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# Journal of the Society of Cosmetic Chemists

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*Journal of the Society of Cosmetic Chemists* **18** 439-453 (1967).

**Synopsis**—Assessment of the efficacy of a treatment for dandruff demands a rigidly controlled methodology, comparable to that employed in other types of clinical testing. Experimental techniques are illustrated and discussed, with emphasis on the need for careful training of observers, who may then be able to derive meaningful results from a study of human volunteers using an antidandruff shampoo.

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N. A. PUTTNAM and B. H. BAXTER.
*Journal of the Society of Cosmetic Chemists* **18** 469-472 (1967).

**Synopsis**—It has been shown that it is possible by the attenuated total reflectance technique to obtain ir spectra from skin *in situ*. The procedure involved the use of a 'V' shaped ATR crystal into which the side of the hand (the hypothenar eminence) was placed. The main features of such spectra agreed with those reported earlier from transmission studies through thin sections of various tissues, but there were relatively small differences in the ratios of the intensities of the absorptions in the wavelength range 1300-1000 $cm^{-1}$ for different individuals, which were not further investigated.

The application of the ATR technique was extended to show that it was possible to detect the retention by the skin of relatively major components of a hand cream applied to the skin.

**The laboratory evaluation of prophylactic dentifrices:** W. H. BULL.
*Journal of the Society of Cosmetic Chemists* **18** 473-491 (1967).

**Synopsis**—The need for laboratory methods of assessing dental prophylactic products is discussed and a review of some of the methods used to evaluate fluorine containing toothpastes given. The techniques mentioned include *in vitro* and *in vivo* solubility studies using chemical, physical and electron microscopical techniques to evaluate the action of the products. The advantages of establishing *in vitro* methods which do not depend directly on acid solubility, e.g. some form of artificial mouth, are explained.

# Journal of the Society of Cosmetic Chemists

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*J. Soc. Cosmetic Chemists* **18** 439-453 (1967) 

# The clinical evaluation of antidandruff shampoos

N. J. VAN ABBÉ and PATRICIA M. DEAN*

*Presented at the Symposium on "Product Testing", organised by the Society of Cosmetic ehdmists of Great Britain in Eastbourne, Sussex, on 15th November, 1966*

**Synopsis**—Assessment of the efficacy of a treatment for dandruff demands a rigidly controlled methodology, comparable to that employed in other types of clinical testing. Experimental techniques are illustrated and discussed, with emphasis on the need for careful training of observers, who may then be able to derive meaningful results from a study of human volunteers using an antidandruff shampoo.

In a previous communication (1), the study of dandruff on volunteer human subjects was described in detail, with emphasis on methods of studying the disorder itself rather than its treatment. Shampoos containing various active constituents represent the accepted means of providing medication for dandruff and several papers (2-4) report clinical investigations of these and other means of applying the active ingredient. In recognition of the need to devise techniques of scientific validity for evaluating potential therapeutic measures, this paper is intended to review some of our own experiences critically.

As before, attention will be confined principally to the manifestation of dandruff as visible scaling, i.e. desquamation in excess of and in fragments larger than that due to a simple shedding of the horny layer of the epidermis. We are not, in the present context, interested in the causation of dandruff, although this must obviously have a bearing on the clinical aspects; our

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concern is to assess the feasibility and reliability of various ways of evaluating potential treatments.

## Principles of clinical testing

Dandruff is a chronic abnormality of the scalp and, as in the case of chronic diseases generally, the prognosis for any individual sufferer at a given point in time is uncertain. In other words, when a treatment is administered to a subject, it is difficult to decide whether future progress (favourable or adverse) is due to the treatment or to spontaneous changes. The pre-treatment phase shown in *Fig. 1* shows how dandruff fluctuates spontaneously; if a course of treatment was initiated at a time when the

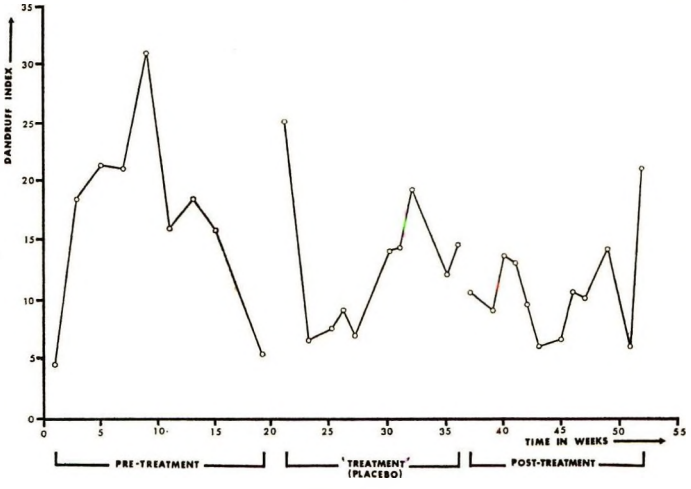


*Figure 1*
Results of 17 weeks' treatment with placebo shampoo on a case of moderate dandruff (Detailed method of inspection).

level was high, there would obviously be a distinct hazard that subsequent inspection would register a reduction in dandruff although this might be wholly independent of the treatment given. Fluctuation of this nature can be taken care of to some extent by using sizeable panels of subjects, so that the purely random effects tend to cancel out. However, if progress under treatment is compared only with pre-treatment levels, there is an indeterminable risk that extraneous factors may be operative during treatment, possibly having a greater influence on the dandruff levels than the treatment itself. Such an irrelevant feature could well be climatic, for an effect of this nature during dandruff treatment has been noticed previously (1).

To overcome this difficulty, it is essential to compare the progress of a treated panel with an untreated panel running concurrently.

Dandruff investigations would be simplified considerably if objective measurements were practical. A technique has, in fact, been published whereby scale samples are taken by means of a miniature vacuum cleaner and subsequently weighed (4). Since this technique may involve the disturbance of scale attached to the scalp which may influence the disorder itself and in the absence of alternatives not showing a similar disadvantage, a subjective method of evaluation is to be preferred.

Clinical trials in which subjective assessments are made always embody the risk of significant observer error and bias. Comparison between treatment and no-treatment panels tends to rule out error and bias due to the subjects themselves, especially if they are allocated to the different panels by a suitable method of randomization; but it is still vital that the observer should not be able to identify whether a subject is receiving treatment or not at the time of inspection. For various reasons it is preferable that this knowledge should also not be available to the actual subject and so the "double blind" technique should be adopted. This involves the employment of a placebo, identical in all discernible respects to the treatment but lacking the active constituents; one half of the subjects receive the treatment and the remainder have the placebo and serve as controls. Whilst it is usually possible to ensure a close resemblance between a placebo and treatment ("control" and "test") shampoo, some ingenuity of the formulator is sometimes needed to achieve this.

To make sure that the identity of test and control materials is unknown to both subjects and observers, whilst also ensuring that the observers do not know or try to guess the allocation of subjects to the various panels, it is desirable to apply several different code-letters both to the test and control products and to allocate them to the subjects by means of a randomization chart. This also helps to prevent subjects comparing notes with one another and possibly influencing their cooperation.

Elimination of bias may also be assisted by stratifying the subjects according to various criteria possibly affecting the dandruff condition, e.g. ensuring that various degrees of initial severity are equally distributed between the panels. Age and sex might well be treated similarly, though the factor next in importance to initial severity is probably the question of prior usage of a medicated shampoo.

"Cross-over" technique would theoretically help greatly to strengthen the validity of a clinical trial; that is, one panel would start with the control

product and later use the test, whilst the other panel would use the two products in reverse order. If, however, the test product has any real effect, it is vital to know how soon the dandruff level reverts to normal when treatment is discontinued, otherwise a "carry-over" effect will be operative during the control phase; for the panel starting with the test product, results during the control phase will also depend on the actual time required to regenerate dandruff by infection or other means. The post-treatment zone illustrated in *Fig. 2* shows that several weeks are required to re-establish the original pre-treatment dandruff level when an active anti-dandruff agent has been used. A practical solution might be to allow a prolonged time-lag between test and control phases, but this is likely to prolong the whole procedure of experiment to an unacceptable extent. A "cross-over" trial ignoring the points noted would tend to underestimate the efficacy of a treatment.

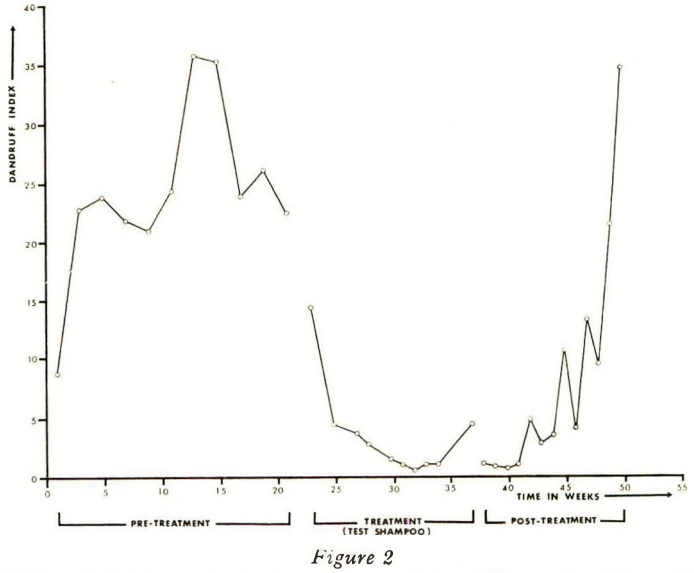


*Figure 2*

Results of 16 weeks' treatment with shampoo containing 2% w/w zinc bis(pyridine-2-thiol 1-oxide) on a case of moderate-severe dandruff. (Detailed method of inspection).

It is almost a truism to suggest that the statistician who will have to determine the significance of the results, should be fully consulted at the planning stage of a clinical trial. It is the authors' view that a carefully-planned and well-conducted trial on a limited panel is far more valuable than a poorly-conceived investigation carried out on a relatively vast scale; an essential feature of sound planning is to ensure that the results will be adequate and in a suitable form for statistical analysis.

## Special problems of dandruff trials

One of the main considerations is the decision on what is to constitute a favourable result for an individual participant. It may, for example, be shown that any shampoo, whether medicated or not, will remove some 70% of dandruff scale during the actual process of shampooing. In the absence of effective treatment, however, dandruff will return to approximately the original level within about five days; what matters to the subject is that this recurrence should not happen, at least to the same extent. Hence an antidandruff shampoo can only be classed as effective if it significantly and consistently reduces the dandruff levels attained five days after shampooing and it is appropriate always to take measurements at this timing as far as possible. It should not be forgotten that clinical trial techniques stand or fall by the extent of cooperation achieved with the participants, and there is an ever-present hazard that some of the subjects may not really have used the test or control products at all, especially if they are unpleasant or complicated in use or deemed to be ineffective. Similarly precise timing of observations after shampooing is a target to be aimed at and it has to be hoped that minor discrepancies will cancel out between the panels.

Recruitment of subjects for dandruff studies presents surprising difficulty. Obviously someone who has no dandruff cannot show any beneficial effect, however efficacious the treatment. It is therefore necessary to recruit at least moderately severe sufferers, though it is also desirable to have some subjects who are only slightly affected in case either the test or control product demonstrates an adverse effect. It is quite impossible to ensure that a sample of subjects in a trial is fully representative of the population as a whole, if panels are to be kept within manageable proportions; for instance, the source of infection in one geographical area may differ from others and may also differ in response to particular treatments (assuming that dandruff results from infection). If the subjects for a trial are recruited from an essentially "closed" community such as a boarding school, the risk of bias due to atypical sampling may be serious. On the other hand, it may be much easier to obtain the desired level of cooperation in such a community and far simpler to make suitable arrangements for conducting inspections. Probably the most satisfactory answer in practice is to utilize several such "closed" communities for the full evaluation of a new product. Subjects who are skin patients attending hospital are not ideal for the purpose, as they may not respond in the same way as "normals".

Some published clinical trials on dandruff appear to have been restricted

to recording the overall impressions of a dermatologist, the findings being expressed in a limited range of descriptive terms such as "cleared", "improved" or "no change"(3). Brief consideration will show that such an approach (especially if it is not accompanied by a strictly "double-blind" routine) leaves much to be desired unless it is only required to distinguish all-or-none efficacy. Important factors, in our experience, are that:—

i. Training and extensive practice in the study of dandruff, making use of the concordance tests previously described (1), are pre-requisites for discriminating and reproducible assessments.

ii. Clear definitions for a gradation of clinical features are necessary, along with a definite system for examining the scalp. Observations may take the form of word descriptions at the time of examination but are preferably transcribed into pre-selected numerical values for subsequent tabulation and analysis.

iii. Intervals between inspections, during which the various treatments are used, need to be programmed on sound lines.

The technique of examining the scalp in 25 sections (1) has formed the basis of much of our experience for several years. If this is used in conjunction with a "double-blind" system of test and control panels, it is possible to compare the average trends for each panel during treatment. Since each physical examination occupies almost 30 min/subject, relatively small panels have to be employed and it becomes arguable whether a less detailed inspection of larger numbers would not be more profitable. Clearly the point could be reached where the technique of assessment became so crude that only the most glaring differences could possibly attain statistical significance; we have therefore sought to test some comparatively simple techniques and are still continuing such investigations.

