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Synopsis—The cooling sensation of menthol on the skin results from a physiological action on the nerve endings. Structure/activity analysis as used in pharmacology has allowed prediction and subsequent synthesis of a large number of compounds which have the cooling action of menthol. The compounds range in chemical type from, e.g. carboxamides to ureas to phosphine oxides. Many are as effective as menthol, and yet do not have the disadvantages associated with its volatility; they are non-odorous and remain longer on the skin. To be effective the compounds need to penetrate the skin, and as expected, the degree of cooling sensation for an area of the body correlates inversely with the thickness of the stratum corneum.

The range of cooling effects (e.g. subjects' descriptions, duration of effect) which have been noted, and the influence of vehicles, is described. Some postulates relating to the ease of skin penetration, and the physical parameters of the molecule are discussed. Future potential applications are briefly described

Effect of a skin cream containing the sodium salt of pyrollidone carboxylic acid on dry and flaky skin: J. D. MIDDLETON and MARESE E. ROBERTS. *Journal of the Society of Cosmetic Chemists* **29** 201–205 (1978)

Synopsis—Humectants added to skin creams can increase the moisture retention of isolated stratum corneum and reduce the incidence of dry and flaky skin *in vivo*. This paper gives results of an investigation into the efficacy of a humectant, the sodium salt of pyrollidone carboxylic acid (Na PCA), which occurs naturally in the corneum. A product containing 5% of Na PCA increased the water-holding capacity of isolated animal corneum. In a consumer trial, with assessment of skin dryness and flakiness by trained assessors, the Na PCA product was more effective than a control product containing no humectant and equally effective as a similar established product with a different humectant system.

An appraisal of the current state of mutagenicity testing: DIANA ANDERSON. *Journal of the Society of Cosmetic Chemists* **29** 207–223 (1978)

Synopsis—Every year many new chemical substances are introduced into our environment. Some of these chemicals may possibly induce genetic damage in populations exposed to them. There is a risk that such damage may accumulate in the gene pool and affect future generations, or even cause cancer in the present generation since a link has now been established between mutagenicity and carcinogenicity.

At present the most appropriate way to determine which chemicals may cause genetic damage is to test them in various *in vitro* or *in vivo* laboratory test systems capable of detecting damage to DNA, chromosomes or the genome. Some of these methods are discussed, as are the factors which determine a mutagenic response, problems encountered when using the test systems, together with data interpretation and assessment of genetic effects.

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New compounds with the menthol cooling effect

H. R. WATSON, R. HEMS, D. G. ROWSELL,* *and* D. J. SPRING
Wilkinson Match Research Division, Poyle Road, Colnbrook, Buckinghamshire

Presented at the Annual Scientific Meeting, Society of Cosmetic Chemists, 1–2 December 1977, New York

Synopsis

The **cooling** sensation of menthol on the skin results from a physiological action on the nerve endings. **Structure**/activity analysis as used in pharmacology has allowed prediction and subsequent synthesis of a large number of compounds which have the cooling action of **menthol.** The compounds range in chemical type, e.g. **carboxamides** to **ureas** to **phosphine oxides**. Many are as effective as menthol, and yet do not have the disadvantages associated with its volatility; they are non-odorous and remain longer on the skin. To be effective the compounds need to penetrate the skin, and as expected, the degree of cooling sensation for an area of the body correlates inversely with the thickness of the stratum corneum.

The range of cooling effects (e.g. subjects' descriptions, duration of effect) which has been noted, and the influence of vehicles, is described. Some postulates relating to the ease of **skin penetration**, and the physical parameters of the molecule are discussed. Future potential applications are briefly described.

INTRODUCTION

The effect which *l*-menthol imparts to the skin and oral cavity is well known and is usually described as 'cooling' or 'fresh'. The compound has long been added to toiletries in order to give a fresh sensation and to pharmaceuticals to alleviate the sensations of inflammation or itch. Because menthol is volatile, its effect on the skin is somewhat transient. In some uses, especially shaving foams, the vapour can give rise to an unwanted cooling sensation in the eyes. In all uses, the dominant odour is almost impossible to mask when menthol is present in a proportion sufficient to give an appreciable cooling effect.

This paper describes compounds which give a cooling effect, but do not have the undesirable characteristics which result from the volatility of menthol. The compounds were synthesised by us between 1971 and 1976, during a search based on concepts of correlation between structure and biological activity. Over 1200 cooling compounds were found; the purpose of this paper is to give a general account of the molecular requirements of cooling, and of the action of cooling compounds on the skin. Details of the synthetic work and other aspects of the study will be published elsewhere, but further description of the compounds is available in published patents (1).

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THE CHEMISTRY OF COOLING COMPOUNDS

THE COOLING ACTION OF MENTHOL

Menthol does not cool by volatilisation. The cooling effect almost certainly results from chemical action at or near those nerve endings which are associated with the sensation of cold. When menthol is placed on the lingual nerve of the cat the cold fibres of that nerve are either provoked into firing or, if already firing, respond with a higher rate of firing (2). Since the neurophysiological bases of the response to cold are essentially the same in all mammals (3–15), including man (16), and since there are many common features in the action of menthol and synthetic cooling agents, as related in this paper, it seems reasonable to assume that all such coolants act by a common mechanism, which is essentially that of a drug-receptor interaction (17).

MOLECULAR REQUIREMENTS OF COOLING ACTIVITY

We have established that four important criteria need to be satisfied for a compound to possess effective cooling activity:

(i) a hydrogen bonding group;
(ii) a compact hydrocarbon skeleton;
(iii) a correct hydrophilic/hydrophobic balance;
(iv) a molecular weight in the range 150–350.

(i) *Hydrogen Bonding*

A hydrogen bonding function is essential for cooling action. We believe that the function must contain an oxygen atom capable of acting as a hydrogen bond acceptor. For greatest cooling activity, strong hydrogen bond accepting capability is necessary. There is no indication that the provision of more than one hydrogen bond accepting group in the molecule enhances the cooling effect; some *p*-menthane-diols are active, but none is as active as *l*-menthol.

Functional groups are listed in *Table I*, together with comments on the groups, and on the compounds which incorporate them.

(ii) *Hydrocarbon Skeleton*

It is assumed that the functional group takes part in hydrogen bonding at a receptor site, and that for cooling the hydrocarbon portion or portions of the molecule must provide a compact hydrophobic region near to the site of hydrogen bonding.

The relationship between the structure of the hydrocarbon skeleton and cooling activity has been most thoroughly examined with N-alkyl carboxamides. *Figure 1* indicates the variety of hydrocarbon structures which, in conjunction with suitable N-alkyl carboxamide groups (−X), give compounds of strong cooling activity. It is noteworthy that the hydrocarbon skeletons are highly branched; this forces a compact configuration, and for many skeletons, allows an apparent requirement that for strong cooling activity the functional group should be attached to a carbon atom which itself is a branching site, or is adjacent to a branched carbon atom. If the functional group is separated from the branching by a $-CH_2-$ group, cooling activity is much reduced, and when separated by a $-CH_2CH_2-$ group, activity is almost lost.

Table I. Hydrogen bonding groups: cooling

High Activity	
Hydroxyl, –OH	The hydrogen bonding group of menthol. Strong hydrogen bonding, which gives some compounds of high activity. Compounds are normally volatile and odorous.
N-Alkylcarboxamide, –CONHR	Probably the most useful group. Compounds are of low volatility, and the many possible variations of the R allow the hydrophilic/hydrophobic balance of the molecule to be tuned finely.
N,N-Dialkyl carboxamide, $-CONR_2$	Compounds reasonably strong but many are volatile.
Sulphoxide, –S(O)R	Gives effective compounds, but their use is limited owing to problems of stability.
Phosphine oxide, =P(O)R	Strong hydrogen bonding groups which give effective, useful compounds. (Although organo-phosphorus compounds in general are suspect on grounds of toxicity, we are not aware of a toxicological effect attributable to the phosphine oxide group; the compounds are thermodynamically very stable and are of low chemical reactivity.)
Medium or Low Activity	
Carboxyl, –COOH	Compounds relatively weak; activity is reduced in alkaline vehicles.
Carboxamide, $-CONH_2$	Compounds relatively weak.
Hydroxyalkyl ester, $-COOC_nH_{2n}OH$	If the hydroxyl group is in the 2-position, moderate–strong activity can result.
Urea, $-\overset{\vert}{N}CONR_2$	Some compounds reasonably active.
Sulphone, $-SO_2R$	Medium hydrogen bonding, but compounds of low activity.
Sulphonamide, $-SO_2NR_2$	Medium hydrogen bonding, but compounds weak and have very bitter taste.
Sulphinamide, $-SONR_2$	Weak compounds, many of which are of limited stability.
Inactive	
Ether, –OR Ketone carbonyl, –C(O)R Ester (simple), –COOR Chloro, –Cl Amine, $-NH_2$ Thioamide, $-C(S)NR_2$	These hydrogen bonding groups appear not to give activity, either because they are not capable of hydrogen bond acceptance, or, if capable, give only relatively weak bonds.

Similar considerations apply to other functional groups, although it should be noted that owing to difficulties of synthesis, we did not explore fully systems with sulphur or phosphorus as the hetero-atom of a cyclic skeleton, and the most active phosphine oxides and sulphoxides prepared have open chain structures.

(iii) *Hydrophilic/Hydrophobic Balance*

For strong cooling activity, a compound must have the correct hydrophilic/hydrophobic balance. This balance is of recognised importance in drug–receptor interactions. The most common measure of hydrophilic/hydrophobic balance is the Hansch log P value, where P is the partition function of the compound between n-octanol and water (18). The log P value is well established as an important factor of the pharmacological activity

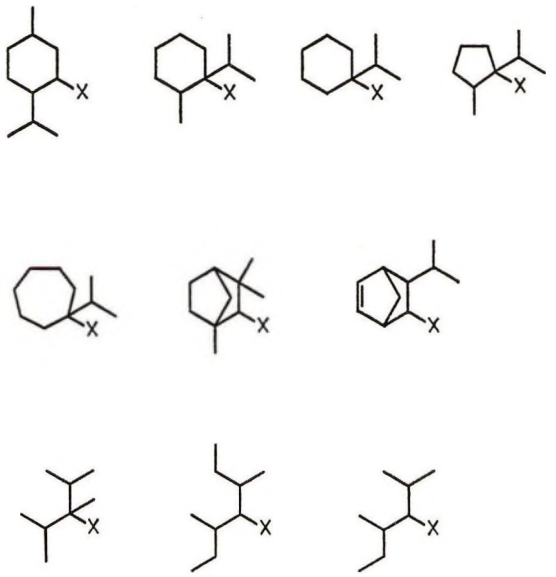

Figure 1. Hydrocarbon skeletons which give strong cooling compounds when –X is a suitable N-alkyl carboxamide group.

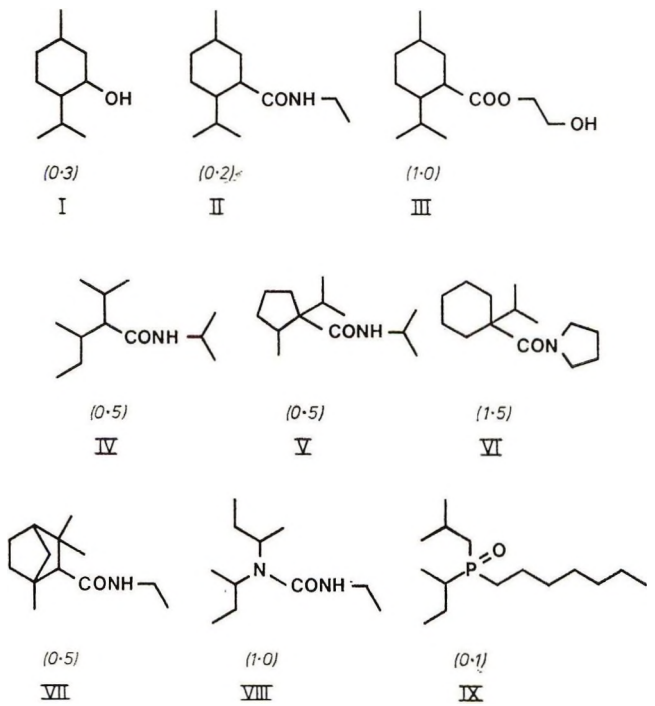

Figure 2. Some structures of cooling compounds. The figure in parentheses is the oral threshold value in micrograms.

of series of related compounds (19–22). It is also recognised as one of the factors which determines the rate of transport of compounds through biological membranes, especially the skin (23).

The log *P* values of cooling compounds were calculated from published tables of the substituent π-values (24). Strong cooling compounds have log *P* values in the relatively narrow range 1·5–4·0 and values for nearly all cooling compounds lie in the range 1·0–5·0. The log *P* value of menthol is 3·1.

(iv) *Molecular Weight*

If a hydrocarbon skeleton capable of giving strong cooling compounds is combined with a strong hydrogen-bond accepting functional group, and if the log *P* value of the resultant molecule is in the correct range, then cooling will be observed if the molecular weight is in the range 150–350. The criterion of molecular weight is more flexible than criteria (i), (ii) and (iii) but it is certainly not possible indefinitely to add balanced hydrophobic and hydrophilic portions to the molecule and to retain a cooling effect.

The observations on the four criteria accord with a drug–receptor interaction (17).

