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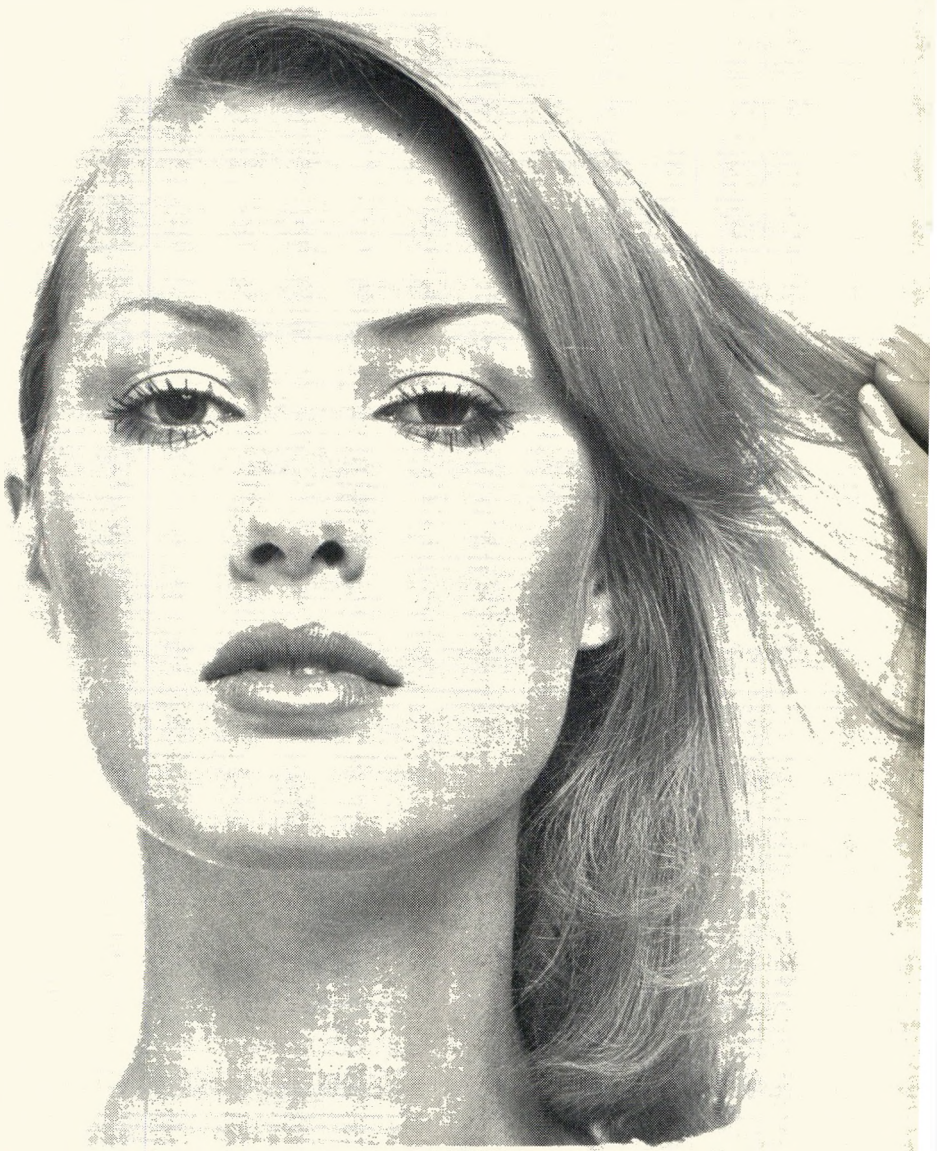

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SYNOPSES FOR CARD INDEXES

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

Local infections—experimental aspects: R. R. MARPLES. *Journal of the Society of Cosmetic Chemists* **27** 449–458 (1976)

Synopsis—The procedures required to induce experimental infections of human skin demonstrate the conditions that must be fulfilled in spontaneous infections. The important variables appear to be the species of micro-organisms inoculated, inoculum size, the degree of hydration of the skin inoculated, trauma, the competition of the normal flora and the immunological state of the host. Cosmetics and toilet products may affect these variables by contamination, by containing antibacterial agents as preservatives or ingredients designed to act on the skin and by changing the microenvironment of the skin.

Dermatocosmetic relations: C. D. CALNAN. *Journal of the Society of Cosmetic Chemists* **27** 459–475 (1976)

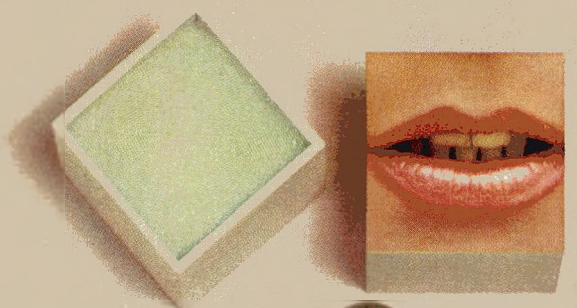
Synopsis—There are a number of different interfaces in the relationship between cosmetic chemists and dermatologists, a relationship which is less developed in Britain than in many other countries. The most evident is in the matter of customer complaints. These may be both subjective and objective. As more active ingredients are used in cosmetics, more diverse types of adverse reactions may be anticipated. Consumer pressure has been responsible for several recent analyses but most of them have been general rather than specific. The detailed investigation and search for the cause of a customer complaint requires active co-operation from the customer, cosmetic chemist and dermatologist.

The dermatologist can help the cosmetic chemist in screening tests and the choice of new materials, based on the experience of his particular speciality. There are encouraging signs that the relationship is steadily improving.

The extraction of vinyl chloride from PVC containers: D. A. TESTER. *Journal of the Society of Cosmetic Chemists* **27** 477–484 (1976)

Synopsis—Polyvinyl chloride (PVC) with a combination of high clarity, toughness, and barrier properties, has become well established in the packaging of toiletries and cosmetics. There has been concern recently over the toxicity of vinyl chloride and its implications for the use of PVC in packaging.

This paper tables the progress made by the PVC producers and fabricators in reducing the level of this residual monomer in PVC. From measurements with a variety of extractants, the factors are shown controlling the extraction of vinyl chloride monomer (VCM) and the levels likely to result from given VCM contents in the PVC. For the VCM levels now pertaining in PVC packaging it is shown that extraction by the contained food, drink or toiletry is minimal if detectable at all.



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Antimicrobial activity—a critical review of test methods of preservative efficiency: R. A. COWEN and B. STEIGER. *Journal of the Society of Cosmetic Chemists* **27** 485–499 (1976)

Synopsis—The United States Pharmacopoeia Preservative Test, Society of Cosmetic Chemists Test and Toiletry & Goods Association Test are compared with a procedure adopted by the authors. Important parameters, including realistic selection of test organisms, inoculum level, growth media, test temperatures, sampling and effectiveness are critically discussed. The use of multi-challenge tests as opposed to single challenge tests is highlighted and the need is stressed for a test procedure which ideally can predict preservative levels suitable for the life of the product.

The effect of irradiation on packaging materials: F. J. LEY. *Journal of the Society of Cosmetic Chemists* **27** 501–507 (1976)

Synopsis—Ionizing radiation, mainly in the form of gamma rays from the radioisotope cobalt 60, is being used increasingly for the inactivation of contaminants in cosmetic and toiletry preparations. The treatment is applied to the product in its final pack, and therefore, it is important to recognize that the properties of the packaging materials could be affected, particularly if high radiation doses are used. The stability of a wide range of materials is discussed.

The nature and extent of chemical and physical changes in plastics very much depends on the presence of antioxidants and other additives, and on the environmental conditions during irradiation. Some useful information is available from research into the use of radiation for the preservation of food—many films and laminates having been cleared for use in the United States. The low radiation doses used for the control of contaminants in cosmetic products are quite unlikely to cause changes in packaging materials which will limit the use of the process, with the possible exception of the discoloration in glass.

Certain radiation induced changes have been used to advantage, as for example with polyethylene, where the melting point can be elevated significantly following high radiation treatment.

J. Soc. Cosmet. Chem. 27 449-457 (1976)

Local infections—experimental aspects

R. R. MARPLES*

Presented at the Symposium on 'Microbiological Safety of Cosmetic and Toiletry Products', organized by the Society of Cosmetic Chemists of Great Britain in Birmingham on 23-25th February 1976.

Synopsis—The procedures required to induce EXPERIMENTAL INFECTIONS of human skin demonstrate the conditions that must be fulfilled in SPONTANEOUS INFECTIONS. The important variables appear to be the SPECIES of micro-organisms inoculated, INOCULUM SIZE, the degree of HYDRATION of the skin inoculated, TRAUMA, the COMPETITION of the NORMAL FLORA and the IMMUNOLOGICAL STATE of the host. Cosmetics and toilet products may affect these variables by CONTAMINATION, by containing ANTI-BACTERIAL AGENTS as preservatives or ingredients designed to act on the skin and by changing the microenvironment of the skin.

Introduction

When the degree of exposure of human skin to potentially pathogenic micro-organisms is considered, exposure is only rarely followed by infection. The human environment is full of organisms and the surface of the body is large. Potential pathogens reach the surface of the body very frequently. In this article we consider the reasons why the inoculum deposited on the skin so seldom results in invasion or other damage and the ways in which cosmetics and toiletries may modify this result. An experimental approach will be used to demonstrate the importance of the different factors that act to determine the survival of an inoculum.

The organism that most commonly is responsible for infections of the skin is *Staphylococcus aureus* but streptococci, enterobacteria and pseudomonads must not be neglected. As is true of nearly all local infections, lesions are caused by a variety of extracellular products, enzymes and toxins but for these to reach a

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locally toxic level a threshold number of cells must be present. An idea of this threshold number comes from studies in which *S. aureus* was injected through the epidermis, bypassing the defences and the opportunities for colonization of the skin's surface. Elek and Conen (1) showed that an inoculum of about 10^6 viable cells of *S. aureus* delivered by intradermal injection was necessary for pus to be formed, the minimum pus forming dose. The general requirements for infection were trauma and a large inoculum. This number could be greatly reduced if a foreign body or a thread was left in the wound. It has been postulated in this, and other examples, that the inoculum must multiply in the tissues to a micro-colony of at least 10^6 before lesions develop. Reducing the inoculum size will reduce the chances of infection when the other factors remain the same. The aim of microbiological quality control should be to minimize the number of organisms present in a product so that inocula from the product are as small as possible and, preferably, contain no *S. aureus* or the other organisms reasonably likely to cause skin infections.

Self-degerming action of the skin

It has been known for a long time that inocula of most bacteria artificially applied to the skin rapidly disappear (2). The skin has been described as having a capacity to degerm itself and investigation of this phenomenon has produced much of interest. A single mechanism whereby bacteria are killed has not been found. It is quite clear that the primary reason why the inoculated bacteria die is because they dry up (3). The horny layer of the skin permits the passage of very little water through the skin and, over much of the body surface, the skin is very dry. Gram-negative rods in particular are susceptible to desiccation but all organisms are affected, counts from different skin sites reflect this (*Table I*).

Table I. Effects of moisture in the micro-environment on the number of organisms in the normal flora at different skin sites*

Site	Micro-environment	Organisms (cm^2) (geometric mean)
Forehead	Slightly moist	30,000
Axilla	Humid	1,350,000
Groin	Moderately humid	19,300
Thigh	Dry	1870
Hip	Dry	596
Toeweb	Wet	7,600,000

* Based on quantitative samples from sixteen healthy adults.

The loss of viability is a simple exponentila decay from the initial inoculum until the skin becomes visibly dry, then other factors such as free fatty acids become important as microbial multiplication commences. A contaminated cosmetic or toilet formulation may dry up in a different way from a simple aqueous suspension; formulation components may prevent the desiccation of an extraneous inoculum or even modify the skin microenvironment so that resident bacteria are favoured. After the phase of desiccation survival of the inoculum may be affected by locally bacteriotoxic compounds. Removing these by lipid solvents may enhance survival and infection (4).

Hydration methods

The opposite of desiccation is hydration which has dramatic effects on the skin flora. Survival of inocula of *Escherichia coli* can be prolonged by spraying the skin repeatedly with water or by changing the relative humidity in an environmental chamber (3, 5). As an example of the experimental exaggeration of the conditions existing in real life we studied the effects of applying a completely occlusive dressing to the skin of the forearm (6). Covering the skin with an impermeable plastic film causes sweating and increases the amount of water in and on the skin without making the horny layer sodden. The number of organisms rose from around 10^3 organisms cm^{-2} to 10^6 or 10^7 suggesting the population was mainly restricted by lack of water. The composition of the flora altered with time becoming similar to that of the naturally moist axilla by the end of a week.

Many cosmetics and toilet products are designed to enhance hydration of the skin and others contain substances that form an occlusive layer. In normal subjects occlusion does not result in infection, although the bacterial growth may cause odour, but we found that two quite minor modifications of our occlusive system did induce infections. Both are likely to be applicable to the case of toilet products.

Superhydration

The simplest modification was adding extra water in the form of a water-soaked felted cotton strip (7). Under these conditions *Pseudomonas aeruginosa* became the most numerous organism and irritant lesions developed (*Table II*). The implications for cosmetics and toilet preparations are obvious but need emphasis. The first is that apparently minor differences from an experimental protocol may have totally different consequences. The addition of an innocuous fluid to a standard dressing was sufficient to change the effect from nothing to a severe and, to many volunteers, an intolerable skin irritancy. A second point is that we still do not know the factors that control the survival and dominance of

Gram-negative organisms on human skin. There is clear evidence that adding water favours Gram-negative colonization but whether it is the addition of water itself or the leaching out of antibacterial substances from the skin that permits infection is not yet determined (7). Clinically, repeated immersion in water facilitates infection of the hand by *Candida albicans* (8) or *Pseudomonas* (9). Rebora, Marples and Kligman (10) showed the distinctive infection of erosio interdigitale blastomycetica could be reproduced experimentally only when the interdigital interspace was truly occluded. In this experimental infection it appeared that the fungus and enterobacteria, both enhanced by moisture, each played a part in producing the signs and symptoms of infection.