Our own staff of observers, who have long experience in the detailed, 30 min method of assessing dandruff, have more recently been trained to conduct a quicker method of inspection which takes about five min. In the detailed method the scalp is partitioned into 25 imaginary sections and each section in turn is scored for severity of dandruff and proportion of area occupied by dandruff; in the rapid method the scalp is divided into four sections instead of 25. The subject is seated under a good diffused lighting and each notional quadrant is examined by parting the hair with a comb at intervals over the area. The estimated amount of scale attached to the scalp (not loose in the hair) for each quadrant is rated by the following verbal descriptions, which are later given the numerical values shown and added together to yield the index for the whole scalp.

| | |
|---|---|
| Nil | 0 |
| Very slight | 1 |
| Slight | 2 |
| Slight to moderate | 3 |
| Moderate | 4 |
| Severe | 8 |

As in the case of the more detailed technique, it is vital to test the concordance between observers, examining the same subjects independently at the same time, as often as possible. Typical results of such concordance tests between a pair of examiners are shown in *Table I*. Whilst these are not as good as those obtained for the more detailed method and reported earlier (1) they nevertheless do show quite good agreement and indicate that a discrepancy of more than 'one place' is unusual. Even when concordance tests show good agreement between observers it is still considered advisable that each subject in a trial should always be examined by the same observer at successive inspections; this will be specially important when some of the observers are relatively inexperienced. An observer is at no time allowed to see the subject's earlier records during an examination

Table I
Concordance between observers

| | | Assessment of dandruff: observer A | | | | | |
|---|---|---|---|---|---|---|---|
| | | Nil | Very slight | Slight | Slight to moderate | Moderate | Severe |
| Assessment of dandruff observer B | Nil | 21 | 6 | | | | |
| | Very slight | 9 | 58 | 5 | 2 | | |
| | Slight | 1 | 10 | 6 | 4 | | |
| | Slight to moderate | | 4 | 4 | 11 | | |
| | Moderate | | 1 | 1 | 4 | 13 | 3 |
| | Severe | | | | | | 5 |

Figures represent number of scalp quadrants

and neither the observer nor the statistician know the breakdown of the product codes before the trial has been completed and analysed.

Our earlier studies to investigate dandruff without particular reference to treatment, involved either weekly or fortnightly examination of subjects over long periods of time. Similarly in clinical trials using the same detailed procedure, we have made frequent inspections over long treatment periods;

indeed, the less effective the treatment, the longer a trial needs to proceed in order to demonstrate a difference between test and control. However, with the records of earlier trials available for study and with more effective products now coming under test, it has been possible to show that the number of inspections during a trial can be greatly reduced without detriment to the validity of the main findings, providing that the panel size is not too small. To make due allowance for any cumulative effect of a shampoo treatment it must be used several times before an attempt is made to measure its efficacy. Our experience suggests that a first examination before using the product should be followed by a second examination after four weeks' use and a third examination after eight weeks. Comparison of the third versus the first reading reveals the main trend, but the second examination is a useful check on placebo effect which may be expected to be substantially the same at the second and third examinations. When using this method we prefer to use panels at least twice the size of those used for the method with more frequent examinations.

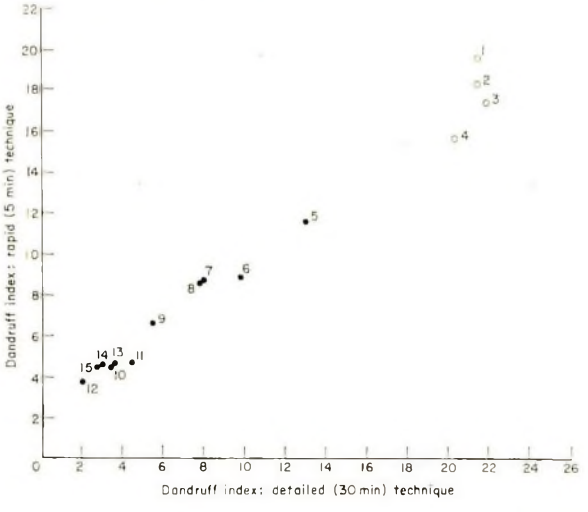


*Figure 3*

Comparison between "5-minute" and detailed "30-minute" technique for dandruff inspection. Numbered points represent successive weekly averages for the same 10 subjects under treatment with (1-4) a non-medicated, and (5-15) an antidandruff shampoo.

## Results

Critical comparison of techniques for the assessment of dandruff ideally requires that the different methods should be used side-by-side on

the same subjects. Correspondence between the rapid (5 min) method and the original detailed 30 min method is illustrated in *Fig. 3.* For levels in the moderate to moderately-severe region, there is little doubt that the two methods are in good agreement. There is a suggestion that the rapid method may be rather more sensitive at low and high levels of dandruff, possibly because the condition is often of patchy distribution and the scores tend to be "diluted" by the zero scores for clear areas in the detailed method.

The distribution of points along the curve in *Fig. 3* reflects the efficacy of the treatment, but this is more readily examined in other ways, e.g. as

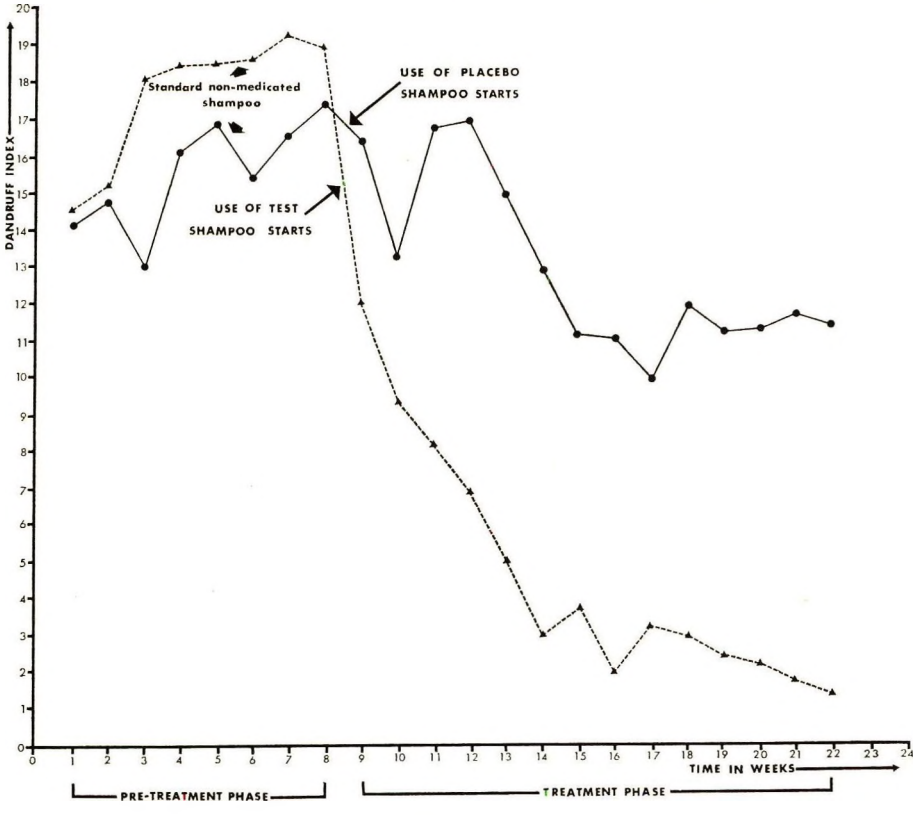


*Figure 4*

Trial of an anti-dandruff shampoo containing 2% w/w zinc bis(pyridine-2-thiol 1-oxide). The points represent average levels for panels of 13 subjects, assessed by the detailed method.

shown in *Fig. 4.* The frequency of examinations helps to show the speed of response to the treatment as well as the maximum benefit obtained.

*Table II* summarizes the data from a trial on the same shampoo, using the rapid inspection technique and the timing pattern discussed above, i.e. before treatment and after treatment for one and two months. The difference between the placebo effect and the treatment is clearly shown, though it should be remembered that expression as percentages does not necessarily accord precisely with the "true" gradations prevailing *in vivo*; the analysis of variance undoubtedly yields a more reliable basis for judging the difference between test and control.

Table II

Trial of an anti-dandruff shampoo using the rapid method of assessment and a reduced number of observations

| | Placebo shampoo | Test shampoo |
|---|---|---|
| Average level at 1st examination for all subjects who reattended for 2nd examination | 9.3 | 10.8 |
| Average level at 2nd examination | 7.3 (38 subjects) | 5.0 (39 subjects) |
| % reduction | 21.8% | 54.3% |
| Average level at 1st examination for all subjects who reattended for 3rd examination | 9.6 | 11.0 |
| Average level at 3rd examination | 7.5 (37 subjects) | 3.2 (32 subjects) |
| % reduction | 22.0% | 70.9% |

Table IIA

Analysis of variance of results in *Table II*

| | | Sum of squares | Degrees of freedom | Mean variance | Variance ratio |
|---|---|---|---|---|---|
| Examination 2v.1 | Between shampoos | 105 | 1 | 105 | 5.8* |
| | Within shampoos | 1370 | 75 | 18 | |
| | Total | 1475 | 76 | | |
| Examination 3v.1 | Between shampoos | 313 | 1 | 313 | 17*** |
| | Within shampoos | 1222 | 67 | 18 | |
| | Total | 1535 | 68 | | |

*Significant at 5%, ***Significant at 0.1%, confidence level

The progress made by individual subjects in a dandruff trial is well illustrated by means of a ternary diagram (*Fig. 5*) showing the cumulative effect of continued usage; this indicates in concise form not only the beneficial effect of a product but also the proportion of subjects whose dandruff level remains unchanged or increases. In this trial the product was considered to have a beneficial effect when the dandruff level after treatment was half, or less, of that before treatment.

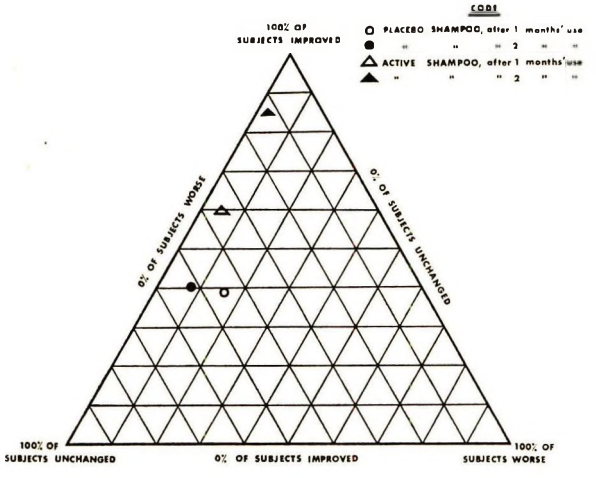


*Figure 5*
Proportion of subjects showing improvement, no change, or worsening of their dandruff (Rapid method of inspection).

The results shown here cannot justifiably be taken to indicate the numbers of subjects necessary in antidandruff trials generally, since this will also depend on the efficacy of the treatment under test; these numbers were nevertheless clearly adequate in the examples quoted.

## Discussion

Experience has taught us that treatments to combat dandruff require to be evaluated no less stringently than medicaments for correcting other chronic disorders. Good organization and, in particular, utilizing the "double-blind" method of clinical trial are exceedingly valuable. Unfortunately, it does not seem to have been appreciated in the past that the clinical impressions of a busy consultant, undertaking a trial as an isolated experiment and with no proper controls, will scarcely do more than

confirm the obvious and confuse the more subtle differences between various treatments. Nevertheless the supervision of a dermatologist is essential to provide guidance on correct diagnosis and to safeguard the interests of the volunteer participants.

For the purpose of taking measurements, however, it is desirable to employ specially-trained observers, the reliability of whose results has been rigorously tested; such observers need not be medically qualified.

Despite the supposedly ubiquitous occurrence of dandruff, we have never found it easy to recruit large numbers of severe sufferers for trials; this may have been a blessing in disguise, insofar as it has inspired us to devise techniques applicable to the available numbers of volunteers. The clinical studies reported here should not, however, be considered to be adequate to establish fully the efficacy of the proposed formulation. It is, for example, necessary to determine whether efficacy is maintained over many months of usage or whether resistance develops; in this particular case, another experiment continuing for 12 months has, in fact, shown that reductions in dandruff levels were satisfactorily maintained but the short term studies are naturally undertaken first, primarily for screening purposes.

It is interesting to note the finding that about 10 weeks are required to re-establish starting levels of dandruff when treatment has reduced it virtually to zero. The true induction period for dandruff is probably rather less than this, however, since the treatment is likely to have exerted some "carry over" effect.

Whenever clinical trials are conducted, according to the patterns discussed here or in other ways, it is important to keep detailed records of any adverse effects (in addition to worsening of the condition under examination). These will generally be confined to mild episodes of transient erythema or itching but the supervising dermatologist will certainly wish to examine anything more than this in some detail. Comparison between test and control products on the incidence of physiological reactions should be made, to show whether irritancy is due to the active constituents or the shampoo base itself or even to establish whether the active constituents may have an anti-irritant effect.

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## *Introduction by Mr. Van Abbé*

Unlike our previous paper on the subject of dandruff, we are now dealing with the clinical evaluation of treatments. It would naturally be preferable to carry out a simple laboratory test but this cannot be done with certainty and so we find ourselves in the field of human subjects and biological variation. The real difficulties arise from the fluctuating character of dandruff itself and a relatively sophisticated technique is necessary for validation of the efficiency of a medicated shampoo.

In this paper, we have dealt with the 25-area method of inspection that appeared in the last paper and with a more rapid technique where the scalp is divided into only four imaginary areas. Contrary to expectation, the quadrant method appears to be rather more sensitive. There is a general parallellism or, in fact, linearity between the two techniques, over most of the range of moderate to severe dandruff but at the level of high dandruff there is a greater spread out on the rapid method and the displacement from zero at the lower end suggests that the rapid method is also more sensitive at low levels of dandruff. We have distinguished between weekly or fortnightly examinations over a fairly long time and a programme of only three examinations, comprising before treatment, after one month's treatment and after two months' treatment. One way of representing the data obtained from the abbreviated programme of inspection is in the form of a ternary diagram which shows not only improvement of the group but changes in the direction of "worse" or "no change".

## DISCUSSION

MR. C. PUGH: When one looks at the ternary diagram, one can see very clearly the proportion of people getting benefit. To my mind this is a most important quantity to examine in trying to make a better shampoo. In *Fig. 5* one can see that the placebo shows virtually no movement, but the test shampoo is moving firmly towards 100% getting better. If the trial had been continued for another month, the treated panel might have reached the apex, although this is unlikely. I would like to stress that this seems to be an excellent method of presentation giving far more valuable information than percentage improvement on an "average head".

MR. VAN ABBÉ: Although curves such as those represented in *Fig. 4* appear to show clearly what is happening week by week, they represent panel averages and they do not show the proportion getting worse or the numbers unchanged. For our ternary diagram, we have arbitrarily chosen a level of 50% improvement or 100% worsening as the level for illustration. However, we could have chosen other levels and shown the appropriate proportions. The actual points on the diagram would differ but the conclusions regarding superiority of treatment-v-placebo would not materially change.

