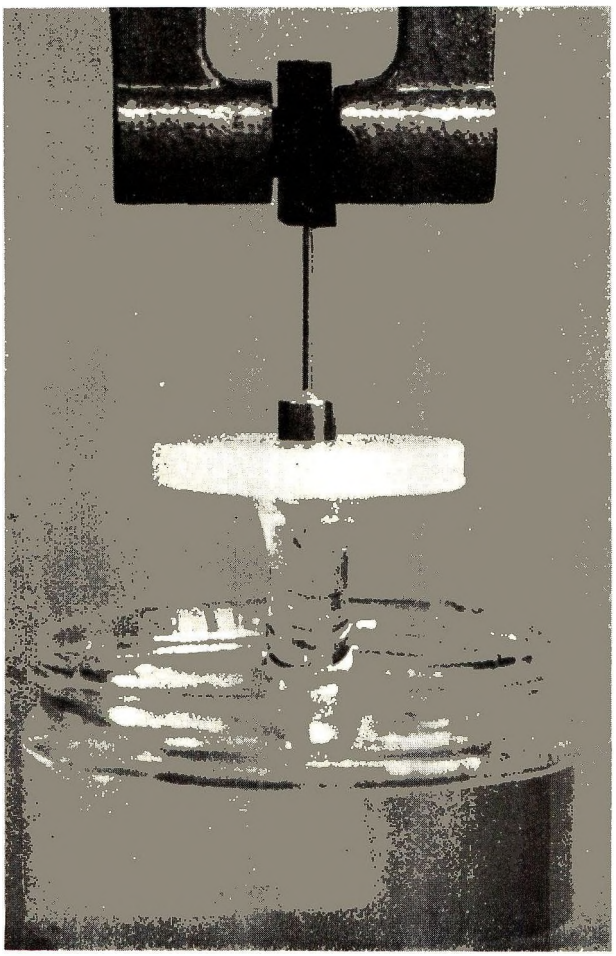

Figure 8. Hesion apparatus—'Necking' of 2% SCMC gum in 70% sorbitol after extension for 50 sec.

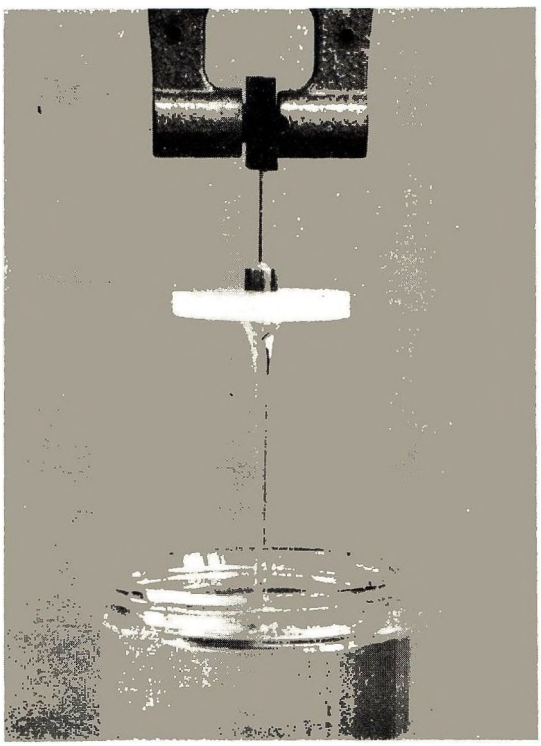

Figure 9. As in *Fig. 8* after 100 sec.

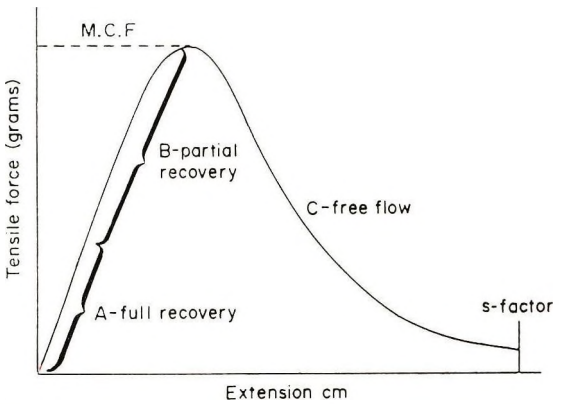

Figure 10. Force-distance curve during adhesion-cohesion.

A — in this elastic region total recovery occurs after deformation;
B — recovery occurs with some deformation;
C — beyond the point of maximum cohesive force, recovery cannot take place and so free flow occurs until the point of rupture, characterized as the stringiness or S-factor.

Henry and Katz (31) pointed out that the area under the force-extension curve was proportional to the work done in extension. The total force reached a maximum value and diminished before the extended semisolid ruptured. Although the tensile force per unit area did not decline, the total force (g $cm^{-2} \times$ area in contact) reached a characteristic maximum value and diminished before rupture.

Results. Table III shows the maximum cohesive force and the stringiness for a number of pastes and mucilages at two crosshead speeds. While the maximum cohesive force increased or remained constant with increasing speed, the S-factor increased in all cases. For all systems studied, the maximum force was reached fairly quickly, indicating that this was the force required to lift the disc over the first few millimetres from the surface. It was found to be dependent upon the time required to develop thixotropy, as shown by successive trials with material from the same jar. *Figs. 11 and 12* show the effects of differing crosshead speeds on the shape of the force-extension curves for two materials. The maximum cohesive force increased with increasing crosshead speed for the mucilage but decreased for the dentifrice.

Discussion. The maximum cohesive force and stringiness were found to be useful parameters for characterizing dentifrices and mucilages, especially

Table III. Maximum cohesive force (MCF) and stringiness at two crosshead speeds

Material	Thickener	MCF		S-factor	
		5 cm min^{-1}	10 cm min^{-1}	5 cm min^{-1}	10 cm min^{-1}
Dentifrice 1	0.3% modified cellulose	240	—	1.2	—
	0.8% Carboxy-vinyl polymer	56	—	3.5	4.4
	1.2% Alginate A	215	295	5.6	7.9
	1.0% Alginate B	80	—	9.9	—
70% Sorbitol	0.5% Carboxy-vinyl polymer	150	185	3.3	—
	1.5% Alginate C	70	95	>16	>16
	1.5% Alginate D	38	45	>16	>16
	1.5% Alginate E	40	40	12	>16
	0.9% Carragheenan A	64	70	2.4	7.0
	0.7% Carragheenan A	58	65	2.8	3.5
	2.0% SCMC C	54	68	>16	14.2
	1.7% SCMC C	20	25	9.2	15.0
	20% silica	44	—	8.8	11.5
	19% silica	24	—	1.3	—
	1.2% SCMC B	—	—	7.5	—

when used in conjunction. Acceptable dentifrices had S-factors below 4 cm at a rate of extension of 5 cm sec^{-1}.

For quality control of pastes and gels a far simpler instrument could be cheaply constructed.

Characterization by measurement of sag

It is a common experience that after extrusion from a tube upon a toothbrush, some dentifrices tend to sag or slump between the bristles. With pastes of low dispersibility, sag would increase the time required to clean the toothbrush after use. The slow ooze of toothpaste from an open tube left in the bathroom is probably also associated with the property of sag. From these considerations, it seems likely that the overall preference for a toothpaste may suffer if the property of sag falls below a certain limit.