Table II. Total count and number of *Pseudomonas* before and after occlusion for 7 days *

	Before occlusion		After occlusion	
Type of occlusion	Total	*Pseudomonas*	Total	*Pseudomonas*
Plain film	436	0	5,568,000	138
Film and wet felt	658	0	6,297,000	5,755,000

* Data from Hojyo-Tomoka, Marples and Kligman (7).

Selective agents under occlusive dressings

Systems in which hydration of the horny layer is the main stimulus to bacterial multiplication may have very different results if a selective agent is added from outside. We applied a neomycin solution to large occlusive dressings maintained in place for 2 weeks (11). Because of the circumstances under which this study was performed, neomycin resistant strains of *S. aureus* happened to exist in the environment and all our volunteers showed colonization of the treated occluded skin by *S. aureus* (*Table III*). In most subjects counts of this organism of 10^6

Table III. Sites dominated by different organisms of sites with or without neomycin under large or small occlusive dressings

	Small dressings		Large dressings	
Organism	Neomycin (10 sites)	Control (10 sites)	Neomycin (10 sites)	Control (10 sites)
S. aureus	1	0	7	0
Yeasts	7	2	3	0
Cocci	2	6	0	4
Diphtheroids	0	1	0	6
Others	0	1	0	0

cm^{-2} or greater grew up on previously normal skin while untreated occluded skin of the other forearm grew only normal skin organisms. In some subjects the overgrowth of *S. aureus* led to the development of a pustular rash in which diffusion of toxins from the surface of the skin seemed the best explanation for the pathology. In other experiments the presence of an antibiotic in an occlusive dressing resulted in overgrowth of *C. albicans* or *Proteus* with the production of lesions of similar etiology. In one volunteer, a user of a medicated soap only, overgrowth of enterobacteria was evident.

S. aureus *model on intact skin*

Singh, Marples and Kligman (12) extended these observations into a method for inducing experimental infections. After demonstrating a large inoculum could overwhelm any interactions with the normal skin organisms, they showed the application of 10^6 or more *S. aureus* cells cm^{-2} and an occlusive dressing could regularly produce a flora containing large numbers of *S. aureus*, which if maintained for a few days could induce lesions. The histology and bacteriology indicated again that the lesions seen were not due to invasion but could be explained entirely on the passage of toxins through the horny layer. Very similar concepts in experimental skin infections with *C. albicans* had led Maibach and Kligman (13) to describe this form of toxic pyoderma as biological contact dermatitis. Although local, this is just as much an infection or intoxication, as is tetanus. We must conclude that if the inoculum is large enough, or competition from the better adapted normal skin organism is prevented then the pathogenic strain can grow to a population sufficient for lesions to be produced on undamaged skin. Infection depends on large inoculum size and occlusion to prevent the desiccation of the inoculum.

Competition with normal skin organisms

Numerous experiments have demonstrated that the most important defence of the body against colonization and infection by pathogenic bacterial strains is the presence of a normal flora. The inoculum required to produce the same severity of lesions in the Singh model can be reduced at least tenfold by destroying the majority of skin organisms before inoculation of *S. aureus* (12).

In the presence of neomycin fewer viable cells of *C. albicans* are required to induce lesions while when the skin flora is made to increase by occlusion *S. aureus*, *C. albicans* and *Pseudomonas* need higher inocula to produce the same effect than without this manoeuvre.

S. aureus *model on traumatized skin*

The integrity of the horny layer of the skin is frequently a critical part of the defences of the body against infection. It is waterproof, physically strong and a major barrier both to diffusion of chemicals and to the possibility of migration of leucocytes (14). Damage to this barrier has major effects on the survival of inoculated organisms. Plucking hairs and covering the site with an occlusive dressing has been used to produce skin infections with *S. aureus* and *Streptococcus pyogenes* (15). A simple scratch is usually insufficient on unoccluded skin (1). The most suitable means of damaging the skin is to remove the horny layer with adhesive cellophane tape. This 'stripping' procedure produces a standard injury only if the horny layer is repeatedly removed until the tape adheres poorly —the 'glistening layer' has been reached. Before this end point the number of cells removed depends too much on the individual, on the local meteorological conditions and on the ardour of those applying the tapes. We found (16) immediate inoculation of *S. aureus* on a site prepared in this way produces a spreading cellulitis too severe for experimental use; delaying the inoculation for a day gives time for sufficient barrier to be formed to give a tolerable local infection. A 2 cm square was stripped and, after 24 h, an inoculum of *S. aureus* contained in 0.01 ml was applied and covered with a 2 cm square of plastic film held in place with cloth backed adhesive tape. After 24 h the clinical severity of the infection was graded on a 5 point scale, quantitative samples were taken and the exudate on the plastic was transferred on to a clean slide by simple pressing followed by staining with Giemsa. We found the inoculum required to infect half the sites was less than 10^2 viable cells but occlusion and trauma were needed (*Table IV*). The infection

Table IV. Comparison of two methods of inducing experimental infections with *S. aureus* on human skin

	Intact skin method	Traumatized skin method
Preparation of skin	Alcohol degerming	Tape stripping of horny layer
Delay before inoculation	None	24 h
Inoculum to infect 50%	1000	20
Practical inoculum	1,000,000	10,000
Occlusion	Necessary	Necessary
Time before lesion appeared	3 days	12 h
Type of lesion	Sterile pustules	Purulent exudate

produced a serosanguinous to purulent exudate from a red denuded lesion. The lesions regressed rapidly after the dressings were removed but it is desirable to treat all the sites with topical antibiotics to prevent dissemination of the organism to the environment.

This model and the simple *C. albicans* model were used to test the activity of antistaphylococcal and antifungal antibiotics in combination with a fluorinated corticosteroid in thirteen volunteers (17). Treatments were applied 6 h after inoculation of six sites with *S. aureus* and occlusive dressings reapplied for 18 h before examination. The treatments were (1) the complete formulation, (2) steroid and antistaphylococcal antibiotics, (3) antistaphylococcal antibiotics alone, (4) steroid alone, (5) cream base and (6), untreated control. The numbers of *S. aureus* recovered from sites 1, 2 and 3 averaged 10^4 while 10^7 were recovered from the remaining sites. All but one of the latter were colonized but no *S. aureus* could be found in twenty-five of the thirty-nine antibiotic treated sites. Sites treated with steroid alone were less inflamed than sites 5 and 6 but still more than the antibiotic treated sites. Neither in this experiment nor in infections induced with *C. albicans* did the steroid potentiate invasion by the pathogens.

Studies of experimental fungal infections carried out by the Letterman group (18) have shown that the lesion and its evolution depends to a large extent on the immunological status of the host. In the subjects not previously exposed to the fungus chronic infections could be induced but in those subjects where the experimental infection was not the first exposure to the organism the lesion induced by appropriate inoculation appeared more rapidly and quickly resolved. Other unpublished studies indicate that steroids may block the desquamation and inflammation, preventing spontaneous cure of the experimental fungal infection and this is a well documented clinical experience.

Conclusions

When considering the role of cosmetic and toilet preparations in the causation of skin infections several factors of differing importance must be appraised. The first factor is the size of the inoculum; if very large then infection is a likely consequence. This may occur by inunction of a contaminated product. It is by no means a certainty but the risk is real and more dependent on the contaminant organism than on any other parameter. Formulations to be applied to human skin should not be heavily contaminated with any microbe.

Probably the next most important characteristic of a formulation is it should not select exclusively from the natural contaminants a dense population of a potential pathogen. This is possible as a result of incorporating an ingredient as a preservative as much as when a component is consciously included because of its antibacterial effect. Because selection is only second in the list, the site of application should be considered. Active antibacterial agents are more likely to be incorporated in products designed for use in the axilla, toeweb or other intertriginous sites yet these are the sites where distortion of the normal flora may have the

greatest effect. Antibiotics are more effective antibacterials than chemical bacteriostats (19) and because of their narrow spectra are more likely to lead to overgrowth of resistant species either of the lowly virulent Gram-negative species or of the yeasts and fungi. We do not know the importance of 'preservatives' in this regard; in most instances no attempt has been made to determine the effects on the skin flora of systems designed to protect a product from microbial spoilage, although the design frequently considers multiple assaults and presumably delivers active compounds to the skin microenvironment, particularly if fresh and unstored.

Next we must consider how a formulation affects the micro-climate of the skin surface to which it is applied. Here, the provision and preservation of moisture is of importance. If the formulation causes hydration of dry skin, bacterial growth will be encouraged; prevention of evaporation will have a similar effect. Hydration will enhance the effect of an antibacterial agent but in the absence of a selective compound the evidence is that the skin will remain undamaged. In the experimental approach occlusive dressings are often emphasized but this is because experiments involve few subjects from whom major lessons are to be drawn. Lesser degrees of hydration are clearly less effective stimuli for microbial growth but are not without effect.

Experimental infections demonstrate the importance of the main variables. (1) The organism has to be one that is able to cause infection; (2) the inoculum must be large enough to overcome the remaining defences; (3) the inoculum must not be killed by drying up or toxic products before an infection can develop—this is usually achieved by occlusive dressings and reduction of the normal flora; (4) the integrity of the horny layer must be disrupted either by trauma or by the action of extracellular toxins; (5) immune and allergic responses must not prevent the development of the infection.

(Received: 27th February 1976)

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The extraction of vinyl chloride from PVC containers

D. A. TESTER*

Presented at the Symposium on 'Recent Advances in the Packaging of Toiletry Products' organized by the Society of Cosmetic Chemists of Great Britain at Bath on 10–12th November 1975.

Synopsis—POLYVINYL CHLORIDE (PVC) with a combination of high clarity, toughness, and barrier properties, has become well established in the PACKAGING of toiletries and cosmetics. There has been concern recently over the TOXICITY of vinyl chloride and its implications for the use of PVC in packaging.

This paper tables the progress made by the PVC producers and fabricators in reducing the level of this residual MONOMER in PVC. From measurements with a variety of extractants, the factors are shown controlling the extraction of vinyl chloride monomer (VCM) from packaging and the levels likely to result from given VCM contents in the PVC. For the VCM levels now pertaining in PVC packaging it is shown that extraction by the contained food, drink, or toiletry is minimal if detectable at all.

Introduction

In recent years there has been a rapidly increasing use of thermoplastics for the packaging of toiletries and cosmetics. Among the general purpose high tonnage plastics, poly vinyl chloride (PVC) with a unique combination of high clarity, toughness and barrier properties, has become the established choice for a variety of applications. During the last 2 years concern over vinyl chloride toxicity has implied constraints on the use of PVC in packaging. Considerable progress has been made in minimizing the incidence of residual vinyl chloride in PVC, and its extraction from PVC containers.

The incidence of vinyl chloride monomer (VCM)

It is accepted that in the packaging of toiletry products, food and medicines, the package should not introduce any significant quantity of toxic or potentially

* ICI Ltd, Plastics Division, Welwyn Garden City, Hertfordshire.

injurious substance to the contents. Poly (vinyl chloride), like most high polymers, is itself non-toxic, and generally inert in this respect, but in its production small quantities of unreacted monomer remain in the polymer, and minute traces can persist in a fabricated PVC package.

Vinyl chloride had been traditionally accepted as presenting no health hazard, at least in the trace quantities, measurable as parts/10^6, remaining in fabricated PVC. However, in January 1974, a link between the inhalation of VCM and angiosarcoma in man was confirmed. Although no evidence existed at that time of any toxicological effects from the ingestion of VCM, as distinct from inhalation, the UK PVC producers took immediate action to implement programmes to reduce the quantities of residual VCM in polymers, feedstocks and food packages (1).

Loss of residual monomer is achieved most readily when the material is in the powder form and VCM is not easily removed once the material has been converted into a melt. To date, the UK PVC manufacturers have already made considerable reductions in the VCM content of their polymers, in addition very significant reductions in the final VCM content of the feedstock can be obtained during the powder mixing process by which the blend with additives is commonly achieved. The technique consists essentially of purging the atmosphere of the high speed mixer with air during the mixing cycle and also operating to as high a temperature as the particular feedstock will allow.

Considerable reductions in the VCM content of polymers have been achieved since January 1974, and work is continuing with the objective of reducing levels still further. These improvements, together with the application of the optimum high speed mixing techniques have enabled manufacturers to produce bottles

Table I. VCM levels in PVC packaging materials (January 1974—March 1976)

Bottles		January 1974 (parts/10^6)	March 1975 (parts/10^6)	March 1976 (parts/10^6)
Level in polymer at time of use		500–1000	100–250	< 50
Level in powder blend at	typical	50	5	< 1
time of manufacture	maximum	100	5	3
Level in bottle wall	typical	50	3	< 1
	maximum	100	5	3
Foil of UK manufacture				
Level in polymer at time of use		800–1500	100–250	< 200
Level in foil	typical	80	8	3
Flexible film (extrusion blown)				
Level in polymer at time of use		400–1000	50–150	< 50
Level in film	typical	1	1	< 1
	maximum	1	1	1
Bottle cap liners	typical	No figures	0.1	0.1

containing less than 3 parts/10^6 VCM and rigid foil based packaging with less than 5 parts/10^6 VCM. Packaging and blend manufacturers in the UK have agreed to monitor their products to ensure these are maximum figures.

Flexible packaging film is made from a plasticized formulation where the choice of polymer and the nature of the film making process combine to give very ready loss of VCM. A typical level of VCM in flexible film as currently made is less than 0.5 parts/10^6.

The reductions in VCM content of PVC packaging materials achieved from 1974 to 1976 are shown in *Table I*. Further progress can be predicted by 1977.