MRS. D. L. WEDDERBURN: You could, perhaps, have omitted a control group in this trial owing to your employment of control and then test and then back on control again. Does your use of a placebo mean that you have found seasonal variations in

dandruff and that you regard it as necessary to have a control group in parallel because the incidence of dandruff fluctuates at different times of the year?

MR. VAN ABBÉ: The dandruff level of untreated subjects does not seem to be dependent on the season but we did see a sign in our earlier work that under active treatment there was a seasonal influence. Other factors might be involved too, such as holidays, examinations and various psycho-somatic influences that could make it difficult to draw conclusions without a placebo.

MISS DEAN: I should like to add that although we carry out concordance tests between examiners, this does not safeguard against all the examiners drifting in the same direction over a period of time. If we run a control panel, the observers do not know which product is control and which is treatment, so it does help to safeguard against any drift of this nature. Of course, using a placebo does put some people off; they realise they are not getting better, and sometimes a proportion of our subjects get discouraged and tend to default if we keep them too long on no-treatment.

MR. K. M. GODFREY: Is the time required to re-establish dandruff affected by residual absorption of the active ingredients?

MR. VAN ABBÉ: Possibly. Zinc omadine (the active ingredient referred to in the paper) is only soluble in water to the extent of about 6 ppm. Nonetheless it is soluble to this extent and may be absorbed or adsorbed; this may contribute towards its efficacy, for some of it may be taken up during treatment and not be eluted for some time afterwards.

MRS. S. M. LUDFORD: Do you use people again for assessment once they have recovered to their pre-treatment level; if so, do they respond differently from new subjects? Do you observe any other effects of antidandruff products or do you only look for effect on dandruff?

MISS DEAN: Occasionally we do use subjects again. We allow a considerable recovery period and, when using them again, we take great care to stratify them between the groups. We have supplemented the clinical examinations by looking for effects on *Pityrosporum* and bacterial counts on the scalp. We pass an applicator through the subject's hair and take plate counts of micro-organisms. Sometimes we find a reduction but so far we have not really demonstrated any definite correlation with dandruff scaling.

MR. VAN ABBÉ: We also record itching and erythema, again without showing any distinct correlations.

MR. K. V. CURRY: Is the response of the moderate/severe group of subjects similar to the response of the "very slight" group? I have a feeling that the majority of people with dandruff have the second type which is really a social nuisance rather than a scalp disorder.

MISS DEAN: On the whole it is easier to show a significant reduction on people who have severe dandruff and for the bulk of our panels we prefer to use such subjects. I think the proportion showing a reduction who start with slight dandruff is nearly as great as for those who start with a severe level but, of course, it is not such a great reduction.

MR. R. CLARK: Is your objection to the use of vacuum cleaner technique purely theoretical or is it based on practical experiments?

MR. VAN ABBÉ: We have carried out some experiments. We did not like the *Hair Vac* (as used by Vander Wyk) in its original state but even when this had been modified by us, we still felt that it was impossible to know whether the course of the condition was being influenced. This is the main difficulty, for one just cannot know and therefore it does not seem to be a desirable technique.

MR. R. CLARK: Did you run a "vacuum cleaner" trial in conjunction with your own subjective method of assessment?

MR. VAN ABBÉ: We have not done so yet, although we have considered the possibility.

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# The evaluation of placebos in clinical trials

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**Synopsis**—A model is proposed for the response of living organisms to the action of drugs when placebo reaction is likely to occur. The estimation of the parameters in the model is discussed together with tests of hypotheses about the parameter values.

## Introduction

The clinical evaluation of drugs and medicaments is as old as medicine itself but it is comparatively recently in the history of the subject that the actions of various drugs have been recorded and compared in a quantitative and scientific fashion.

The value of a treatment can only be expressed in meaningful terms by reference to the state of affairs when the treatment is not used, either because of an alternative treatment or because of the absence of any treatment.

If a disease is severe so that the mortality rate for sufferers from the disease is high and if no treatment is currently known it is clear that any substantial reduction in the mortality rate is a sufficient indication of the value of a new treatment. A more common state of affairs is the case of a well established treatment for a disease where two questions may arise:

i. Is the customary treatment more effective than no treatment?

ii. Is the customary treatment less effective than one or more new possible treatments?

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The second question may usually be answered by comparison in a clinical trial but ethical considerations require that new treatments are admitted to a trial only if there are good grounds for expecting them to be as least as effective as the old treatment. The first question is more subject to ethical difficulties in the answering, since only in the mildest diseases may treatment be stopped in order to study the effect of no treatment.

The ethical considerations referred to above are not unequivocably interpreted, as has been pointed out by Hill (1), since the only well established treatment may actually be inferior to the absence of treatment, as in the use of anti-coagulant in cerebrovascular disturbances.

No scientist is completely free from the ethical implications of his work and this applies to the statistician as much as to any other scientist. We may, however, consider the scientific aspects of a problem without reference to its ethical context.

When attempting to answer the first of the two questions above, a placebo is frequently administered instead of merely neglecting those patients who are to receive no treatment. One of the original meanings of *placebo* is derived from the same root as *placate*, that is the placebo is given in order to please the patient or to keep him from the knowledge that he is being neglected. Lasagna (2) has given several meanings for *placebo*; we are at present concerned with the administration of a dummy treatment (3), which may also and incidentally please the patient either consciously or in more subtle ways. There is little to be gained by distinguishing formally between placebos and dummies (4).

## Quantitative responses to drugs

When a drug or medicament is administered to a patient the response is likely to be quantitative if the treatment has pharmacological properties but it may be quantitative or qualitative if the effect of the treatment is psychological or psychosomatic. The effect of a pharmacological preparation on a living organism must depend on the potency of the preparation and on the dose used but the mode of observation of the effect may either be quantitative as well or it may only be possible to observe success and failure of the treatment. The psychological results of the administration are likely to be qualitative; the treatment either pleases or it does not, although degrees of pleasure may sometimes occur.

Both pharmacological and psychological effects are probably present in most clinical trials, each to a greater or lesser extent. If the observed

response is quantitative we may suppose that pharmacological factors predominate although this is not always the case since, for example, Wolf *et al* (5) found it possible to affect the eosinophile count by placebo action.

Such quantitative observations would occur, for example, in a trial to test the effects of treatments by various fruit preparations on the vitamin C content of the blood. These effects can only be measured against a standard which in this case consists of the absence of treatment. However those persons receiving the fruit preparations are thereby also receiving extra water, sugar and other components besides vitamin C so that the absence of treatment should be interpreted as receiving equivalent amounts of water, sugar, etc., and perhaps even sulphur dioxide, rather than as complete neglect. At the same time any psychological factors will be equalized between the active treatments and the blank treatment or placebo. The function of the placebo experiment, in such a trial is to avoid bias in the results just as in, say, the direct determination of oxygen in rubber by the Unterzaucher method (6) it is necessary to do a blank determination, or as in absorption spectrometry the intensities must be corrected for the background absorption. An important additional characteristic of the clinical trial is the pronounced variability between individuals receiving the treatments. It is therefore particularly important to design the trial so as to control and reduce sampling error especially by the random allocation of treatments and by ensuring that the individuals studied form a random and representative sample of the population for which the treatments are intended. A comprehensive account of the application of statistics to the design and analysis of clinical trials has been given by Hill (7) and the general principles of experimental design have been discussed nonmathematically by Cox (8).

## Quantal responses to drugs

If the observed response of an individual to drugs or other medicaments is *success* or *failure* of the treatment the response is termed quantal. The object of the treatment of serious diseases is normally the survival of the patient, and chronic conditions usually require the alleviation of pain or other symptoms. In the first case *success* denotes survival, and in the second improvement, versus death and no improvement respectively.

The reaction of a patient to the administration of pharmacologically inert substances is usually referred to as placebo reaction. This reaction is normally quantal so that in clinical trials where there is a possibility of

placebo reaction as well as of pharmacological action the observed response is likely to be quantal.

The distinction between graded and quantal responses to the action of drugs is not absolute since any quantitative response may be made quantal. Conversely, quantal data may be made numerical by the use of scores, particularly if there are more than two possible outcomes. Hewlett and Plackett (9) have studied the relation between the quantal and graded responses to a drug in terms of a bivariate Normal distribution of graded response and critical graded response.

Since the placebo reaction is usually observed quantally it is appropriate that the corresponding drug action is also discussed in the same terms, although it might be possible in a further investigation to consider a trivariate distribution of graded response and critical graded response to drug action jointly with quantal response to placebo action.

## The effect of placebo reaction on drug action

In clinical trials as reported in the literature, where placebo reaction is considered, the usual design is equivalent, in the simplest case of one treatment, to allocating individuals to a group of placebo reactors or to a group of nonreactors according to the results of tests with placebos. Each of the groups is then separately tested for responses to a given dose of a drug.

In a suitably designed trial the observed proportion of placebo reactors is an unbiased estimate of the proportion in the population. However placebo reactors are not always consistent, for example Lasagna (10) found that 55 per cent of patients receiving the placebo were inconsistent, that is 79 per cent of reactors to the placebo did not react on every occasion they received the placebo. Similarly the response to drug action is not consistent in given individuals; Berkson (11) has compared this variability with the variation in the weights of human beings from time to time and Hewlett and Plackett (9) have stated that this variation does not invalidate the interpretation of drug action on a particular occasion. It follows that if the groups are allocated by means of single observations on the placebo then each group may be contaminated by the other and so the difference in drug action on the two groups is liable to be underestimated.

Moreover even if individuals in a trial are correctly allocated as reactors or not, the difference in response to an active drug will depend on the dose given in general. This is illustrated in *Fig. 1* for a high dose $A$ with a small difference between the groups and for a low dose $B$ with a large difference between them.

It is clear that in order to evaluate drug action in the presence of placebo action it is necessary to study a range of doses, preferably spanning from zero level to a dose corresponding to about 95 per cent response. In view of the difficulty in separating the placebo reactors from the non-reactors it is proposed to analyse data from mixed groups.

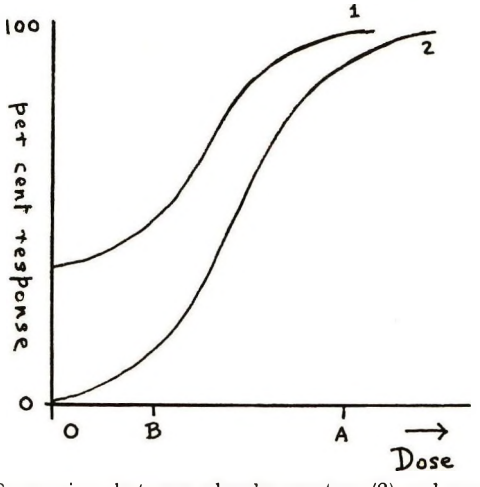


*Figure 1* Comparison between placebo reactors (2) and non reactors (2)

## A MODEL OF PLACEBO REACTION

The population of persons to be treated by drugs for a particular disease or condition may be regarded as composed of a proportion $\Pi_1$ of placebo reactors, I, and a proportion $\Pi_2 = 1 - \Pi_1$ of nonreactors to placebo, II.

A common model for the quantal response of living organisms to given doses, $\chi$, of a drug is the probit or normit transformation (12) defined by

$$\theta = \Phi\left(\frac{\chi - \mu}{\sigma}\right) = \Phi(\alpha + \beta\chi) \qquad \text{I}$$

$$\alpha + \beta\chi = \Phi^{-1}(\theta) = y_p - 5 = y \qquad \text{II}$$

where $\theta$ is the probability of a response to a dose $\chi$, $\Phi$ denotes the standard normal probability integral and $\mu$, $\sigma$ are two parameters which are often transformed to $\alpha$, $\beta$. The empirical probit of an observed proportion p of successes is given by $y_p$ and the normit by y when $\theta$ is replaced by p. The parameters $\mu$ and $\sigma$ are sometimes interpreted as the mean and standard deviation, respectively, of an underlying normal distribution of tolerances to the drug in the population but this interpretation is not essential to the use of the probit method.

The normit model may be extended to the two classes I and II separately so that in the mixed population

$$\theta = \Pi_1 \theta_1 + \Pi_2 \theta_2 \qquad \text{III}$$

$$= \Pi_1 \Phi(\alpha_1 + \beta_1 \chi) + \Pi_2 \Phi(\alpha_2 + \beta_2 \chi) \qquad \text{IV}$$

where $\Phi(\alpha_1) > \Phi(\alpha_2)$.

The quantities of especial interest to be determined by a clinical trial are

i. the proportion $\Pi_1$, of placebo reactors in the population,

ii. the dose, $\chi_0$, such that, say, 99.9 per cent of nonreactors to the placebo will be successfully treated by the drug,

where $\chi_0 = (3{\cdot}09 - \alpha_2)/\beta_2$. V

If these quantities can be estimated satisfactorily then the potency of a drug may be assessed without the complications that a drug may be rejected if the proportion $\Pi_1$, of placebo reactors is high and that the effective dose of an accepted drug may be underestimated for the same reason. Lasagna *et al* (10) have stressed the importance of these complications and have also suggested that the presence of placebo reactors may alter the dose response relationship and so alter the sensitivity of the clinical trial.

If $\beta_1$ is non zero then the placebo reactors are also subject to the pharmacological action of the drug whereas if $\beta_1 = 0$ then the placebo reaction is independent of the dose and there is no advantage in administering the drug to this group.

## Modifications to the model

If the population contains any placebo reactors then $\Pi_1 \Phi(\alpha_1)$ will not be zero and therefore $\Pi_1 \Phi(\alpha_1 + \beta_1 \chi)$ is not negligibly small for all negative values of $\chi$. Although the model may fit the data from a clinical trial satisfactorily it is difficult to interpret the model when $\chi$ is negative. This objection may be overcome by putting

$$\Phi = \Pi_1 \left\{ \tfrac{1}{2} + \int_0^{\alpha_1 + \beta_1 \chi} (2\Pi)^{-\frac{1}{2}} \exp(-t^2/2)\, dt \right\} + \Pi_2 \Phi(\alpha_2 + \beta_2 \chi), \qquad \chi \geqslant 0. \qquad \text{VI}$$

The dose response relationship for many drugs is given by the probit transformation in terms of the logarithm of the dose as metameter (12, p 23). If this is the case then either $\chi$ may be replaced by $\log(\lambda + \chi)$ for some $\lambda > 0$ or $\beta_1$ may be deleted from the model.