COOLING COMPOUNDS

The variety of types of molecule which give rise to cooling is shown in *Fig. 2*. All of these we have rated as medium-strong to strong cooling agents; their average oral threshold, recorded in μg (determined as described below), is given in parentheses. Compound I is, of course, menthol. Compound II has received particular attention as being of merit for oral and topical use; it is FEMA GRAS listed.

The variety of effective N-alkyl- or substituted N-alkyl-carboxamide functional groups is worthy of note. *Table II* gives oral thresholds of compounds where an N-alkyl-carboxamide group is substituted for the hydroxyl group in *l*-menthol.

Table II shows examples of molecules where a large hydrophobic N-alkyl group is balanced by the presence of a further hydrophilic group. Note also an indication that the hydrogen bond accepting function of the carboxamide group is influenced by the presence of other electron withdrawing atoms. All the *p*-menthane-3-carboxamides listed in *Table II* have the same stereochemical configuration as natural *l*-menthol.

Table II. N-alkyl carboxamide functional groups

N-Alkylcarboxamide	Oral threshold, μg
$-CONHCH_3$	1·1
$-CONHCH_2CH_3$	0·2
$-CONHCH_2CH_2CH_3$	0·8
$-CONHCH(CH_3)_2$	0·45
$-CONHCH_2CH_2CH_2CH_3$	1·4
$-CONHC(CH_3)_3$	0·4
$-CONHC_6H_4OCH_3(p)$	0·2
$-CONHCH_2CH_2OH$	5·0
$-CONH(CH_2)_3OH$	2·7
$-CONH(CH_2)_6OH$	1·1
$-CONHCH_2CH_2OCH_3$	2·7
$-CONHCH_2COOC_2H_5$	0·13
$-CONHCH(CH_3)COOC_2H_5$	0·4

Just as *l*-menthol has the greatest cooling activity of the eight stereoisomeric menthols, we believe that carboxamides such as II (*Fig. 2*) with the same stereochemistry also possess higher activity than any of their isomers. Thus, *d*-menthol is 45 times less active than its natural enantiomer *l*-menthol (as determined by the oral threshold method), and the *d*-enantiomer of Compound II is 70 times less active than II. These observations are consistent with menthol and the synthetic coolants acting at common receptor sites, the sites being associated with the nerves responsible for the sensation of cold. We believe that where stereoisomerism is possible in a molecule it is a determinant of activity.

GENERAL PROPERTIES OF COOLING COMPOUNDS

Apart from the cooling effect, and a degree of flavour potentiation and odour modification,* there is no common property of cooling compounds. For instance, there is no association between minty smell and cooling.

There being such a wide range of chemical type, the physical properties of the compounds are varied. Most are solids, although liquids are not uncommon when the functional group is hydroxyl, N,N-dialkylcarboxamide, or phosphine oxide. Most are readily soluble in common organic solvents, but owing to the requirements of the log *P* value, all are of very limited solubility in water (although it should be noted that saturated aqueous solutions have readily perceptible cooling effects). The alcohols and N,N-dialkylcarboxamides are in general volatile, and are odorous, the odour types ranging from minty to fruity to earthy to camphoraceous. Compounds of other classes have low or very low volatility and are non-odorous.

Many of the N-alkylcarboxamides, phosphine oxides and ureas are virtually involatile, and are odourless and tasteless. Chemical and thermodynamic stability follow the known characteristics of the constituent groups. The simple alcohols, acids, amides and phosphine oxides are of very high stability. This includes the long-term stability in aqueous environments which is essential for most projected end uses. Sulphur compounds and ureas are less stable and careful consideration is needed when matching these compounds with usage compositions.

HUMAN SUBJECTS AND THE COOLING EFFECT

TESTING OF RELATIVE ACTIVITY

Intrinsic Activity – Oral Thresholds

A reliable test of the intrinsic cooling effect of a compound was essential for development of any theory of relationship of structure to activity. The tongue is very sensitive to the cooling effect, it offers a convenient test site and, in particular, appears to present relatively little physical barrier to the compounds. Although there are a few exceptions, cooling compounds applied to the tongue appear to penetrate rapidly to the cold nerve

* It appears that when cold receptors are chemically excited, there is an associated mild response from adjacent odour and taste receptors, and modification of these two senses is almost certainly a general characteristic of cooling compounds (25). (David Kendall of Arthur D. Little, Boston, has tested over 100 compounds of our series; all modified flavour.) The property is in accord with that of pungent or 'warming' compounds; pungent components of peppers, chillies etc. are well established as flavour modifiers.

receptors. For this reason, we believe that the oral activities are a reasonably close approximation to the intrinsic activities of the compounds. Oral equipotency tests, in which trained panelists would derive equivalent cooling effects by balancing different concentrations of test and control solutions would have been the most scientifically satisfying, but such tests are very time-consuming and in view of the large number of compounds involved, were not considered for our own studies. (Trained panelists at Arthur D. Little, Boston, derived the same order of ranking of effectiveness which results from the threshold method described below when testing 20 of the compounds by an equipotency method.)

A threshold method was used for all determination of oral activity, and the values of oral threshold derived from it are treated as (reciprocal) measures of intrinsic cooling activity. Filter paper (1 × 1 cm) was impregnated with a known amount of compound by application of a measured volume (1–5 μl) of a solution in redistilled petroleum ether (40–60°). After 30 sec, the paper was placed on the tongue of the subject, who was required only to report presence or absence of a cooling effect. After an interval, the procedure was repeated, adjusting the amount of compound as required, until the threshold was ascertained. Usually, a sufficiently accurate estimate could be obtained with as few as five to ten measurements, and although there was a variation of ± 30% on repeat tests, this was acceptable because differences between compounds were usually quite considerable.

Individuals differ in their sensitivity to the cooling effect, and the personal thresholds of a group of subjects are likely to cover a considerable range. For example, the menthol thresholds of 23 subjects, chosen at random, were in the range of 0·02–10 μg. Also it is a general feature that the distribution is not simple Gaussian. Most panelists had thresholds reasonably near the minimum, there being a long tail to the value representing lowest sensitivity.

Even though the individual members of a panel may have had a wide variation in their menthol threshold, they ranked a series of compounds in the same order. Thus, although the basic sensitivity to a cooling effect varied from subject to subject, the relative effects of a series of compounds were equivalent for all panelists. All strong compounds appeared relatively strong to all subjects, and all weak compounds appeared relatively weak. In the absence of this feature, ranking of intrinsic effect would have been much more difficult.

In view of the wide variation between panelists, arithmetic means of threshold results were obviously of no value. Geometric means gave useful ranking, but it is believed that a more accurate comparison of compounds was achieved by comparing a panelist's threshold for a test compound with his personal threshold for a standard compound. The average ratio for the whole panel was then derived as the arithmetic mean of the individual ratios, and the mean threshold was calculated by reference to the known mean threshold (geometric mean of 23 subjects) of the standard substance. Menthol was not chosen as the standard substance because it gives occasional inaccurate values owing to its volatility; N-ethyl-*p*-menthane-3-carboxamide (II, *Fig. 2*) is our standard.

The above method appears to be reliable. Panelists were routinely checked for standard response by normal cross-check methods, and it is noteworthy that their threshold for a given cooling compound does not change even after several years of practice.

Oral thresholds recorded during the work spanned the range 0·08–50 μg. A compound with a threshold of less than 0·5 μg is regarded as 'strong'. Thresholds of greater than 50 μg were not quantified; at that level a compound shows exceedingly weak activity, and

undesirable side effects such as an unpleasant taste, tingling and burning usually predominate. Large numbers of compounds were examined in our study. When the method described above had become well established, a small panel of four to six subjects, chosen from the larger panel for their near average sensitivity was used for rapid initial screening of compounds.

Topical Testing

Like oral threshold determination, measurement of topical thresholds is complicated by the fact that individual sensitivities to cooling compounds differ considerably. Skin thresholds were measured as follows: 0·2 ml of a solution in petroleum ether (40–60°) was measured on to a circular area (10 cm^2) of skin surface on the inside of the forearm, midway between the wrist and elbow. After 30 min, the subject was required to respond whether or not a cooling effect had been noticed during the period. (Some compounds were slow to act, but the main reason for the delay between dose and question was to allow the panelist to differentiate the chemical cooling effect from the inevitable but transient physical effect of the solvent evaporation.) Since degreasing the skin with solvent before application of the cooling compound did not change the threshold, and washing may have caused hydration, the skin was not treated before a threshold test.

A panel of 50 subjects, tested by the above method, had thresholds for menthol of between 0·5 and 100 mg. As an example of the non-Gaussian grouping towards the sensitive end, the thresholds of 32 of the subjects lay between 2·0 and 10 mg, and only six subjects had thresholds of greater than 25 mg.

It should be recalled that the oral threshold method was used extensively because it appeared to give the best measure of intrinsic cooling activity. Skin threshold levels were not of value for that purpose, and in addition they were confounded by the manner in which the compound deposited on the skin following evaporation of the solvent. Some compounds even crystallised, and therefore clearly gave spuriously high 'threshold' results. For these reasons, our topical testing did not rely on threshold methods.

The aim of the topical testing was (a) to find, in general terms, those compounds which were most suitable for application in topical products and (b) to match compounds with product types so that the overall effect of the addition of a particular cooling compound to a product could be judged. In view of the difficulties implicit in skin threshold testing, and the inevitable relationship between compound effectiveness and the medium in which it is applied, we concentrated on tests based on application in the product type. Thereby (b) above, for a particular composition, was satisfied directly, and (a), the relative general characteristics of the compounds, gradually emerged as the number of tests in different media increased. This part of the study is on-going. The pattern of product oriented tests is exemplified by the following, which was used for aerosol shaving foams. The panel consisted of five men who were experienced in the effects of cooling compounds. The subjects applied approximately 0·5 g* of the shaving foam across the trigeminal/cheek area, wiped the area after 5 min, and a trained observer recorded their comments during (normally) the first 10 min following application. The observer took

* Unlike threshold testing, direct effectiveness testing is less influenced by the quantity of material applied. The concentration of the cooling compound in the medium is important, but compositions such as shave foams, which are applied in excess, are best tested in excess, it being implicit that they do not dry out during the period of observation.

particular note of time of onset, degree of cooling, and 'quality' (the meaning of the term 'quality' in this context is described below).

The degree of cooling effect was scored on an 8-point scale (no effect, threshold, weak, weak–moderate, moderate, moderate–strong, strong, very strong). Repeat tests on different days were desirable; a subject's repeat scores could vary by as much as 3 points on the scale. It was important that, as much as possible, the subject's skin was in the same state of hydration on repeat tests. (As an extreme example, the face is considerably more sensitive immediately following shaving than at other times of the day.) It was also desirable frequently to include as a control the base composition with no cooling compound; this acted as a test on the panelists, and also permitted weighting of the scores since the base composition invariably caused the subjects to record sensations. Similarly, compositions of known very strong cooling effect were included as positive controls.

SENSITIVITY OF DIFFERENT PARTS OF THE BODY

Different parts of the body differ greatly in their sensitivity to the cooling compounds. No attempt has been made to determine the relative sensitivities in absolute terms, but the order of sensitivity is:

> eye > > tongue > interior buccal region > ano-genital area > lip > trigeminal area > other face areas > axilla > inside forearm, breast > other arm areas, thigh, back > hands, feet > > palms,* soles.

The eye is extremely sensitive, with thresholds probably measurable in nanograms, and to an extent that a simple test for cooling effect in a volatile compound is to hold the opened bottle near the eye. This test with menthol gives a sharp and very obvious sensation, which accords with consumers comments on the effect of mentholated shaving foam on the eye.

Like menthol, synthetic cooling compounds also give a cooling sensation in the lungs (when added to cigarettes or inhaled as an aerosol spray) and to the gastrointestinal tract (when ingested).

The order of sensitivity clearly follows a general order of increasing thickness of stratum corneum (26), and it seems probable that the sensitivity of an area is determined mainly by the ease with which the compound can penetrate this barrier. Further indication is provided by the fact that abraded or hydrated forearm skin has a reduced threshold compared to intact skin. Although the barrier role of the stratum corneum is probably dominant (27), it is likely also that the number of cold-sensitive nerve endings per unit area, and the efficiency with which the central nervous system processes nerve signals, vary with the location on the skin (3, 28). (During a search for an equipotency method based on mirror imaged body areas, the menthol thresholds of sixteen subjects were measured on the left and right forearm. Six were more sensitive on the right, and six on the left forearm. Four had no difference of threshold. There was no correlation with the side of the dominant hand of the subjects. Similar differences between left and right sides of the face have been noted in threshold tests. The study has not been taken further, but it seems possible that these differences are related to the efficiency of neural signal processing. At suprathreshold levels these bilateral differences are not detectable).

* Some subjects have recorded a response when strong solutions are applied to the palms but our current view is that the effect is insufficiently distinct to be recorded positively.

All subjects in this study were Caucasians. Using 50 subjects, we sought to relate forearm threshold values to age, gender, hair colour and skin type (as pale or dark, greasy or dry), but on this small population no relationship has been noted.

DOSE–RESPONSE RELATIONSHIPS FOR COOLING COMPOUNDS

In compositions designed for usage the concentration of cooling compound is many times the threshold concentration, and although threshold measurements are a useful guide to relative activities a study of the relationship between dose and the subjects' response is of more relevance to practical formulations.