Laboratory studies of VCM extraction from PVC packages by foodstuffs and food simulants

From the start of concern over VCM in PVC packaging, it was clear more knowledge of the nature and rate of VCM extraction from container to contents was required. Prolonged storage trials were set up with a range of foods and food simulants in miniature (50 ml) bottles of known VCM contents (VCM determinations were carried out by a gas chromatographic method, employing head space analysis). From the information obtained it was possible to assess the rate of extraction of VCM and the maximum levels likely to be built up in a range of substrates, from containers with a wide range of residual VCM levels. This knowledge, confirmed by data obtained for commercial PVC packs and their contents, provides a firm basis for the prediction of long term VCM extraction from containers now being produced with very low VCM levels. Recently, measurements of VCM extraction by water, under laboratory conditions, from a PVC container with very low residual VCM, has confirmed these predictions. The emphasis in this work, for obvious reasons, was on foodstuffs. It is considered, the results are highly relevant to the packaging of toiletries, for reasons that will be outlined.

The results of the VCM extraction tests with food simulants are shown in *Table II* and results for three systems of particular relevance are plotted in *Figs 1–3*. Results for extraction by water from a container with a very low level of residual VCM are shown in *Table III*. Several general conclusions may be drawn from this work.

Although results are undoubtedly affected by the nature of the extracting medium, the levels of extraction are of the same order for a wide range of foodstuffs and simulants.

The maximum extraction levels for any foodstuff or simulant after long periods indicate a partition between the PVC container and its contents, so the concentration is always much higher in the PVC. This effect over-shadows the weight ratio of contents : container and determines that only a very minor proportion of the residual VCM ends up in the foodstuffs. This is not unexpected from a

Table II. VCM extraction tests with food and cosmetic simulants

VCM in bottles (parts/10^6 w/w)	Storage temperature	Storage time	Measured VCM in extract (parts/10^6 w/w)					
			Water	3% Aqueous acetic acid	15% Aqueous ethanol	50% Aqueous ethanol	Maize oil	Orange squash
15	23	7 days	0.009	0.009	0.011	0.011	0.005	—
		42 days	0.044	0.037	0.042	0.050	0.018	0.011
		3 months	0.050	—	0.055	0.075	0.060	0.015
		12 months	0.080		0.070	0.210	0.060	0.022
30	4	7 days	0.003	0.003	0.004	0.004	0.005	0.001
		21 days	0.003	0.006	0.008	0.006	0.005	0.002
		3 months	0.022	—	0.024	0.025	0.025	0.010
		6 months	0.029		0.040	0.002?	0.011	0.010
30	23	3 days	0.010	0.009	0.009	0.010	0.004	0.003
		7 days	0.017	0.019	0.020	0.021	0.007	—
		21 days	0.036	0.027	0.050	0.050	0.017	—
		42 days	0.020	0.055	0.080	0.080	0.031	0.019
		3 months	0.070	—	0.095	0.120	0.100	0.045
		6 months	0.100	—	0.115	0.210	0.070	0.031
		12 months	0.140	—	0.100	0.340	0.100	0.032
30	49.5	28 days	0.007	—	—	—	—	—
		42 days	—	—	—	0.365	0.019	—
30	60	3 days	0.115	0.125	0.105	0.240	0.065	0.080
		21 days	0.100	0.105	0.115	0.355	0.195	—
86	23	7 days	0.054	0.042	0.070	0.070	0.035	0.055
		42 days	0.060	0.180	0.222	0.330	0.010?	0.075

All experiments were carried out using miniature bottles weighing approximately 9 g and containing 50 ml. Bottles were so manufactured as to give batches of a given VCM content. Fresh bottles from each stored batch were opened for each VCM determination, and values quoted are the mean of results for two bottles.

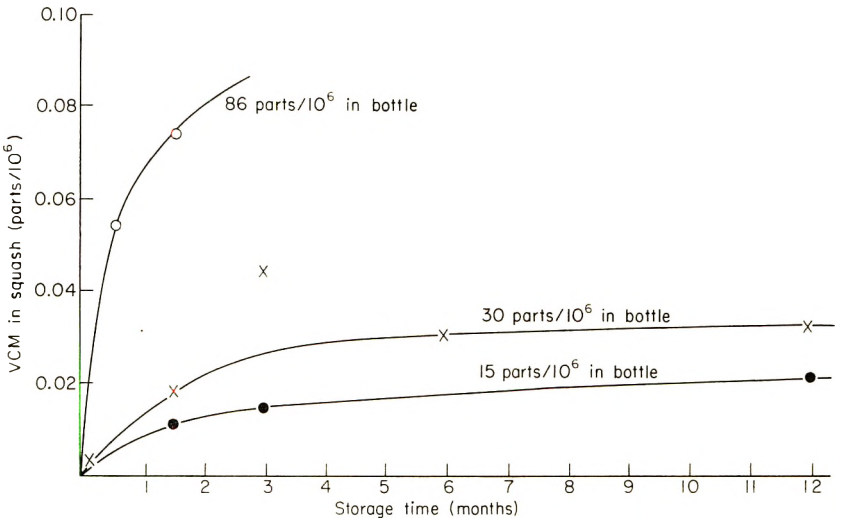

Figure 1. Extraction of VCM from PVC bottles by orange squash. Laboratory study 23°C.

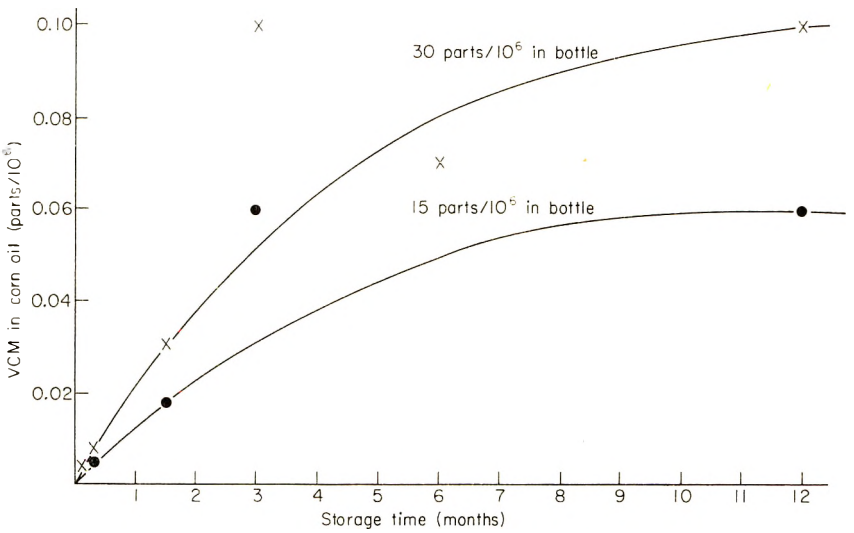

Figure 2. Extraction of VCM from PVC bottles by corn oil. Laboratory study 23°C.

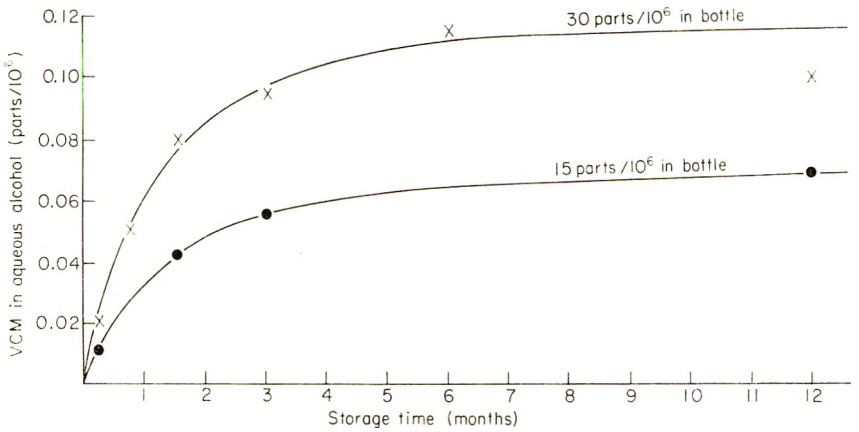

Figure 3. Extraction of VCM from PVC bottles by 15% aqueous alcohol. Laboratory study 23°C.

Table III. VCM extraction by water, from a 1 litre bottle containing approximately 1 part/10^6 of VCM

Storage temperature (°C)	Storage time	Measured VCM in water (parts/10^6 w/w)
23	3 weeks	< 0.001
	7 weeks	0.001
	12 weeks	0.001
	24 weeks	< 0.001
	32 weeks	< 0.001
60	3 days	0.002
	7 days	0.002
	10 days	0.0015
	21 days	0.001

consideration of the likely affinity of VCM for its own polymer, in competition with aqueous systems or vegetable oils, and is of course of vital significance in assessing the effects of residual VCM in containers.

The rate of extraction, and to a lesser extent the levels ultimately obtained, increase with increasing temperature for all the systems studied.

Under given conditions, the level of extraction is directly proportional to the level initially present in the container.

Significance for the packaging of toiletries

The extractants studied included corn oil and a number of diverse aqueous systems, and with the exception of the 50% ethanol solution, the results were remarkably similar. It is reasonable to suggest that other aqueous and oily systems, including those containing minor proportions of ethanol, will not behave very differently. Aggressive systems that might give a considerable shift in the partition of VCM between container and contents would be unsuitable for packing in PVC, in any case. One would thus expect PVC containers with 5 parts/10^6 or less of residual VCM, as now produced, to give 0.001–0.01 parts/10^6 in typical toiletries after prolonged storage, as for various foodstuffs. From the concentration levels recorded for foodstuffs, it is possible to gain an appreciation of the maximum intake of VCM now likely to obtain in the average diet in the United Kingdom. Precise calculations must depend on the assumptions made as to individual diet but it can be readily demonstrated that only minute quantities of vinyl chloride can exist in the average diet—of the order of one part in five billion of the food consumed (2). Since toiletries and cosmetics are not generally ingested, except by accident, any introduction of VCM to the human system by this route would be presumed to be largely through the skin. Very little is known about the possibility of introducing VCM to the body by contact with extremely dilute solutions in oils or water. It can be assuredly predicted however that the potential introduction of VCM to the community through the packaging of toiletry products in PVC is even lower than the minute quantity calculated for diet.

Toxicological significance

The only knowledge of a carcinogenic hazard to humans relates to the inhalation of VCM at relatively high concentrations in air, but before this evidence had been obtained, animal studies on the effect of the ingestion of VCM had already been initiated. However, these first experiments of Professor Maltoni at the Bologna Institute of Oncology were designed to give positive results, and deliberately high feeding rates were used to achieve this. Interim reports of this work indicate some carcinogenic effect at levels of at least a million times greater than the most generous estimate of the level in human diet (3, 4). More recently, animal feeding trials have been initiated with much lower VCM levels.

Future objectives

Efforts to reduce the level of residual VCM in packaging polymers are continuing and there is every reason to believe these levels will be still further reduced,

with consequent reductions in the packaging also. This in turn will result in even lower levels extracted into the contents. 'Nil extraction' can hardly have real meaning in scientific terms, but it may be expected at this point the VCM levels will either escape detection by even sophisticated analytical techniques, or will be so low that quantitative results must be of doubtful significance.

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Antimicrobial activity—a critical review of test methods of preservative efficiency

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Presented at the Symposium on Microbiological Safety of Cosmetic and Toiletry Products, 23–25 February 1976, Birmingham, organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—The United States Pharmacopoeia Preservative Test, Society of Cosmetic Chemists Test and Toiletry & Goods Association Test are compared with a procedure adopted by the authors. Important parameters, including realistic selection of test ORGANISMS, INOCULUM level, growth MEDIA, test temperatures, sampling and effectiveness are critically discussed. The use of MULTI-CHALLENGE TESTS as opposed to single challenge tests is highlighted and the need is stressed for a test procedure which ideally can predict PRESERVATIVE levels suitable for the life of the product.

Introduction

Most modern toiletry and cosmetic products provide an environment for bacterial or fungal growth, unless they are adequately preserved. Apart from the possibility of the contaminating organism posing a health risk, microbial growth can lead to quite dramatic changes in the formulation including splitting of emulsions, change in odour, or viscosity, discoloration and formation of gas. As a result of the greater awareness of these problems there has been, and there continues to be, a marked interest in the microbiological safety standards being

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applied to toiletry and cosmetic products. This has led to an increasing demand for adequately preserved products which can cope, not only with contamination introduced at the source of manufacture, but, subsequently, with consumer contamination.

As a result, a number of test structures have been proposed for measuring the efficacy of preservatives. These differ significantly from orthodox antimicrobial tests made on preservatives *per se* which are useful in making an initial choice for preservative assessment. Thus, minimum inhibitory concentration tests (agar diffusion and tube dilution test) and killing dilution tests are quite acceptable for initial selection of preservative systems, but they may have little bearing on the activity a preservative displays in the final formulation. The final assessment of preservative activity can only come from a study of the way in which an inoculum is reduced when added to the preserved product in the final pack. Hence the need for specific type tests.

Early preservative tests described by Rdzok *et al.* (1) and Kenney, Grundy and Otto (2) relied on the measurement of the loss of viability of an inoculum when added to a product containing preservative. Because of the difficulty in making viable counts their methods were confined to homogeneous fluid preparations. Baker (3) and Gucklhorn (4) extended the methodology to rather more complex formulations by using a sterile blender or removal of the oil phase, before making viable counts, in an attempt to overcome the problems inherent in determining the numbers of viable organisms in gels, creams, thick suspensions and semi solid products.

The United States Pharmacopoeia XVIII edition contained a preservative test based upon the above principles, which were subsequently modified in the United States Pharmacopoeia XIX edition. Although aimed essentially at pharmaceutical type products in multiple-dose containers, it was recognized that the method was applicable to other types of product including toiletries and cosmetics.