The tendency to sag is a characteristic property of a semisolid and may be measured simply without reference to a container or to a particular procedure for extrusion. This has been done in the paint industry. In the paint laboratory, many different coatings, which may vary in their requirements as regards resistance to sag, are being tested and the chemist requires a simple, rapid and reproducible method for specifying and testing resistance to sag. The method should have good precision, with test results preferably expressed in unequivocal numbers (32).

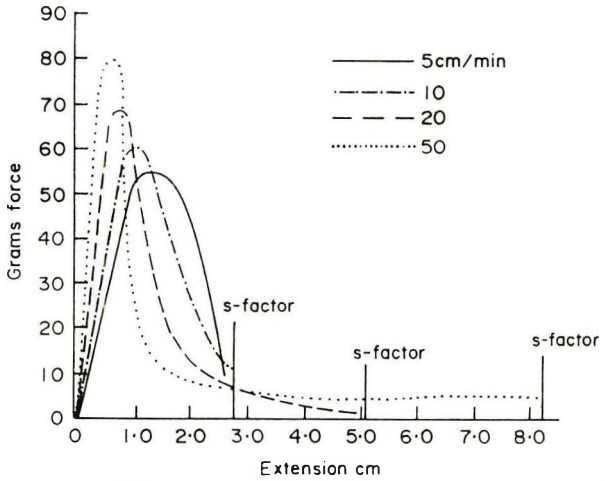

Figure 11. Force-distance curve for carragheenan A in sorbitol syrup.

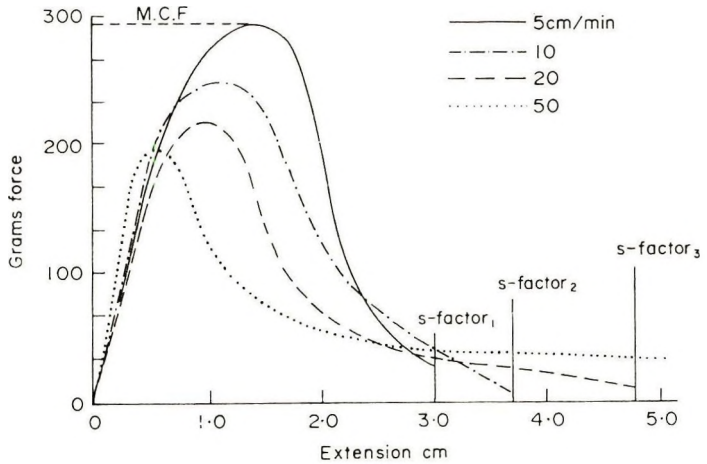

Figure 12. Force-distance curve for a dentifrice.

Reynolds and Larsen (33) devised a sag index blade for the purpose and this was improved by Schaeffer (32). It consists of a doctor blade with a series of wide notches of varying clearance which is used to lay down a series of parallel stripes of paint of uniform width but increasing height. Sagging is related to the height of the stripe. The blade is commercially* available in three sizes: 1–6 mils for spraying and dipping enamels, 3–12 mils for trade sale type paints and 14–60 mils for coatings of high film thickness

* The Leneta Company, Ho-Ho-Kus, New Jersey, U.S.A.

(1 mil = 10^{-3} inch). Its use is mandatory for measuring resistance to sag in some US Federal Specifications.

Description and procedure. For use on dentifrices, a simplified sag meter in the range 60–120 mils was constructed (*Fig. 13*). The brass doctor blade has 11 notches, each $^1/_4$ in. wide with intervening gaps of $^1/_{16}$ in. The heights of the notches are 60, 70, 80, 90, 100, 120, 140, 160, 180 and 200 mils. The last notch is a thin, shallow marker.

Toothpaste is placed upon a horizontal, glazed glass plate along the recessed side of the meter, which is drawn at a slow, steady rate along a straight edge. A series of equidistant, parallel stripes of uniform width and varying heights is obtained. The plate is placed in a vertical position with the stripes horizontal, the fattest stripes being lowest.

A slightly roughened surface is required to prevent slip of the stripes of toothpaste.

Results. Fig. 14 shows the results of a typical sag test. The five fattest stripes have sagged, obscuring the intervening gaps. (The lowest stripe is the shallow marker.) The sag number is therefore 5. A series of pastes can be compared easily in this way, sag ceasing after about 30 min.

For a series of more fluid pastes, the time taken for the most mobile one to show a sag number of 1 may be noted. The other pastes can then be ranked in order of sag number, using the same time span. *Fig. 15* illustrates the good reproducibility of the test. Each horizontal line shows the

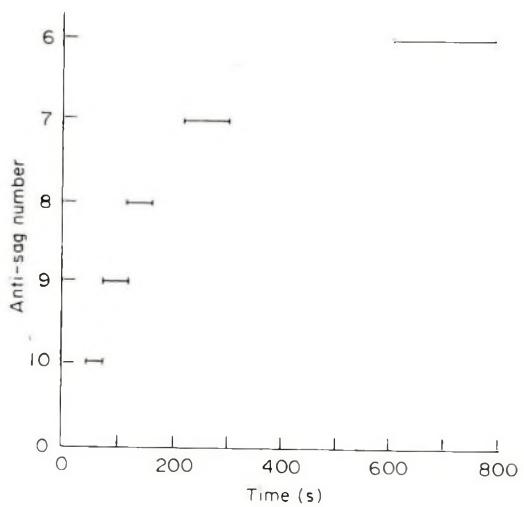

Figure 15. Span results for five trials on one paste. Final sag number for all trials 5.

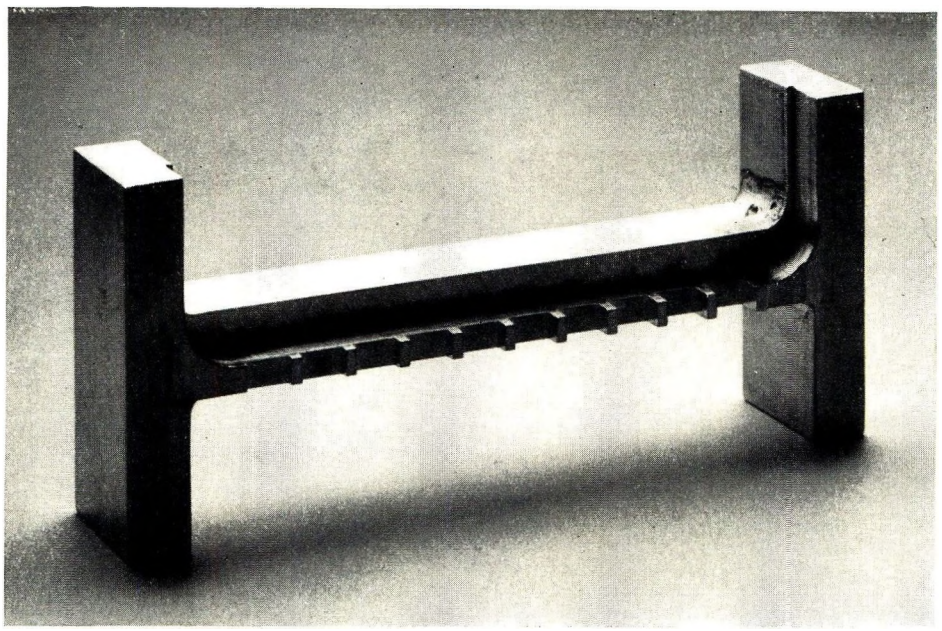

Figure 13. The toothpaste sag meter.