Ideally, we would have preferred to use the well established psychophysical techniques of magnitude estimation and cross modality matching (29) to evaluate the form of the power law (30) which would relate the sensation produced by the cooling compounds to the degree of stimulus (that is, the dose) applied. The number of formulations and the variety of compounds which we wished to cover necessitated a more rapid technique, that of the magnitude rating described earlier. This gave results which afforded valuable information on the relationship between dose and response, and which allowed comparison between different compounds when used in the same formulation. *Figure 3* shows typical response patterns for two compounds in a shaving foam formulation.

It is important to note that at suprathreshold levels of cooling compounds in compositions the variation of response from one individual to another is much less than the

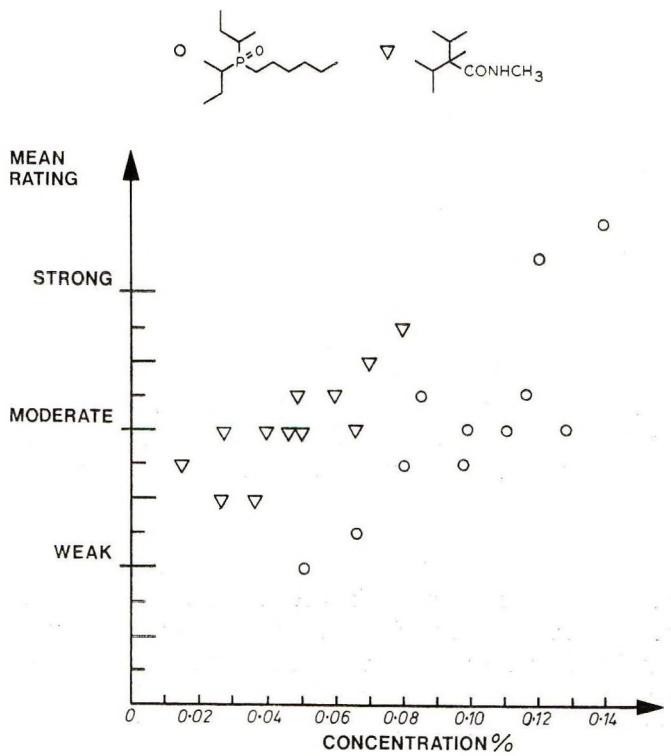

Figure 3. Plots, for two compounds, of subjects' description of degree of cooling against concentration of compound in a shaving foam formulation.

variation of threshold levels. (If this were not the case, then it is unlikely that menthol would be used so widely in toilet preparations.)

The duration of the cooling effect on the skin is related to the dose, higher doses resulting in a more prolonged effect. Also, at a given concentration, the more active the compound then the more prolonged the effect; this pertains regardless of compound type. Obviously, volatility is an important factor in determining duration. The cooling effect of volatile compounds, including menthol, is relatively transient and is rarely recorded for more than 15 min. The duration of involatile compounds is considerably longer; some produce cooling for more than 4 h.

After the initial perception of cooling effect has ceased, it is frequently observed that the cooling sensation is further perceived, but for shorter periods. This effect is more noticeable with strong compounds at high doses, and normally follows washing or sweating. The long duration of involatile compounds, and the repetition of the cooling effect, is consistent with the finding that compounds can still be detected in the stratum corneum 24 h after application. Thus, when Compound II (*Fig. 2*) was applied as a 10% solution in ethanol to a 5×5 cm area of the forearm, half of the area being stripped with pressure-sensitive tape after 30 min, and the other half after 24 h, 1·5 mg of compound was detected in strippings 3–6 after 30 min and 0·3 mg in stripping 3–6 after 24 h.

OTHER SENSATIONS PRODUCED BY COOLING COMPOUNDS

All cooling compounds produce sensations other than pure cooling. They are most readily described by reference to a strong peppermint candy, which produces tingle in the mouth and, if strong enough, a burning sensation. Compounds differ in the relative degree of cooling and side effects; those that produce little effect other than cooling are, in our terminology, of high 'quality'. The relative degree of cooling and side effect appears to correlate to some degree to compound type; for instance, phosphine oxides and hydroxyesters tend to have particularly low levels of side effect. The side effects are dose sensitive; at low doses with most compounds, or at medium doses with compounds having a low level of side effects, they are not apparent. In general, increasing the dose of the cooling compound beyond a certain limit gives no apparent increase in cooling, but causes an increase in side effects, finally to a degree where the side effects dominate the cooling effect.

Descriptions of side effects in the mouth, normally tingling, stinging, and burning, are paralleled by descriptions of side effects on the skin, and compounds prone to give oral side effects give side effects on the skin. In general, the more active the compound the less noticeable are the side effects at a given level of perceived cooling.

SKIN PENETRATION

Hydration or partial stripping of the stratum corneum aids penetration of the cooling compounds considerably, thus according with the established pattern reported by the other workers (27). The brief account of skin penetration which follows is directed to the relationship between the nature of the compounds and their ability to penetrate skin, and refers to non-occluded application where the skin has had no previous treatment, with relative humidity at between 40 and 60%. When a composition containing a cooling

compound is placed on the skin, three processes must occur before a cooling effect is perceived:

(i) molecules of the compound must transfer from the vehicle and must penetrate the surface of the stratum corneum.
(ii) molecules must diffuse through the skin.
(iii) molecules must interact with the receptors.

Each of the three processes is influenced by the properties of the compounds, but the properties of the vehicle, in relation to its interaction with the compounds, are also of considerable influence on process (i).

Polarity is the most important parameter of the vehicle. This is apparent from results obtained from usage formulations, and also from direct vehicle trials. Nine compounds with the *l*-menthol stereochemistry, either carboxamides chosen from compounds listed in *Table II* or hydroxyesters, were tested as 0·25% solutions in eight vehicles which represented a polarity range from olive oil to aqueous ethanol, using the face test detailed above for shaving foams. Regardless of their intrinsic activity, all compounds gave higher cooling ratings in the more polar vehicles. Indeed, no cooling was noted from solutions in the two vehicles of lowest polarity, olive oil and hexyl laurate.

In a corresponding test the Compound III (*Fig. 2*) was tested as a 0·4% solution in different ethanol-water mixtures (25; 33; 50; 67% w/w of water). The cooling score increased progressively from 'weak–moderate' (25% water) to 'moderate–strong' (67% water). Onset of cooling effect occurred in less than 2 min, and it is believed that skin hydration had little or no influence on the increase of score.

The process of transfer of compound from the vehicle to the outer skin layers may be viewed as a partition between the vehicle and the protein/lipid material which represents the stratum corneum. The cooling compounds are relatively hydrophobic ($\log P > 0$) and therefore they will partition only little from less polar vehicles into the skin: increase in polarity of the vehicles will shift the equilibrium in a direction which will favour absorption into stratum corneum. This effect is well established; for example, hydrophobic drugs are absorbed better from hydrophilic than from hydrophobic vehicles (31–33).

Once a cooling compound has been released from the vehicle into the stratum corneum, then its ability to diffuse to receptor sites is of prime importance. It is recognised that the size of molecule is a parameter of skin diffusion, and it is noteworthy that of the compounds of our series, those that give effective topical cooling all have molecular weights lower than 250.

A degree of water solubility as well as lipid solubility appears essential for skin diffusion, and it is known that for most drugs the limiting parameter is water, rather than lipid, solubility (23). This is also apparent with cooling compounds, where in general compounds with lower log P values are more likely to be effective on the skin. A simple correlation of log P value with cooling effect is not expected, because the log P value influences intrinsic activity, but in those (short) homologous series which we have been able to study, the log P value of the compound with the highest topical score is displaced by approximately one unit, in the direction of more hydrophilic, when compared to the log P value of the compound of highest intrinsic effect.

Oral threshold values are of limited use for prediction of topical effects. *Figure 4* shows a plot of oral threshold against mean activity scores for 64 compounds in shaving foam (0·15% concentration). There is clearly no well-defined correlation, and the only

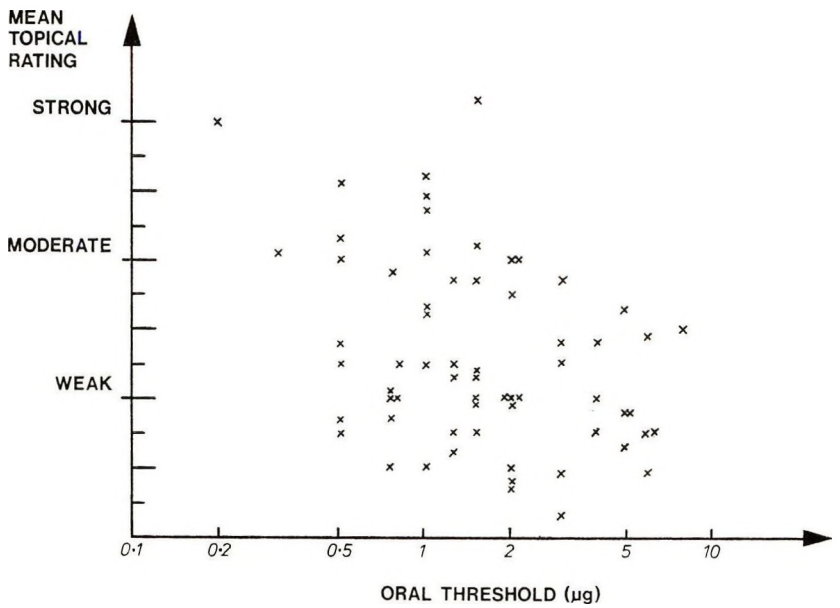

Figure 4. Plot of oral threshold of 64 compounds against cooling activity rating for 0·15% concentration in shaving foam on face.

deduction to be made is that a reasonable oral activity (threshold <2 μg) permits, but does not guarantee, a high topical activity. Poor penetration will considerably reduce the topical effectiveness of a compound of high intrinsic cooling activity, but good penetration characteristics will not give a strong topical cooling compound if the compound is not of high intrinsic activity.

As described previously, the four molecular requirements for intrinsic activity relate to hydrogen bonding, compact hydrocarbon skeleton, hydrophilic/hydrophobic balance, and molecular weight. As evolves in this section, for topical use of the compounds the last two criteria named are modified by requirements of skin penetration. The limit of molecular weight is reduced from 350 to 250, and the optimum range of log P value is shifted from $3{\cdot}0 \pm 1{\cdot}0$ to $2{\cdot}0 \pm 1{\cdot}0$.

COOLING COMPOUNDS IN TOPICAL COMPOSITIONS

Many compositions containing the synthetic cooling compounds have been prepared, and only an outline is possible in this paper, together with notes on instances where their use is contra-indicated. As might be expected the compounds are very effective in products for use in the mouth (e.g. toothpastes, mouthwash etc.) and, apart from occasional problems with absorption on solids, matching the cooling effect to the flavour profile is more important that the effects due to the formulation.

For topical products, the influence of the vehicle is important. Various compounds have been examined in compositions for topical use; derivatives of *p*-menthane-3-carboxylic acid, acyclic carboxamides and phosphine oxides have received the most attention. Comments on the various types of formulation are presented in *Table III*.

Table III. Coolants in compositions

Nature of composition	Approximate proportion of coolant, % w/w	Comments
Alcohol solution	(0·3–0·5)	Gives effective cooling. Compounds residing on the face can be transferred via finger to lips, giving cooling effect hours later. This is viewed as undesirable, therefore slightly volatile compounds are preferred. Note that the effective vehicle of an aftershave is often the mixture of non-volatile components, i.e. the fragrance oil and emollients. For this reason it is better to use polar emollients such as propylene glycol than non-polar emollients such as isopropyl myristate.
Shaving foam	(0·1–0·2)	Very effective, with cooling noticed 10–30 sec after application. Little residue left on face, hence no transfer problems. Cooling remains 5–15 min after removal.
O/W emulsion creams, lotions, balms.	(0·2–0·5)	Effective. Perception may be slow, e.g. 60 sec after application. With suitable compounds, duration is long.
Solid cologne	(0·3–0·7)	Very effective. Much recurrence of cooling experienced on, e.g. the forehead.

Contra-indications normally relate to the different sensitivities of different body areas:

Aerosol sprays: if aerosols are inhaled, the cooling effect is likely to be noticed in the throat and lungs (although in air fresheners a very small proportion of compound gives a pleasant fresh effect).

Shampoos: accidental ingress of the resulting foam to the eyes gives an intense level of cooling.

Talcs: the concentration of compound which is needed to provide effective cooling on most skin areas gives intense effects in the ano-genital area.

Toilet Soaps: as talcs.

Deodorants, antiperspirants: very effective, but the subject associates coolness in the axillae with the wetness of perspiration.

CONCLUSION

In this paper only a general description of the work has been possible. In the future, we hope to publish in greater detail aspects of structure/activity correlations, and certain aspects of topical effects. We recognise that the wide range of compounds which is now available offers possible new tools for study of skin penetration and neurophysiological effects; we are unlikely to extend our brief inspection of these areas, and invite specialists in these fields to take the study further.

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Effect of a skin cream containing the sodium salt of pyrollidone carboxylic acid on dry and flaky skin

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Synopsis

Humectants added to **skin creams** can increase the **moisture retention** of isolated **stratum corneum** and reduce the incidence of dry and flaky **skin** *in vivo*. This paper gives results of an investigation into the efficacy of a humectant, the sodium salt of **pyrollidone carboxylic acid** (Na PCA), which occurs naturally in the corneum. A product containing 5% of Na PCA increased the water-holding capacity of isolated animal corneum. In a consumer trial, with assessment of **skin dryness** and flakiness by trained assessors, the Na PCA product was more effective than a control product containing no humectant and equally effective as a similar established product with a different humectant system.