Olsen (5) used a slightly different approach by titrating products with different levels of micro-organisms and reported a correlation between the ability of a system to inactivate the highest level of organisms and to maintain a product consistently sterile in a practical situation. In a modification of this method Barnes and Denton (6) used the basis of the capacity test, which was initially introduced for disinfectant testing, to evaluate preservative efficacy and concluded that a technique might be evolved which emulated the challenge to a preserved product in practice.

The Toilet Goods Association Test (7) employed the capacity principle in so far that the method introduced a rechallenge of the product 7 days after successfully reducing the level of the original contaminating inoculum to an acceptable level. Likewise the test suggested by the Society of Cosmetic Chemists (8) also employed, for a number of products, a rechallenge with a further inoculum after the product had dealt satisfactorily with the initial inoculum.

Comparison of methods

The United States Pharmacopoeia, Toilet Goods Association and Society of Cosmetic Chemists tests, whilst similar in many circumstances, make interesting comparison. The purpose of this paper is to critically examine these three methods in relation to the test in use in the authors' laboratory.

Selection of test organisms

The type of micro-organisms representing the challenge is obviously a major factor in determining the assessment of a preservative. To be meaningful test strains must be chosen for their known association with product contamination, high level of resistance to preservatives, opportunistic contamination capacity, low nutrient demand and adaptability. In addition it is usual to include, as standard, a range of primary or potential pathogens which might contaminate an inadequately preserved product.

Evans, Golden and Bruch (9) and Dunningan and Evans (10) have shown that principal isolates from cosmetics, topical drugs and raw materials used as constituents rest heavily with Gram negative bacteria, principally *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Achromobacter* and *Flavobacter* species. Gram positive cocci, Gram positive rods, as well as organisms of the *Candida*, *Penicillium*, *Aspergillus* and *Cladosporum* genus, have also been isolated.

It is noticeable that *Staphylococcus aureus* does not feature amongst the isolates, probably because of its demanding nutrient requirements. Similarly, the ubiquitous and highly resistant Gram positive spore bearers do not figure prominently and an explanation may be sought in the susceptibility of their vegetative forms to the action of preservatives. Nevertheless, both organisms are used routinely in preservative testing. *S. aureus* is employed because it poses a threat from consumer contamination of frequently used products, whilst *Bacillus subtilis* is an example of the Gram positive spore bearers. Normally inadvertent contamination of an adequately preserved system with Gram positive spore bearers results in a slow decline in the numbers of bacteria over a period of a few days to a few weeks, but in a product not adequately preserved such organisms may survive, germinate and actively proliferate. For this reason a member of the *Bacillus* genus is included in the selection.

A selection of the above named organisms appears in most of the major preservative test systems.

The United States Pharmacopoeia Test relies heavily on five culture collection type organisms, *Candida albicans*, *Aspergillus niger*, *Escherichia coli*, *S. aureus* and *Pseudomonas aeruginosa*, although option is given for use of other micro-organisms where these may represent likely contaminants. The Toilet Goods

Association Test advocates a similar range of culture collection micro-organisms, but in addition, employs *Penicillium luteum* and *B. subtilis*. In contrast the Society of Cosmetic Chemists Test recognizes that culture collection type organisms may not necessarily represent strains of typical resistance or growth characteristics, and therefore underlines the use of vigorous strains of contaminants which have been isolated either from the product or factory environment in addition to culture collection type organisms. Emphasis on selection is placed on those organisms which will grow in the unpreserved product and, furthermore, choice of organism is varied and enlarged according to product class (see *Table I*).

The range of micro-organisms recommended in the latter is comprehensive and there seems little reason to extend the range beyond those listed. The authors certainly work within this particular list. However, Bruch (11) proposes the inclusion of *Herpes* virus and *Enterovirus* for toothpaste challenge, but the inclusion of such organisms makes very considerable extra demands on the facilities available.

Whilst the Society of Cosmetic Chemists Test recognizes the need for use of resistant strains, the particular selection methods may not, in themselves, be sufficient to ensure challenge with an organism in its most resistant state for survival and multiplication in a preserved product. The authors have experienced situations in which organisms recovered from a spoilt product have failed to establish themselves in a non-contaminated sample of the same preserved product even though the organism in question has only been subjected to a single overnight growth in a nutrient medium. The choice of medium in which the organisms are propagated is important.

Selection of growth medium

Cowen (12) has already reported the variation in resistance of *P. aeruginosa* towards phenolic antimicrobial agents which can be achieved by minor changes in growth medium and has shown that *S. aureus* is equally susceptible to change in medium.

Many of the offending micro-organisms are capable of slime layer or capsule formation which is dependent on the balance of carbon energy and nitrogen source in the environment in which they are growing. A change in this balance may occur for example by transfer of an organism from its source of isolation to a laboratory medium. This can result in a complete reversal in the resistance of an organism to many preservative agents and other substances found in cosmetic products.

Flawn, Malcolm and Woodroffe (13) showed that pre-treatment of organisms with levels of detergent up to 13% gave the inoculum added resistance and allowed rapid establishment of the population in the product, which the authors believed to be due to slime covering the bacteria. Similarly, Favero *et al* (14) reported that *P. aeruginosa*, when grown in water, showed increased resistance to various

Table I. Test organisms employed in various preservative tests

United States Pharmacopoeia XIX ed.	Toilet Goods Association	Society of Cosmetic Chemists	Authors
C. albicans	*C. albicans*	Toothpaste	*C. albicans*
		Product contaminants	*Penicillium* spp.
A. niger	*A. niger*	Shampoo	*A. niger*
		Gram negative factory	
E. coli	*E. coli*	contaminants product	
		spoilage.	*E. coli*
P. aeruginosa	*P. aeruginosa*	*Pseudomonas* spp.	*P. aeruginosa*
		Creams and lotions	*Pseudomonas* spp.
S. aureus	*S. aureus*	*S. aureus*	*Enterobacter* spp.
		S. faecalis	*P. vulgaris*
Product contaminants	*B. subtilis*	*P. aeruginosa*	
		P. fluorescens	
	P. luteum	*E. coli*	*S. aureus*
		Klebsiella spp.	*Staphylococcus epidermidis*
	Product contaminants	*Proteus* spp.	*S. faecalis*
		A. niger	
		Penicillium spp.	
		Cladosporum spp.	Product contaminants
		Alternaria spp.	
		Fusarium spp.	
		Mucor spp.	
		Rhizopus spp.	
		Phoma spp.	
		Trichoderma spp.	
		Verticillium spp.	
		C. albicans	
		Saccharomyces cerevisiae	
		Eye cosmetics	
		P. aeruginosa	
		P. fluorescens	
		Micrococcus luteus	
		S. faecalis	
		Fresh saliva	

antimicrobial agents including: chlorine dioxide, quaternary ammonium compounds, acetic acid and activated glutaraldehyde compared with the same strains grown on trypticase soya agar.

Soya-bean casein digest medium, nutrient agar and saboraud agar are commonly used media both for storing and propagating bacteria and fungal species for use in preservative testing, but as Goldman (15) observed the present practice of growing bacterial challenge organisms for preservation studies in conventional microbiological media is open to question, a view supported by the authors.

In order to ensure adequate preservation Cowen (12) suggested the possibility of propagating the organism in a suitable medium to which has been added the preservative or product in low concentration, and proposed the method of Basset, Stokes and Thomas (16) as a means of achieving this.

Single versus mixed cultures

In an attempt to limit the amount of testing, mixed rather than single cultures are sometimes used. Criticism has been made that such challenges exhibit greater susceptibility to preservatives when contrasted with a single cell inoculum. The authors can cite at least one instance where a mixed *Staphylococcus*/*Streptococcus* population showed less resistance than the pure culture, but, in general, their experience would support the Cosmetic Toiletry and Fragrance Association comparative trial (17) of pure versus mixed culture challenges in which no significant difference was found in the results. As a result the authors' employ pools of broadly related types of organisms, e.g. Gram positive spp., Gram negative spp. and mixed fungal and yeast suspensions, as a means of reducing experimental effort in preference to limiting the range of test strains and/or number of tests.

Inoculum size

Within the span of the United States Pharmacopoeia, Toiletry Goods Association and the Society of Cosmetic Chemists preservative test systems, the recommended inoculum size lies somewhere in the range from 10^5 to 10^7 organisms g^{-1}. As is to be expected, variation in inoculum size influences results. Increasing the number of organisms will, on the assumption of a natural Poisson distribution of resistance, introduce a greater number of more resistant cells and make the challenge correspondingly more severe. Secondly, the use of larger numbers of organisms results in a greater inactivating effect on the preservative, the extent of which depends on the nature of the preservative/bacterium interaction.

The influence of inoculum size can most easily be identified by a series of challenge tests, and is illustrated admirably by the titration method of Olsen (5).

Argument must range as to the most appropriate level, but as a generalization based on the authors' experience 10^6–10^7 organisms g^{-1} provide an acceptable level. If adequate preservation is to be achieved, the larger inoculum obviously has merit, but this is dependent on whether further challenges are planned.

The authors' test system utilizes a series of four challenges at minimum weekly intervals and it is interesting to note the cumulative failure rate of products subjected to four sequential challenges is 9.8, 13.4, 18.2 and 20.9%. Since these challenges are carried out at short intervals, the increase in failure is most likely due to the increase in total inoculum level rather than loss of activity for other reasons. Proof of this is found in samples stored for 12 months before being subjected to the standard four challenges, the failure rate in this case being 7.8, 11.7, 13.7 and 15.7%. There is a significant increase in the failure rate as the inoculum level is increased from 2×10^6 organisms g^{-1} to 8×10^6 organisms g^{-1}. The extent of the failure will depend on the nature and mode of action of the preservative and in addition on the preservative concentration.

It is a matter of some debate as to whether four separate inoculum levels of 2×10^6 organisms g^{-1} are equivalent to a single inoculum level of 8×10^6 organisms g^{-1}. One advantage of a four inoculum system is that a failure somewhere between the first and fourth inoculum gives some measure of the degree of preservation available and offers appreciable guidance as to the remedy necessary for a satisfactory preservation. A failure in a single step inoculum system does not allow any assessment of the situation to be made.

Number of challenges

No fixed pattern for rechallenging products with micro-organisms has so far emerged from preservative tests. The United States Pharmacopoeia Test uses a single challenge, whilst the Toilet Goods Association Test uses two challenges 7 days apart. The Society of Cosmetic Chemists Test varies conditions according to product groups, but tends to recommend a second challenge for a number of products.

The authors favour a multi-challenge test and introduced the method into their testing several years ago, initially because it seemed to represent more closely the practical situation relating to possible consumer contamination. In addition the need was seen for a test which could predict that preservation was adequate over the life of the product.

Whilst it may be argued that the use of multi-challenge test can lead to excessive preservation of the product, this is not necessarily the case. It will depend upon the preservative system under investigation and the number of challenges made.

There will be a decrease in activity of the preservative system with age of the product for a variety of reasons, including: instability of the preservative; loss of

preservative through pack; interaction of preservative with components of product; interaction of preservative with pack; interaction of preservative with foreign matter. An ideal test system should make allowances for such factors and enable predictions to be made of preservative levels which will ensure adequate, but not excessive, preservation of the product over its normal shelf life.

However, since the factors affecting preservative activity vary from agent to agent and for a given preservative from product to product and, furthermore, since these aspects are for the most part completely independent of any parameters included in a test structure, no direct account can be taken of them, except for regular testing of the product over its shelf life period.

At best any preservative test performed early in the life of a product can only take account of the likely loss of preservative activity at later stages by building into the test a degree of severity which will match the likely loss in preservative efficiency.

Success in achieving the correct balance between over-preserving and under-preserving a product will be dependent, for a multi-challenge system, on the number of challenges used and this can only be judged after a period of experimentation. The authors initially selected four challenges as offering a realistic balance. The success or failure of this choice can be judged by the fact that 64% of products passing the initial test have a totally satisfactory preservative system after storage for $1\frac{1}{2}$ years.

Figure 1 shows the cumulative failure of products at the 6th, 12th and 18th month stage of storage on the basis of the results of the four challenge tests carried out at each period. The success rate would be somewhat higher were it not for the fact that one of the preservative systems widely employed in the test is thought to work through breakdown and release of formaldehyde. Clearly the task of ensuring satisfactory preservation over a 3 year period is extremely difficult and much more work is needed before a totally satisfactory preservative test system can be worked out. Our results to date would suggest the four challenge system is slightly underestimating preservative loss, particularly with those agents which breakdown to release formaldehyde.

Temperature

There is a tendency when choosing the test conditions to select temperatures which reflect the optimum for the particular bacterial or fungal species employed. For bacteria this is generally in the region 32–37°C and for fungi 27–32°C.

These temperatures are not a realistic representation of the temperatures to which the majority of products are normally subjected (except those destined for tropical countries) and, whilst such temperatures favour bacterial growth this may be more than offset by the enhanced performance of the preservative agent.

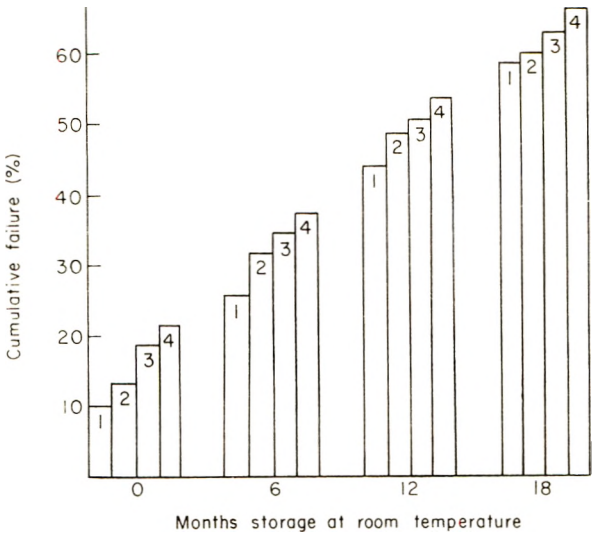

Figure 1. Cumulative percentage failure of products subjects to the four challenge test system after storage for zero period of time, 6, 12 and 18 months at room temperature.