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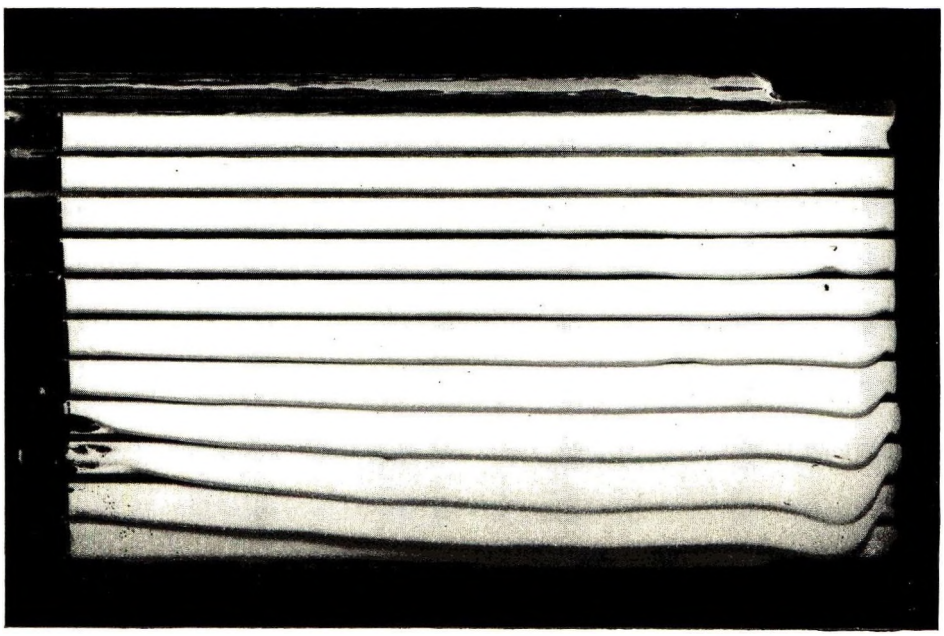

Figure 14. Typical sag test. This paste has a sag number of 5.

span of time taken to reach a given sag number in five successive trials on one paste.

Conclusions

The measurement and control of the complex and rather ill-defined rheological properties of semisolids are becoming increasingly important to industry and to the consumer to-day. It is hoped that the methods described will prove useful in factories and in other laboratories for research, development and quality control. In the absence of expensive, multipurpose equipment, adequate measurements may still be made with rather simple devices.

This work has been based on the research of scientists from the United Kingdom, South Africa, Israel and the United States on materials as diverse as cooking fats, butter, Napalm and paints.

Acknowledgment

I wish to thank Mr J. A. Cristofides for his assistance during an industrial training period.

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Percutaneous absorption of Triclosan from toilet preparations

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Presented on 26–30th August 1974 in London at the IFSCC VIIIth International Congress on 'Cosmetics—Quality and Safety' organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—The absorption of [^{3}H) TRICLOSAN (*Irgasan*® DP300) through RAT SKIN treated with SHAMPOO containing 0.05% (w/v), and with AEROSOL DEODORANT containing 0.1% (w/v), has been measured. The products were applied in a manner designed to simulate consumer use, and the penetration was calculated from the amount of radioactivity excreted by the animals. From the shampoo, the penetration was 0.197 μg cm^{-2} which increased as the concentration of [^{3}H] Triclosan was increased but which was independent of duration of contact with the skin for a given concentration of [^{3}H] Triclosan. BLOOD LEVELS at 48 h after treatment were proportional to concentration of applied [^{3}H] Triclosan and for 0.05% (w/v) were less than the equivalent of 0.1 μg ml^{-1}. From the aerosol deodorant the penetration was 6.85 μg ml^{-1} and the blood level reached a maximum, equivalent to 0.26 μg ml^{-1}, at 6 h after a single application.

The calculated absorption by the human is an extremely low proportion of the no-effect level in rats.

Introduction

It has previously been established that small amounts of the germicide, hexachlorophene, can penetrate through intact skin (1) and can also be identified in blood, adipose tissue, brain and other body organs (2, 3). The compound 2-hydroxy, 2^1,4,4^1-trichlorodiphenyl ether, now called Triclosan and formerly known as *Irgasan*® DP300 (Ciba Geigy Ltd), has some chemical features in common with hexachlorophene and has been shown to be effective in reducing both Gram positive and Gram negative

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bacteria on the skin (4). It has the advantage over hexachlorophene of being of low toxicity (4). Nevertheless, it is important to know the degree to which it could be absorbed through skin from a variety of skin products. We have previously reported on the percutaneous absorption in guinea-pigs of Triclosan from soaps (5) and this present report examines its absorption through rat skin treated with shampoo containing various concentrations of [^{3}H] Triclosan and with aerosol deodorant containing 0.1% (w/v) [^{3}H] Triclosan. The possible absorption by the human of 0.05% (w/v) Triclosan in a shampoo and of 0.1% (w/v) Triclosan in an aerosol deodorant is calculated from the rat data.

Methods

Materials

Tritiated Triclosan was prepared and purified as described by Black, Howes and Rutherford (5) and had a specific activity of 44.86 μCi mg^{-1}.

Samples of [^{3}H] Triclosan were accurately weighed out and the shampoo base was added to give concentrations of 0.05, 0.1, 0.5, 1.0 and 2.0% (w/v) of [^{3}H] Triclosan. The mixtures were stirred at 50°C for 1–2 h to ensure complete solubilization of the germicide. Aliquots of each preparation were counted to determine the precise amount of [^{3}H] Triclosan applied to the rats.

A single can of aerosol deodorant was prepared by dissolving [^{3}H] Triclosan in ethanol and adding the other ingredients to give a concentration of [^{3}H] Triclosan in the product of 0.1% (w/v). The can was sealed and the propellant added through the valve from another cannister.

Animals and treatment

Subcutaneous turnover of Triclosan

Twelve female Colworth-Wistar rats (120 g) were injected with 0.5 ml of [^{3}H] Triclosan solution in 50% aqueous polyethylene glycol (BDH, Poole, Dorset) under the loose skin over the upper thorax. The animals were placed immediately in the individual metabolic cages for up to 96 h and 24-hourly samples of the separated urine and faeces were collected for determination of tritium. Some rats were killed at 24, 48, 72 and 96 h after being anaesthetized and heart blood taken.