The two classes of patient taken separately may not have a linear relationship between the probit and the dose and it may be necessary to consider polynomial forms. In this case it is preferable to use the logit transformation.

$$y = \log p - \log (1 - p) \qquad \text{VII}$$

where p is the observed proportion of successes and then to put

$$y = \alpha + \beta \chi + \gamma \chi^2 \qquad \text{VIII}$$

for example.

The logit transform is equivalent to taking

$$\theta = e^y/(1 + e^y) \qquad \text{IX}$$

where y may be given any one of a number of functional forms, possibly different in the two classes.

## The estimation of the parameters in the model

If the population contains only one homogeneous class the parameters for the probit or normit and the logit models may be estimated by the method of maximum likelihood, by the method of minimum chi-square (13), and by weighted least squares (14).

In the case of a population consisting of two classes the maximum likelihood method is the most appropriate since the other methods lose any computational advantage they may have in the generalization to two groups.

The method of maximum likelihood will be applied to the model given in equation (IV); the various modifications may be treated similarly.

Let the observed frequencies of successes be $r_i$ when $n_i$ individuals are treated and the dose is $\chi_i$, for $i=1,2, \ldots, k$. The likelihood is the joint probability of the observed results taken as a function of the unknown parameters.

The maximum likelihood is found by solving the following equations for the estimates $\hat{\alpha}_1$, $\hat{\beta}_1$, $\hat{\alpha}_2$, $\hat{\beta}_2$, $\hat{\Pi}_1$:

$$0 = \frac{\partial L}{\partial \alpha_1} = \Pi_1 \sum_{i=1}^{k} \frac{r_i - n_i \theta_i}{\theta_i (1 - \theta_i)} Z_{1i} \qquad \text{X}$$

$$0 = \frac{\partial L}{\partial \alpha_2} = \Pi_2 \sum_{i=1}^{k} \frac{r_i - n_i \theta_i}{\theta_i (1 - \theta_i)} Z_{2i} \qquad \text{XI}$$

$$0 = \frac{\partial L}{\partial \beta_1} = \Pi_1 \sum_{i=1}^{k} \frac{r_i - n_i \theta_i}{\theta_i (1 - \theta_i)} Z_{1i} \chi_i \qquad \text{XII}$$

$$0 = \frac{\partial L}{\partial \beta_2} = \Pi_2 \sum_{i=1}^{k} \frac{r_i - n_i \theta_i}{\theta_i (1 - \theta_i)} Z_{2i} \chi_i \qquad \text{XIII}$$

$$0 = \frac{\partial L}{\partial \Pi_1} = \sum_{i=1}^{k} \frac{r_i - n_i \theta_i}{\theta_i (1 - \theta_i)} (\theta_{1i} - \theta_{2i}) \qquad \text{XIV}$$

where $\theta_i$, $\theta_{1i}$, $\theta_{2i}$ are the values of $\theta$, $\theta_1$, $\theta_2$ when $\chi = \chi_i$, respectively, and $Z_{1i} = (2\Pi)^{-\frac{1}{2}} \exp(-(\alpha_1 + \beta_1 \chi_i)^2/2)$ XV

$$Z_{2i} = (2\Pi)^{-\frac{1}{2}} \exp(-(\alpha_2 + \beta_2 \chi_i)^2/2) \qquad \text{XVI}$$

for L = log likelihood =

$$\text{constant} + \sum_{i=1}^{k} r_i \log \theta_i + \sum_{i=1}^{k} (n_i - r_i) \log (1 - \theta_i) \qquad \text{XVII}$$

There are no explicit solutions for equations X through XIV and it is necessary to use a method of successive approximations.

The following equations have been obtained by a generalization of the Bliss and Fisher solution of the probit equations (12). If $\hat{\alpha}_1, \hat{\beta}_1, \hat{\alpha}_2, \hat{\beta}_2, \hat{\Pi}_1$ are approximate solutions then better solutions are obtained by taking

$$\hat{\alpha} + \Delta\alpha_1, \hat{\beta}_1 + \Delta\beta_1, \hat{\alpha}_2 + \Delta\alpha_2, \hat{\beta}_2 + \Delta\beta_2, \hat{\Pi}_1 + \Delta\Pi_1,$$

where $\Delta\alpha_1, \Delta\alpha_2, \Delta\beta_1, \Delta\beta_2, \Delta\Pi_1$ are obtained as the solutions of the equations:

$$\sum \frac{r_i - n_i \hat{\theta}_i}{\hat{\theta}_i (1 - \hat{\theta}_i)} \hat{Z}_{1i} = \Delta\alpha_1 \hat{\Pi}_1 \Sigma \hat{\omega}_i \hat{Z}_{1i}^2 + \Delta\alpha_2 \hat{\Pi}_2 \Sigma \hat{\omega}_i \hat{Z}_{1i} \hat{Z}_{2i} + \Delta\beta_1 \hat{\Pi}_1 \Sigma \hat{\omega}_i \hat{Z}_{1i}^2 \chi_i + \Delta\beta_2 \hat{\Pi}_2 \Sigma \hat{\omega}_i \hat{Z}_{1i} \hat{Z}_{2i} \chi_i + \Delta\Pi_1 \Sigma \hat{\omega}_i \hat{Z}_{1i} (\hat{\theta}_{1i} - \hat{\theta}_{2i}) \qquad \text{XVIII}$$

$$\sum \frac{r_i - n_i \hat{\theta}_i}{\hat{\theta}_i (1 - \hat{\theta}_i)} \hat{Z}_{2i} = \Delta \alpha_1 \hat{\Pi}_1 \Sigma \hat{\omega}_i \hat{Z}_{1i} \hat{Z}_{2i} + \Delta \alpha_2 \Pi_2 \Sigma \hat{\omega}_i \hat{Z}^2_{2i} +$$
$$\Delta \beta_1 \hat{\Pi}_1 \Sigma \hat{\omega}_i \hat{Z}_{1i} \hat{Z}_{2i} \chi_i + \Delta \beta_2 \hat{\Pi}_2 \Sigma \hat{\omega}_i \hat{Z}^2_{2i} \chi_i$$
$$+ \Delta \Pi_1 \Sigma \hat{\omega}_i \hat{Z}_{2i} (\hat{\theta}_{1i} - \hat{\theta}_{2i}) \qquad \text{XIX}$$

$$\sum \frac{r_i - n_i \hat{\theta}_i}{\hat{\theta}_i (1 - \hat{\theta}_i)} \hat{Z}_{1i} \chi_i = \Delta \alpha_1 \hat{\Pi}_1 \Sigma \hat{\omega}_i \hat{Z}^2_{1i} \chi_i + \Delta \alpha_2 \hat{\Pi}_2 \Sigma \hat{\omega}_i \hat{Z}_{1i} \hat{Z}_{2i} \chi_i +$$
$$\Delta \beta_1 \hat{\Pi}_1 \Sigma \hat{\omega}_i \hat{Z}^2_{1i} \chi_i + \Delta \beta_2 \hat{\Pi}_2 \Sigma \hat{\omega}_i \hat{Z}_{1i} \hat{Z}_{2i} \chi_i$$
$$+ \Delta \Pi_i \Sigma \hat{\omega}_i \hat{Z}_{1i} \chi_i (\hat{\theta}_{1i} - \hat{\theta}_{2i}) \qquad \text{XX}$$

$$\sum \frac{r_i - n_i \hat{\theta}_i}{\hat{\theta}_i (1 - \hat{\theta}_i)} \hat{Z}_{2i} \chi_i = \Delta \alpha_1 \hat{\Pi}_1 \Sigma \hat{\omega}_i \hat{Z}_{1i} \hat{Z}_{2i} \chi_i + \Delta \alpha_2 \hat{\Pi}_2 \Sigma \hat{\omega}_i \hat{Z}^2_{2i} \chi_i +$$
$$\Delta \beta_1 \hat{\Pi}_1 \Sigma \hat{\omega}_i \hat{Z}_{1i} \hat{Z}_{2i} \chi_i^2 + \Delta \beta_2 \hat{\Pi}_2 \Sigma \hat{\omega}_i \hat{Z}^2_{2i} \chi_i^2$$
$$+ \Delta \Pi_1 \Sigma \hat{\omega}_i \hat{Z}_{2i} \chi_i (\hat{\theta}_{1i} - \hat{\theta}_{2i}) \qquad \text{XXI}$$

$$\sum \frac{r_i - n_i \hat{\theta}_i}{\hat{\theta}_i (1 - \hat{\theta}_i)} (\hat{\theta}_{1i} - \hat{\theta}_{2i}) = \Delta \alpha_1 \hat{\Pi}_1 \Sigma \hat{\omega}_i (\hat{\theta}_{1i} - \hat{\theta}_{2i}) \hat{Z}_{1i} +$$
$$\Delta \alpha_2 \hat{\Pi}_2 \Sigma \hat{\omega}_i (\hat{\theta}_{1i} - \hat{\theta}_{2i}) \hat{Z}_{2i} +$$
$$\Delta \beta_1 \hat{\Pi}_1 \Sigma \hat{\omega}_i (\hat{\theta}_{1i} - \hat{\theta}_{2i}) \hat{Z}_{1i} \chi_i +$$
$$\Delta \beta_2 \hat{\Pi}_2 \Sigma \hat{\omega}_i (\hat{\theta}_{1i} - \hat{\theta}_{2i}) \hat{Z}_{2i} \chi_i +$$
$$\Delta \Pi_1 \Sigma \hat{\omega}_i (\hat{\theta}_{1i} - \hat{\theta}_{2i})^2 \qquad \text{XXII}$$

where $\hat{\omega}_i = \dfrac{n_i}{\hat{\theta}_i (1 - \hat{\theta}_i)}$

The calculations are iterated until the estimates do not change and a goodness of fit test may be used as a criterion of convergence as in probit analysis (12). The complexity of the calculations makes it necessary to use a digital computer when solving these equations.

## Initial approximations for the solutions

In order to apply the method of successive approximations given above it is necessary to choose suitable starting values. These may be

obtained graphically from an arithmetic probability plot of the observed responses. The case of fifty per cent reactors with the two groups differing only in the value of $\alpha$ which is sufficient to clearly separate the groups is given in *Fig. 2*. It can be seen that the curve for the mixed groups tends to the first line for low dosage and to the second for high dosage. *Fig. 3* illustrates the behaviour when the two groups are not so clearly separated

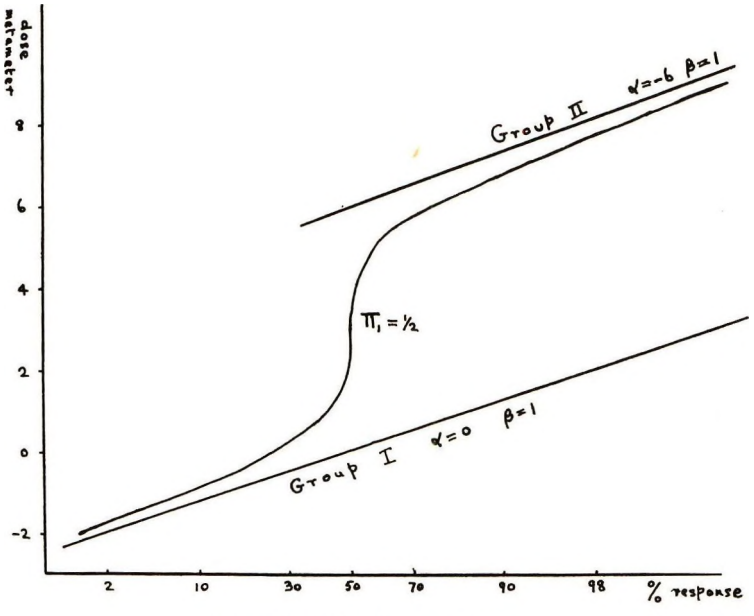


*Figure 2* 50% placebo reactors.

and where the values of $\beta$ are different. In this case also the mixture curve tends to the separate groups at the extreme values of the dosage.

The initial values for $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$ may be obtained by drawing lines through the points on the A.P. plot at the ends of the range of dosage and these values used with an intermediate point to estimate $\Pi_1$. It is advisable to take $\alpha_1 \neq \alpha_2$ and $\beta_1 \neq \beta_2$ since otherwise equations XVIII through XXII become identical with the equations for fitting a single straight line to the A.P. plot and the above method is liable to converge to the wrong solutions.

When the observed responses are plotted the points will be scattered about the population curve and straight lines should be drawn so that the points lie close and slightly below line II and above line I.

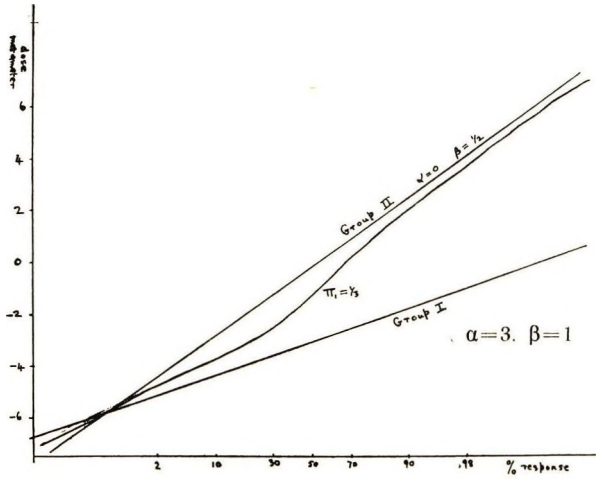


*Figure 3* 33% placebo reactors.

## Tests of hypotheses about the parameters

If it is desired to compare two models for their applicability to a given set of data, for example, to test whether $\beta_1 = 0$ in the model of equation IV the likelihood ratio test (15) should be used. In this test the maximum likelihood estimates of $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$, $\Pi_1$ are used to calculate the likelihood and then the whole procedure is repeated after deleting $\beta_1$ from equations XVIII through XXII. The ratio of the second likelihood to the first may be entered in a chisquare test by taking $-2$ log (ratio of likelihoods), with one degree of freedom in this case.