It is not inconceivable that either a lethal or static activity may be achieved at the higher temperatures which is not apparent at lower temperatures. Such an occurrence has been observed by the authors in a mouth-wash employing cetylpyridinium chloride as active agent. At room temperature *P. aeruginosa* not only survived, but actually multiplied in the product whilst at 37°C the product proved lethal to this particular organism within 24 h.

Whenever possible test temperatures close to normal storage temperatures should be employed, and to this extent some of the temperatures recommended by the Society of Cosmetic Chemists Test are more realistic than either the United States Pharmacopoeia or Toilet Goods Association Test.

Preservative concentrations

It is usual to examine more than one preservative system and employ a series of concentrations where possible. Concentrations should be evenly spaced and the authors prefer to use geometric progressions as a means setting realistic levels.

Methods of sampling

The pour plate agar count is the method for the estimation of survivors specified in the majority of protocols. Serial dilutions of the samples are prepared

with a diluent containing appropriate neutralizing agents to antagonize the preservative and preclude carry over of residual antimicrobial activity. Alternatively, or in addition, neutralizers are often incorporated into the pour plate agar. 0.1% Tween 80/Peptone water is recommended as diluent for water based emulsions, whilst Lubrol W is recommended for water-in-oil products. Membrane filtration techniques are advocated for recovery of small numbers of organisms from emulsions, using diluents similar to those recommended for agar counts, except that water/oil emulsions are first diluted in sterile isopropyl myristate.

Problems encountered in obtaining accurate viable counts from other than homogeneous aqueous based products have led to a decline in the use of viable count sampling techniques, and to the substitution of absence of micro-organisms as a criteria for satisfactory preservation. In this connection it is significant that Hart and Ratansi (18) showed the membrane filtration technique achieved only 56% or less recovery of *P. aeruginosa* from oily creams whilst Berens, Romond and Lemaire (19) showed that pour plate counts gave a significantly lower count than surface counts, no doubt due to the oxygen requirements of obligate aerobes.

As a consequence whilst the United States Pharmacopoeia Test recommends assessment based upon viable counts, tests which have followed, e.g. Toilet Goods Association Test and Society of Cosmetic Chemists Test depend on an evaluation based essentially on the absence of bacterial/fungal growth at periods ranging from 1, 2, 7, 14, 28 and 56 days, although the Society of Cosmetic Chemists Test does recommend viable counting in addition.

The Cosmetic Toiletry and Fragrance Association (17) showed that a simple agar streak method of sampling and assessing counts was valid and produced comparable results to conventional counting procedures. In view of this, the authors have adopted a technique of sampling with a standard bacteriological loop so that a total sample 0.025 g is taken and spread over an agar plate. Although, at first sight, criticism may be levelled at this technique on the grounds of the low recovery volume statistics show that with an average number of survivors of four per loopful there is a 98.2% chance of picking up a positive. Bearing in mind the initial inoculum level, this represents a better than 99.99% reduction in micro-organisms. Subsequent challenge and sampling will lead to an overall improvement in probability of recovery should viable organisms remain.

Controls

Wherever possible products without preservatives should be examined in parallel with the samples containing the preservative system. Alternatively, or in addition, formulations of known susceptibility should be used to monitor the resistance of the test challenges.

Frequency of sampling

There is a lack of agreement in the various test procedures on the period between inoculation and sampling. Most tests run for at least 28 days and samples are taken at intervals during this period. Certain procedures, notably those of the United States Pharmacopoeia and Society of Cosmetic Chemists sample at weekly intervals, whilst the Toilet Goods Association recommends an extra sample 1–2 days after inoculation. The necessity for sampling over extended periods is the result of two separate possibilities.

(1) Some preservative systems are essentially bacteriostatic and, consequently, bring about a slow reduction in viable microbial level. Bacteriostasis is an unstable state and the aim is to achieve a reduction in viability. Change can take place within a formulation which alters the activity of the bacteriostatic agent sufficiently so that microbial proliferation is possible.

(2) Certain bacterial species, in particular *Pseudomonads*, whilst succumbing to the action of bactericidal agents almost totally, do survive in very small numbers and can multiply as they gain resistance to the antibacterial agent. Maurer (20) showed that *P. aeruginosa* introduced into disinfectant solutions often fell to very low numbers over the first day or so, but subsequent multiplication resulted, 7 days later, in levels which were not significantly lower than the initial inocula.

Quite apart from the need to ensure that the product reaches the consumer with an acceptable micro-biological purity, there is also the need to ensure the product can deal with subsequent microbial contamination introduced by the consumer. Sampling at weekly intervals is unlikely to give an indication of the suitability of the preservative for this purpose, particularly if the product is used frequently. The product should be capable of dealing with such contamination in a period which is less than the frequency of use. The Toilet Goods Association method of sampling 1–2 days after inoculation is to be recommended and even earlier sampling is preferable for certain products. The Society of Cosmetic Chemists Test recommends sampling after 1 and 6 h for eye cosmetics.

Effectiveness

The United States Pharmacopoeia Test in its original form in the 18th Edition called for a reduction in viable bacteria of at least 99.9% at two sampling periods 7 days apart; the concentration of viable yeast and moulds to remain at, or below, the initial inoculum level during the first 14 days, and the concentration of each micro-organism to remain at, or below, these designated levels during the remainder of a 28 day test period. Bruch (21) believes the test needs to be strengthened for topical products not only by increasing the initial inoculum level to

10^6 organisms ml^{-1} or g^{-1}, but by demanding a 99.99% reduction during a 28 day period. According to Bruch (21), certain products would also be expected to achieve a 99% reduction in the number of yeasts and a 90% reduction in the number of moulds. In addition re-inoculation at a reduced level is prescribed.

The Toilet Goods Association Test makes no formal recommendation with regard to preservative efficacy, but indicates that a formula which has a relatively high recoverable count of the insult organisms after the 7th day examination may be considered inadequately preserved for long periods of product storage. Final judgment, however, is not recommended until after 28 days.

By comparison the Society of Cosmetic Chemists Test is more demanding. In the case of shampoos, products should prove self sterilizing within 1–7 days. For creams and lotions a drastically reduced count, or the demonstration of an apparently sterile product which remains sterile on three successive weekly samplings, is considered indicative that the preservative will give good protection, whilst toothpastes should show a reduced count. Rechallenge is recommended in the case of shampoos where an immediate decrease to zero count is maintained for two consecutive weeks. Eye cosmetics are sampled at 1 and 6 h, but no guidance is given on the minimum reduction considered necessary, although it is recommended that the product is bactericidal to *P. aeruginosa*.

In our view none of the recognized test methods specify or demand preservative efficiency in keeping with today's requirements. In particular, no test takes into account preservative loss on storage and in this respect the authors' test is more severe.

Quite apart from requiring a reduction to at least 99.99% or greater from four challenges introduced at minimum weekly intervals, products are challenged at regular periods after storage by a test regime identical to that adopted for the initial test and similar reductions in micro-organism must be achieved for a product to be considered as satisfactorily preserved.

Tests are normally carried out at the equivalent of the 6th, 12th, 18th, 24th and 36th month stages at room temperature. The product, prior to carrying out the test, is stored in the final pack so that any interaction between pack and preservative is highlighted. Accelerated as well as ambient temperature storage tests are carried out so that products can be launched with a minimum of delay.

Conclusion

Although there is general acceptance at the present time of the challenge test as the most practicable and meaningful means of assessing preservative efficiency, there is considerable divergence in the parameters of the various challenge procedures and little, if any, guarantee that a preservative system fulfilling requirements will provide adequate preservation over the expected shelf life of the product.

These observations clearly emerge from the comparative review made of authoritative test procedures advocated by such bodies as the Cosmetic, Toiletry and Fragrance Association, Inc. and Society of Cosmetic Chemists and in the United States Pharmacopoeia XIX. The lack of unanimity in test temperature requirement, magnitude and number of microbial insults, frequency and mode of sampling, duration of test period and above all in the definition of effectiveness, no doubt, is a reflection on the uncertainty of the experimental significance to be attached to these test conditions. It emphasizes the need for a long term detailed investigation to evaluate these and other factors in order to build them into a test which gives a realistic measure of preservation of the expected life of the product.

The authors give details of a more severe four cycle challenge regime adopted by them in an effort to meet some of the disadvantages held to be inherent in the official procedures. This test is not claimed to solve the problems associated with preservative testing, but points to some of the factors which clearly need further study.

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APPENDIX

Authors' method of assessment of preservative capacity

Test organisms

The following were employed routinely as test organisms: *P. aeruginosa*, NCTC 1999; *P. aeruginosa*, ex-product contaminant; *Enterobacter aerogenes*, ex-product contaminant; *Escherichia coli*, NCTC 86; *Proteus vulgaris*, NCTC 4635; *S. aureus*, NCTC 7447; *S. epidermidis*, NCTC 7944; *Streptococcus faecalis*, NCTC 8213; *Aspergillus niger*, ex-product contaminant; *Penicillium* spp., ex-product contaminant; *Candida* spp., ex-product contaminant.

Preparation of test challenges

Each week the bacterial and Candida strains were taken off stock slopes and propagated in Oxoid Nutrient Broth. The broth cultures were incubated at 27°C for 48 h to give a density of *ca.* 2×10^8 cells ml^{-1}. The fungal challenge was prepared from malt extract agar slopes which had been incubated for 7 days or longer by washing the spored growth with 0.85% saline, shaking the suspension with glass beads in a shaker until uniform and, finally, filtering the suspension asceptically through glass wool or coarse sinters, if mycelial mat aggregates were present. The suspension was then adjusted to give a spore count of *ca.* 2×10^8 ml^{-1}.

Finally, three pools of mixed cultures were prepared by mixing equal volumes of broadly related test strains of: Gram negative rods; gram positive cocci; fungi and *Candida*. These three mixtures formed the test challenges.

Inoculation and sampling

Ten gramme samples of product were separately challenged with 0.1 ml amounts of each of the three test challenge mixtures. After shaking or mixing with

a glass rod the three contaminated product samples were incubated for 48 h at 27°C.

Approximately 0.025 g of product is then sampled by means of a 4 mm diameter loop and inoculated onto nutrient agar plates of malt extract agar in the case of the fungal challenge, each containing suitable neutralizer. All plates were incubated at 27°C and scrutinized for growth after 48 h and in the case of fungi and yeast up to 5 days.

If growth was produced, further samples were taken twice a week for a period of up to 3 weeks. In the absence of growth the product was challenged again after an intervening minimum interval of 1 week. This cycle was repeated until the product had received a total insult of four challenges.

Controls

Wherever possible products without preservatives were examined in parallel with the samples containing the preservative system. Alternatively, or in addition, formulations of known susceptibility were used to monitor the resistance of the test challenges.

Tests following storage

All products were stored in the final pack at both room and elevated temperature and examined by the above procedure after 3 months, 6 months and 12 months storage. Room temperature samples were also examined at 18 months and 2 years by the same procedure and at 3 years by a single inoculum challenge.

Assessment of effectiveness

The product satisfied the test provided no growth was produced from each of four challenges made with mixed pools of Gram negative, Gram positive and fungal cells and that each challenge was eliminated within a week to 10 days. Furthermore the product had to satisfy the requirement at least at the 3rd and 6th month storage stage at elevated or the equivalent period at room temperature. Further tests were carried out as described above after 2 years and 3 years of storage at room temperature as a check on the efficacy of the preservative over extended periods.

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The effect of irradiation on packaging materials

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Presented at the Symposium on 'Recent Advances in the Packaging of Cosmetic and Toiletry Products' organized by the Society of Cosmetic Chemists of Great Britain at Bath on 10–12th November 1975.

Synopsis—IONIZING RADIATION, mainly in the form of gamma rays from the radioisotope cobalt 60, is being used increasingly for the INACTIVATION of CONTAMINANTS in cosmetic and toiletry preparations. The treatment is applied to the product in its final pack, and therefore, it is important to recognize that the properties of the PACKAGING MATERIALS could be affected, particularly if high radiation doses are used. The stability of a wide range of materials is discussed.

The nature and extent of chemical and physical changes in plastics very much depends on the presence of ANTIOXIDANTS and other additives, and on the environmental conditions during irradiation. Some useful information is available from research into the use of radiation for the preservation of food—many FILMS and LAMINATES having been cleared for use in the United States. The low radiation doses used for the control of contaminants in cosmetic products are quite unlikely to cause changes in packaging materials which will limit the use of the process, with the possible exception of the discolouration in glass.

Certain radiation induced changes have been used to advantage, as for example with POLYETHYLENE, where the melting point can be elevated significantly following high radiation treatment.

INTRODUCTION

Ionizing radiation in the form of gamma rays from the radioisotope cobalt 60, is now widely used on an industrial scale for the sterilization of medical products. Such products include disposable, plastic, hypodermic syringes, needles, rubber gloves and catheters and also some pharmaceuticals, including antibiotic preparations and surgical dressings. In recent years, the process has

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been extended to the control of contaminants in cosmetic preparation or their raw materials. Information on the radiation inactivation of micro-organisms pertinent to the processing of such products has been given in a previous paper by Ley (1) and this also includes a detailed description of the radiation facilities in use and the control procedures employed.

The high penetrating power of gamma radiation combined with the fact that there is only a very small rise in the temperature of the target material during its treatment, makes radiation processing applicable to the product in its final pack and often without removal from its transport container. It follows that the direct effect of radiation on packaging materials requires study, as well as effects on products, and furthermore, it will be of interest to note that packaging materials themselves are often radiation sterilized prior to use in aseptic filling operations. It is important to appreciate at the onset that there is no question of either the materials or the products becoming radioactive as a result of the irradiation, since the photon energy level of this radiation is too low to affect the nucleus of any element. However, there remains the possibility that some purely chemical change could be induced, leading for example, to alterations in physical properties such as strength or sealability or colour.