Shampoo application

Thirty-two female Colworth-Wistar rats (110–130 g) were clipped and the exposed lumbar skin was pre-washed with a 20% (v/v) solution of the shampoo base, rinsed and dried. Twenty-four hours later the animals were lightly anaesthetized with cyclopropane, oxygen and carbon dioxide gas mixture, and an area of 7.5 (or 15) cm^2 of skin was marked. 0.1 ml (or 0.2 ml) of the test shampoo was applied to the skin (7.5 or 15 cm^2 respectively) and spread over the marked area with a rounded glass rod. 0.1 ml (or 0.2 ml) of water was applied to the marked area and lathered with the glass rod for 1 min. The solution was left for a further 4, 9 or 19 min (5, 10, or 20 min total) in contact with the skin before thoroughly rinsing the skin with distilled water. The rinsings were collected and monitored for tritium and the treated area of skin was dried by lightly dabbing with paper tissues. The treated area of skin was protected with a non-occlusive patch, which was composed of three layers of surgical gauze (3.0×3.5 cm for a 7.5 cm^2 area of skin) covered with 100 mesh stainless steel gauze and the whole wrapped around the trunk of the animal with perforated (5–10 holes/cm^2) '*Sleek*' tape (Smith & Nephew Ltd). The animals were placed in individual metabolic cages and excreta were collected separately in 24-hourly batches. At the end of the experiment the animals were anaesthetized, terminal heart blood taken and then sacrificed. The protective patch and treated area of skin, which was frozen until required, were monitored for tritium.

Deodorant application

Six female Colworth-Wistar rats (120 g) were prepared as described above. Twenty-four hours later the animals were anaesthetized and a protective card screen with a 7.5 cm^2 window was placed over the back. The aerosol can was aimed centrally over the window area and a 2-s spray applied with the can held approximately 15 cm away from the skin. The screen was removed, the treatment area marked with a felt-tipped pen and covered with a protective patch. The animals were then placed in separate metabolic cages and treated in the same way as the animals washed with shampoo.

Topical application of Triclosan in ethanol

A solution of [^{3}H] Triclosan in ethanol was applied to the skin of 12 female Colworth-Wistar rats (120 g). The treated area of skin was air-dried for about 20 s after which time the ethanol had apparently evaporated. The treated area of skin was covered with a protective non-occlusive patch

and the animals placed in separate metabolic cages. The animals were killed at various times up to 96 h after application and excreta, blood, skins and protective patches were monitored for tritium.

Rats on all treatments were fed on pellets of *Spital* diet (BOCM/Silcock, Process Development Department, Bromborough, Cheshire) and given water *ad libitum*.

Analysis of biological samples for tritium

Urines were made up to 50 ml and 2.0 ml aliquots were counted in 18.0 ml of *Triton* X-100 : Toluene (1 : 2, v/v) liquid scintillator containing 5.0 g PPO and 0.2 g POPOP/1. The samples were thoroughly mixed and counted in a Packard 4322 liquid scintillation spectrometer. A channels ratio technique was used to determine the counting efficiency and [1,2-^{3}H] *n*-hexadecane was used as an internal standard. All other aqueous samples (skin rinsings, standards and washings) were monitored in a similar manner.

Faeces were freeze-dried and the sublimate was monitored for tritium. Aliquots of up to 350 mg of the residue were combusted in a Packard 305 sample oxidizer to determine the tritium content as tritiated water. Recoveries of greater than 98% were recorded from standards with counting efficiencies of up to 25%.

Full depth, 1 cm diameter punch autopsies of frozen skin were monitored for tritium either after combustion in a similar way to the faecal samples or after solubilizing in *Soluene* (Packard Instruments Ltd) at 40°C overnight and neutralizing with an excess of solid CO_2.

The protective patch was soaked in 50 ml ethanol at room temperature overnight and further extracted with 50 ml ethanol. The extracts were combined and aliquots were counted to determine the tritium content of the patch. Recovery of radio-activity by this method was better than 99%.

Blood tritium levels were determined by combusting up to 0.5 ml aliquots of blood in the Packard 305 sample oxidizer.

Results

Turnover of subcutaneously injected Triclosan

The recoveries of tritium in the urine and faeces of female rats during 4 days are presented in *Table I*, from which it can be seen that 89.2% of the dose was recovered, some 33% being present in the urine. The faeces

Table I. Turnover of Triclosan injected subcutaneously

Time (days)	Tritium recovery (dpm × 10^{-6})		
	Urine	Faeces	Total
1	17.487 ± 5.079	23.210 ± 6.112	40.697 ± 8.722 (4)
2	3.589 ± 1.303	12.155 ± 4.011	15.744 ± 6.041 (3)
3	1.126 ± 0.840	2.289 ± 1.037	3.415 ± 1.149 (2)
4	0.857 ± 0.611	1.170 ± 0.610	2.027 ± 1.021 (2)
Total	23.059	38.824	61.883

Four female Colworth-Wistar rats (120 g) were injected with 0.5 ml of a 50% aqueous polyethylene glycol 400 solution containing 69.453×10^6 dpm [^{3}H] Triclosan. The rats were placed into individual metabolism cages and the excreta collected separately every 24 h for 4 days. The tritium content of the faeces was determined by combustion and scintillation counting and that of the urine by counting directly.

always contained the greater proportion of radio-activity throughout the 4 days. Using these data, a semi-logarithmic plot of total excreted tritium against time indicated a biological half-life of approximately 14 h. In the first two days 81.3% of the dose was excreted, and this figure is used to correct the recoveries in 2 days from rats treated topically with [^{3}H] Triclosan in shampoo or aerosol deodorant. The level of tritium in blood of subcutaneous-injected rats ranged from the equivalent of 0.2 μg ml^{-1} at 0.5 h to a maximum of 5.2 μg ml^{-1}, at 6 h after injection and decreased over 24 and 48 h to approximately 0.3 μg ml^{-1} at 96 h.

Application of [^{3}H] Triclosan in shampoo

Some 85% of the [^{3}H] Triclosan applied to rat skin at various concentrations in the shampoo base was recovered in the rinse water and the patch, with additional smaller amounts remaining in the treated skin (*Table II*). The amount of [^{3}H] Triclosan residing in skin, at 48 h after the shampoo, was proportional to the concentration of [^{3}H] Triclosan in the shampoo applied to the skin.

The extent of penetration of [^{3}H] Triclosan through rat skin washed with shampoo was calculated from the amount of tritium in the excreta during the 2 days after treatment. Penetration of [^{3}H] Triclosan through rat skin increased in proportion to the concentration in the shampoo (*Table III*) but was independent of the duration of contact of the shampoo with the skin for periods up to 20 min before rinsing (*Table IV*). The concentration

Table II. Recovery of Triclosan from shampoo-treated rats

Application (%)	Application (μg/7.5 cm²)	Recovery at 48 h: Rinsings (μg)	Recovery at 48 h: Patch (μg)	Recovery at 48 h: Skin (μg cm⁻²)	Rats (No. of ♀)
0.05	89 (15 cm²)	76.5±5.6	<1.0	0.15	12
0.10	103	93.7±1.7	2.8±0.6	0.48	3
0.50	474	410 ±63	7.4±3.4	1.40	3
0.75	671	563 ±38	6.6±4.6	1.75	8
1.0	880	879 ±51	18.6±3.4	2.40	3
2.0	1664	1717 ±65.6	26.9±7.9	3.24	3

To 7.5 cm² (or 15 cm²) clipped dorsal female rat skin, 0.1 ml (or 0.2 ml) shampoo containing various concentrations (w/v) of [³H] Triclosan was applied followed by 0.1 ml (or 0.2 ml) H_2O and spread over the skin for 1 min. After a further 9 min the rats were rinsed, dried, a protective patch applied and transferred to individual metabolism cages. At 48 h after treatment the rats were killed and the patch and treated area of skin removed for analysis.

in blood, based on the radioactivity expressed as μg Triclosan, was very low and increased as the concentration of [³H] Triclosan in the shampoo was raised (*Table III*), but it remained constant when the contact time was increased (*Table IV*). For a shampoo containing 0.05% [³H] Triclosan the observed penetration was 0.16 μg cm⁻² during a 48 h period. On adjustment for an 81.3% excretion of a subcutaneously-injected, known dose the corrected penetration figure is 0.197 μg cm⁻². The total amount of [³H] Triclosan penetrating through the treated area of skin is, therefore, 2.95 μg, or 3.3% of the applied amount. For the other concentrations, the extent of penetration ranged from 2.8% to 4.1%.