INTRODUCTION

The integrity of the surface layer of skin, the stratum corneum, depends upon its ability to adapt without cracking to the forces applied during normal body movement. A lack of adequate flexibility and extensibility will result in corneum surface cracking and flaking, a condition commonly seen in dry and cold conditions on exposed skin.

One factor of importance in maintaining corneum extensibility is its water content (1, 2). Many skin creams are, therefore, formulated with the objective of increasing the water content of the corneum.

It is now well established (2–5) that an adequate corneum water content depends upon the presence within the corneum of hygroscopic water-soluble materials which can hold water in drying atmospheres. The water held by these hygroscopic substances is responsible for much of the extensibility of the corneum (2).

The hygroscopic water-soluble material within the corneum contains a mixture of substances (3). Amongst the more important hygroscopic components of the mixture are the sodium salt of 5-pyrollidone-2-carboxylic acid (Na PCA) (6) and lactate (3). Sodium lactate and lactic acid in a skin cream have been shown to be effective in increasing the water-holding capacity of isolated animal corneum and in reducing the incidence of dry and flaky skin as judged by trained assessors in consumer trials (7). This report gives an account of a similar investigation into the efficacy of Na PCA in a skin cream.

EXPERIMENTAL

MEASUREMENT OF WATER CONTENT OF ISOLATED CORNEUM IN HUMID ATMOSPHERES

Corneum was obtained from the rear footpads of guinea pigs. It was separated by incubating the whole footpad in 0·1 mol tris buffer, pH 7·2, containing 2 mol urea and

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0·5% pancreatic trypsin for 18 h at 37°C (2). After this, the softened underlying tissues could be scraped away and the resulting corneum was washed by immersion in distilled water for 2 h. It was solvent-damaged by immersion in diethyl ether at ambient temperature for 18 h, followed by immersion in distilled water for 6 h. This procedure removes lipids and hygroscopic substances and reduces the water-holding capacity in humid atmospheres (2). The solvent-damaged corneum was used in experiments designed to increase the water-holding capacity towards the level for intact corneum.

The technique for measuring water-holding capacity has been described previously (2). Briefly, it consisted of equilibrating pieces of corneum at 81% r.h. and ambient temperature to constant weight. The pieces of corneum were then transferred to a dry atmosphere over self-indicating silica gel and re-equilibrated before weighing. Both equilibria took about 6 days to attain. The water-holding capacity was expressed as mg water held 100 mg^{-1} dry weight of corneum at 81% r.h.

The effect on water-holding capacity of a skin cream containing 5% Na PCA (ACO Fukträm, ACO Läkemedel AB, Solna, Sweden) was compared with that of a cream with the same formulation except that it contained no Na PCA. The corneum was treated by rubbing the cream for 90 seconds into both sides of a piece of corneum with the fingertips while wearing rubber gloves. Excess cream was removed by wiping with tissues and the treated corneum equilibrated at 81% r.h. before weighing.

No attempt was made in these experiments to quantitate the amount of cream deposited on the corneum, as preliminary gravimetric experiments showed this to be a highly variable quantity owing to the loss of loose corneum flakes while applying the cream. It was not considered that more elaborate experimentation to determine the quantity deposited would be justified as the *in vitro* technique was regarded as a simple screening test only.

Results were obtained on the corneum from 15 guinea pigs. One piece from each animal was treated with the test cream containing Na PCA and the other with the control cream containing no Na PCA. The mean values for water-holding capacity for each cream were compared statistically by Student's 't' test for paired observations.

EVALUATION OF SKIN DRYNESS AND FLAKINESS IN CONSUMER TESTS OF HAND CREAMS

The effects of three hand creams were investigated in a 6 week home-use trial in which groups of panelists used each cream under test for consecutive 2-week periods in all possible orders of use. Trained assessors evaluated the degree of hand skin dryness and flaking after the use of each cream.

The three hand creams were (a) the test cream containing 5% of Na PCA, (b) the control cream with the same formulation but containing no Na PCA and (c) an established marketed cream with an alternative humectant system containing urea.

One hundred and fifty women took part in the trial. Each woman used each of the three creams for a period of 2 weeks. There were approximately equal numbers of women using the creams in each of the six possible sequences. Only women with some degree of hand skin dryness and flaking were selected to take part. In order to obtain a reasonable degree of dryness and flakiness, the trial was carried out in southern Scotland during the late winter and early spring of 1976.

The method of assessing hand skin dryness and flaking has been reported previously

(7, 8). It consisted of a trained technician using a simple numerical scoring system to assess six areas of each hand according to the following scheme:

0=no relevant visible damage,
1=slight dryness,
2=marked dryness and/or slight flaking,
3=severe dryness and/or marked flaking,
4=severe flaking and/or slight cracking,
6=severe cracking.

The areas of the hand assessed were:

back of hand,
thumb web,
other webs,
back of fingers,
palm,
front of fingers.

The 12 areas on each panellist were summed to give a total hand score.

For convenience, the panel was divided into two halves and each half saw a different assessor. Each panellist saw the same assessor throughout the trial.

Panellists were allocated to one of the six possible sequences of hand cream usage according to their initial total hand score. The allocation was carried out so that there were equal numbers of women in each of the six sequences and so that the mean and range of hand scores in each sequence was approximately the same. The allocation was carried out separately for each assessors' half of the total panel.

After allocation to one of the six sequences, each panellist was given the appropriate cream and asked to use it at home after wet operations and before going to bed. They were asked to return for assessment after 2 weeks and collect the next cream. They were instructed not to use any cream on the assessment days. Residues of cream obscure dryness and flaking and absence of cream from the hands during assessment means that any effect of cream is on the skin itself and is reasonably long-lasting and does not reflect the ability of the fatty components of the creams to stick down any scales and obscure dryness by optical effects.

The assessors were not aware which panellist had been using which product. The panellists themselves received coded products and did not know which creams contained humectant.

After the end of the trial, results were examined for statistically significant differences between creams by analysis of variance. Because the trial was balanced for initial hand scores and because all possible sequences of cream usage were employed, it is reasonable to calculate mean changes from previous hand scores at each assessment and for each cream. These changes were calculated and used as the data for the analysis of variance. The results for each assessor were calculated separately and then combined to give an overall mean.

RESULTS

WATER-HOLDING CAPACITY OF ISOLATED CORNEUM

Table I shows the mean water-holding capacity at 81% r.h. of guinea pig corneum treated with the cream with and without Na PCA. The cream with Na PCA resulted in

the greater water-holding capacity. Statistical analysis by Student's 't' test gave a value for 't' of 4·14 with 14 degrees of freedom indicating that the difference between creams was highly significant ($P < 0{\cdot}001$).

Table I. Comparison of the effects of cream with and without 5% Na PCA on water-holding capacity at 81% r.h. of solvent-damaged guinea pig footpad corneum

Cream	Water held (mg 100 mg^{-1} dry corneum)
With Na PCA	19·4 (15)
Without Na PCA	17·2 (15)

Figures in brackets indicate number of replicates

EFFECT ON HAND SKIN DRYNESS AND FLAKINESS IN A CONSUMER TRIAL

One hundred and forty eight panellists completed the trial. *Table II* shows the mean total hand scores for all panellists in each of the six sequences and at each assessment. These figures show a considerable fluctuation of scores between sequences and assessments and the effects of creams are not easily distinguished.

Table II. Mean total hand scores in consumer test for each product sequence at each assessment

Cream sequence	No. of panellists	Assessment No. 1	2	3	4
abc	24	11·4	11·1	11·8	11·5
acb	24	12·7	14·1	10·6	15·3
bac	24	11·5	12·8	11·7	13·8
bca	23	11·2	13·4	12·2	13·5
cab	27	12·3	13·2	11·9	16·7
cba	26	12·5	13·8	14·9	15·6

a = cream with Na PCA.
b = cream as (a) but without Na PCA.
c = urea cream.

Statistical analysis was carried out on the mean changes in total hand score for each cream. *Table III* shows the mean changes for each cream during each of the three use periods. The overall means for each period and for each cream are also given together with the difference between creams required for statistical significance.

The statistical analysis showed that the cream containing 5% Na PCA resulted in significantly lower hand scores, i.e. in better hand skin condition, than the control formulation containing no Na PCA There was no significant difference between the cream containing Na PCA and the cream containing urea. The urea cream resulted in a lower hand score than the control cream.

Table III. Mean changes in total hand score in consumer trial for each cream during each period of use

Cream	Period no. 1	2	3	Cream mean
a	0·5	−1·2	1·0	0·11
b	1·7	0·9	4·8	2·47
c	1·1	−2·3	0·9	−0·07
Period mean	1·1	−0·9	2·3	

Difference between creams required for statistical significance ($P = 0{\cdot}05$) = 1·56

a = cream with Na PCA

b = cream as (a) but without Na PCA

c = urea cream.

DISCUSSION

The results of the study on isolated animal corneum showed that the addition of 5% Na PCA to a skin cream could result in a measurable increase in water-holding capacity of the corneum. In order to determine whether or not this increase in water content would be of any significance when the cream with Na PCA was used in the normal manner, the consumer trial was carried out.

The consumer trial showed that the increased corneum water content did result in an improved hand skin condition as judged by trained assessors This improved skin condition caused by the Na PCA cream was equal to that resulting from the use of a cream containing urea.

The results of these experiments, therefore, demonstrate that the presence of Na PCA in skin creams can reduce the incidence of dry and flaky skin under normal use conditions.

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An appraisal of the current state of mutagenicity testing

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Synopsis

Every year many new chemical substances are introduced into our environment. Some of these chemicals may possibly induce **genetic damage** in populations exposed to them. There is a risk that such damage may accumulate in the gene pool and affect future generations, or even cause **cancer** in the present generation since a link has now been established between **mutagenicity** and **carcinogenicity**.

At present the most appropriate way to determine which chemicals may cause genetic damage is to test them in various *in vitro* or *in vivo* laboratory test systems capable of detecting damage to DNA, **chromosomes** or the genome. Some of these methods are discussed, as are the factors which determine a mutagenic response, problems encountered when using the test systems, together with data interpretation and assessment of genetic effects.

INTRODUCTION

The problem of the possible induction of genetic damage after chemical exposure is worrying both to the scientific community and to the population at large The increase in number of different chemical substances and biological synthesis products in man's environment is due to the rapid strides made by scientific and technical progress. Each year new chemical substances are introduced in the form of medical preparations, pesticides, food additives and industrial compounds and some of these could induce mutations. There is a risk that such mutations may accumulate in the population and affect future generations. In recent years many substances shown to be mutagens have also been shown to be carcinogenic, with 80–90% correlations between mutagenicity and carcinogenicity with certain organic chemicals (1–8). These data are in conflict with similar studies made in the early 1950s on the mutagenicity of chemical carcinogens which showed little correlation between carcinogenic and mutagenic activity. These early experiments led to the premature demise of the somatic mutation theory of cancer; metabolic activation systems were not used and it was not realised that certain strains of bacteria might detect only specific types of genetic alterations (9). Nowadays, with the new-found interest in the somatic mutation theory of cancer, a study of the genetic effect of chemical substances may be useful not only for protecting future generations but also for preventing cancer in present generations.

Little is known of the practical use of results obtained from mutagenic studies, how these results might apply in the field of toxicology in general, or how relevant they are to man. Whilst cancer in man has a definitive end-point in the production of a malignant

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tumour and is a very emotive issue, the end-point of genetic damage in man is more obscure. With current techniques we are able to distinguish between molecular changes in the gene and small chromosome aberrations. Most human traits are inherited as Mendelian units which can be divided into autosomal dominant, autosomal recessive and X-linked recessive units. There are also X-linked dominants and there may be a few Y-linked traits but these are numerically insignificant (10). McKusick (11) lists such traits. The dominant expression of a mutant gene is sufficient to cause a recognisable abnormality or disease and there are also some well-established recessive traits. However, whilst a change in a dominant trait is immediately evident in the next generation, recessive traits may take very many generations before they are expressed. Therefore, the effect of chemical mutagenesis at this level of the gene may not show immediate results and the data generated by mutagenic tests lose their impact.

Chromosome abnormalities may arise by errors in the distribution of chromosomes leading to abnormalities of chromosome numbers such as non-disjunction, where the effect is seen in the next generation, or by the consequence of chromosome breakage. Consequences of chromosome damage are physical or mental abnormalities, sterility or embryonic death. With regard to the last condition, epidemiological evidence suggests that there is an increased incidence in foetal wastage in wives of workers exposed to vinyl chloride (12, 13, 14). There is however, a conflict concerning the statistical interpretation of the data (15), and as yet there is thus no unequivocal epidemiological evidence that vinyl chloride can cause germ cell mutations in man. Married women anaesthetists were claimed to have a higher incidence of spontaneous abortion when they worked than when they did not work and the incidence was also higher by comparison with non-anaesthetist married women doctors (16); but, it is difficult to determine if the abortions were due to genetic events or embryotoxicity because of systemic involvement after exposure of the women.