Quite apart from the relevance of radiation effects on packaging materials to product sterilization, there lies the rôle of radiation as a tool in the deliberate production of new and useful properties in plastics. A good example is in the manufacture of 'shrink-wrap' film, this and other examples will be described which indicate the kind of chemical changes which can be induced in polymers, associated with packaging.

Nature of chemical change

The process by which the high energy radiation is absorbed in matter is extremely complex and involves the loss of energy from the gamma ray photons by ejection of electrons from atoms. Most chemical and physical effects in a medium, are caused through the agency of these fast electrons which dissipate most of their energy in matter by causing excitation and ionization. The yields of radiation induced reactions are expressed as G values, where G is the number of molecules changed, whether formed or destroyed, per 100 eV of energy absorbed. The official unit of absorbed dose is the rad, which is defined as 100 erg per g, this is equal to 6.242×10^{13} eV per g.

Modifications of the properties of polymers by radiation are the result of a few basic processes, involving cross-linking, analogous to dimerization, degradation analogous to main chain scission and gas evolution. The character of change varies with the polymer involved, but one of the processes predominates. If cross-linking predominates, the ultimate effect of irradiation will be to produce a network polymer in which all molecules are joined to each other. If degradation

predominates, the molecules become smaller and the material loses its polymeric properties. Steric factors may be involved in the behaviour of different polymers. For example, with vinyl polymers, it can be seen that cross-linking predominates when the formula is $(—CH_2—CHR—)_n$, but degradation predominates when the formula is $(—CH_2—C(CH_3)R—)_n$ and this is probably because the methyl group introduces a steric strain into the molecule enhancing main chain scission. *Table I* summarizes *G* values of cross-linking for a variety of polymers and of chain scissions for others.

Table I

Polymer	*G* (cross-linking) @ 20°C	*G* (scission) @ 20°C
Polyethylene	2.0	
Polypropylene	0.6	
Polystyrene	0.04–0.06	
Natural rubber	1.3	
Polybutadiene	2.0	
Polyacrylonitrile	1.4	
Poly(methyl acrylate)	0.5–1.1	
Poly(vinyl chloride)	0.2–0.5	
Poly(vinyl acetate)	0.28	
Poly(dimethylsiloxane)	2.5	
Poly(methylphenylsiloxane)	0.8	
Polyamides	0.3	
Polyisobutylene		3.0
Poly(methyl methacrylate)		1.9
Cellulose		10.0
Poly(α-methylstyrene)		0.25

Another important chemical change is the formation of gas. The major product is usually hydrogen, together with low molecular weight hydrocarbons. Chlorine containing polymers yield hydrogen chloride as with poly(vinyl chloride).

Improvement in properties at high doses

The amount of chemical change induced in a polymer will depend only not on the total radiation dose involved, but also on environmental conditions during irradiation, particularly with respect to the presence or absence of oxygen and the temperature. In some instances, very high doses, in the order of 20 Mrad, are used to cause striking changes in physical properties. For example, low density polyethylene melts at about 115°C, but after sufficient cross-links have been introduced, it no longer converts to a liquid on heating. This property is used to advantage in a number of applications where a thermal treatment is required, such as in heat-sealing bags and wrappers. The same property greatly facilitates the soldering of insulated electric wire, since the insulating coating does not melt

even if locally overheated. Again with polyethylene is the interesting 'memory effect'. The polymer is irradiated, then heated and expanded and cooled while still under stress. If the material is now placed round an object and then heated, it reverts to its original shape forming a tight sheath and hence a 'shrink-wrap'.

Stability of various materials

Considerable experience of the radiation stability of packaging materials and associated accessory products, such as adhesives and printing inks has been accumulated in the course of the application of radiation for sterilization purposes. In the medical and pharmaceutical field, the radiation dose normally used is 2.5 Mrad and rarely exceeds 4.5 Mrad. In contrast, in applications aimed at the control of contaminants in cosmetic products or their raw materials, doses are usually much less and within the range: 0.25–1.0 Mrad. There are few packaging materials which are unsuitable for use, even at the highest dose mentioned. It should also be stressed that stability is often influenced by the size and environment of the article being irradiated, and by the nature of additives intended to aid processing or to improve technical properties.

Although it is unlikely that instability of packaging materials will limit the use of radiation to treat cosmetic products, the general guide given in *Table II*, to the effects noted usually at very high doses, has proved useful. It was compiled mainly from observations made in the course of the development of radiation processing at the Wantage Research Laboratory of the United Kingdom Atomic Energy Authority.

Radiation stability guide based on average observations

Table II

(See footnotes on page 506)

Material	Stability	Comments
Rubbers		Stability influenced by the nature of the antioxidants present
Polyurethane rubber	*	The most radiation stable rubber
Natural rubber	†	Good stability
SBR Butadiene styrene rubber	†	Good stability
Nitrile rubber	†	Good stability
Silicone rubber	‡	Usually polydimethylsiloxanes-methyl phenyl silicones. More stable
Neoprene rubber	‡	Hydrogen chloride evolved—beware of corrosion
Butyl rubber	**	Becomes fluid at comparatively low doses

Table II (*continued*)

(See footnotes on page 506)

Material	Stability	Comments
Thermoplastics		
Polystyrene	*	The most radiation stable common moulding plastic
Polyethylene (high and low density)	†	Melt flow index drastically reduced, stress cracking improved
Nylon 6 and 6 : 6	†	Hardens at high doses (10^8 rad). Much less stable in film form
Polyester-Mylar or Melinex	†	Turns brown. Much less stable in film form
Polyvinylchloride PVC and copolymers	‡	HCl evolved. Turns brown. Polyvinylidenechloride less stable
Polycarbonate	‡	Tendency to become brittle at high doses
Cellulose esters—acetate and nitrate	‡	Acetate slightly more stable than nitrocellulose (Celluloid)
Polypropylene	§	Becomes brittle, especially on storage
Polymethylmethacrylate—'Perspex' 'Diakon'	§	Turns brown, becomes brittle and may crack
PTFE Teflon or Fluon	**	Acids evolved—much more stable in the absence of air
Polyacetal-polyformaldehyde and copolymers	**	Becomes brittle and loses strength
Thermosets		These materials are much more stable when filled with fibre
Epoxy resins	*	Very stable especially when cured with aromatic amines
Phenol formaldehyde 'Bakelite'	†	Good stability—sometimes colour changes
Urea formaldehyde UF resins	‡	Sometimes colour changes
Polyesters—styrene modified	†	Good stability—resins may be cured using radiation
Textiles		
Polyester—Terylene or Dacron	‡	The most radiation stable textile material
Cellulose acetates—Dicel and Tricel	‡	Loss of strength—acetic acid produced
Acrylic yarn—Orlon, Acrilan and Courtelle	‡	Small amount of cross-linking at low doses
Wool	‡	Loses strength and solubilizes. Silk is less stable than wool
Viscose Rayon	‡	Much more stable than cotton
Nylon 6 and 6 : 6	‡	Very sensitive to air. Degradation in air 5× that *in vacuo*
Cotton	§	20% loss in strength at 5×10^6 rad and yellowing

Table II (*continued*)

Material	Stability	Comments
Adhesives		
Structural adhesives—epoxies and phenolics	†	Araldite (epoxy based)
Vinyl type, e.g. polyvinyl acetate	‡	Inorganic fillers improve the stability
Pressure sensitive adhesives	**	Oxidative breakdown at doses $>10^6$ rad giving excessive tack
Miscellaneous		
Paper and cardboard	‡	Eventual loss of mechanical strength
Metal foils	*	Of outstanding stability
Glass	**	Colour change to brown even at 10^5 rad
Cork	†	Very stable
Wood	‡	Loses mechanical strength, but serviceable
Printing ink	†	Very occasionally a change of shade

* Satisfactory up to a dose of at least 5×10^8 rad (500 Mrad).
† Satisfactory up to a dose of at least 10^8 rad (100 Mrad).
‡ Satisfactory up to a dose of at least 10^7 rad (10 Mrad).
§ Satisfactory up to a dose of at least 2.5×10^6 rad (2.5 Mrad).
** Very unsatisfactory, particularly at doses greater than 10^6 rad (1.0 Mrad).

Storage effects

All plastics age on storage and this effect may be magnified following exposure to radiation due either to the consumption of antioxidant during irradiation and/or the production of more oxidizable groups in the polymer structure. An additional storage effect usually associated with plastics containing crystalline regions, for example, polypropylene, is due to the presence of trapped 'free radicals'. These molecular fragments slowly diffuse from their traps and cause further chemical changes. High temperature annealing removes these radicals, but may also accelerate the ageing process.

The discoloration of PVC which is generally to a light brown, appears to proceed during storage in the light. Although there is no change in mechanical properties even at a comparatively high dose, the colour change is aesthetically displeasing and there is a need to find a suitable stabilizer system. Conjugated double bonding is shown by infrared measurements to be the major reason for discolouration. Investigations of stabilizers with reference to PVC and polypropylene have been described in some detail by Plester (3).

In contrast, the discolouration observed in irradiated glass, which browns to violet depending on composition and dose, fades during storage—the process

being accelerated by heat or light. The colour is due to the trapping of displaced electrons at impurity centres; the displacement of atoms introduces extra defects which give rise to additional absorption bonds.

Toxicity

In both the medical and food areas, attention has been given to the question of the inertness of packaging materials in that there should be no migration of chemicals into the pack contents during storage. The problems concern potential toxic hazard and damage to the product. In general, there has been little or no problem, although the release of HCl from PVC packs at sterilization doses has caused some concern in regard to corrosion of metal contents or even toxicity in regard to irradiation of transfusion sets, etc.

Studies specifically directed to the safety for use of irradiated packaging in the food industry has been made in the United States (4), as part of the wider research programme aimed at the application of the process to food preservation. Food simulating solvents such as distilled water, acetic acid and *n*-heptane, were used in an attempt to extract chemicals from irradiated films. The nature and quantity of extractives were determined by fine analytical techniques, and the results compared with those obtained from the same films non-irradiated. It was concluded that there was little difference. A substantial number of materials have already been approved by the United States Food and Drug Administration, for use with irradiated foods. A similar study of extractables from irradiated plastics using pentane as solvent is reported in the paper referred to earlier (3).

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Dermatocosmetic relations

C. D. CALNAN*

The 1976 Medal Lecture by Professor C. D. Calnan, Consultant Dermatologist, St. John's Hospital for Diseases of the Skin, delivered before the Society of Cosmetic Chemists of Great Britain at the Royal Society of Arts on 4 March 1976, with Dr. F. G. Brown, President of the Society in the Chair.

Synopsis—There are a number of different interfaces in the RELATIONSHIP between COSMETIC CHEMISTS and DERMATOLOGISTS, a relationship which is less developed in Britain than in many other countries. The most evident is in the matter of customer complaints. These may be both subjective and objective. As more active ingredients are used in COSMETICS, more diverse types of ADVERSE REACTIONS may be anticipated. Consumer pressure has been responsible for several recent analyses but most of them have been general rather than specific. The detailed investigation and search for the cause of a customer complaint requires active cooperation from the customer, cosmetic chemist and dermatologist.

The dermatologist can help the cosmetic chemist in screening tests and the choice of new materials, based on the experience of his particular speciality. There are encouraging signs that the relationship is steadily improving.

In spite of the so called special relationship between Britain and the United States and of our current membership of the European Economic Community, there is no doubt that Britain remains distinct, an island in many other ways than geographically, whether one thinks of our universities, our schools or our Health Service. We are also distinguished from Europe and the United States in the relationship between the two disciplines of dermatology and cosmetology. In fact, one could argue that there is almost no relationship at all between them. This contrasts markedly with the situation in the major European countries and in the United States. It is likely that 95% or more of dermatologists in

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Britain have never met a cosmetic chemist. Their European and American counterparts, at least in University departments, mix easily with cosmetic chemists, as the volumes of the Journal of your Society will show.

What are the reasons for such continued isolationism? Although many could be quoted, the most important is probably ignorance. During the two world wars, for reasons which I need not go into now, dermatology was a relatively backward speciality in Britain. Even with the post-World War II scientific revolution the advance of British dermatology into the scientific age was slower than that of Europe and the United States. Three other factors are also relevant; first, the rather rigid regulations of the General Medical Council concerning ethical behaviour by doctors; secondly, the framework of the National Health Service which required consultants to devote their time to clinical patient care; and thirdly, the absence of even a single academic university department of dermatology. To many dermatologists membership of the Society of Cosmetic Chemists appeared as a dubious move, not to be inserted in either the Medical Directory or Who's Who. Even today dermatologists in Britain are liable to regard cosmetic chemists as swashbuckling adventurers seeking the secrets of nirvana and a pot of gold.

How different is the reality? Cosmetic chemists, when found, are not the pin-striped salesmen of the salon but surprisingly similar in dress and attitudes to the lecturers of university departments whom the dermatologist has known at medical school; and even more so nowadays they are ready and able to discuss problems of physiology, pharmacology and pharmacy in a rational scientific manner. The difficulties are those of xenophobia or any other form of prejudice based on ignorance. In my view the difficulties can only be overcome by greater contact. Unfortunately, the opportunities for such contacts are all too few. Clinicians in Britain have a heavy daily work load, with more emphasis on illness than on health.

Cosmetologists and dermatologists have different purposes in life. The primary function of the cosmetic chemist is to produce products to beautify, which involves both increasing the advantages and diminishing or masking the disadvantages in a person's appearance. The primary role of the dermatologist is to treat disease and relieve physical and mental suffering. Each, however, can provide skills for the other.