Application of [³H] Triclosan in aerosol deodorant

The mean amount of spray used per application was 1.45±0.36 g which at a concentration of [³H] Triclosan of 0.1% meant that 1.45±0.36 mg [³H] Triclosan was sprayed at the shielded rats.

The mean concentration of [³H] Triclosan in the 1 × 15 cm² of shield around the treatment window was 13.2±6.2 μg cm⁻² which was taken as a good measure of [³H] Triclosan applied to the treated area of skin. At 48 h after treatment, the protective patch contained 41 μg [³H] Triclosan and the residue in the treated area of skin was 9.7±4.8 μg cm⁻². Recovery of tritium in the excreta was equivalent to 41.7±11.48 μg [³H] Triclosan for six rats,

Table III. Effect of concentration on penetration of Triclosan from shampoo

Application (%)	Application (μg/7.5 cm²)	Blood (μg ml⁻¹)	Urine (μg/2 days)	Faeces (μg/2 days)	Total (μg/2 days)	Penetration (μg cm⁻²)	Rats (No. of ♀)
0.05	89 (15 cm²)	0.044 ± 0.003	0.98 ± 0.19	1.42 ± 0.71	2.40 ± 0.50	0.16 ± 0.06	12
0.10	103	0.025 ± 0.005	0.98 ± 0.21	2.79 ± 0.45	3.77 ± 0.42	0.50 ± 0.06	3
0.50	474	0.038 ± 0.070	2.85 ± 1.62	8.40 ± 2.26	11.25 ± 0.89	1.50 ± 0.12	3
0.75*	695	0.047 ± 0.011	6.79 ± 2.40	10.41 ± 3.23	17.20 ± 0.38	2.30 ± 0.35	8
1.0	880	0.051 ± 0.013	8.45 ± 1.87	20.61 ± 5.15	29.06 ± 5.11	3.87 ± 0.67	3
2.0	1664	0.107 ± 0.031	12.65 ± 3.30	37.00 ± 5.61	49.65 ± 5.80	6.62 ± 0.77	3

To 7.5 cm² (or 15 cm²) clipped dorsal female rat skin 0.1 ml (or 0.2 ml) shampoo containing various concentrations (w/v) of [³H] Triclosan was applied followed by 0.1 ml (or 0.2 ml) H_2O and spread over the skin area for 1 min. After a further 9 min the rats were rinsed, dried and transferred to individual metabolism cages. Urine and faeces were collected separately every 24 h for 2 days when the rats were killed and blood radioactivity measured.

* Mean data from *Table IV*.

Table IV. Effect of contact time on penetration of Triclosan from shampoo

Duration (min)	Rinsings (μg)	Patch (μg)	Skin residue (μg cm⁻²)	Blood μg ml⁻¹	Urine (μg/2 days)	Faeces (μg/2 days)	Total (μg/2 days)	Penetration (μg cm⁻²)
1	481	9.9	1.84	0.047	8.54	8.18	16.72	2.23
5	575	6.5	1.75	0.057	7.53	10.70	18.23	2.43
10	561	5.5	1.95	0.043	6.12	12.60	18.72	2.50
20	583	4.6	1.43	0.042	4.95	10.17	15.12	2.02

To 7.5 cm² clipped dorsal female rat skin 0.1 ml shampoo containing 0.75% (w/v) [³H] Triclosan (671 μg) was applied with 0.1 ml H_2O. After 1, 5, 10 or 20 m contact, the skins were rinsed and dried and the rats transferred to individual metabolism cages. The excreta were collected separately every 24 h for 2 days when the rats were killed. Samples were taken for determination of radioactivity as in methods section. Results are mean of two animals.

thus the observed penetration was equivalent to 5.57 μg cm^{-2}. Adjustment for an 81.3% excretion of a known dose given subcutaneously, gives a corrected penetration figure of 6.85 μg cm^{-2}.

Application of [^3H] Triclosan in ethanol

It was difficult to spray a standard dose of aerosol deodorant on to rat skin. Thus, ethanol, which is one of the main ingredients of the aerosol formulation, was used as a solvent in which to apply a more accurately known amount of [^{3}H] Triclosan to experimental animals. The results of the penetration studies from ethanol are recorded in *Tables V and VI.*

Of the [^{3}H] Triclosan applied to skin, a high proportion was recovered from the patch lint or in the treated skin area. It can be seen (*Table V*) that in some instances the treated skin contains the larger amount of [^{3}H] Triclosan, while in other cases the patch lint contains the greater proportion. The level of tritium in the blood calculated as ppm Triclosan (i.e. μg ml^{-1}) rises to a maximum at about 6 h after application and falls steadily thereafter.

Based on the recovery of tritium in the urine and faeces during the first 48 h of the experiment (*Table VI*) the equivalent of 36.3±5.88 μg [^{3}H]

Table V. Penetration of Triclosan from ethanol

Rat	Time after application (h)	Residue (μg) in Patch	Skin (7.5 cm^2)	Tritium in blood (as ppm Triclosan)
1	1	21.06	163.63	0.294
2		138.98	27.56	0.070
3	2	14.73	166.73	0.194
4		21.04	167.50	0.362
5	4	128.16	27.65	0.114
6		133.67	23.85	0.190
7	6	93.08	38.48	0.301
8		112.32	29.96	0.228
9	24	81.26	25.75	0.241
10	48	79.36	21.84	0.119
11	96	69.84	17.74	0.072
12		63.43	16.33	0.074

To 7.5 cm^2 clipped dorsal female rat skin was applied 0.2 ml ethanol containing 162 μg [^{3}H] Triclosan. The alcohol was allowed to dry and the skin protected with a non-occlusive patch. The rats were placed in individual metabolism cages, killed and the patch, treated skin and blood, urine and faeces were collected separately every 24 h and analysed for tritium.