Many constitutional and degenerative diseases such as epilepsy and schizophrenia may be caused by other irregularities of the gene expression or arise from multiple genes (10). The effect of chemical mutagenesis both on multiple gene effects as well as at the level of the cromosome may also not be immediately obvious.

Nevertheless, since the world population is rapidly increasing the chance for natural selection in civilised communities is being sharply reduced, and whilst it is recognised that not all mutagens are harmful and some are necessary for evolution, any increase in the mutagenic factors in the environment could cause a potential risk to the population. It is estimated that more than 4% of the population are affected by genetic anomalies and about 1% of children born each year have chromosomal mutations (17). Mutations that cause sterility or early embryonic loss are detrimental in the Darwinian sense but have little impact on society. Mutations that are more fit biologically may be a heavy burden to society if the affected persons require medical or institutional care (18). Thus it is necessary to monitor potential chemical mutagens which could increase the percentage of genetic aberrations in the population, and although a great number of relatively simple and practical methods are available the evaluation of mutagenic effects is a complex and extremely difficult task.

FACTORS DETERMINING A MUTAGENIC RESPONSE

The mutagenic activity of chemical substances has been studied in a number of organisms, using different methods for calculating both gene and chromosome mutations in somatic

and germ cells. Systems from micro-organisms, plants, insects, mammalian and human cells *in vivo* and *in vitro* have been used, and whilst the mutagenic effect of a chemical may be detectable in one system it may not be in another or even in different organs of the same system. There may be many interactions between a chemical and an organism, all of which may determine whether genetic damage is expressed – unlike ionising radiation, which is immediately active in terms of its mutagenic potential. With radiation extremely short-lived free radicals are generated randomly in cells and tissues very close to or within the genetic target molecules. There are many factors that determine whether a chemical compound reaches and reacts with the critical genetic targets. Among these factors in mammals are chemical structure of the compound, duration of treatment, route of administration, absorption, distribution, excretion, drug-protein binding, metabolic transformation, pharmacogenetic make-up (species, strain, sex) membrane barriers, numbers of SH groups, etc (19). The following examples will illustrate such factors: Vinyl chloride, a known carcinogen (20), requires metabolic activation to exert its effect (21). Vinyl chloride exposure causes mutations in micro-organisms (22) and in some test systems involving higher organisms. It causes chromosome damage in human peripheral lymphocytes (23), but no effect is seen in the dominant lethal assay in mice (24). This suggests that various tissues metabolise the substance to differing extents and maybe the testes metabolise the substance least. This is certainly the case with dimethylnitrosamine, another indirect mutagen, where there is less alkylation in the testes than in other organs (25). This compound also gives a negative result in the dominant lethal assay (26), and in a host-mediated assay with yeast cells, mutagenic activity is high in the liver, moderate in the lungs, but only very weak in the testes (27, 28). Other compounds such as methylnitrosoguanidine, a direct mutagen, are metabolised rapidly in mammals to non-mutagenic activity and 4-nitroquinoline-1-oxide has high mutagenic activity in yeast cells recovered from the lungs and slight activity in the liver but is not active in the testes (28). Another possibility is that such compounds may be detoxified by organs where little mutagenic activity is detected.

Other factors determine whether the damage in the target molecules is expressed as genetic damage. Such factors are the innate susceptibility of the cell (e.g. repair capacity), numbers of susceptible cells, type of genetic target of the genetic target locus, germinal and somatic selection processes involved, mode of inheritance etc (19). It has been found with ethyl methanesulphonate (EMS) for example, a compound which can alkylate all tissues directly (29), that at doses below 100 mg/kg a dominant lethal effect is not observed (30) but at higher doses an effect is clearly manifest in CD-1 mice at 150 mg/kg body weight (31), confirmed by Anderson *et al.* (32, 24, 33). Sperm proteins and sperm DNA are alkylated by EMS at doses as low as 3 mg/kg body weight (34). Such discrepancies may occur because the numbers of animals screened in a dominant lethal test may be statistically too small to detect a weak effect at low doses, or unknown factors may affect the expression of damage in germ-cell development preventing the production of mutations (19).

The factors described above as influencing a mutagenic response complicate mutagenicity testing. Many compounds that would require testing will not behave in the same way as model mutagens, which bind covalently with biologically important molecules. Many will react reversibly with receptors by mechanisms such as hydrogen bonding and electrostatic binding. Such compounds may not react with the genetic material unless they become metabolised to highly reactive forms. Other factors such as temperature, ageing, pH, hypoxia and viral infections can also produce genetic damage (19). Chemicals

may, therefore, disturb normal bodily functions producing a change in one of these parameters to cause a genetic effect indirectly.

TEST METHODS AND RECOMMENDATIONS FOR THEIR USE

Geneticists have a large number of experimental methods at their disposal to assess the mutagenic action of chemicals. No single method, however, gives any conclusive information about the genetic risk to a person who has been exposed to a mutagenic substance. Thus it would seem necessary for a number of test systems to be used. Bochkov *et al.* (17) have listed the effects, and given a useful précis of the results of different types of chemicals such as cytostatics, antibiotics, pharmaceutical preparations, food additives and admixtures, chemosterilants. fungicides, insecticides, herbicides and industrial chemicals in the different test systems of micro-organisms, plants, fruit fly (*Drosophila*), mammalian cell cultures, bone marrow and dominant lethal systems of mammals and human cells in cultures and human lymphocytes.

Various recommendations for combinations of test systems have been put forward (35–46, 17). All approaches have their advantages and disadvantages. In the more recent reports Bridges (43) proposed that assessment at each stage of testing should take into account the population exposed to the substance, its economic value and the possibility of substituting a non-mutagenic grouping for the mutagenic grouping in the structure of a substance. However, non-mutagenic substitutions are not always possible without altering the desired biological activity of a product. The testing scheme proposed by Flamm (45) includes tests for the detection of heritable translocations and specific locus mutations. Both of these tests require large numbers of animals, are not always economically feasible, and in any case do not necessarily generate sufficient information to assess the genetic risk to man.

Various governmental agencies, such as those of America, Japan, Britain and other EEC countries, are now preparing guidelines for testing methods. Some of the more recent ideas indicate the need for flexibility. This should permit changing to new and improved methods as soon as their usefulness has been substantiated. However, there is anxiety that any significant deviation from generally accepted guidelines may be questioned by regulatory agencies and that the guidelines that will satisfy the most demanding health authority may become the generally accepted procedure.

With all the recommendations in the communications already in the literature (listed above), those put forward in this present communication will be *brief*, and it must be remembered that testing organisations, if allowed flexibility, will have their own ideas regarding scientific protocols, etc.

The problem with the present testing methods is that not all systems are equally reliable and reproducible. Even the systems in which most compounds have been tested, such as the *Salmonella typhimurium* plate incorporation mutagenicity assay with metabolic activation (5), give different results for some compounds in different laboratories (47). Ideally, a system for assessing chemicals for mutagenic activity should:

(a) detect the potential of the test substance to induce gene and chromosome mutations in somatic and germ cells,
(b) provide quantitative data for extrapolation to man,
(c) be capable of detecting metabolic products of the compound which have mutagenic potential,

(d) be reliable and reproducible,
(e) be economically viable and quick.

No one test system at present meets all these demands.

Below the test systems that are available are considered (References are given for the test systems only where systems are not generally discussed later.)
Gene mutations may currently be detected by:

(a) micro-organisms with metabolic activation *in vitro* and *in vivo*,
(b) *Drosophila melanogaster*,
(c) cultures of somatic mammalian and human cells,
(d) specific locus tests in mammals (48, 49).

Cytogenetic effects in somatic cells can be detected by:

(a) the micronucleus test,
(b) conventional metaphase analysis or sister chromatid exchange analysis (50) of bone marrow cells from mammals and mammalian and human lymphocyte cultures.

For detecting chromosomal damage in spermatogenesis and oögenesis, the dominant lethal or heritable translocation test (30, 51, 52) can be used. *Drosophila* can also be used for detecting chromosomal damage, and since *Drosophila* can also detect gene mutation it is a useful 'catch-all' system (53, 54) but extrapolition to man is difficult from such an organism.

In addition to methods that detect gene mutations and chromosome aberrations, the potential of a chemical to induce primary DNA damage can be detected by measuring stimulation or inhibition of repair (55, 56, 57), or recombinational or gene conversion events (58, 59).

It is not feasible for all chemicals to be tested by all methods, so a system of priorities should be arranged. Not all substances are used by man in the same way: there are some substances to which man is chronically exposed and others to which man is only subjected by acute exposure under exceptional circumstances. Thus the genetic risk of a substance will depend on its mutagenic potency, where it is known, the extent to which people come into contact with it, and on the individual susceptibility of a person. We have to accept the fact there is variation in individual susceptibility but we can differentiate our test systems according to levels of exposure of the population to a chemical. Exposure will depend on a combination of two parameters: the number of people exposed and the dosage to which the people are exposed.

If the product of the parameters is low then the chemical is a low exposure chemical: if the product is high then the chemical is a high exposure chemical.

Low exposure chemicals, such as very low tonnage industrial chemicals including some intermediates, non-ingested substances and substances known not to accumulate in the environment or body, may be subjected to a simpler screening programme, which should, however, cover the induction of both gene mutations and chromosome aberrations, e.g. a test on micro-organisms with metabolic activation *in vitro* and a cytogenetic examination of bone marrow cells, or an *in vitro* cytogenetic examination with metabolic activation.

High exposure chemicals, such as high tonnage industrial chemicals, pesticides, widely-used medicine, food additives, ingested products and substances known to accumulate, should be subjected to more rigorous testing with at least the two tests

mentioned above and a relevant whole mammal test in germ cells. Other tests may be added if the chemical warrants it, such as cytogenetic analysis of human lymphocytes of exposed workers, or any others that may be considered relevant.

Substances found positive in the first screening, depending on their economic or medical importance, could be subjected to more rigorous testing. According to Matter (19), however, very few compounds (0·1%) originally produced in pharmaceutical research ever have a chance of being marketed. He suggests that extensive early mutagenicity testing of drugs is of little help since benefit/risk decisions cannot be made at that stage, and that such testing should be concentrated on those compounds scheduled for clinical trials or commercial introduction. However, it would seem better to avoid mutagenic products at an early stage where possible.

Before a substance is tested, consideration should be given to its chemical structure, to determine if it is related to compounds which already have known mutagenic, carcinogenic, teratogenic or general toxic effects. Such a consideration might give an indication of its mutagenic potential and this may be useful in setting testing priorities. If it is suspected of having high mutagenic potency, then this factor may override the other two parameters which determine exposure.

COMMENTS ON SOME OF THE METHODS

A great deal has already been published about recommended protocols, e.g. (17, 60). However, some general rules apply for all testing systems. All assays should be run with concurrent negative and positive controls. Positive controls should, where possible, be structurally or mechanistically related to the compound under test.

MICROBIAL METHODS

Much work in different laboratories has been carried out on the bacterial *Salmonella typhimurium* mutagenicity system with rat liver microsomal activation, and as such it is well validated both for the detection of some mammalian and human carcinogens and bacterial mutagens. However, it is not well validated for human germ cell mutagens since none are unequivocally known. This does not mean that other microbial systems would not be equally valid for detecting mutagens/carcinogens if as much work had been undertaken with them. The *Salmonella typhimurium* strains of Ames (3) are able to detect base-pair substitution and frameshift gene mutations. A drawback to the system is that it is not quantatitive and the potency of mutagens cannot be ranked. However, the system is useful for a qualitative answer. The system of *Salmonella typhimurium* and other systems such as that of the bacterium *Escherichia coli* (61) and yeasts (62, 58, 59) can provide a quantitative answer if used in liquid cultures in combination with viability studies. Yeasts, however, are used to measure not only gene mutation but more commonly gene conversion and mitotic recombinational events. A very sensitive test for measuring gene mutation is claimed when *E. coli* is used in a fluctuation assay (63).

Generally, compounds in a screening programme should be tested both with and without microsomal activation. Strains to detect both base-pair and frameshift mutations should be used, as well as at least five concentrations of the test compound over a wide dose range in order to maximise the chance of obtaining a response, with the highest dose if possible inhibiting the growth of the microbes. This ensures that the compound has entered the microbes. Replicate experiments should be undertaken to determine a

reproducible response. Simpler 'spot' tests measuring the degree of differential killing in repair-deficient microbial strains by comparison with wild type strains (64, 65) provide an indirect answer to the problem of mutation testing, since they detect damage to DNA but do not measure gene mutation directly. Such tests at the present time are not considered particularly sensitive, however.

MAMMALIAN CELL SYSTEMS

Cell 'transformation'/mutation assay

In this laboratory, concurrent with the Ames' test the 'cell transformation' assay system of Styles is used (8, 66). It is based on the ability of mammalian cells transformed by a carcinogen to form colonies in soft agar and this is only one of the accepted criteria for cell transformation. A recent paper (67) suggests that the ratio between transformation and mutagenesis for ouabain resistance in normal diploid cells is about 20 : 1 (after treatment with benzo(a)pyrene and its 7,8-dihydrodiol), which suggests that any one of 20 genes may be involved in transformation as opposed to 1 for mutation. This ratio is also apparently substantiated for normal hamster embryo cells treated with benzo(a)-pyrene. If transformation is, therefore, a genetically based system, it may be useful for preliminary genetic monitoring. We have found it to be equally predictive for carcinogenicity as the Ames' test, and by inference, therefore, it is equally predictive for mutas genicity. Baby hamster kidney (BHK 21/C12) and either human diploid lung fibroblast-(WI 38) or human liver cells (Chang) were treated as described (47). Five doses of compound were used with the S-9 mix of the Ames' test. Survival was assessed independently and a transformation frequency calculated. The test is not as rapid as the Ames' test, but in our 'blind' study for 120 compounds with these two tests in combination the tests only missed detecting as carcinogens/mutagens diethylstilboestrol and vinyl chloride. This latter compound was later detected when tested in the gaseous phase. This therefore seems a very promising system for a preliminary mutation screen.