## Summary

Since placebo reactions are usually quantal and drug responses may be observed quantally if desired, a suitable model for the action of drugs in a mixed population of placebo reactors and nonreactors is given by a linear combination of normal probability integrals with different parameters or by the corresponding logit model. This model allows for the possible inconsistency in placebo and drug response.

The maximum likelihood method is applied to obtain estimates of the parameters in the model but it is expected that the calculation of the estimates will require the use of a computer.

In order to test whether or not the model gives satisfactory evaluations of placebo effects it will be necessary to carry out clinical trials in which

several dose levels of each drug are administered in place of the customary trials in which each drug is used at only one or two levels.

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## *Introduction by the lecturer*

If the two populations are not sufficiently separated it may be difficult to distinguish between the curve for the mixed population and the corresponding straight line approximation in view of the random fluctuations which will occur. However, there is less practical importance in the separation into two groups for this case since a straight line approximation will give results which are very similar to the hypothetical curve. For example, if $\alpha_1 = 0$, $\alpha_2 = -2$, $\beta_1 = \beta_2 = 1$, and $\pi_1 = \frac{1}{2}$, then the following results are obtained by means of a straight line approximation drawn by eye.

| x | | −1 | 0 | 1 | 2 |
|---|---|---|---|---|---|
| percent | exact | 8.0 | 26.1 | 50.0 | 73.9 |
| response | approx. | 9.0 | 24.8 | 50.0 | 75.0 |

The conclusion is that, for a mixture of two distinct groups of reactors and non-reactors with only a small difference in their reaction to drugs, it is still possible to fit the data and obtain satisfactory results.

One characteristic of most clinical trials on placebo reaction in the past is the use of only two, or at most three, dose levels including zero. Inherent in the proposed method is the use of sufficient points in the effective dose range to determine the shape of the curve. The increase in the amount of information from a clinical trial is dependent on a considerable increase in expenditure.

At the moment, a student at Brunel University is writing a programme for this method and also to simulate some data, because one of the things when you introduce a new model is to find out whether it works reasonably in terms of the assumptions. Then you can apply it to real data to determine whether the assumptions hold in practice.

## DISCUSSION

MR. N. J. VAN ABBÉ: Since a range of dose levels, as such, is not normally applicable to cosmetics and toiletries, would it be correct to interpret your model in terms of either (a) a range of concentrations of active constituent in the base, or (b) a range of increasing time intervals for observation?

THE LECTURER: Although I have spoken primarily in terms of drug action, I am really concerned with medicaments and X does not have to be interpreted as the dose; the normal procedure is to use a so-called dose metameter which is often the logarithm of a dose because it so happens that in the majority of situations one gets closer to a straight line on A.P. paper if the log of the dose rather than the dose itself is plotted. X only denotes some characteristic which, when it varies, will produce a variation in the probability of response and it can be the duration of treatment, or the concentration in some base, or both. I suspect that the random fluctuations would be much too large to make much use of a model with several such variables but there is no objection in principle. Instead of X we could use concentration and time of treatment as two variables, providing, of course, that we have a consistent way of determining successes. If we have prolonged treatment then we have to look at its success or failure at the end rather than at stages. Otherwise it is necessary to use sequential analysis and I believe that this was discussed at a previous meeting. There is no attempt in my model to introduce sequential ideas.

DR. K. H. R. WRIGHT: Why is there a difference between $\beta_1$ and $\beta_2$?

THE LECTURER: I based this more or less on a desire to be as general as possible and the most general case would be that the two betas are different. In one paper in the literature it was pointed out that it appeared that placebo reactors did respond differently with respect to the level of dose, but there is nothing to say that one can not restrict the model to equal betas, Another way in which one can restrict the model is to assume that the placebo reactors do not react to the drug at all, in which case we will put $B_1$ equal to zero. All these things are possible and the equations can be modified accordingly. I have suggested that one can formally make this a matter of statistical test as to whether $\beta_1=\beta_2$ or whether one of them is zero. I think that we want some experience with the method before we get too sophisticated. It might be a good idea to start off with $\beta_1=\beta_2$. We are just trying to carry out some clinical trials, observe the proportion of responses and match it by means of a model so that for different values of dose we can predict the response in the general population. Any model which fits our data well can be used for this, irrespective of whether it reflects some underlying physiological or pharmacological action. You can certainly make $\beta_1=\beta_2$, particularly if there is some justification for doing so.

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# Spectroscopic studies of skin *in situ* by attenuated total reflectance

N. A. PUTTNAM and B. H. BAXTER*

**Synopsis**—It has been shown that it is possible by the attenuated total reflectance technique to obtain ir spectra from skin *in situ*. The procedure involved the use of a 'V' shaped ATR crystal into which the side of the hand (the hypothenar eminence) was placed. The main features of such spectra agreed with those reported earlier from transmission studies through thin sections of various tissues, but there were relatively small differences in the ratios of the intensities of the absorptions in the wavelength range 1300-1000 $cm^{-1}$ for different individuals, which were not further investigated.

The application of the ATR technique was extended to show that it was possible to detect the retention by the skin of relatively major components of a hand cream applied to the skin.

## Introduction

Several reports have been published of the application of ir spectroscopy to study various tissues (1–3). These studies have been carried out by transmission through thin sections of the dried tissue, mounted onto silver chloride discs. Absorption studies of skin, in the wavelength range 2 to 15 microns, have however been rather limited (2, 4). The development of attenuated total reflectance (5), by which it is possible to obtain high contrast spectra from surfaces which are equivalent to those obtained by transmission through very thin films, suggested the possibility of obtaining spectroscopic information from skin *in situ*. Such a possibility has also been pointed out by Scheuplein (4), and more recently Hermann (6) has obtained ATR spectra from freshly removed organs, e.g. liver and kidney.

The purpose of the present communication is to show that ir spectra can be obtained from skin *in situ* by attenuated total reflectance, and

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further, that by this technique it is possible to study the retention, by the skin, of components present in products applied to the skin.

## Experimental

The ATR spectra were recorded on a *Unicam* SP 200 infrared spectrometer with increased slit width and gain. The ATR attachment, which is shown in *Fig. 1*, was equipped with a 'V' shaped analysing crystal supplied by *Research and Industrial Instruments Co.* This shape of analysing crystal,

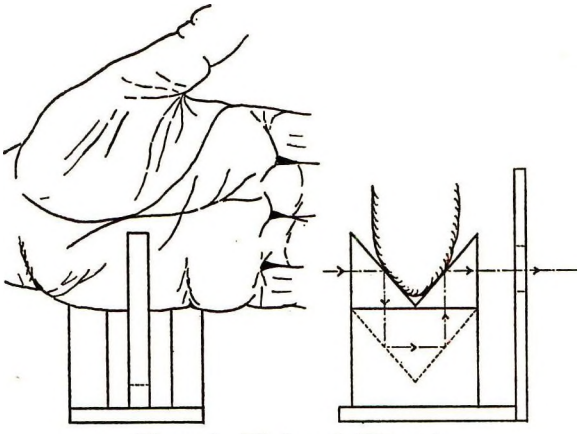

*Figure 1* 'V' shaped ATR unit.

which was originally suggested by Harrick (7), allowed two reflections from the skin with the incident radiation striking the crystal/skin interface at angles of 45°.

In order to record the ATR spectra, the ATR unit was placed in the sample compartment of the spectrometer and the reference beam attenuated to an absorbance value of approximately 0.1 at 1900 $cm^{-1}$, where the sample showed no absorption, since the transmission of the ATR crystal was only about 30%. The fleshy part of the right hand, the hypothenar eminence, was then placed into the 'V' of the ATR crystal using normal hand weight pressure, and the spectrum recorded over the wavelength range 650–5000 $cm^{-1}$.

In *Fig. 1* is shown schematically the ATR unit, together with the position of the hand during the recording of the spectrum.

## Results and discussion

The ATR spectrum shown in *Fig. 2(a)* was typical of those obtained from skin *in situ*. Its main features, which agreed with those reported by Blout and Mellors (1) for various tissues, are:– (i) the broad absorption centred at *ca.* 3400 cm-$^{1}$ due to moisture, which probably obscured any absorptions due to NH groupings, (ii) the relatively weak C-H absorptions at 2950 and 2860 cm$^{-1}$, (iii) the very weak carbonyl absorption at 1740 cm$^{-1}$, due to an ester, (iv) the strong absorption at 1640 cm$^{-1}$, which was due to a combination of a moisture absorption and the polypeptide amide

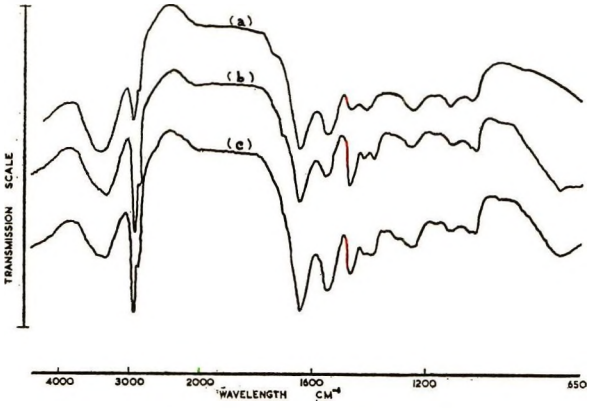


*Figure 2* ATR spectra of skin.

I absorption, (v) the polypeptide amide II absorption at *ca* 1540 cm$^{-1}$, and (vi) absorptions due to $CH_2$ and $CH_3$ groups at 1460 and 1380 cm$^{-1}$.

There were relatively small differences in the ratios of the intensities of the absorptions in the wavelength range 1300 to 1000 cm$^{-1}$ for different individuals and a very significant difference in the intensity of the moisture absorption at *ca.* 3400 cm$^{-1}$. This latter effect was probably due to variations in the perspiration rate between individuals; the former variations have not been investigated further.

The spectrum shown in *Fig. 2(b)* is of the same hand after a hand cream, the major components of which were mineral oil, fatty alcohol, water and stearic acid, had been applied in the usual manner. Comparison of this spectrum with that shown in *Fig. 2(a)* shows a very significant increase in the intensities of the absorptions at 2950, 2860, 1460 and 1380 cm$^{-1}$. These absorptions are due to an aliphatic hydrocarbon grouping and the increased intensities indicated retention of the mineral oils and fatty alcohol by the

skin. There was also the appearance of a weak absorption at 1720 $cm^{-1}$ in *Fig. 2(b)* which was due to the retention of the stearic acid. At the same time there was a marked decrease in the intensity of the moisture absorption at *ca* 3400 $cm^{-1}$. This indicated a fall in the moisture content of the surface of the skin, probably due to the cream exerting a barrier effect.

From *Fig. 2(c)* it can be seen that after the hand was washed with soap and water and dried, traces of the cream were still retained by the skin. This is shown by the persistence of the absorptions at 1720, 1460, 1380 and 1010 $cm^{-1}$.

These results show that the retention of relatively major components of a product by the skin can be demonstrated by this technique. The degree to which a component can be detected depends, of course, on the actual intensities of the component's infrared absorptions. It should be pointed out that when the retention of a component has been shown it is not known whether the component is actually on the surface of the skin or absorbed into the surface layers. From previous studies it is known, however, that the depth of sample from which ATR spectra are obtained is of the order of a few microns.

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# The laboratory evaluation of prophylactic dentifrices

W. H. BULL*

*Presented at the Symposium on "Product Testing", organised by the Society of Cosmetic Chemists of Great Britain in Eastbourne, Sussex, on 14th November, 1966*

**Synopsis**—The need for laboratory methods of assessing dental prophylactic products is discussed and a review of some of the methods used to evaluate fluorine containing toothpastes given. The techniques mentioned include *in vitro* and *in vivo* solubility studies using chemical, physical and electron microscopical techniques to evaluate the action of the products. The advantages of establishing *in vitro* methods which do not depend directly on acid solubility, e.g. some form of artificial mouth, are explained.

## Introduction

From writings which have survived from the Ancient World it is apparent that dental products designed to care for teeth have been made by man from earliest times. In Chinese and Indian records of ca. 3000 B.C. and in Egyptian manuscripts of ca. 1500 B.C. there are references to dental topics including anatomy, treatments with drugs and by acupuncture, dentifrices, etc. The Romans were also versed in such matters and Pliny records the formula for a dentifrice which includes the ashes of oxen hooves, myrrh, burned eggshells, pumice, etc. The formulator of today may not recognise all of these as ingredients which he might want to use in toothpaste but such a mixture is likely to have an abrasive, and therefore cleaning, action on teeth. That cleaning the teeth is desirable for other than social reasons has been shown by Fosdick (1) and Mansbridge (2) who found that children about 12–14 years of age, practising good oral hygiene, i.e.

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regular tooth brushing, experienced a lower caries incidence than those with poor habits in this respect.

The greatest contribution so far made by manufacturers of dental products has been to provide the public with toothbrushes and dentifrices which are reasonably efficient and pleasant to use. However, with increase in knowledge and in sophistication additional attributes, such as sweet breath, healthy gums and freedom from caries, have been claimed. These have been based, usually, upon some scientific fact or hypothesis but their efficiency has not necessarily, in the past, been proved rigorously. A genuine desire on the part of the manufacturer to make his product as effective as possible and the explicit demand of bodies such as the Independent Television Authority that his claims be related to user experience has resulted in industrial organisations undertaking *in vitro* and *in vivo* studies on problems of oral health and hygiene.

The only satisfactory method for testing products designed to improve dental health is a clinical trial. Because the changes achieved by prophylactic agents may be difficult to assess and indeed may be small, large numbers of subjects are required if a statistically significant result is to be obtained. Thus a full scale clinical trial to demonstrate, say, the effectiveness of a caries inhibitory agent will be expensive, not only in terms of time and money, but also in the numbers of highly trained research workers required to conduct it. The thorough screening of potential agents by whatever laboratory means are available or can be devised, is therefore essential if we are to be sure that these expensive, cumbersome and sometimes scarce facilities are used to the best advantage.