Table VI. Excretion of tritium from rats treated with [^{3}H] Triclosan in ethanol

Time (days)	Tritium recovery (dpm × 10^{-6})		
	Urine	Faeces	Total
1	0.702 ± 0.216	1.233 ± 0.353	1.935 ± 0.240 (4)
2	0.406 ± 0.095	1.277 ± 0.481	1.683 ± 0.410 (3)
3	0.234 ± 0.064	0.437 ± 0.153	0.671 ± 0.089 (2)
4	0.197 ± 0.016	0.189 ± 0.047	0.386 ± 0.062 (2)
Total	1.539	3.136	4.675

Four female Colworth-Wistar rats (120 g) were treated topically with 0.2 ml ethanol containing 16.09×10^6 dpm [^{3}H] Triclosan over 7.5 cm^2 clipped dorsal skin. Non-occlusive patches were fixed in position and the rats placed into individual metabolism cages. The excreta were collected separately every 24 h for 4 days. The tritium content of the faeces was determined by combusion and scintillation counting and that of the urine by counting directly.

Triclosan were recovered. This gives an observed penetration of 4.84 μg [^{3}H] Triclosan per cm^2 skin, which on adjustment for an 81.3% excretion of a subcutaneous dose gives a corrected penetration of 5.96 μg cm^{-2}. The total amount of [^{3}H] Triclosan penetrating through the treated area of skin is therefore 44.68 μg or 27.6% of the applied amount.

Discussion

In the present report, some 33% of the dose given subcutaneously to female rats was recovered in the urine during 96 h. The difference from a previous study (5) in which only 8% was excreted in the urine of male rats injected intraperitoneally with [^{3}H] Triclosan was shown by subsequent experiments (unpublished data) to be due to sex and not to the route of administration.

The half life of both intraperitoneally and subcutaneously-injected [^{3}H] Triclosan was approximately 14 h whereas after topical application some 23 h were required to excrete 50% of the absorbed dose, an increase which reflects the reservoir effect of the stratum corneum (6). The fact that there may be such a reservoir is supported by the finding that the same amount of [^{3}H] Triclosan is absorbed through skin despite an increase in the duration of contact with the skin of the shampoo.

The method of application of the shampoo and deodorant was selected to reproduce the way in which the consumer would use these types of

products. Thus the shampoo was applied to the skin, diluted with water and the excess, after varying periods of contact, was rinsed away. The aerosol on the other hand was applied and allowed to dry on the skin. The penetration of [^{3}H] Triclosan from the shampoo was 0.197 μg cm^{-2} and was 6.85 μg cm^{-2} from the aerosol, or some thirty times more in favour of the aerosol at these particular product concentrations of [^{3}H] Triclosan. Even at comparable concentrations of [^{3}H] Triclosan (i.e. 0.1% (w/v)) the penetration is still some eleven times greater from the aerosol. Thus, the composition and mode of use of different products containing [^{3}H] Triclosan is very important in determining the extent of penetration of the germicide.

Based on the work described by Feldman and Maibach (7), Maibach *et al.* (8) and Bartek, Labudde and Maibach (9) together with the data collated by Tregear (10), we consider that the permeability of rat skin may be similar to that of human scalp and axilla. Using the experimental data obtained from the present rat experiments together with a no-effect level from a 3-week target organ test in the rat of 200 mg kg^{-1} day^{-1} (unpublished observations) we can make calculations of the safety-in-use of Triclosan for the average woman of 55 kg body weight. Assuming the area of the scalp and hands is 1350 cm^2, then for a penetration of 0.197 μg cm^{-2}, the absorbed dose is 4.8 μg kg^{-1}, which is 42 000 times less than the no-effect level observed in the target organ test. The highest concentration of [^{3}H] Triclosan gave a minimum of 1000 times less than the no-effect level.

From the aerosol deodorant if the spray is used twice daily for 2 s on each axilla of 50 cm^2, then the absorbed dose, at 6.85 μg cm^{-2}, is 24.9 μg kg^{-1} which is 8000 times less than the no-effect level in the target organ test.

Thus, we conclude on the basis of the percutaneous absorption and toxicity data available, that extremely small proportions of the no-effect level of Triclosan are likely to be absorbed through adult human skin treated with shampoo containing 0.05% (w/v) Triclosan or with an aerosol deodorant containing 0.1% (w/v) Triclosan.

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A parametric test to measure the cleaning power of toothpaste

W. B. DAVIS and D. A. REES*

Presented on 26–30th August 1974 in London at the IFSCC VIIIth International Congress on 'Cosmetics—Quality and Safety' organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—A sensitive *IN VIVO* method was developed to quantify the cleaning power of dentifrices. Volunteers built up natural STAIN on their TEETH over a period of 5 weeks by replacing their usual TOOTHPASTE with a non-abrasive paste. The estimated % area of the incisors covered by stained pellicle was observed to increase, especially towards the end of this build-up period. The natural stain was progressively removed by a series of 10 s BRUSHING periods using an electric TOOTHBRUSH and the pastes to be tested. The logarithm of the area of stain was plotted against the duration of brushing with the test paste and found to conform to a linear trace. Thus an equation for stain removal may be of the form $S=ae^{bt}$ with the coefficient b being an expression of the ability of the test paste to remove stain.

Introduction

Over thirty years ago, Manly (1) observed that the 'brown pellicle' which formed regularly on the teeth of some people who did not use dentifrice abrasives could be readily removed by one or two brushings with a dentifrice grade calcium carbonate or phosphate but not by brushing with a dry or moistened brush without a dentifrice.

Lobene (2), and later Van Abbé *et al.* (3) who slightly modified Lobene's technique, employed direct visual observation to assess the removal of stained material from teeth using a toothpaste under controlled brushing conditions. Wilkinson and Pugh (4) used both photographic recording and

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a photometric method to assess the extent of stain on teeth cleaned for 2 weeks with a test toothpaste, after an initial scale and polish.

These techiques detected differences in the stain-removing properties of toothpastes, but employed non-parametric data. Moreover, stain colour and intensity, and the presence of plaque complicated the assessment.

A technique has now been developed which uses quantitative measurement on a parametric basis, to generate a value of cleaning power for toothpaste. It is uncomplicated by stain colour and intensity or plaque and gives repeatable results.

Experimental

Formation of stain for study of cleaning power

When volunteers used a non-abrasive toothpaste for cleaning their teeth, a natural stain, not related to their smoking habits, developed visibly in approximately one-third of the subjects and increased in area during the course of 1 month. Satisfactory plaque removal and oral hygiene was achieved, however, with no adverse effect on the gingivae.

Quantitative assessment of stain

By examining the incisors of each volunteer through a horizontally mounted Nikon stereoscopic binocular microscope (model SMZ-2), fitted with double spotlight tungsten illumination, the stained areas were easy to identify.

Ektachrome X photographic transparencies of the teeth were taken with a Miranda Sensomat camera through the binocular microscope using crossed polaroid screens over the Multiblitz photographic flash* and objective lens. This method of illumination eliminated specular reflection and permitted clear identification of the stained areas, although giving a slightly out-of-focus effect.

Examiners were trained to estimate visually the percentage area of the labial surfaces of the eight incisors covered by stain and their estimates were found not to differ from the planimeter-derived measurements by more than one-sixth.

The percentage area of the labial surfaces of the eight incisor teeth covered by stain was calculated from planimeter measurements of boundary

* Dr Ing. D. A. Mannesmann, GmbH & Co. KT, Porz-Westhoven, West Germany.