The limitations of using these two test systems for a simple screening programme have been discussed elsewhere (8).

Lymphoma Cells in Culture

Whilst human cells or normal diploid cells are obviously desirable for mutation assays in culture, they are more difficult to handle from a screening viewpoint than malignant cells in culture due to their low plating efficiencies and lack of perpetual proliferation. Lymphoma cells, which grow in suspension, are even easier to handle than cells which grow as a monolayer. They do not require trypsinisation, are very easily subcultured and they are not subject to metabolic co-operation which can cause a loss of mutants. Also they can be used in a host-mediated type assay (68) or in a fluctuation test (69).

Lymphoma cells in culture can readily detect direct-acting mutagens and carcinogens (70–78) and may also be used in combination with S-9 mix to detect indirect acting mutagens and carcinogens (Anderson, unpublished). The lymphoma cell mutation system is manageable in that induced mutation frequencies are readily detectable and vast numbers of plates do not have to be used to detect a spontaneous frequency (e.g. 10^5 cells per petri dish in soft (0·3%) agar and selective medium gives a background frequency of about 2–20 colonies in P388F cells with selective media containing 5-iodo-2-deoxyuridine and excess thymidine respectively). Induced frequencies can increase from 10- to 100-fold above these

levels. Absolute increases in induced colony numbers are observed over some of the dose range above control values – which is not always the case for human cell systems, where mutants may be very sensitive to the inducing agent and are being killed more rapidly than non-mutant cells (79, 70).

Bone marrow in Mammals

This is a useful system in that no auxiliary metabolising system is required, animals can be dosed with compounds directly and bone marrow cells do not need stimulation to divide since they have a high proliferative cell activity (80–83). Rats are suitable species to use but others will do. With rodents, groups of 5–8 animals, 8–10 weeks of age, should be used per concentration of the test substance and positive control substance. A larger number of animals should be used in the negative control group to provide a better data base for comparison. A minimum of 50 cells, preferably 100, from each animal should be analysed from coded slides to avoid observer bias when scoring chromosome damage. The higher the background frequency, the higher the number of cells needed to be examined in order to detect a statistically significant effect (Buffler, personal communication).

A maximum tolerated dose of compound should be used, together with a more realistic dose related to human exposure and an intermediate value. In a preliminary screen only the maximum tolerated dose need be used – or even a micronucleus test may be used (84, 85).

Animals can be sacrificed at various time intervals after exposure. From our own studies and those of others (86, 81, 60) 6 h seems to be suitable time after multiple chemical exposures and 24 h after single exposures. However, these times may not be suitable for all chemicals.

The most relevant route of administration of the compound should be used. If man is exposed to the chemical by inhalation, then an inhalation route should be used. If he is likely to ingest a pesticide sprayed on crops, then an oral route is recommended either by gavage or in the diet. Rarely does man receive intraperitoneal or intravenous injections of compound except in the case of some medicines.

Positive control animals should be housed under identical conditions to those dosed with the test compound of animals even if identical routes of administration of test compound and positive control substances cannot be achieved.

Cells should be scored for all type of chromosome aberrations including chromosome gaps. We have shown with several positive control mutagens such as ethyl methanesulphonate, mitomycin C, benzene and vinyl chloride that gaps are at least as sensitive an indicator of damage as other types of damage: they may indicate a toxic event as opposed to or in addition to a genetic event (Anderson and Richardson unpublished).

Dominant Lethal Mutation Assay

This is one of the whole mammal tests on germ cells (87). The tests should be carried out on random-bred rodents of 8–10 weeks of age. The comments which apply to dosing regimes in bone marrow cells also apply to dominant lethal tests, i.e. a maximum tolerated dose should be used together with a dose related to human exposure levels and an intermediate value. Again, the most relevant route of administration should be used, and animals treated with the positive control substance sould be housed under identical conditions to those of animals exposed to the test compound.

Each test group should consist of at least 10–20 males if males are exposed. (Dominant lethal effects in females are difficult to separate from systemic effects.) A pre-treatment fertility test should be undertaken to check fertility of the animals used and to determine a background dominant lethal frequency. Each male is bred with 2–4 females once a week, for 8 weeks in the case of mice or 10 weeks in rats, to sample all stages of the spermatogenic cycle. Negative control animal numbers should be larger, again to provide a better data base for comparison. (The size of the negative control group can be determined by the square root of the number of treatment groups, i.e. if there are 9 treatment groups each of 10 animals, then there should be 30 in the control group (88).)

The statistics used in the dominant lethal test are complicated; various statistical methods can be used, each with its own bias. The greater the number of statistical methods used, the greater the chance of producing a false positive result (Anderson, unpublished).

Different evaluation methods are used for dead implant data. The US Food and Drug Administration has analysed all data on dead implants in two ways (both on dead implants/total implants/female basis and on dead implants/female) and infer that equally significant data are provided by both (89). If late deaths are eliminated from the analysis of the above parameters a more sensitive index of dominant lethality is obtained.

Preimplantation losses can be indicated by comparing values of total implants in females mated with treated males and those mated with control males, as suggested by Epstein (90) instead of counting corpora lutea. Decreases in total implants which represent increases in preimplantation losses without a corresponding increase in early deaths may not represent a mutagenic event. Other than genetic factors can explain a preimplantation egg loss. Fertility effects can also be determined in the dominant lethal test if fertile animals only are used for the study. The dominant lethal test is best conducted with at least three concentrations of test compound in order to try to obtain a dose–response relationship from which extrapolation of risk might be made. Clear-cut genetic effects are best claimed when a dose dependent increase in post-implantational foetal deaths is evident.

Other methods such as the heritable translocation test detect transmitted damage and are useful is this respect. However, such a test is extremely expensive, as is the specific locus gene mutation test, requiring very large numbers of animals, extensive housing and maintenance.

A negative result in any one of the systems so far discussed may mean that the wrong species of animals or microsomes has been used, or a result may be negative for any one of the reasons discussed earlier, or it may be a true negative.

Human Peripheral Lymphocytes from Exposed Workers

This sort of study is usually carried out retrospectively after a compound has been identified as a hazard, witness the many publications on workers exposed to vinyl chloride (91). Before such a study can be undertaken. many ethical problems have to be taken into consideration, e.g. if the exposed work-force need to be told the results of the study and what the results mean in the light of current knowledge. Such a study and decisions relating to it involve negotiations with medical officers, workers, unions, etc. Negotiation may be more difficult on a prospective basis where compounds present an unknown hazard. If a positive response is obtained, while it is possible to show a correlation between exposure and level of abnormal cells on a groups basis, the range of

individual values within groups of exposed people is wide and overlaps the ranges of adjacent or control groups. In retrospective studies where exposure has occurred for up to 20 or 30 years, it is often difficult to know exact exposure levels and exposure can often be only estimated roughly from occupation.

We are investigating whether such a study is useful in trying to determine safe exposure levels. If the exposed population is not significantly different in terms of chromosome damage from the control population, then it might be assumed that safe exposure levels have been achieved, because a negative result suggests that the chemical concerned is not a mutagen. However, there are limitations to this approach, since the exposure level may be too low to produce the chromosome damaging effect but may still cause sister chromatid exchanges or gene mutations which are not detected by conventional chromosomal analysis.

Controls should be taken from both on and off site where possible, and should be age and sex matched. Cells are generally cultured for 48 and 72 h and we have found no significant differences in data at these two times after vinyl chloride exposure (91), although after irradiation this is not the case (92) and 48 h cultures are desirable. At least 100 cells per individual should be analysed from slides coded to avoid observer bias.

Whilst there is a correlation bteween carcinogenesis and mutagenesis (earlier references), a review paper by Harnden (93) puts the correlation for clastogenic and carcinogenic effects into perspective, as does the book edited by German (94). There appears to be a fairly good but non-quantitative correlation.

Other techniques such as sister chromatid exchange may be useful on exposed workers, but effects are much more short-lived (95, 96). and may to some extent have disappeared before culturing is possible.

Once it is established that a chemical is clastogenic, regular population monitoring of the work force may be initiated, in which the workers are monitored both pre-exposure and during employment The results of the monitoring will be useful for checking plant hygiene, and an increase in absormal cells in an individual could be used as an indication that the worker should not continue to be exposed to that chemical. If an individual is found to have a chromosomal abnormality linked to a certain disease he can be advised through appropriate medical channels of the risk of inheritance of the disease in any children he may have.

If all workers in chemical plants were monitored as part of a routine medical surveillance service, then many of the difficulties involved in initiating prospective studies would disappear.

Computerised microscopic techniques are available which can reduce the time spent by a technician by about 40%. At present, however, metaphase spreads are merely located and the amount of damage still has to be assessed visually. Initial cost of purchasing a computer microscope is very high.

Other techniques for direct application to man are available, such as the use of urine or blood plasma from exposed workers in combination with a microbial assay (97), electrophoretic monitoring of enzymatic markers in man (98), detection of variants in haemoglobulin molecules (99–100), investigations of sperm morphology (101) and an increase in the presence of YY bodies (102).

INTERPRETATION OF DATA

Assuming that experiments are reproducible, in well-conducted experiments using

adequate testing protocols, interpretation of data may still be difficult especially with results within the control range or on the border-line of statistical significance. Interpretations may differ depending on whether results from treatment groups are compared with historical (accumulated) or concurrent controls, the type of statistical analysis or whether results are compared at equitoxic doses. If the experiments are repeated, similar equivocal results may be obtained and if not there is the problem of whether the first or repeat experiment is correct. The difficulty is in proving and accepting negative data. By comparison the handling of positive data is much more clear-cut.

Systems giving negative results are often considered insensitive, e.g. the dominant lethal test. However, such a test might truly reflect the situation in the germ cells and the testes may really be incapable of metabolising many compounds to their active intermediates. Microbial systems need large concentrations of compounds to detect a positive result by comparison with concentrations expected to be present in whole mammals, and thus could equally be considered insensitive.

ASSESSMENT OF GENETIC EFFECT

Making a quantitative risk evaluation for man from data obtained in testing procedures is an even more difficult task. First it must be determined if the data are biologically and/or statistically significant. Chemicals with unknown mutagenic potential are probably more difficult to assess than data for radiation or known mutagens (many of which are 'radio-mimetic' agents) unless there are clear-cut dose responses, since chemicals behave differently with different cells, organs and organisms.

One of the recommended ways of assessing risk is by comparing the values obtained from chemical data with corresponding radiation data. Crow (10) recommended that the population genetic effects of clinical mutagens be assessed, taking radiation as an equivalent and equating the population dose of chemical mutagens to the radiation dose admissible for that population. Bridges (43, 44) in his papers describing the three-tier system also recommends the principle of a radiation-equivalent dose. With this system only those substances which have passed through the first two tiers and are of great social, medical and economic importance are subjected to a quantitative assessment. Chemicals that have shown a positive effect in the first two tiers should, if not widely used, be prohibited or used with a non-mutagenic derivative substituted for the mutagenic radical. Those that are positive in the third tier should be expressed as the equivalent of a radiation dose producing the same effect, and this makes it possible to standardise the level of chemical mutagens to the limits of the level of ionising radiation. However, with extrapolation back to very small doses it would be diffcult to decide which is the line of best fit, and with some chemicals there may be shoulders of varying size on dose response curves due to permeability problems with a chemical or other unknown factors.

Yet another 'radiation-equivalent concept', the ABCW model (101), is based on the hypothesis that the radiation induced mutation rate per radiation unit and gene locus in a variety of organisms from microbes to mammals is proportional to their genome size. This model has been extended to chemical compounds (104). As a result it would seem possible to estimate risks for man on the basis of an upward extrapolation. However, this model has been criticised by Sankaranarayanan (105).

The problem with these radiation concepts is that chemicals may behave differently from radiation induced free radicals and often have species type specificity. This problem has been highlighted by Auerbach (106) and Sobels (107).

Bochkov (17) recommend that the assessment should be based on individual and population prognoses. The individual prognosis should be determined by the quantity of chemical and its mutagenic activity. The population prognosis should be determined by the number of persons of reproductive age who are in contact with the chemical mutagen and the average quantity of this substance for each of them. To make such an assessment. various factors need to be considered:

(a) data on the test specimen for which the highest and clearest quantitative dependencies are obtained,

(b) the quantity of substance with which an individual comes into contact over a period of a year,

(c) the fraction of the population up to the age of 30 who are subject to the action of a mutagen,

(d) the mean population dose of substance which can be calculated from the above data,

(e) the limit for the admissible level of genetic changes.

They suggest prohibiting a substance with mutagenic activity, replacing it with a non-mutagenic compound, or restricting its use to persons of non-reproductive age in cases where its mean population dose causes an increase of 0·1% above the spontaneous level and a doubling of the spontaneous level on an individual basis.