The crucial factor, of course, is the selection of appropriate tests, the results of which will correlate with user experience. In this field the results which will permit correlation are acquired only slowly and so at the start of a project the experimenter is left with the problem of selecting tests which, on his knowledge at that time, are likely to be most relevant to the mode of action of the active ingredient. As this knowledge is often scanty, a battery of tests to cover all possible modes of action, whether likely or not, may be the safest way of dealing with this problem.

A further requirement for laboratory methods is the need to test hypotheses which might involve the use of materials which could never be employed in the mouth for reasons of toxicity but which might establish principles and therefore, lead to the development of safe derivatives.

Prophylactic toothpaste means any product which has a specific action

against caries, gingivitis, calvulus, etc., but only products intended to inhibit caries are considered here.

To test the caries inhibiting properties of a product efficiently two requirements are necessary:

(a) Knowledge of the aetiology of the carious process.
(b) Knowledge of the mechanism by which the product is intended to interfere with this process.

If these were known, specific tests could be designed to check that the product does, in fact, perform its allotted function. Although Miller's acidogenic theory of caries is largely accepted as being true, the detail is by no means established.

However, the wide acceptance of the acidogenic theory and the undoubted effect of fluoride in reducing dental decay has led to the popularity of the study of enamel solubility in acid in the investigation of potential cariostatic agents.

## Enamel solubility

In essence the method is simple in that the solubility of enamel in a suitable acid buffer – usually lactate or acetate – before and after treatment with the test material is determined. Practical difficulties arise, however, in that no two teeth seem to behave in the same way even when efforts are made to standardise conditions. The use of ground enamel, prepared by the method of Manly and Hodge (3) was introduced to overcome this problem.

By this technique a large batch of ground enamel, free from fines and graded by sieving to give a standard material, can be prepared and its solubility characteristics in acid buffers determined accurately. A large number of tests can then be run on the rest of the batch of enamel. Further, if a suitable mesh size is selected (e.g. 25–72 mesh) the enamel can easily be separated from toothpaste solids by sedimentation and this avoids tedious filtration procedures. In determining solubility changes, some of the early work was performed on a weight-loss basis, e.g. Manly and Bibby (4), but most studies now depend on a determination of calcium, usually by a microtitration with ethylenediamine tetraacetic acid (EDTA), or of phosphorus, by a colorimetric procedure. Radiotracer techniques have also been used (5).

Numerous criticisms and drawbacks to powdered enamel are apparent, e.g. a large surface area is exposed compared with intact enamel and

agitation during treatment may cause attrition of the enamel pieces with the result that minute particles appear in suspension: these are difficult to remove and affect results. The use of intact enamel would certainly be more realistic.

Of the many studies made to investigate the solubility characteristics of tooth enamel, those of Gray (6, 7) in which he showed that the rate of solution is predominantly diffusion controlled, are probably the most detailed. Using pieces of enamel of constant area and by careful control of the experimental conditions, particularly agitation, he proved that sound enamel from different teeth dissolved in acid buffer at a similar rate, provided the surface enamel was first removed. This is necessary because of adsorption or incorporation into surface enamel of salivary calcium phosphate, salivary organic material and numerous trace elements which tend to reduce its solubility.

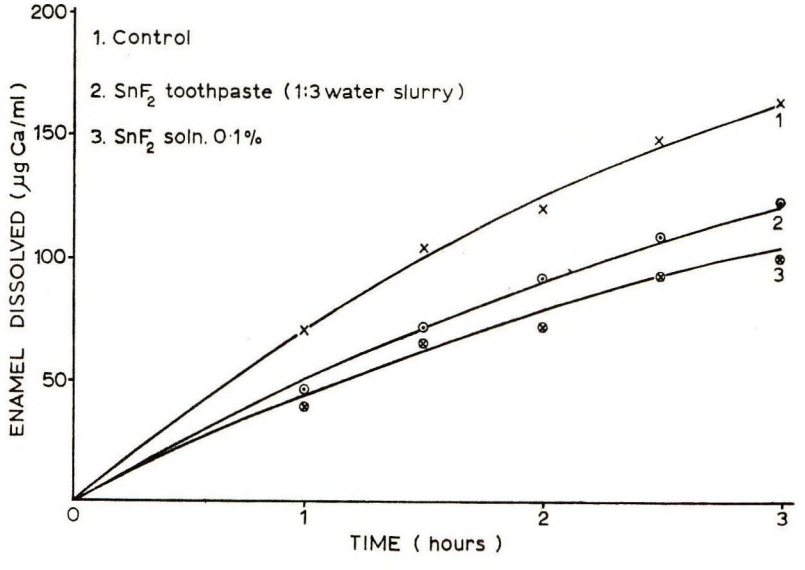


*Figure 2.* Solubility of untreated and treated tooth enamel (continuous immersion in acid).

The equipment shown in *Fig. 1* was constructed to carry out this type of work and it consists of a constant speed motor (1300 rpm) geared to drive six *Perspex* rods. The tubes which hold the acid have a baffle at the bottom of each to limit vortex formation. The apparatus spans a constant temperature water bath so that the lower two thirds of the tubes are

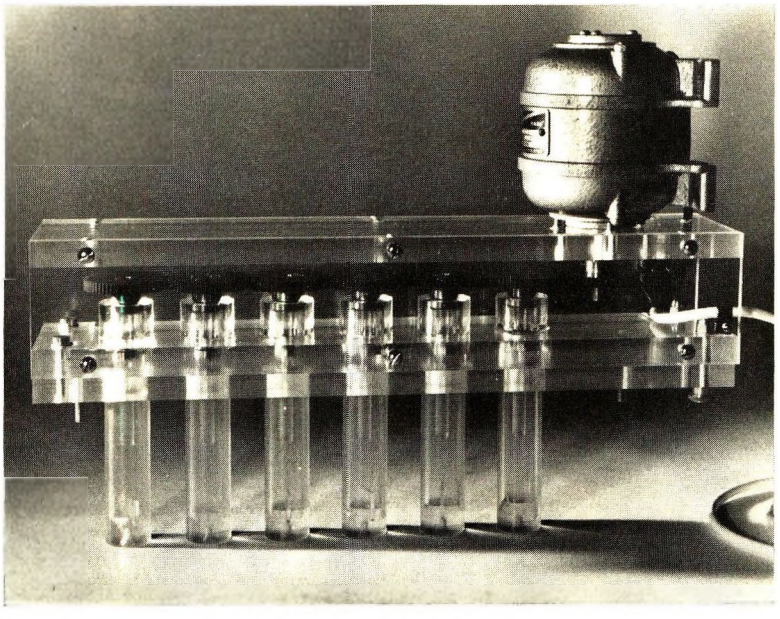

*Figure 1.* Apparatus used to determine the solubility of intact tooth enamel.

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immersed. The pieces of enamel are attached to the ends of the stirrers with blue inlay wax, only the required area of sound enamel being exposed.

The results obtained with the equipment are shown in *Fig. 2*. The upper curve is that for untreated enamel while the lower one, indicating a lower solubility rate, is for enamel treated with a 0.1% solution of stannous fluoride. The third line represents the effect of a stannous fluoride toothpaste.

A slightly different technique, also mentioned by Gray, is that of immersion of a tooth or piece of tooth in successive aliquots of acid buffer.

*Fig. 3* is reproduced from Gray's paper and it is apparent that the protection provided by $SnF_2$ survived only the first two exposures to acid.

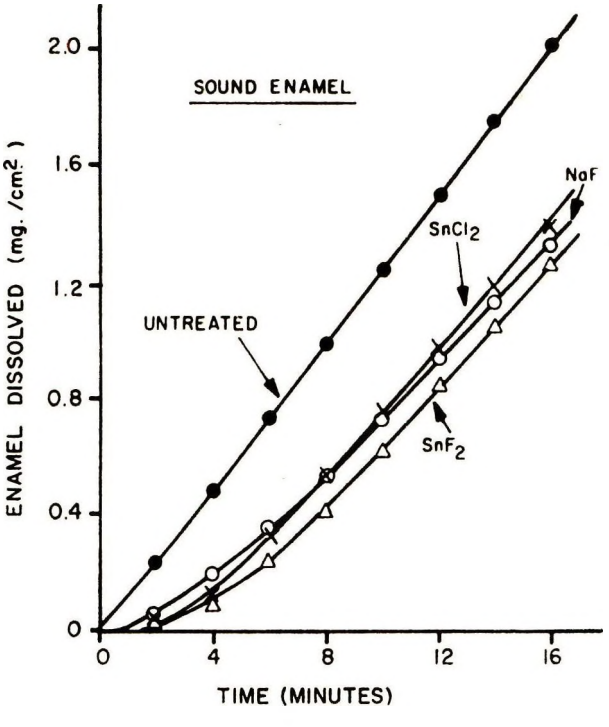


*Figure 3.* Solubility of untreated and treated tooth enamel (successive immersion in acid).

Thereafter the slopes of this and the control sample become parallel, indicating that the rates of solution are the same and that the protective effect of $SnF_2$ is confined to the surface of the enamel.

## Electron microscopy

The application of electron microscopy to the assessment of dental products provides a fascinating insight into what is happening on the tooth surface although interpretation of the results requires considerable care.

*Fig. 4* shows a polished tooth surface which, in this case, is rather featureless except for surface scratches. *Fig. 5* is of a similar tooth after exposure to 0.2M lactate buffer (pH=4) for 15 min and shows a roughened, etched appearance. The enamel prisms are very obvious. *Fig. 6* shows a tooth surface, half of which was masked during immersion in sodium fluoride solution (0.2%: pH=4). The masked area can be seen to be similar to the polished surface while the treated area is covered with a deposit of calcium fluoride crystals. This is undesirable as it means that erosion of the enamel is taking place. This is apparent in *Fig. 7* where the sodium fluoride treated area has been brushed to remove the calcium fluoride and reveal the etched region below. Treatment with stannous fluoride on the other hand (*Fig. 8*) results in no apparent change in the enamel structure and exposure to lactate buffer now has apparently little effect (*Fig. 9*). However, if this surface is now brushed with a toothbrush and re-examined it is seen that etching has occurred (*Fig. 10*). Thus stannous fluoride has deposited a film, probably of hydrated tin oxides, which is quite adherent to the tooth because it can still be found after several replicas have been taken from the one area but which is permeable to acid. *Fig. 11* shows a tooth in which part of the enamel was masked while a stannous fluoride treatment was given to the exposed area. The mask was then removed and the whole surface exposed to lactate buffer. The micrograph was taken by a reflectance technique, i.e. the elctron beam was directed at an angle onto the enamel surface and the reflected electrons produced the micrograph. There is obviously a marked difference between the etched surface and the stannous fluoride treated surface. The critical brushing experiment has not been done so that it cannot be said with certainty that the 'cliff' effect is not due entirely to the film of hydrated tin oxides; the other chemical evidence strongly suggests that this film reduces the rate of penetration of the acid to the tooth surface.

## Fluoride in enamel

The pattern of distribution of fluoride in tooth enamel has been studied by a number of workers. Early results were based on analyses performed

on bulked enamel which suggested that the average fluoride content of enamel is low but more refined techniques used by Jenkins and Spiers (9), Brudevold *et al* (10, 11), Spiers (12), Weatherell and Hargreaves (13, 14) and Mühlemann (15) have proved that fluoride acquired by enamel from natural sources is concentrated in the outer layers.

The technique devised by Weatherell and Hargreaves is elegant and worthy of description. A tooth is covered with nail varnish except for the particular area on which fluoride is to be determined. This can conveniently take the form of a rectangular area on, say, the labial surface of an incisor. A small spot of nail lacquer is also placed within the test area.

The area is then dipped in strong (6N) perchloric acid for a predetermined time (e.g. 5 sec), rinsed with a drop or two of water and dried. A second spot of nail varnish is applied beside the first one and the etching process repeated in fresh acid. This sequence of masking and etching is repeated as often as desired. A section cut through the row of spots gives a histological picture from which can be derived the depth of each layer from the surface. The amount of fluoride in the acid solutions is determined colorimetrically using the SPADNS reagent of Wharton (16), provided the fluoride is first separated from the components of the tooth mineral also present. This can be accomplished very neatly using the Conway diffusion principle.

Fluoride in strong perchloric acid will volatilise as hydrogen fluoride and can be absorbed quantitatively in an alkaline medium. Although numerous variations have been proposed, the original Conway design of diffusion cell, provided it is made from an unreactive material such as polyethylene or better, polypropylene is probably the most convenient apparatus in which to carry out the diffusion. Sodium hydroxide solution (about 1.5N) is placed in one well and the acid fluoride in the other. The lid, suitably greased to prevent leakage, is fitted on top and the unit is then incubated at 60°C for 24 hr during which time the fluoride is transferred quantitatively to the alkaline reagent. The colorimetric procedure can then be carried out on this solution. The amount of fluoride found can be related to the amount of enamel dissolved at each stage either by determining phosphorus in the acid residue from the diffusion stage or by weight loss.

The results quoted by Weatherell show that 500 to 2500 ppm of fluoride may be present in surface enamel and that this dwindles to about 50 ppm in the deeper layers. There is a nice correlation in Weatherell's results between depth of the etch as seen on the photomicrograph and fluoride content (*Fig. 12*).

This technique is obviously applicable to the study of the possible uptake of fluoride by enamel from a fluoride-containing product and some preliminary results which were obtained to prove the feasibility of the method can be described.

Upper central incisors free from caries were selected and cut in half, one half to act as control and the other as test. The test halves were attached to the circumference of a disc fitted to the end of a stirrer spindle. When the stirrer motor was switched on, the test surfaces were arranged to brush lightly against the tips of toothbrush bristles set in the bottom of the beaker which contained the test solution. In the results which follow, the treatment was continued for 24 hr in an effort to ensure that some fluoride would be incorporated in the enamel.