A cosmetic chemist produces acceptability in the form of high quality bases, perfumes and covering creams. A dermatologist-physiologist produces drugs to diminish excessive sweating, deodorants, sunscreening and depigmenting agents, relief of acne, and hormones. Both are involved in understanding the bacteriology, physiology and biochemistry of the skin, the principal area of study being the horny layer of the skin surface. The post-War decades, however, have shown considerable overlapping of these roles, mostly to the advantage of the clients of both specialities.

Customer complaints

The field of customer complaints is an important one for a cosmetic company and each will handle them in their own way. Such complaints fall into two main categories—product faults and adverse reactions. Almost no manufacturing company seems immune from product faults. The village doctor's surgery with its own dispensary is not exempt, and patients may want to know how an earwig got into their bottle of medicine. A meat processing and packing company will have metal detectors better than any at London Airport, and their electromagnets are rightly proud of their display case of trophies to rival that of any football club. For the cosmetic company even one grain of sand in a face cream can end up on the legal department's desk.

Subjective

But it is in the sphere of adverse reactions unexpected by the customer, that a dermatologist's help may be needed. The types of complaint vary enormously and it is by no means easy to evaluate them from the evidence of a letter alone. Cosmetics are designed to have a subjective as well as an objective effect, and not surprisingly the majority of the complaints are subjective. Few husbands have escaped the demand to examine an invisible painful and itchy rash on the face which never reaches the doctor's surgery next morning. I do not wish to imply that such complaints are figments of imagination. But they often defy explanation and investigation. The usual types of skin test procedure are negative. Application to a site other than the face is without effect, and later re-application to the face may also fail to produce a recurrence. I have seen this phenomenon with face creams and lotions and with lipsticks and eye products. Many explanations are put forward such as variable susceptibility at different phases of the menstrual cycle and psychogenic factors, but I remain sceptical. They appear to be more frequent in fair skinned, blonde or rufus people than in brunettes and may be part of the well recognized increased vulnerability of such people to soaps, detergents and so on, as well as to ultraviolet light. A smaller group of complaints are those with a visibly evident change in the skin. These may be of all grades of severity from the trivial to the most alarming. By no means all of them seek medical help, and of those who do go to their general practitioners only a small proportion are seen by a dermatologist.

Objective

The visible changes may be of a wide variety and can be classified as dermatitis, acne, pigmentatary disturbance, systemic effects and structural defects.

Dermatitis

This is a rather general term for a type of inflammation of the skin which can be produced by many different mechanisms. It also has many different manifestations, such as redness, swelling, scaling, dryness, roughness and cracking of the horny layer, as well as a range of 'spots', blisters and exudation, and crusted patches. With these ingredients the range of individual pictures or clinical appearances can be almost infinite.

Acne

In this context acne essentially means blackheads. It is well known that mineral oils particularly are the commonest general agents to produce occupational acne. The oils on babies' skin or clothing used to be quite a frequent cause of infantile acne; and acne from the use of oils in brilliantines and similar preparations are still occasionally seen. Some other substances can also produce this effect, for which Kligman and Mills (1) have coined the term acne cosmetica. Fulton, Bradley and Black (2) have now put forward suggestions for non-comedogenic cosmetics.

Pigmentary disturbances

These are also much less frequently seen now. The best known is the so called berlocque dermatitis from oil of bergamot in eau de colognes. (This is not a dermatitis but a form of hypermelanosis, although in high concentrations bergamot oil can produce dermatitis.) The effect is a photosensitizing one now known to be associated with bergaptene (3). Synthetic bergamot can be made without it. It is similar to the well known effect of certain tar derivatives which, in the 1920s, were thought in Central Europe (wrongly) to be the cause of a condition called Riehl's melanosis, as a result of impurities in fats used in cosmetics.

The types of pigmentation from chronic exposure to lead, silver and mercury salts in cosmetics are now mostly anachronisms.

Skin lightening creams based on hydroquinone which are used on a huge scale in Africa and Asia can produce not only dermatitis but unfortunate pigmentary effects. Post inflammatory hyperpigmentation is not uncommon, but the most worrying is an irreversible hypopigmentation of a highly characteristic confetti like pattern. A recent study in South Africa (Bentley-Phillips and Bayles (4) states that hydroquinone is very much safer than the monobenzyl ether of hydroquinone; this latter compound has now been prohibited in South Africa. A yet more serious complication called colloid milium was reported by Findlay, Morrison and Simson (5) last year.

Systemic effects (or internal disturbances)

These may ensue from absorption of chemical substances through the skin,

and must be a constant source of anxiety. The toxic and even fatal potential of hexachlorophene has been sufficiently well published not to merit further mention. Boric acid also has lost its place in toiletries for the same reason. Little publicized, however, have been the deaths from surface absorption of hexylene glycol and pentachlorphenol, although they were used in higher concentrations and in circumstances not likely to be applicable to cosmetic use. An unexpected effect of absorption encountered in recent years has been that of persulphates in hair bleaches (Calnan and Shuster (6)). Ammonium persulphate can produce an alarming syndrome of histamine liberation and shock with unconsciousness although I am not aware of any deaths. Some people may be surprised that persulphates are still used, while their addition as improvers in flour is now forbidden in many countries.

Structural defects

These are almost always considered serious by the customer. They usually involve the hair and nails. Hair straighteners and cold permanent wave solutions when used to excess will make the hair inelastic and brittle like flax and it will break off. Fortunately this is a temporary effect and the hair eventually grows out normally. Plastic nail coverings, artificial nails, and other nail treatments based on formaldehyde or acrylate compounds can produce equally alarming effects. It is not always realized that the nail plate is porous and chemical substances readily penetrate them. This can result in a dermatitis of the nail bed which disrupts its growth.

A most extraordinary effect is the so called 'Bird's nest hair' produced by shampoos. I have seen several such cases, all from a cetrimide shampoo (Dawber and Calnan (7)). During a shampoo the hair becomes inextricably tangled or matted into a large mass which has to be cut off. Scanning electron microscopy shows what has happened (*Figure 1*). Where hairs cross they are 'welded' together by a thick viscous material. The shampoo itself is not the only factor; the surface scales of hairs become imbricated together, rubbing of fibres produces felting, and there is electrostatic attraction between hair fibres with an anionic surface in the presence of a cationic detergent. It can be produced experimentally in normal hair.

Incidence

There are very few published reports of customer complaints which are of value for independent assessment. This is entirely understandable. Weissler (8) gave details of such complaints sent to the Food and Drug Administration (FDA). They appeared to be rising in frequency (*Table I*).

Table I. Number of yearly complaints to FDA

Year	Number
1970	227
1971	314
1972	377
1973 (5 months only)	300

As regards the individual items responsible, there is a variable yearly incidence. Between 1970 and 1971 complaints about bath preparations and dentifrices, for example, had decreased while those about shampoos, deodorants/antiperspirants and hair dyes had increased (*Table II*).

Table II. Incidence of complaints about individual products in 1970 and 1971

	1970 (%)	1971 (%)
Bath preparations	11·1	8·6
Dentifrices	9·4	3·8
Shampoos	8·5	14·0
Deodorant/antiperspirants	7·0	11·8
Dyes and rinses	3·5	9·9

It must be appreciated the figures given are percentages of total complaints and are without meaning unless they can be related to the quantities or units used. A recent study of antiperspirants and deodorants has been published by the Consumer's Association in 'Which' (9). In the summer of 1975, nearly 1800 people who use these preparations regularly were questioned about skin problems from them; 429 (23.8%) reported some complaint; 264 were examined in detail. One in six had not followed instructions and used them directly on shaved skin. Of the rest a third recovered within 24 h after washing, 10% had trouble which lasted longer, but only 7% had not recovered after a month.

A quarter of the complaints were with unperfumed products, and over half of the complainants admitted to having a sensitive skin or previous skin trouble. These findings support the view that most antiperspirant/deodorant complaints are non-specific. One must recognize the conditions of use in a warm moist armpit do facilitate penetration and sensitization by allergenic germicides and perfume ingredients.

In an attempt to obtain better data the FDA and a Task Force of the American Academy of Dermatology co-operated in a study over September, October and November 1974; 36,000 users in 10,000 American households were studied. A total of 703 (1.9%) complaints were recorded, and 589 were confirmed by a

dermatologist. They were classified as follows: mild, 86%, requiring no treatment; moderate, 11%, involved disability or loss of time from work; severe, 2% requiring medical attention; unclassified 1%.

The products responsible in order of frequency were chemical depilatories, antiperspirant/deodorants, moisturizers and lotions, bath toiletries, hairsprays, eye products, hair colourants and bleaches, face creams and cleansers and nail polishes. Again, the criticism can be voiced that they are not related to units used. One knows that the popularity or fashion for particular types of product changes with time and in different societies.

Investigation

This study is laudable, but one should remember the investigation of cosmetic consumer complaints is not an easy task even for a qualified dermatologist. His function may appear simple—to make a diagnosis and determine the nature of the causative agent. But special experience with this kind of problem is required to arrive at a reasonably correct answer. Certain other essentials, which are not always easy to obtain, are necessary for success: information from the cosmetic chemist; co-operation of the patient; physician's time. Many dermatologists have told me they are unable to obtain the necessary help from the product manufacturer. I have rarely failed to obtain such help. The usual cause, I think, is the dermatologist either does not make personal contact with the cosmetic chemist or fails to explain the problem in detail to him. A telephone conversation is often more valuable than a letter. Dermatologists do not always appreciate that cosmetic chemists have a scientific training like themselves and have a similar interest in obtaining the truth.

The co-operation of the patient is essential, but she does not always realize what is required of her. She often believes a mere analysis of the product and an interview is enough. But it usually requires skin tests and trials involving a number of visits.

Equally important, the dermatologist must be prepared to give the necessary time and interest. Even in 1976 only a minority of dermatologists carry out patch tests regularly in their practice; and without repeated usage the patch test is by no means the simple procedure it appears to be. Unless he has a lot of experience of this type of complaint he may become discouraged at not being able to obtain a satisfactory answer (for him) or at least the answer he is expecting. Some of the possible pitfalls in diagnosis have been termed pseudo-cosmetic reactions by Fisher (10).

It is not always appreciated by dermatologists that the majority of dermatitis cosmetic complaints are of the so-called irritant or non-allergic type (in the medical sense). A look at the products listed in the FDA—Task Force Survey

mentioned previously will quickly confirm this. Although most products contain at least a potential allergen, it is not usually found to be the cause. For example, chemical depilatories designed to soften and dissolve hair will obviously have a similar effect on the keratin of the horny layer, causing irritation. Deodorant/antiperspirants are used by women on the (often recently) shaved armpit skin. Unfortunately it is by no means easy to distinguish between irritant and allergic reactions on purely clinical grounds without skin testing, especially as the adverse reaction has often subsided by the time the patient sees a dermatologist. But unless he sees the patient and knows the content of the product he is unlikely to be able to help.

I have never thought it is any part of the dermatologist's role to come between the customer and the cosmetic company. His function is to make a diagnosis and if possible determine which product, if any, is responsible for the adverse reaction, and proceed to identify which particular ingredient is responsible. This information can then be made available to the manufacturer and to the customer if she wishes, after which his role should end. I do not believe that it is a doctor's role to placate the customer, and I am pleased to say that I have never felt any pressure to do so placed upon me by any manufacturer.

I should add that I am assuming the dermatologist has the necessary knowledge and experience to avoid the pitfalls in diagnosis and skin testing. For example, a number of cosmetic preparations can produce a positive reaction under the conditions of a 48 h closed patch test, but are perfectly safe to apply to normal skin when used according to instructions. This is especially true of shampoos, cleansers, fresheners, some creams and lotions and mascaras. Eye products containing a high content of soaps or deodorized kerosene will frequently produce such a false positive test.

Allergens

A good personal review of cosmetic allergy is given by Schorr (1). He studied seventy instances of proven dermatitis over a period of 5 years. Each patient was patch tested and submitted to a usage test. The agents responsible are shown in *Table III*.

An important point is that about 40% of the patients had a previous history of skin trouble, and some of these might be regarded as pseudo-cosmetic reactions by Fisher (10). The responsible agent could not be satisfactorily identified in every case, but the ones found are shown in *Table IV*.

Screening tests

I do not need to emphasize the special difficulties involved in screening new

Figure 1. Matted hair from the scalp of case 1.

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Table III. Causes of cosmetic reactions in seventy patients (11)

Creams and lotions	31
Perfumes and colognes	9
Hair dyes and colour rinses	6
Deodorants	6
Eye make-up	5
Face make-up	5
Multiple cosmetics	4
After-shave lotions	3
Shampoos	2
Nail preparations	1
Skin fresheners	1

Table IV. Specific allergens found in seventy cases of cosmetic dermatitis (11)

Parabens	8	Eugenol	1
Sorbic acid	4	Isoeugenol	2
Formalin	3	Oil of Bergamot	1
Mercurials	5	Oil of Clove	1
Dichlorophene	6	Cinnamic aldehyde	3
Hexachlorophene	1	Lavender oil	2
Ethylenediamine/EDTA	2	Balsam of Peru	3
Lanolin	5	Oil of Eucalyptus	1
Brij 30 and 72	2		

substances for use in cosmetics—both irritants and sensitizers. There are two separate problems. and different test procedures are required for detecting irritants and sensitizers.

Allergenicity

Everyone knows that there are standard procedures laid down to satisfy the allergenic safety requirements of the U.S. Food and Drug Administration. But some doubt has been cast in recent years as to whether these procedures will always detect potentially dermatitic agents. It was this doubt which led Kligman and Mills (1) to evaluate the prescribed tests with known allergens and they found them inadequate. Eventually Magnusson and Kligman (12) devised their guinea-pig maximization test; and this test is now increasingly widely used by research workers in the cosmetic and toiletry fields. Few will doubt it is superior as regards its degree of sensitivity to the former Draize test. (Whether it is necessary for routine use is a separate question.)