Again, this approach depends on the reliability of the experimental data obtained and being able to determine information concerning the above-mentioned factors. Auerbach (106) and Sobels (107) also criticise the concept of the doubling dose.

Considering the difficulties of interpreting results obtained in a test system, any single test can hardly provide absolute answers. Negative results are often underweighted. Results from several practical test methods should be processed and decisions based on the biological and statistical significance of all the results observed, having regard for the normal range of control values in the test systems used. If a socially and economically useful compound is found to be 'hazardous' for man, then a detailed examination can be made of levels to which workers are exposed and attempts made to improve plant hygiene where the product is manufactured. Further investigations can be made to determine if and how much other groups or people or the general population are exposed, e.g. in the case of vinyl chloride, manufacturing plant exposure levels have been reduced and attention has been focused on whether any free monomer occurs in plastic food wrappings, etc. Auerbach (106) feels that in the benefit/risk calculations of a compound, on the benefit side the calculation should carry a correction factor for the special economic, social or medical situation of the country concerned, e.g. in a country where millions suffer from malaria, the benefits of an efficient preventive or curative drug should be weighted accordingly, or in countries where there is famine problem, pesticides should be considered similarly.

CONCLUSIONS

Both academic and industrial scientists are well aware of the need for safety evaluation in general toxicological testing and this is certainly true in the field of genetic toxicology. We still do not understand if positive or negative results in a laboratory model test system are really relevant to man because of man's unique metabolism and because of the absence of any convincing 'no-effect' level data for animals or man. Epidemiological evidence for

germ cell mutations after chemical exposure (or in fact any agent) is sadly lacking. In industrial areas it is often hard to pinpoint the exact chemical or agent which may be causing the problem. Unbiased abortion rates are difficult to obtain in interview by comparison with control or unexposed populations. Even if they are taken from hospital and medical practitioners' records not all abortions are recorded. Auerbach (106) does not believe that we shall even be able to identify potential human mutagens with complete confidence and even less shall we be able to feel confident about such quantitative features as thresholds, dose–effect curves and comparisons between mutagens in the human environment. She thinks this is unavoidable when we as a species are both the subject and the object of such investigation.

However, we do have thousands of untested chemicals in our environment and some attempt must be made to identify those potentially hazardous to man. The limitations of the simple short-term tests which are more concerned with the concept of somatic mutation are becoming better understood and the problem of false positives and negatives has been discussed earlier (8). At present, we can only do our best with the test systems available and hope that as research progresses our understanding and techniques will improve so that results generated in our model systems will become unequivocal in terms of hazard to man. To achieve this goal, attention will have to be given to studies aimed at assessing the significance to man of positive mutagenic responses produced by a test system for a given chemical, in addition to the search for better assay procedures.

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J. Soc. Cosmet. Chem. **29** 225 (1978)

Society of Cosmetic Chemists of Great Britain 1978 Medal Lecture Presentation

The 1978 Medal Lecture of the Society of Cosmetic Chemists of Great Britain was given by Professor M. N. Naylor, *R.D.*, B.Sc., B.D.S., Ph.D., F.D.S., R.C.S.(Eng), of the Department of Periodontology and Preventive Dentistry, Guy's Hospital Dental School, University of London. Professor Naylor's lecture was entitled 'Dental Health – a Community Responsibility' and was presented on Thursday, 2nd March, at the Royal Society of Arts, London, before an audience of Society members, their friends and colleagues.

When making formal presentation of the Silver Medal immediately prior to the Lecture, the President of the Society, Mr K. V. Curry, reminded the audience that Professor Naylor regularly contributed to Society activities and especially as the Lecturer on Dental Disease and Dental Hygiene at the Society's Postgraduate Courses in Cosmetic Science.

The vote of thanks was proposed by the Society's Hon, Treasurer, Mr G. L. Banks, who expressed his gratitude that the Society should honour, for the first time, an eminent member of the dental profession. Professor Naylor's subject was of particular interest to those many members of the Society who were involved with oral care and oral products. This was a sentiment shared by the audience who showed their appreciation by their warm reception of the lecture.

J. Soc. Cosmet. Chem. **29** 227–233 (1978)

Dental health — a community responsibility

M. N. NAYLOR *Department of Periodontology and Preventive Dentistry, Guy's Hospital Dental School, London Bridge SE1 9RT*

The 1978 Medal Lecture by Professor M. N. Naylor, R.D., *B.Sc., B.D.S., Ph.D., F.D.S., R.C.S.(Eng), delivered before the Society of Cosmetic Chemists of Great Britain on the 2nd March 1978 with K. V. Curry Esq., President of the Society in the Chair.*

It is perhaps almost as difficult to define what is meant by the term 'dental health' as it is to measure the level of dental health in any community. In 1965 the World Health Organisation defined dental health as 'a state of complete normality and functional efficiency of the teeth and supporting structures, and also the surrounding parts of the oral cavity and of the various structures related to mastication and maxillo-facial complex'. This is not a very helpful definition for there is no attempt to specify 'normality' or 'functional efficiency'.

A much easier situation to describe is 'dental ill-health' due to the two major oral diseases, decay or caries, and periodontal disease. Both of these conditions have such an extremely high prevalence that for practical purposes they must be regarded as universal in civilised man. It has been estimated that by the age of 15 years, 97% of British children have decay; in Norway this proportion is 99% whereas at age 21 years, only one in a thousand is free of disease. In 1973 a survey of 13,000 children in England and Wales showed that approximately 75% of 5-year-old children had decay and that, on average, every child had four affected deciduous teeth. In spite of previous care, 20% of children aged 5–8 years and 10% aged 9–15 years had five or more teeth needing treatment. By age 15 years on average, 10 of the 28 teeth were either decayed, filled or extracted. These data are confirmed by our own studies in London, the Isle of Wight and Hampshire.

It is interesting to note that in the year prior to this survey, i.e. 1972, 7·8 million courses of treatment were provided under the NHS for children of school age.

The situation in adults is no less depressing. In 1963, 37% of a carefully generated random sample of adults over the age of 16 years, in England and Wales was found to have had a complete clearance of all teeth. In Scotland the figure was 44%. A recent survey reported by Dowell and Beal has shown that by 1977 the situation had improved in England and Wales, the percentage of toothless adults having fallen to 31%.

Periodontal disease is no less prevalent. Some years ago, we examined 120 11-year-old children in a smallish London comprehensive school. Only one child was entirely clear of overt gingivitis. At this stage the condition is entirely reversible but the evidence supports the view that steps are not taken, generally speaking, to achieve this.

In adults, periodontal destruction becomes the major reason for extraction in people over the age of about 30 years.

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It is an unfortunate fact that the public generally does not place good dental health high on the list of priorities. This is not surprising since most people remain entirely unconcerned about their general state of health – until of course, they become seriously ill. Testimony of this are peoples' smoking habits, the prevalence of obesity and the scant regard paid to even the simplest forms of exercise. Small wonder that dental health is ignored when people are prepared to set themselves onto a head-on collision course towards coronary heart disease, malignant neoplasia and other equally devastating conditions resulting from personal abuse.

At all levels of society dental diseases are accepted as inevitable. Dental treatment, whether it be based upon regular routine visits to a practitioner or when driven to seek extractions because of pain, is likewise regarded as an unpleasant inevitability.

There are still people who regard a full clearance of all natural teeth and the provision of full upper and lower dentures as the sole criterion for good dental health. Not so long ago a girl's marriage prospects were regarded as being greatly enhanced when she became the proud possessor of full dentures!

Despite the fact that both decay and periodontal disease can be prevented, most people accept as normal, bleeding and inflamed gums, and the inevitable bad breath, decaying teeth and the various types of pain which accompany the different phases of the disease.

Compared with some of the 'killer' diseases with which our medical colleagues have to deal, dental diseases may seem but minor considerations. Nevertheless, dental diseases have such a high prevalence that the cost of NHS treatment is running at over £140,000,000 per annum; a conservative estimate of the working days lost due to dental disease has been put at 0·6 million. Finally, despite its relatively minor nature, dental diseases do have a mortality rate and it has been estimated that about 12 people per year die either from dental disease, the consequences of the disease or treatment. Some of these are anaesthetic deaths and others are associated with sub acute bacterial endocarditis of dental origin. It is quite impossible of course, to quantify the pain and distress which people suffer from dental diseases.

Periodontal disease, when it has reached a certain stage in its natural history, and dental decay, are both irreversible conditions.

When the periodontal disease is confined to the gingival margins and not involving the deeper periodontal structures, the situation can be reversed and normal health restored. However, when the deeper structures are involved, namely the periodontal membrane and the alveolar bone, the condition cannot be reversed and the best that treatment can do is bring the inflammation under control and prevent further destruction of tissue. If the destructive processes proceed inexorably, eventually the teeth become loose and finally are exfoliated. The onset of destruction and indeed the rate of destruction varies considerably from patient to patient. Unfortunately, the reasons for this variance are not clear.

Caries, certainly when an established lesion is present, is irreversible and the only treatment available is surgery which takes either the form of extraction of the tooth or excision of the diseased tooth substance and its replacement by means of one of several filling materials. There is evidence that very early lesions which have not involved a breach of the surface continuity of the tooth can be reversed or arrested. Examples of this are, reversal by sodium fluoride rinses of experimentally induced very early lesions, and the common clinical experience of an early lesion becoming arrested when stagnation is eliminated by extraction of the neighbouring tooth.

Since both caries and periodontal disease have destructive components which loom large in their natural history, the only logical approach to management is prevention. To achieve this a working understanding of their natural history is essential.

The common factor in both diseases is dental plaque. Plaque is a dense microbial deposit which adheres firmly to the surfaces of the teeth and gingivae. It tends to accumulate in stagnation areas between and around the necks of the teeth and in the gingival crevice. Plaque is not readily recognised but when a disclosing agent is used its presence and distribution are vividly identified. Structurally plaque comprises micro-organisms of many kinds which colonise on the surface of teeth and gums and are bound together in a matrix of polysaccharides which give it the sticky and slimy character.

The bacterial composition of plaque varies from individual to individual but generally it is true to say that young plaque is made up predominantly of gram positive, aerobic cocci, whereas older plaque comprises gram negative, anaerobic rod-shaped forms.

The micro-organisms undergo metabolic activity, the nature of which depends upon the substrate provided by the dietary intake. Periodontal disease is mainly brought about by the adsorption into the tissues of toxic metabolic end products – endotoxins – which cause irritation and inflammation of the tissues surrounding the tooth. The toxins enter the tissues through the mucosa especially that of the crevice causing the chronic inflammatory response which leads to destruction of the more specialised tissues and their replacement by granulation tissue. Such specialised tissues include the periodontal membrane and the alveolar bone. This leads to pocket formation around the teeth and, with loss of the supporting bone and attachment, the tooth sooner or later becomes loosened and eventually is exfoliated.

Sugars, of which sucrose is the most important, play a major role in the onset of decay.

Plaque bacterial enzyme systems metabolise sucrose in two ways. First, the sucrose is broken down anaerobically to form lactic acid which is capable of lowering the pH at the plaque-tooth interface to levels at which dissolution of enamel occurs. Second, sucrose units can be polymerised to form polysaccharides, mainly of the stable glucan type. These polysaccharides provide substrate for the micro-organisms between food intake and cause the plaque to thicken. Plaque is relatively impermeable to salivary buffers but it is even less so the thicker it becomes. It is for this reason that salivary buffer systems are unable to neutralise the acid at the plaque-tooth interface.

Decay begins as a sub-surface demineralisation and it is only when the lesion is quite extensive that the surface collapses and cavitation actually occurs.

It is clear therefore that plaque is the common feature of both periodontal disease and decay and it is reasonable to suppose that if it can be eliminated totally, then neither disease would occur. This is far easier said than done! There are chemotherapeutic drugs which will have a profound effect on plaque organisms, either killing them or preventing reproduction but for very good reasons, plaque control by this means is to be condemned.

There are a number of mechanical methods of removing plaque but of these the toothbrush is by far the most efficient and popular. The brush is only effective on accessible surfaces; the mesial and distal aspects of the teeth in a complete dentition are not reached by the bristles. In recent years the use of dental floss has become increasingly advised by practitioners. However, it is only recently that actual evidence of the value of sustained immaculate oral hygiene has become available. A study carried out in Sweden on school children, the test groups of which – and their parents – were subjected to a continuous programme of oral hygiene instruction supported by regular two-weekly

sessions with a hygienist. The results after 3 years showed major reductions in gingivitis, decay and plaque when the test groups were compared with the controls.

Returning to periodontal disease, there is overwhelming evidence that the basis of all treatment is plaque control. All the complex and sophisticated surgical procedures which are available today are doomed to failure unless supported by regular and effective removal of microbial deposits.

An important advance in the provision of dental care is the widespread introduction of Dental Hygienists, first trained by and employed in the dental branch of the Royal Air Force during World War II. After the war hygienists were employed within the Hospital Service only, but in 1957, following the passing of the 1956 Dentists Act, they were allowed to work in general practice. The number of training school for hygienists has increased and the number of girls applying for the 1-year course far exceeds the number of places available.

At Guy's Hospital, the School of Dental Hygienists started in 1963 with five trainees per year. We now have 20 per year and have no difficulty selecting high quality trainees from the many applicants. Many colleagues in general practice, the community service and in hospitals regard these girls as an indispensible feature of the service they provide to their patients. Not only do they carry out scaling and plaque control instruction, but they play a vital role in the provision of dental health education in its widest sense. To contemplate a preventive and periodontally orientated form of practice without hygienists' support would be rather like contemplating Heaven without Angels.