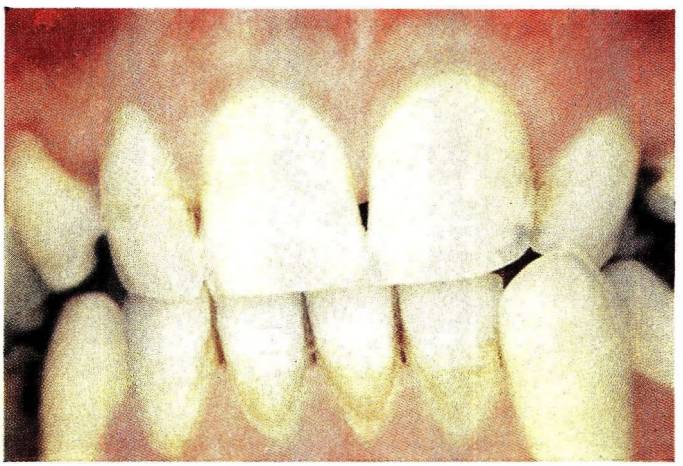

Figure 1. Naturally stained incisors. A cross-polarized photomicrograph of the stain built up on a volunteer's incisors after using a non-abrasive toothpaste for 1 month.

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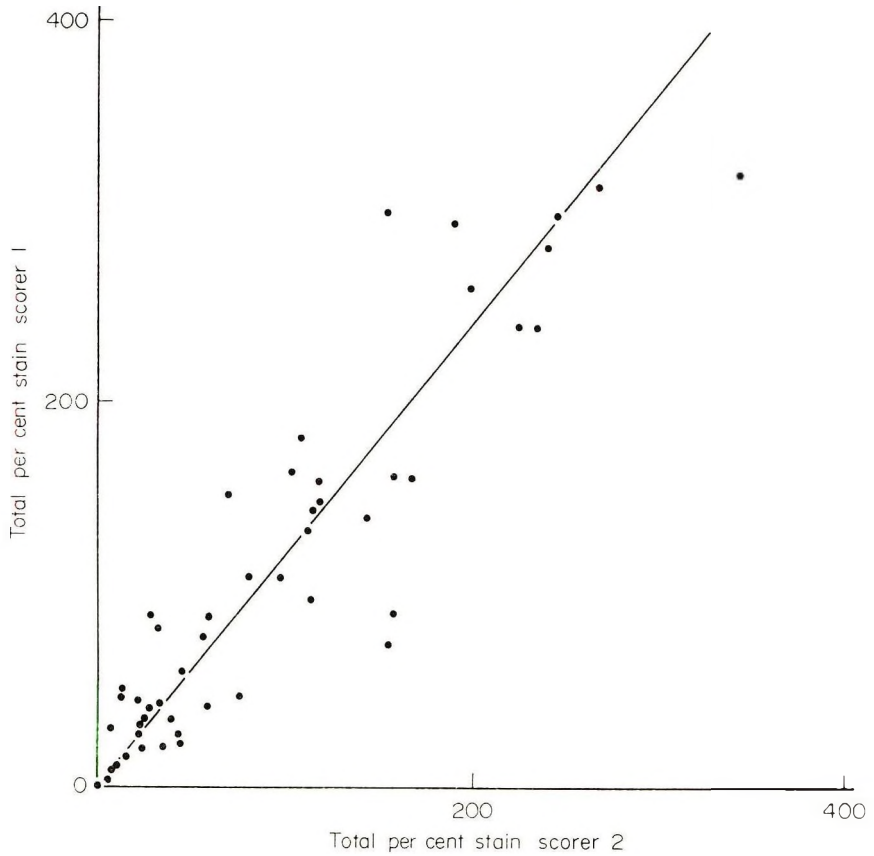

Figure 2. Scorers' concordance.

tracings drawn from projected images of the transparencies. After training examiners by repeatedly comparing their estimates with planimeter measurements of traces, good agreement was found between examiners' scores as shown in *Fig. 2*. The slope of line of best fit was 1.2 (complete agreement would be indicated by a slope of 1.0).

Measurement of stain removal on a single volunteer from a group

A hygienist removed fresh plaque by brushing for 15 s with a non-abrasive toothpaste applied with a Touch-Tronic electric toothbrush (Teledyne, Aqua-Tec, U.S.A.). After independent assessment of stain area on the labial surfaces of the eight incisors by two examiners, the hygienist brushed the incisors for 10 s, using a test toothpaste applied with the electric

toothbrush. The volunteer rinsed with a flavoured mouthwash to mask recognizable toothpaste aroma and stain was reassessed. The sequence was repeated for a further 10 s brushing and then followed by three periods of 20 s brushing with intervening assessments.

At each assessment interval, the percentage stained area on each of the eight incisors was recorded and totalled, giving a value S which represented *total* percentage stained area (maximum value of $S = 800\%$).

Tables I and II present typical data using the technique described.

Cleaning power assessment

Those volunteers producing an appreciable amount of stain after 1 month's use of a non-abrasive toothpaste were allocated to groups in a way which approximately balanced the initial levels of stain. To each group of volunteers a test paste was randomly allocated, then subjects were presented to the examiners in a random order of treatment; neither the subjects nor the examiners knew which toothpaste was used on any individual.

In all these tests so far, when $\log_{10}S$ was plotted against brushing time, t, and the data were fitted to straight lines using the least squares method, Pearson correlation coefficients were found to be mainly in the range 0.75–0.99, confirming the $\log_{10}$/linear relationship found graphically.

The gradient of the mathematically-fitted straight lines, designated b, was a measure of the brushing time, in seconds, required with a particular toothpaste to remove a given portion of the initial stain. The reciprocal of the magnitude of the gradient, b, was the time in seconds required to remove 90% of the initial stain.

The data could be presented mathematically in the form of an equation.

$$\log_{10}S = \log_{10} a + bt \qquad (1)$$

where 'a' is the constant representing the interpolated initial level of stain.

Cleaning Power (B) for a dentifrice is defined as $-\bar{b}.\ 10^3$ for the subjects allocated to this preparation, $\bar{b}$ being the average gradient.

Results

Data accumulated from several tests appear in *Fig. 3* and *Table III*. From these results it can be seen that the cleaning powers of the dentifrices were dependent on the mineral used as the polishing agent.

Table I. A typical raw data sheet for stain removal

Test Product Code Number .....*P*.....
Volunteer..... *Mr Smith* .....

Oral Hygiene..... *Brushes twice daily* .....
Usual Toothpaste* .....
Date of Last Scale & Polish... *Not known* ...
Next Dental Appointment ..... *None* .....

Smoking Habits..... *20 daily* .....

Cleaning time (s)		0				10				20				40				60				80			
Scorer (1)	Upper stain (%)	45	60	25	15	45	10	18	10	45	5	15	5	40	0	5	5	35	0	3	3	35	0	2	3
	Lower stain (%)	75	80	70	65	70	45	65	60	50	60	55	50	40	55	60	40	30	45	50	15	50	45	48	20
Scorer (2)	Upper stain (%)	80	20	15	30	40	15	10	50	40	10	8	25	35	10	5	20	35	5	5	15	30	5	5	10
	Lower stain (%)	50	50	50	50	50	40	40	40	40	40	40	25	35	40	35	35	35	35	30	15	35	40	30	15

* The brand name or other identifying name is entered here.