A light brushing with distilled water was given before the fluoride determination was carried out as described above. Three consecutive 10 sec etches were given and the fluoride in each determined. In some experiments

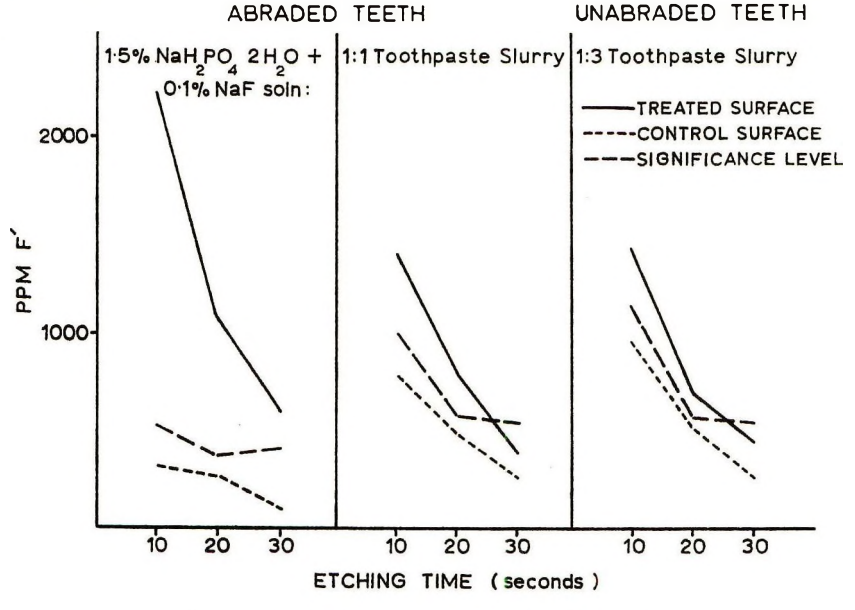


*Figure 13.* The uptake of $F^-$ on enamel surfaces.

the surface of the teeth was abraded to remove the layer of high fluoride content and so help to achieve more consistent results. Typical results, the average of six teeth per treatment, are shown in *Fig. 13*. The treatment in the first experiment was with a sodium fluoride/sodium phosphate

solution as suggested by Brudevold (17). This is obviously more effective than when incorporated in a dentifrice though even then there is a significant uptake compared with the controls. The dotted line indicates the fluoride level above which the test teeth have acquired a significant increase in fluoride.

### Tin uptake by enamel

A similar type of experiment can be performed to demonstrate the presence of tin derived from stannous fluoride preparations though the analytical procedure is not so simple. The technique of electron probe microanalysis is applicable and the results can be obtained either as a photograph in which the atoms of tin appear as white spots on a dark ground or as a recorder trace obtained as the electron beam traverses the selected area. An example of the latter is shown in *Fig. 14.* A polished

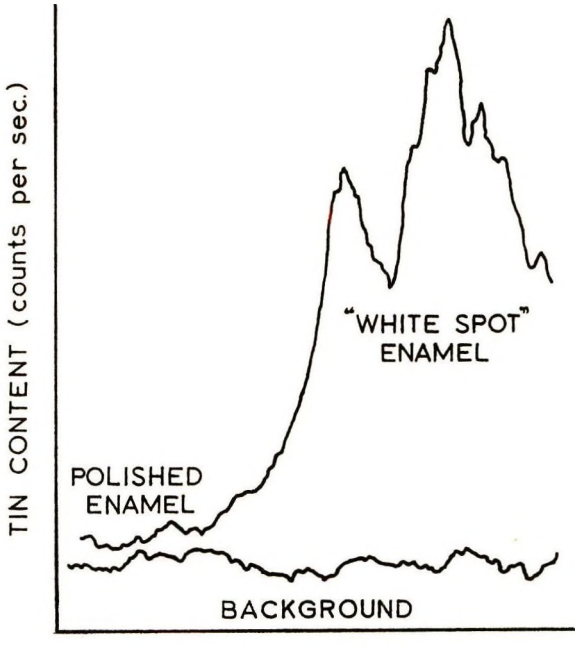


*Figure 14.* Tin uptake on polished and on etched enamel.

tooth surface was etched with dilute acid to produce a white spot. A stannous fluoride treatment was given and, after rinsing, the area was

scanned. The peaks on the trace indicate the presence of tin on the tooth surface and it can be seen that greatest uptake occurs in the white spot area. This may well be due to the increased surface available on this etched region rather than to any specific affinity of white spot enamel for tin.

## *In vivo* methods

All the tests mentioned are test-tube experiments and however much the conditions are varied in an effort to test the permanence of the treatment, they give no unequivocal indication of what will happen in the mouth.

Walter (18) was the first to describe a feasible technique which went some way to answering this criticism, in that during the period of the trial, the treated teeth were exposed to all the dynamic conditions normally present in the mouth. His test was again dependent upon solubility changes of tooth enamel but on humans it was conducted as follows:

A 2mm diameter filter paper disc impregnated with an acid base indicator and with 2μl of 0.3N HCl, was placed on the selected tooth surface after it had been dried with a tissue. The time taken by the indicator to change colour (Colour Reaction Time) is a function of the tooth's 'resistance' to the acid attack. In a test using a panel of school children Mühlemann (19), using this method, reported the effectiveness of certain amine hydrofluorides.

In our hands this test gave a positive result for a topical stannous fluoride treatment but even with half the amount of acid used by Mühlemann it had a (literally) marked effect on the subjects' teeth and had to be abandoned.

An alternative method was then developed in which a weak acid was used in place of hydrochloric acid and the change in pH was recorded after a predetermined time. The technique is described by Middleton and Holmes (20) and Morley and Holmes (21), and depends upon clamping a well to an upper central incisor so that leakage cannot occur (*Fig. 15*). With the well clamped in position, 0.1 ml acetic acid (pH=3.4) is added from a syringe and is stirred by a stream of bubbles from the air line during the 3 min of the test. At the end of this time the air stream is stopped and a sample of the acid is sucked into the microcapillary glass electrode for pH measurement. This is a rather elaborate micro-electrode supplied by Messrs. Pye of Cambridge.

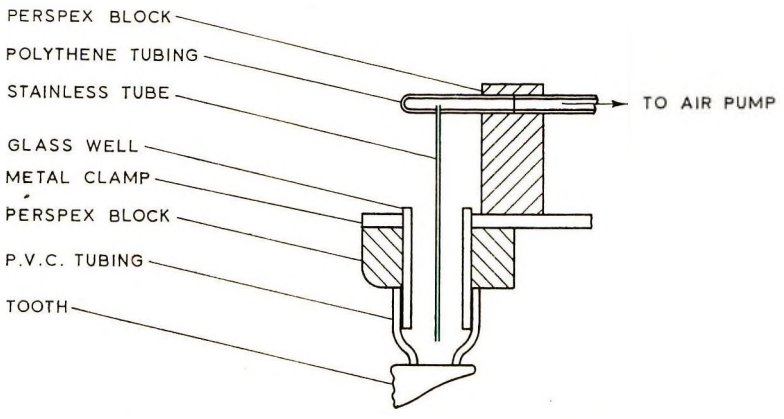


*Figure 15.* Apparatus used to maintain a weak acid solution in contact with a tooth surface

The results obtained from a panel of about 50 people, who used a proprietary stannous fluoride toothpaste and a control, are illustrated in *Figs. 16–19*. *Fig. 16* shows the design of the panel test. All used the control paste first and, after 5 weeks, base line measurements were made. Half the subjects were then given the test paste while the remainder continued with the control paste and measurements were taken after 4 to 8 weeks. The pastes given to the two groups were then interchanged and after a further 4-6 weeks, final measurements were made.

The results for the subjects on the sequence–control, fluoride, control are given in *Fig. 17* and it is evident that the rise in pH is much less after the use of the test paste and that after a further spell on the control paste, there is a trend towards the original values. For those on the sequence-control, control, fluoride (*Fig. 18*) the trend continues towards small decreases in rise of pH at each stage. The overall effects can be summarised in graphs of the population distribution of pH values (*Fig. 19*). The shift in the peak populations towards lower pH values denotes a reduction in apparent enamel solubility. Statistical analysis proved these differences to be significant.

Herd and Overell (22) report results obtained for a monofluorphosphate paste using a somewhat similar well to maintain acid in contact with a tooth but their estimate of the amount of enamel dissolved was based on phosphorus determinations. They claimed a significant reduction in enamel solubility after one brushing with the test paste.

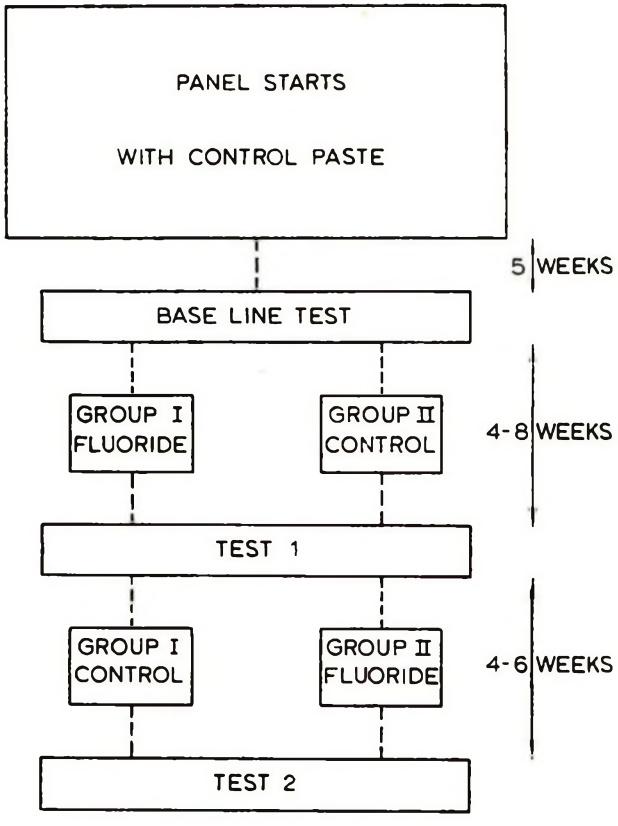


*Figure 16.* Design of panel trial.

Most of the tests mentioned depend upon assessing changes in enamel solubility. There are probably numerous reasons why this principle is so popular, e.g. Miller's acidogenic theory of caries emphasises the destructive role of acid; naturally occurring fluoride affects the structure of teeth – excessive amounts cause mottling – and these teeth are more resistant to caries. However, some agents have been found which produce a marked reduction in solubility, e.g. lead, indium and zinc, but which have no effect on caries (23). The ability of fluoride to reduce enamel solubility is well documented, but the really critical caries inhibiting effect may not be this at all. Indeed, Jenkins (24) quotes a number of ways in which fluoride could inhibit the carious process, viz:

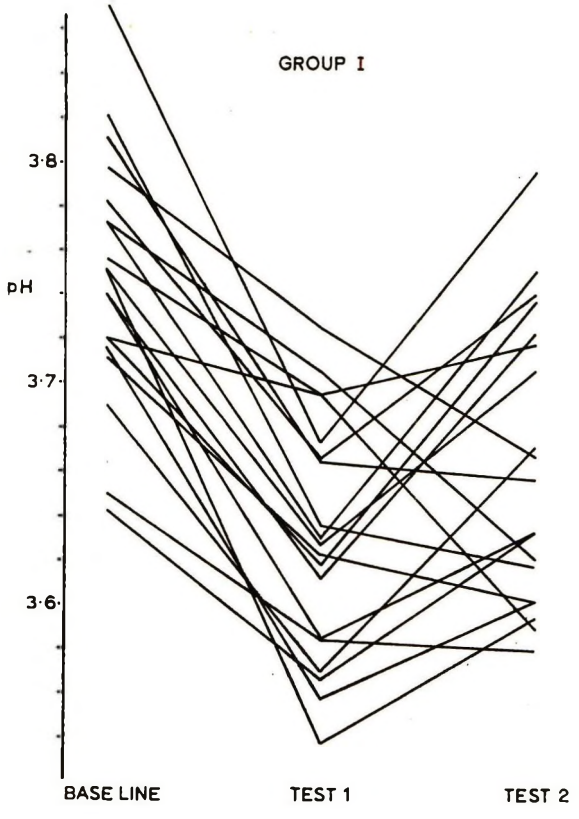


*Figure 17.* 19 persons on sequence—control, fluoride, control.

1. Cause morphological changes in teeth resulting in shallower fissures.
2. Slow decalcification and accelerate recalcification.
3. Promote apatite formation and improve crystallinity.
4. Inhibit the production of polysaccharide and acid by plaque bacteria.

The second and fourth processes could possibly be achieved by a topical application. That bacteria are essential to the carious process is known from studies on germ-free animals and so this is obviously an important area to consider when techniques are being selected to evaluate cariostatic agents.

The need for a method to assess the effect of agents on the carious process as a whole is shown by the various attempts which have been made

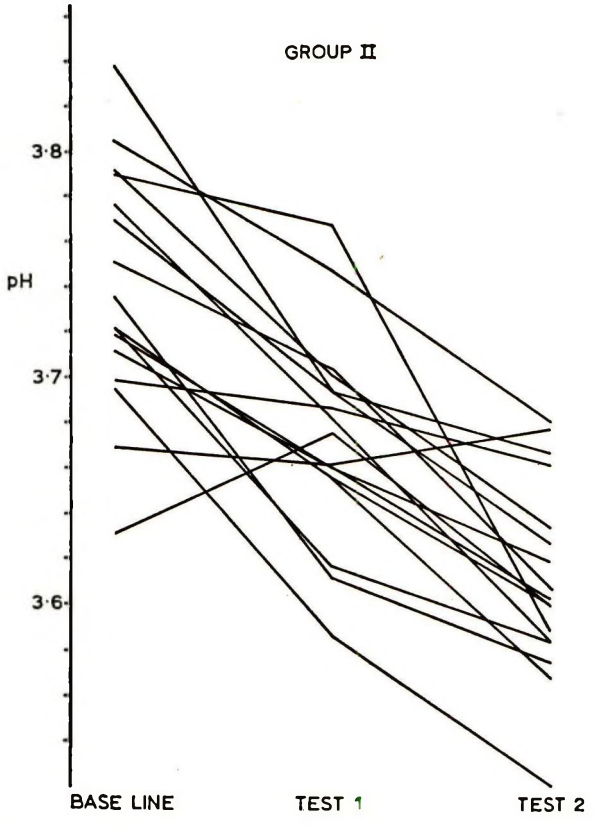


*Figure 18.* 17 persons on sequence – control, control, fluoride.

to produce caries under controlled conditions. Von der Fehr (25) described a technique in which he induced lesions in premolars which were to be extracted for orthodontic reasons. The buccal surface of the tooth was pumiced, rinsed with water and dried. One half of the surface selected as the control was covered with wax while the other was treated with the test solution. The wax was removed and a gold onlay cemented in place over the test and control areas in such a way as to leave a space about 0.5 to 1 mm wide between the enamel surface and the gold. The teeth were extracted after periods of three to five weeks and the test areas examined micro-

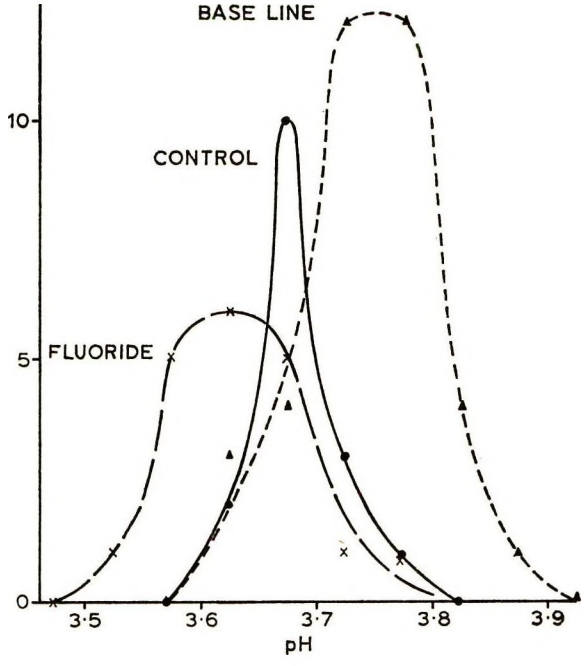


*Figure 19.* pH distribution of fluoride and control users.

scopically and macroscopically to assess the degree of carious attack. This seems a valid method but is impracticable for studying large numbers of test materials.