However, the Magnusson–Kligman (12) technique demands the most meticulous attention to detail, and small errors in technique can produce major

differences in results. Every scientist knows that to repeat another worker's experiment and obtain the same result is rarely easy. This is particularly true of the guinea-pig maximization test. I heard recently of a scientist who learnt the technique in Magnusson's department but failed to obtain any positive reactions when he repeated the experiment in his own laboratory. A return to the teacher soon uncovered the single error in technique which was responsible. When I recounted this event to a colleague, his comment was that if success or failure depended on such a small step in experimental technique the test should not be regarded as of great value. But surely this is typical of all biological work. Enzyme actions may depend on small changes in pH; just as small differences in temperature, pressure, catalysts and physical conditions can alter many chemical reactions. It took nearly 30 years after the discovery of melanin synthesis from tyrosine to appreciate that light was necessary to activate human tyrosinase. Why could Marion Sulzberger not sensitize guinea-pigs in New York after learning the technique in Zurich, until he had guinea-pigs sent from Switzerland? We now know the genetic strain of a guinea-pig is of paramount importance.

Not only is meticulous detail in experimental method required, but considerable differences in recording results can produce different answers. I cannot recommend strongly enough to all workers in this field to link up with other groups to try to achieve uniformity of method and recording. This is not, of course, only a problem in animal experiments. It is equally true of human patch testing—my own experience constantly leaves me asking, how can one doctor record a test result as positive and another negative?

We all know that research workers come to know and trust their own method, the one of which they have most experience, just as doctors tend to keep using the drugs they know best and surgeons use the operative techniques they know best. For this reason I consider the mammoth work of Schultz and Noster (13) of great value. They chose two known allergens, dinitrochlorbenzene and chlormethylimidazoline hydrochloride, and tested each of them by eleven of the published guinea-pig assay methods on ten animals. Their results showed that the Magnusson–Kligman (12) test was the best, followed by that of Maguire and Chase (14).

Screening test procedures are carried out by dermatologists and by chemists with two quite different objectives in mind. The dermatologist wants to know if a particular chemical has any allergenic potential at all, and submits it to the most vigorous and stressful test possible. Since allergenic potential is directly proportional to concentration, he can then decide at what dilution it might be safe to use such a substance. The chemist, however, wishes to know that the concentration at which he chooses to use it is free of allergenic hazard. Hence he carries out tests in the usage concentration.

There are many examples which illustrate this point. Over 40 years ago methyl heptine carbonate in a perfume was found to be the cause of some cases of

lipstick dermatitis (15). Landsteiner and Jacobs (16) showed guinea-pigs could be readily sensitized to it by topical application. One might have thought that its use would be excluded by these events. Not at all: I understand from perfumers that it is still widely used, but at a sufficiently low concentration; and no further cases of dermatitis have, as far as I know, been reported.

The colouring agent Quinazoline Yellow (D and C Yellow 11) has been quite extensively used over many years in colognes or similar preparations. But when it was used in lipsticks and rouge sticks, cases of severe allergic dermatitis followed (Calnan (17), Larsen (18)), and sensitization could be readily produced with a 1% concentration. This does not mean the colour should be banned—only that it should be used in very low concentrations.

It has been said that the more rigorous screening tests favoured by dermatologists are not relevant to practical usage experience. An attempt to relate the two has recently been made by Marzulli and Maibach (19). They showed good correlation in regard to benzocaine, formaldehyde, ethylenediamine and paraben esters. Their predictive tests probably overstated the risks of paraphenylene diamine and underestimated the potential of neomycin.

Irritancy

An area in which dermatologists and cosmetic chemists need more active research is that of irritancy. Although so called irritant or non-allergic dermatitis is recognized to be much more frequent than allergic contact dermatitis, dermatologists know almost nothing about its mechanisms, and test procedures are abysmally inadequate. Part of the trouble is that we do not know what is the defect for which we are trying to test. The primary change is thought to be some form of physico-chemical damage to the horny layer, but whether it is to cells, cell membranes or intercellular material is not certain. It is not as dependent on pH as was once thought, for the horny layer has a good buffering mechanism. Alkali neutralization and alkali resistance tests are now almost universally devalued, except in some Eastern European countries.

Many of the recommended tests are really too complex to be of practical value. Kligman and Wooding (20) devised a meticulous procedure for estimating irritancy potential. Probably the most sensitive is the estimation of water permeability, but to perform it accurately requires very expensive equipment and a great deal of experienced skill (Thiele and Malten (21)).

At the ordinary level of patch testing Bjørnberg (22) has shown in an extensive study how unreliable such methods can be. Cruickshank (23) suggested tissue culture methods but they have not been used by cosmeticians probably because the effects in which one is interested are on the dead cells of the horny layer and not on living cells in culture.

Some idea of the difficulties involved are shown in van Abbé's (24) investigation into the cause of consumer complaints following the use of a male hairdressing preparation. All the usual types of screening tests were carried out prior to marketing, with satisfactory results. However, when 170,000 units were sold in 10 months, twenty complaints of eye irritation were received. An unusual aspect was that in all of them the product had been used prior to exposure to rain or snow. Hence it was conceivable that the gel could have been dissolved in rain water and trickled down into the eyes. The compaints were of itching, stinging, pain and blurred vision. Ophthalmologists who examined some of the patients described pitting of the corneal epithelium—a punctate keratitis.

Routine rabbit eye tests were negative, but rinsings from human hair after use of the hairdressing did cause irritation in the rabbit eye, with pitting of the cornea. The individual ingredients of the preparation were then tested separately; only the 13-mol ethoxylated oleyl alcohol produced a positive reaction, and it had a similar effect on rabbit skin. A 5-mol ethoxylated oleyl alcohol was much less irritant. Finally, thin layer chromatography showed considerable differences between the 5-mol and 13-mol ethoxylates, especially as regards their hydrophilic character.

This study reveals the thoroughness of investigations by van Abbé (24) and his colleagues and is a credit to the cosmetic industry. By publishing such a study the industry can only gain more respect from dermatologists and the general public.

Choice of materials

No doctor who has had contact with cosmetic chemists will envy them the task of choosing whether or not to use particular chemicals. Brauer (25) has criticized dermatologists for abusing the results of patch testing. This may be true, but it is the function of dermatologists to identify chemicals as allergens in the human environment and report their results. There are many reference books and papers providing this information. Hardy (26) has recently given such a list in this journal.

It is important to remember, however, that many different factors can influence whether or not substances cause allergic dermatitis in practice. The following are some of them.

Total usage

The criteria required differ for usage according to the market. Some years ago I saw a single instance of dermatitis from a germicide in talcum powder. It was present in an elite expensive product with limited sales, and I know of no other cases. If it had been incorporated into a mass market cheap product, the chemical may have been unusable.

Chemical environment

It has long been my feeling that a certain level of exposure in the population has to be reached before a dermatitis problem appears. It is rare for products containing a new chemical to cause dermatitis soon after marketing. (I am excluding the new product phenomenon.) The same is true of drugs for topical use. Allergy to ethylenediamine is almost unknown in countries where a particular therapeutic product containing it is not used. But when it was introduced into Britain, several years passed before allergy to ethylenediamine was detected and several more passed before it rose to the top of the 'allergen league table'. It is at the top of the 'table' in the United States, where the cream containing it is now said to be the most frequently used cream in its class.

It is well known that the antibiotic neomycin is one of the most frequent topical therapeutic allergens in Britain. In Bristol the bacteriologists became very concerned at the rising incidence of neomycin resistant organisms being isolated in the department. They asked the medical staff to stop prescribing it and to substitute gentamycin. Within a short time the incidence of neomycin resistant organisms fell. At the same time the dermatologists noted the number of positive patch tests to neomycin markedly diminished, but allergy to gentamycin appeared for the first time. There are analogous experiences for cosmetic chemists with products such as Chloracetamide, and it is possible that the same phenomenon may be seen with the more recently introduced triclosan (Irgasan DP 300).

Bases

Particularly in Africa but also in the United States and more temperate zones, skin lightening creams based on hydroquinone and its derivatives have been used on an enormous scale. For many years the dermatitis hazard remained at a low and presumably acceptable level. In 1973/1974, however, in South Africa the number of cases of leukomelanoderma traceable to skin lightening cream rose sharply. Dogliotti *et al.* (27) saw 347 patients over a period of 10 months. They followed a product change in a particular marketed product, which had been sold in open mouthed jars. Ammoniated mercury was replaced by monobenzyl ether of hydroquinone (2%) to which salicylic acid (2%) was often added. The open necked jar in which it was sold allowed atmospheric oxidation of the monobenzone.

The climate

One is constantly informed that a product which has caused dermatitis or other ill effects in one country has been safely marketed in another without any trouble ensuing. Is this valid and is there any explanation for it? Are the people in one country different from those in another in their dermatitic capability?

Experimental work on contact sensitization in man does confirm that there are differences in the human population. Caucasians are more sensitizable than

mongoloids and both more than negroes. And the skin is more readily sensitized in summer than in winter. Animal experiments show that genetics are important. It is no use doing the guinea-pig maximization test on animals who are not sensitizable. But are these the reasons for our observed differences? When certain identical products were marketed in Britain and the United States, the level of consumer complaints was ten times higher in Britain. When an identical hand cream was marketed in both countries, the consumer complaints were many times greater in Britain. Is the explanation to be found in a study of British female skin? I doubt it. One is only looking at customer complaint files and not looking at the customers. The factors which lead a customer to make a complaint to a cosmetic company will vary considerably from one country to another. Even major complaints are not always made. When I recently saw a series of women with severe sensitization from Quinazoline Yellow almost none of them had written to the manufacturer (Calnan, 1976).

Experimental population

I am sure that the majority of cosmetic companies carry out some form of consumer testing even if they do not embark on costly market research and test market studies. For convenience the company's own employees are often used. But such a choice may influence the results obtained. Are the company's own employees necessarily representative of the general public? I know of at least two disturbing episodes.

Some years ago the Akron Rubber Company in Ohio decided not to use a certain rubber additive because human sensitization studies had shown that it was a major sensitizer. The research department at another company were sufficiently interested to repeat the experiments with this chemical but failed to produce sensitization. When the Akron Rubber workers heard this they decided to repeat their own experiment under fresh conditions. They used the personnel of a local college. No sensitization resulted. They had previously used their own company volunteer personnel. When the individual records were studied, it was found the individuals sensitized were among those who had been used most frequently in previous experiments. One man had been exposed by such tests to dozens of different chemicals. Always be careful of the ready volunteer!

The reverse may also happen. A few years ago the staff members of a company used a nail preparation without any ill-effects being apparent but when marketed customer complaints were so numerous and severe that the product had to be withdrawn within 3 months.

Perfumes

The whole subject of allergy to perfumes is a complex one. Some of the difficulties seems to be due to separation of perfumers from cosmetic chemists.

Even within a company the perfumer may appear as a man apart. Inevitably this makes for suspicion. A customer complaint is frequently first attributed to the perfume by the company, while the perfumer takes the view that every other possibility should be considered first. How often is a customer satisfied by receiving an unperfumed replacement? I do not know, and I know of no follow up study to find the answer. Absence of a second letter of complaint does not imply satisfaction or that the perfume was originally responsible.

The problem is not insuperable. There are plenty of published examples of a particular single ingredient being found responsible. The first is the report on methyl heptine carbonate in lipstick already mentioned. Another is that of benzoin in a lipstick perfume (Hjorth, personal communication). A number of patients gave a positive patch test to the lipstick, its perfume, balsam of Peru and benzoin. When the benzoin was removed no further cases occurred.

In another instance, a deodorant was marketed under one label but with two perfumes from different manufacturers. I investigated a number of the customer complaints and was able to show that all of them were associated with allergy to one of the perfumes only. Whether pre-market test procedure could have detected this difference I do not know, but it would have been an invaluable piece of information for the manufacturer.

There are now lists available of perfume ingredients which have caused allergic dermatitis, and the Research Institute for Fragrance Materials (RIFM) has made some recommendations about their use; it listed a small number of materials which should not be used at all. Personally, I do not support such a view. I would not advise against the use of any single ingredient, but should prefer to place an upper limit on the concentration used. I sense this is also the view of most perfumers in practice.

In my experience perfume allergy is not a very common cause of cosmetic dermatitis. Over a period of 20 years at St John's Hospital we patch tested 25,683 patients and found 102 with reactions to a perfume. The preparations to which our patients reacted are shown in *Table IV*. These might be regarded as proven cases and are much less than the numbers of women who believe that they are allergic to perfumes.

In a separate enquiry, of 295 women questioned, forty-two (14%) told me

Table V. Sources of perfume allergy in 102 patients

Perfume	76	Moisture cream	2
Deodorants	14	Mascara	2
Eau de Cologne	11	Foundation	1
Talcum powder	7	Sun tan oil	1
After-shave	7	Cleansing creams	1
Hand cream	2	Freshener pads	1
Lipsticks	2	Tonic lotion	1

they reacted to one or more perfumes. In a similar survey Magnusson (personal communication, 1973) found fourteen (3.7%) of 377 women gave the same evidence, and three (0.8%) reported asthma, hayfever or conjunctivitis.

Conclusions

In this short survey I have done no more than touch on some of the points of contact between dermatologists and cosmetic chemists, and include those which have been of special interest to me personally. I have deliberately avoided those concerning legislation, both in Britain and in the European Economic Community, although I know that many of you are greatly occupied by such problems at the present time. It is a time of great change, and one hopes that Britain will come to play a leading part in any legislation which comes to be enacted.

In conclusion it is a pleasure to tell you how much I have enjoyed the many years of contact which I have had with your industry, and I have especially appreciated the tolerance and understanding which I have experienced in my own relations with cosmetic chemists and your distinguished and respected Society.

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