Table II. Summation of stain area on Mr Smith

	Brushing time (s)					
	0	10	20	40	60	80
Examiner 1	435	323	285	250	181	203
Examiner 2	345	285	228	215	175	170

Each score was total recorded stain for eight incisors.

Because, in any cleaning experiment, the examiners would expect a progressive decline in scores, some volunteers were returned at random to examiners for a repeat scoring, without a further brushing. By doing this, the expected tendency of the observers to record lower scores could be quantified. Of 58 repeat observations, 36 were lower and 22 higher than the previous reading and a 9% mean fall in estimated stain area was recorded. This proved to be of the same order as the assessment made when cleaning throughout was done with a non-abrasive gel toothpaste.

The initial tests reported here, carried out to prove the technique, used only ten to twenty subjects per group. Despite such small group sizes coefficients of variation for cleaning power were nevertheless within the range 0.4–0.7, except for the non-abrasive paste which had the lowest cleaning power. Even with these small groups of volunteers, differences between the cleaning powers for non-abrasive and chalk-based toothpastes could still be detected.

Chalk-based toothpastes were generally the best cleaning agents and as effective as a highly abrasive pumice-based prophylaxis paste. A silica-based toothpaste was almost as efficient with respect to cleaning power as the least effective chalk-based toothpaste. The non-abrasive toothpaste was significantly lower in cleaning power, especially if the quantified expectancy element was taken into account.

Discussion

The development of the parametric test for dentifrice cleaning power was aimed at producing a test that was quantitative, realistic and reproducible while also being capable of detecting small differences between products. By using this technique on small groups of volunteers, the technique appears of fulfil all these requirements. Furthermore, the training of new operators was a simple process; since the expectancy of the scorers could be quantified,

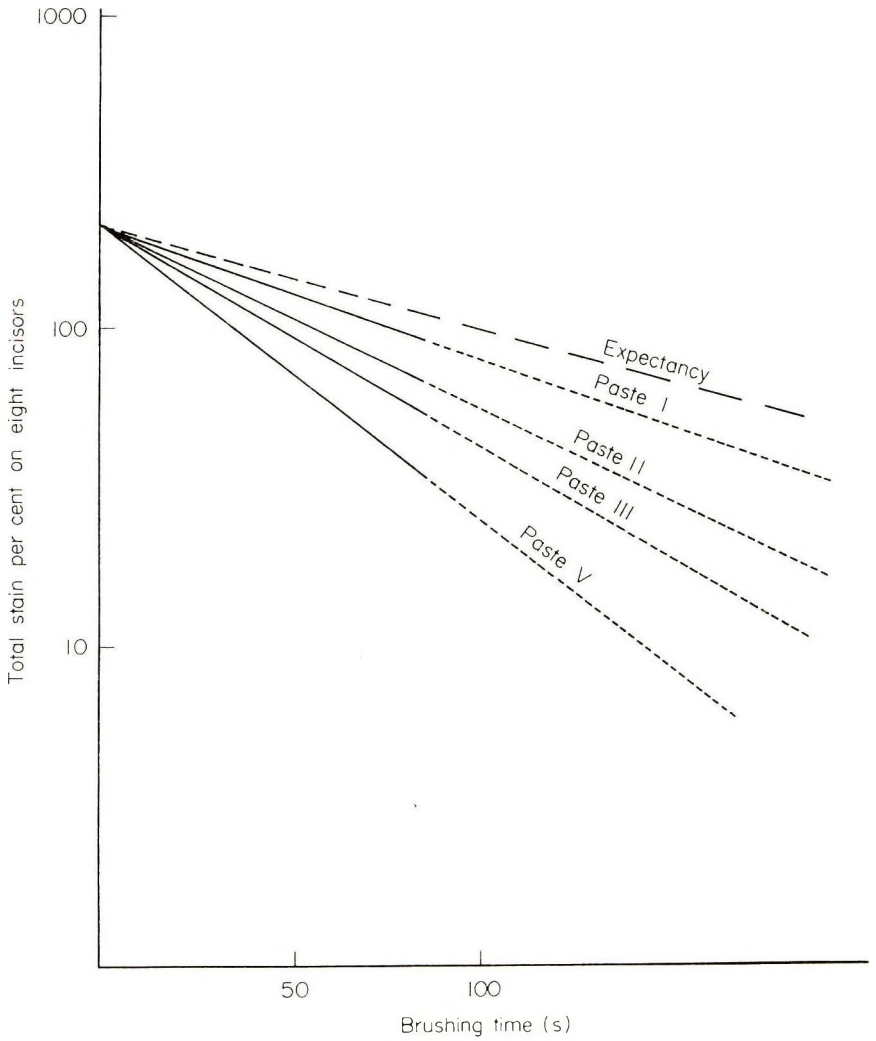

Figure 3. Total stain/brushing time. The straight lines represent the average least squares fit lines for stain reduction with the dentifrices tested. The broken line indicates the scorers' expectancy. (Paste IV is not shown as the line almost coincides with that for Paste II.)

Table III. Cleaning powers B of six pastes

Paste type	Non-abrasive I	Silica II	Chalk III	Chalk IV	Chalk V	Pumice VI
Cleaning power	2.8	5.5	6.1*	5.6*	8.0	6.0

* These data are from two separate tests and confirm the reproducibility of the technique using the same toothpaste.

influence of the unavoidable human elements in the trials could be minimized and the test could be used to compare scorers one with another, as well as for comparing different products in numerical terms.

As the basis of measurement was parametric in this series of tests, the number of volunteers required to permit statistical differentiation of the cleaning powers of test dentifrices could be estimated before starting the test. The information required for this calculation was (a) typical test data variance deduced from earlier tests, (b) the marginal difference in cleaning powers that the test was required to detect, and (c) the approximate cleaning powers of the pastes to be tested.

The reproducible quantitative form of the data generated by this method permits statements such as Paste A is two to three times better in cleaning power than Paste B with statistically-reinforced confidence. The quality of toothpastes may also now be directly related to the duration of brushing required to remove stain.

The current status of investigations on abrasivity/cleaning power relations does not suggest any numerical relationship between these functions at present, although the evidence shows that stains build up when a subject's normal dentifrice is replaced with a non-abrasive toothpaste and that the cleaning power of a non-abrasive toothpaste is probably less than one-quarter of that of a typical chalk-based dentifrice.

Summary

A reliable *in vivo* method of quantitatively measuring the cleaning power of toothpastes has been developed. Natural stain is allowed to build up over a period of 1 month by volunteers using a non-abrasive toothpaste.

The stain is progressively removed by a hygienist using an electric toothbrush and the test pastes for 10 or 20 s periods. The progress of stain removal is quantified by measuring the percentage areas of stain remaining on volunteers' incisors. When the logarithm of this parameter is plotted against the total brushing time, a series of straight lines are produced, the gradients of which are a numerical measure of the cleaning power of each specific test paste.

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A Lecture at 7 p.m. on 24th April, 1975
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SAFETY OF AEROSOLS

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