As indicated previously, the composition of the diet is extremely important in determining decay experience. Sugars, especially sucrose, have long been regarded as being heavily implicated in the initiation and progressing of decay but it was not until the mid-1950's that it was appreciated that the relationship between intake and disease was not a simple one. A human study carried out at the Vipeholm Hospital at Lund in Sweden demonstrated that both the form of the sugar food and its pattern of consumption were of crucial importance. Sucrose consumed at mealtimes only, in whatever form, caused little or no new decay, but continuous between-meal consumption of sticky toffees profoundly increased decay experience. Subsequent animal studies and human dietary investigations have confirmed these findings.

It must be clear therefore that dietary regulation is an important aspect of any preventive programme. Dental health education which emphasises the differences between right and wrong patterns of sugar consumption, is much more likely to be successful than trying to banish dietary sugar which anyway is an important and relatively cheap form of calories.

Perhaps the one and only truly community measure to prevent decay is fluoridation. The inverse relationship between the decay experience in a community and the fluoride ion content of the drinking water supplies has been known since the early 1930s. In 1937 Trendley Dean carried out a survey of the decay levels of children living in 21 U.S. cities with different levels of fluoride in the drinking water. The results clearly demonstrate that when the fluoride ion level of the water supply was 1 ppm (i.e. 1 mg/litre) the decay experience was considerably reduced. Studies in other parts of the world including the U.K. have confirmed these findings. In 1945 the first programme to adjust the fluoride level to 1 ppm was initiated at Grand Rapids, Michigan. After 10 years the reductions were entirely similar to natural fluoride areas. Thus began the water fluoridation story. Many areas have been fluoridated in countries throughout the world, amongst which are Canada and the U.S., S. Ireland and Hong Kong and, to a disappointing extent, the U.K.

The main inhibiting factor has been a small and vociferous minority, who oppose fluoridation on the grounds that it is a hazard to health and that it is unethical to tamper with water supplies. Two years ago, the Royal College of Physicians published a report of a Committee which had spent nearly 3 years inquiring into possible health hazards. The unequivocal conclusion was that fluoridation is safe and constitutes no hazard. Regarding the ethical aspects, this is very much an emotional and personal view; my view is that it is unethical to withhold this proven health measure.

Fluoridation exerts its effect systemically, the fluoride ions being absorbed intestinally and, via the bloodstream, becoming incorporated into the crystalline structure of the tooth enamel during the development period. In this way the proportion of fluorapatite is increased thus decreasing the solubility of the enamel acids.

Tablets containing fluoride are believed to be just as effective but, their regular daily ingestion for the first 14 or so years of life does require intense dedication and motivation. Tablets provide an individual preventive measure but water fluoridation includes the community as a whole and requires no individual effort or responsibility.

Topical fluorides using dentifrice, or mouthwash as the vehicle are becoming increasingly popular. In fact, over 90% of toothpaste sold in the U.K. contains either sodium monofluorophosphate or stannous fluoride. The actual amount of paste sold however, suggests that large numbers of the population do not use toothpaste at all. Almost all these toothpastes have been tested by means of a traditional 3 year Clinical Trial and shown to be effective in reducing decay. Taking into account the natural history of the decay process and the limited duration of the trials, it should surprise no one that the absolute values of the reduction in decay are somwhat small. However, there can be no doubt that regular and conscientious use of a fluoride dentifrice, both in terms of specific fluoride effect and the plaque removal function, is an important feature of any preventive programme.

As far as mouthwashes are concerned, the early studies carried out in Sweden, indicated that fluoride mouthwashes used as infrequently as at monthly intervals, caused a significant and clinically relevant reduction in the number of fillings required.

In recent years an increasing number of practitioners have been providing 'preventive packages' which comprise dental health education, dietary advice, topical fluorides including dentifrices. Whilst we support wholeheartedly this approach and the philosophy behind it, we are very doubtful of the need for multi-topical fluoride treatments.

Our own work in recent years has been concerned with this matter and we have already published the report of one study and the second is about to appear very soon. The first study was carried out in Croydon and was designed to determine the effect on 2-year decay increments of supervised daily use at school of an acidulated phosphate fluoride rinse and a sodium monofluorophosphate toothpaste used singly and in combination. Statistically significant reductions in 2-year caries increments were observed in all three experimental groups, i.e. fluoride rinse alone; fluoride dentifrice alone; and both together – when compared with the control. However, there were no significant differences between treatment groups.

Our second study was carried out on the Isle of Wight and in London and was designed to test the decay prevention of twice per year professional applications of APF gel and the unsupervised home use of a monofluorophosphate-calcium carbonate based dentifrice used either singly or in combination. The findings were similar to the Croydon Study.

The APF gel and flouride paste used singly or together, produced significant reductions in decay but, as in Croydon, there was no significant difference between treatment groups.

From these studies it would certainly seem that little or no advantage is to be gained from a multiple topical fluoride approach.

Since fluorides, whether systemically or topically applied exert their maximum effect on the smooth non-biting surfaces of the teeth, it would obviously be desirable if some procedure could be devised to protect the biting surfaces. Fissure sealing is one possibility. This notion is not especially new; as long ago as the 1920s T.P. Hyatt was advocating the sealing of fissures with amalgam prior to the onset of decay. Hyatt estimated that the chances of a fissure becoming decayed were 2,500 : 1. Whether these odds are valid today it is difficult to say, but it is certainly true that few fissure surfaces escape. It is therefore a reasonable approach to pre-empt the decay and seal the fissure immediately the tooth erupts through the gum. Sealing by means of amalgam which requires a very shallow cavity to be cut, has been superceded by a group of resinous materials which are polymerised *in situ* either by chemical means or ultra-violet light. Unfortunately, for such sealing to be successful an extremely meticulous technique is needed and a very co-operative subject. It is because of these very precise clinical requirements that the assessments of sealants, both in terms of retention and decay prevention have been so variable. Of course, far worse than losing the sealant, is the situation where the sealant remains *in situ* but leaks, thus allowing bacteria and sugars to accumulate beneath and start the caries process which can remain undetected for a very long time. Whilst present sealants can be successfully applied to selected patients, we shall have to await the development of material which demand a much less precise technique before sealing can be regarded as a community measure.

For many years the notion of elaborating a vaccine against dental diseases has waxed and waned. It is not surprising that, with the relatively recent advances in knowledge of the microbiology of decay and periodontal disease we should see a resurgence of interest in the possibility of developing a human vaccine. Whatever anyone may have read in the tabloid Sunday newspapers, no effective human vaccine is in existence today. There is considerable work in progress in the U.S. and here in the U.K. at the Royal College of Surgeons and at Guy's Hospital Dental School. Primate studies have been most interesting, and encouraging. Nevertheless, there are many problems to overcome, not least the problem that these diseases are multi bacterial; they are not due to a single organism like the recognised immunisable diseases. Furthermore, if and when a vaccine is developed, it will be necessary to convince the public that the risks associated with vaccination are justified, especially since there are other effective ways of prevention.

From what has been said so far, it is clear that good dental health is within the reach of all. The problem is motivation and the creation of the right attitudes. Certainly, in my professional lifetime, which covers the years since the end of World War II, there has been tremendous progress. Undoubtedly, the availability of dental care as part of the NHS has played a great part in this.

Other factors of equal importance are the advances in treatment techniques – local anaesthetics, the high speed air-turbine handpiece, low seated, four handed dentistry – are but a few. Advertising of dental products, notably toothpastes on commercial television, have done a superb job in bringing dental health right into the homes of the public. Together these factors have taken much of the fear and pain out of the visit to the dentist.

Having said all this it is salutary to remind ourselves that, despite all the measures that can be taken to prevent disease, the advances in practice techniques and changing attitudes towards dental health, only about 40% of the public seek regular dental care, that in 1977 an estimated 31% of the public over age 16 years wear full dentures, that less than 10% of the public drink water containing the optimum level of fluoride ions, that a high but unknown proportion of the population do not own a toothbrush, let alone use it, and that in the U.K. we buy approximately half the number of tubes of toothpaste per family, per annum, compared with the U.S.

Despite the fact that nearly two thirds of the population fail to seek regular care, the dental profession is stretched almost to breaking point. If only a small proportion of the erstwhile 60% decided to seek regular care the profession would reach the point when it couldn't cope. It is obvious that the whole approach to the provision of dental care has to change; it has to change from an approach which is essentially reparative to one which is preventive. The profession alone cannot bring about that change. It requires everyone's participation – the profession, government, industry and the public. Indeed it is a community responsibility.

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Notices

AN INTERNATIONAL SYMPOSIUM ON BIO-ENGINEERING AND THE SKIN

An International Symposium on 'Bio-engineering and the Skin' will be held at the Welsh National School of Medicine, Heath Park, Cardiff, on the 19th, 20th and 21st July, 1979. It is intended to hold the meeting over the three days with four formal sessions (including poster and workshop sessions and exhibition). Progress made in understanding the physical properties of the skin and in the instrumentation for these measurements will be reviewed and original work on these themes presented.

Further details and abstract forms may be obtained from the co-organisers:

Dr R. Marks,
Reader and Consultant in Dermatology,
Department of Medicine,
Welsh National School of Medicine,
Heath Park, Cardiff.
CF4 4XN

or

Dr P. A. Payne,
Head,
Bioengineering Unit,
University Hospital of Wales,
Heath Park, Cardiff.
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THE EPIDERMIS IN DISEASE

An International Symposium will be held at the Welsh National School of Medicine, Heath Park, Cardiff, on the 18th, 19th and 20th April 1979.

Session Subjects and Chairmen

Dr E. Beutner (USA)
Immunopathology of epidermal disease
Professor M. Greaves (London)
Pharmacological aspects of epidermal disease
Professor E. Wilson Jones (London)
Reaction patterns of epidermal disease
Professor M. Prunieras (France)
Epidermal symbionts
Professor E. Christophers (Kiel)
Disorders characterised by increased epidermopoiesis
Dr R. Marks (Cardiff)
Disorders of keratinisation

Further details and registration forms can be obtained from the co-organisers:

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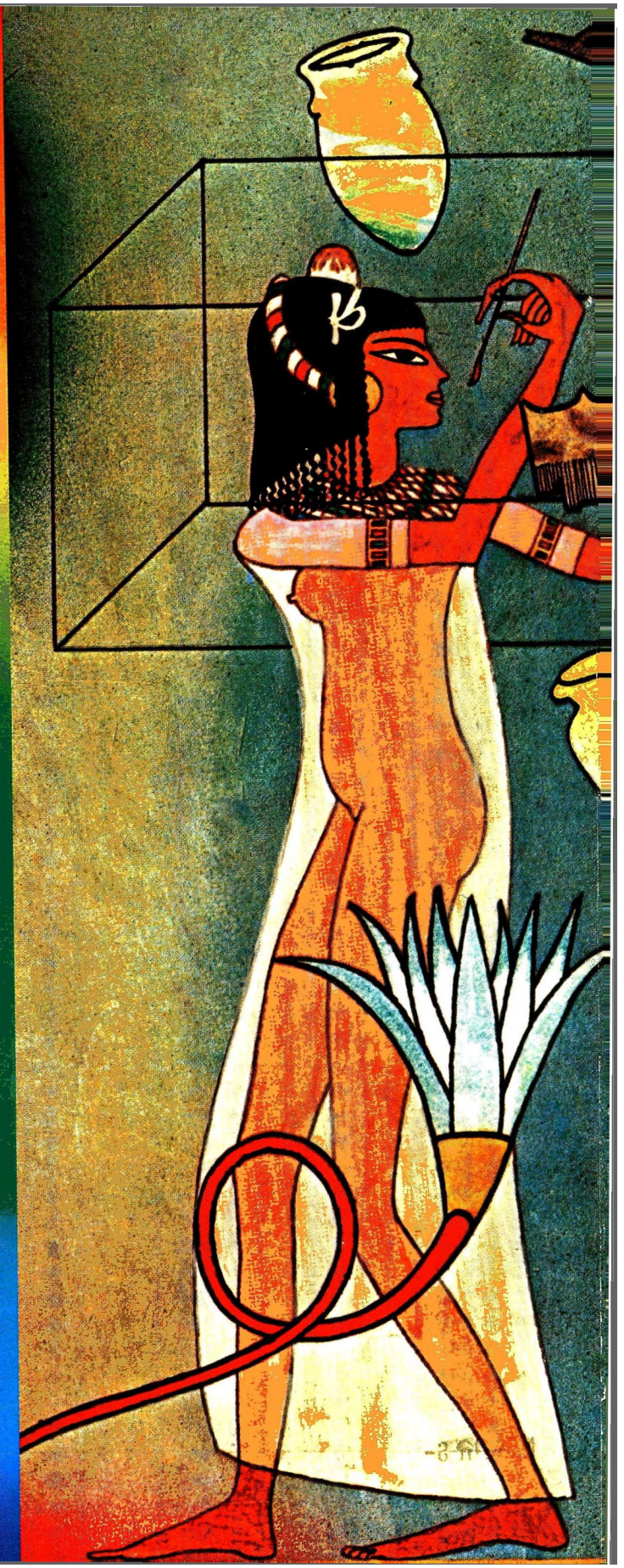

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