## The artificial mouth

Numerous attempts have been made to produce carious lesions in an artificial mouth system for the purpose of studying the carious process. Pigman *et al* (26) quote some twenty references, the first dating from 1878, to work involving the formation of carious lesions in 'a laboratory apparatus'. Some report merely general decalcification while others claim to produce lesions identical to natural ones.

Such a system is attractive as an assessment technique in that the overall effect of an agent, whether it be on enamel structure, enzymes or bacteria, could be assessed without understanding in detail the mechanisms involved. Pigman (27) has developed an artificial mouth apparatus in which a nutrient medium is fed to a culture of oral organisms in contact

with teeth. The medium was dripped into a two part acrylic box which held the teeth in the lower compartment. A piece of muslin impregnated with plaque derived from saliva was in contact with the teeth while the upper part of the box maintained a pool of nutrient in contact with the plaque and teeth. In this paper Pigman used enamel hardness measurements to assess the effect of topical treatments but other workers, for example Francis and Meckel (28), have reported the production of lesions in this type of equipment.

## Conclusion

The techniques mentioned have been used to assess products containing fluorides which became popular as a topical treatment because of the observed reduced caries experience of people living in areas with fluoride in the water supply. This effect was observed and exploited before the mechanism of its action was fully understood and it is possible that, based on the suggestions of Jenkins referred to previously, biochemical or bacteriological tests would have been at least as appropriate as the chemical ones described.

Any new caries inhibiting agents which may be proposed are likely to be based on knowledge derived from a study of the carious process. The point at which they are intended to attack this process will be known and so specific tests will be defined to study their efficiency. The laboratory assessment procedures will consequently be realistic so that, in the clinical trial which will still be necessary, these expensive and scarce facilities will be used to test only those materials likely to have a marked beneficial effect on dental health.

## Acknowledgements

I should like to thank Mr. A. Saxton for the use of the electron micrographs, some of which are to be published shortly in another journal.

I also gratefully acknowledge permission by the following, to reproduce illustrations—

*Figure* 3 – The editor, *Journal of Dental Research;*

*Figure* 12 – The editor, *Advances in fluorine research and dental caries prevention;*

*Figure* 15 – The editor, *British Dental Journal.*

(*Received: 20th February 1967*)

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## DISCUSSION

MR. N. J. VAN ABBÉ: You mentioned that you use pH measurements rather than phosphate determinations to assess *in vivo* tooth solubility. I wonder how far the spread of results that you have, which seemed to be unaccountable, was due to trying to measure pH values accurately on small quantities of weak acids.

THE LECTURER: We are fairly satisfied that, with attention to detail, we can achieve a good degree of reproducibility in determining pH values. We do, of course, rely on our statistician to show that the differences reported are statistically significant.

MR. N. J. VAN ABBÉ: One has seen an occasional statement that the case hardening effect due to fluoridation makes it more difficult to recognise a cavity with the probe. Is there any suggestion that this can be true in the case of a fluoride toothpaste?

THE LECTURER: The recognition of what is a carious lesion is a vexed one in the context of clinical trials and has been the subject of much learned discussion. The phenomenon of 'reversals' in clinical trials shows that the development and recording of lesions is not a simple matter. If the suggestion were true then the apparent benefit derived from fluoride toothpastes would be inflated.

MRS. H. BUTLER: You were suggesting that fluoride in water prevented dental caries. I wondered if you were referring to work that has been done since the war in areas in this country where fluoride has been added to water or whether you were basing it upon areas known to have natural calcium fluoride in the water.

THE LECTURER: My understanding of the position is that if fluoride is present at the right concentration in the water supply irrespective of whether it occurs natrually or is added, then a reduction caries incidence will be obtained in that area.

MRS. H. BUTLER: I should like to counter by saying that before the war in areas in this country where fluoride occurred at more than 5 ppm there was evidence of very bad dental caries in the children, and that it might be a bad thing to perpetuate this fiction. I should like to know what positive results have been obtained in areas where 1 ppm has been put into the water.

THE LECTURER: A fairly high incidence of mottling of tooth enamel would be expected with 5 ppm of fluoride in the water supply. I have not read reports which suggest that this would lead to the high level of caries you mentioned. A British mission studied several areas in the U.S.A. where fluoride occurred naturally or was added to the water supply. They found no health hazard associated with the presence of about 1 ppm of fluoride in water while the caries rate in teeth which had developed in this environment was much less than in non-fluoride areas. The Ministry of Health report on the five year studies conducted in this country records a marked improvement in the condition of teeth which had developed during these experiments and confirms that 1 ppm of fluoride, whether natural or added, is nothing but beneficial.

MR. R. CLARK: Extensive data has been collected in the U.S.A. in epidemiological studies where fluoride occurs naturally. The results are very significant and show that where 1 ppm or thereabouts of fluoride occurs in water, the incidence of caries is some 60% less than that where fluoride falls to about 0.2 ppm. This has been confirmed by extensive studies, again in America, where fluoride has been added artificially to water previously low in fluoride. When you have a high level of fluoride in the water supply you get tooth mottling and things like that but this is not the intention of those who want to fluoridate water. The evidence is incontrovertible.

DR. K. H. R. WRIGHT: Do you have any evidence of improved mechanical properties of an enamel surface after treatment with a fluoride toothpaste?

THE LECTURER: I have no results of my own on this topic but there are references in the literature to the remineralisation of enamel which can occur, or rather which is accelerated, in the presence of fluoride. This has been shown by hardness measurements on partly demineralised enamel. The hardness increased after exposure to fluoride solutions.

DR. K. H. R. WRIGHT: How much more effective is a toothpaste than a simple mouthwash?

THE LECTURER: I have no knowledge of *in vivo* tests which would answer this. In *in vitro* tests, fluoride is more effective in a simple solution in reducing enamel solubility than when mixed with other ingredients, e.g. toothpaste constituents.

MR. J. M. BLAKEWAY: Have electron microscopic studies been made on the effect of monofluorophosphate on enamel on the same basis as for stannous fluoride and sodium fluoride?

THE LECTURER: Not by us. The electron micrographs (*Figs. 4–11*) were taken at an early stage of the development of a fluoride toothpaste. We have done chemical studies on the action of monofluorophosphate on hydroxyapatite which showed that the fluorophosphate ion seems to be incorporated into surface enamel.

# Book reviews

## OIL, DETERGENTS AND MAINTENANCE SPECIALTIES. Vol. 1. B. Levitt. Pp. vii + 280 + Ill. (1967). *Chemical Publishing Co., New York.* $13.75.

The emphasis of this book is in the basic practical application of knowledge acquired by the author over many years of experience in the manufacture of oils, soaps and detergents. The book in many ways resembles E. T. Webb's classic work *Soaps and Glycerine Manufacture* in which the same subjects are presented in a similar lucid manner devoid of excessive theoretical and technical discussion. It is very easy in this type of informative writing to become sidetracked by the hundreds of excellent scientific specialist papers which can only distract without satisfying the reader's interest in the main issues involved. The author has fortunately avoided these pitfalls, and each chapter is dealt with in a masterly and facile style, which can readily be followed by semi-technically trained personnel. The book is divided into nine chapters comprising the Introduction, Animal Fats and Oils, Vegetable Fats and Oils, Fatty Acids, Fatty Alcohols, Glycerol, Surfactants and Surface Activity, Production of Fats and Oils, Soap Manufacture, Synthetic Detergents, and finally Analysis of Oils and Detergents.

The second chapter deals concisely with the rendering of animal fats for soap making and the refining of fish oils such as herring, menhadden, salmon, pilchard and sardine oils for use in paint, linoleum and printing ink industries. The therapeutic values of the fish liver oils, followed by butter, margarine, lanolin and its derivatives, sperm oil and spermaceti and their applications are fully discussed.

The third chapter deals in like manner with about twenty of the better known vegetable oils and fats finalised in a chemical and physical constants table of 76 oils and fats each with their sources of origin.

Fatty acids, alcohols, and glycerol, their synthesis, characteristics and uses, are treated effectively in chapter four, followed by surfactants and surface activity and the importance of biodegradability of detergents in chapter five.

The production of fats and oils solvent extraction, continuous centrifugal refining, Solexol, Furfural, and Emersol processes, followed by a description of hydrogenation, sulphonation, the fat splitting processes and the production of glycerol, concludes an interesting and factual sixth chapter.

Perhaps the most important chapters in the books are the 7th and 8th which deal fully with the various aspects of soap and detergent manufacture which hold a mass of facts, figures and formulations most useful to the technician or chemist working in these fields. L. CHALMERS.

## AEROSOL SCIENCE. Editor: C. N. Davies. Pp. xviii + 468 + Ill. (1967). *Academic Press, London, New York.* 115s. $10.50.

It must be stated at the outset that the average cosmetic chemist will not find in this book an easy, intelligible introduction to the science of aerosols that will help him to bridge the gap between the technology and the fundamental science. "Aerosol Science" makes few concessions to a popular or even technologically based approach. It is a thorough, well edited account of the present state of this branch of colloid science. The book was almost devoid of direct reference to the more familiar manifestations and applications of the aerosol state, for example, fogs, smokes and atomizers were not discussed in practical detail.

Nevertheless, "Aerosol Science" is a very able compilation of up-to-date information, written by the leading practitioners of this subject. Physicists, colloid chemists and chemical engineers can derive considerable enlightenment from a study of the various chapters of this work. The first chapter of this book, dealing with the generation of aerosols is worthy of study of those practising a variety of scientific and technical disciplines. Chapters V and VI, which deal in detail with the transport influences on aerosol particles, namely thermophoresis, diffusiophoresis and photophoresis will appeal to colloid chemists. An excellent chapter by R. G. Doorman on the filtration of aerosol particles would repay study by chemical engineers and others concerned in the processing and classification of powders. Chapter IX, on the adhesion to surfaces of particles is of general interest, if only because of the descriptions of elegant techniques for the measurement of this property. These techniques, incidentally, owe much to Tabor and his school at Cambridge.

To conclude, many industries which are more science-based than our own could derive immediate value from "Aerosol Science". Is it too much to hope that in the near future we may find available to us "An Introduction to Aerosol Science" in whose pages aerosol science and aerosol technology would be more strongly integrated?

F. J. MOTTRAM.

## MODERN PRINCIPLES OF ORGANIC CHEMISTRY. J. L. Kice and E. N. Marvell. Pp. ix + 449 + Ill. (1966). *Collier-MacMillan, London.* 70s.

This claims to be the "first brief text" presenting a flexible mechanistic treatment of modern organic chemistry. Brevity it certainly has: the authors cast a wide meshed net over a large proportion of the high seas of natural and synthetic organic substances, sufficiently superficially to require only 420 large-type pages; there is also one final chapter encompassing the diagnostic importance of the spectral properties of molecules.

An unusual feature of the presentation is the liberal use of brown type to emphasize key substituents or direct attention to particular reacting species or the nature of rearrangements. One must also praise the clarity of the structural formulae, wherein atoms other than carbon and hydrogen are set in heavy black type against the finely drawn hexagons, etc. There is also use of a paler brown background to set off, somewhat luridly, important tables or reaction schemes. Each chapter carries its own crop of graded problems although the answers are left to the class tutor.

The pattern of the book, admittedly reminiscent of the inestimable 'Cram & Hammond', is at first to introduce the necessary principles of nomenclature, structure and key reactions, before passing to the fundamentals of mesomerism, stereochemistry and structure correlation with reaction rate and equilibrium; these 10 chapters provide a reasonable framework for developing a general analysis of organic reactions. In the remaining five chapters the preceding treatment is applied to various classes of relatively sophisticated natural and synthetic products: carbohydrates, peptides, heterocyclics, terpenes and steroids, and macromolecules (including synthetic polymers). These chapters frequently degenerate into tables of illustrative formulae of arguable paedogogic value but the examples chosen are in many cases undeniably topical. One welcomes a brief but readable account of the principles, as currently held, of the theory of acetate-mevalonoid biogenesis of isoprenoids.

The ubiquity of contemporary deployment of spectrometric investigations has prompted a further chapter which briefly explains the absorption of electromagnetic radiation and then refers to frequencies for characteristic vibration (ir) and electronic (uv) transitions – the treatment of the latter being restricted to $\pi$-conjugated systems. There are three simple examples of diagnosis from ir spectra. Surprisingly the presentation of nmr spectrometry is rather less terse than for the more conventional techniques: chemical shifts and first order spin-spin coupling are briefly explained and exemplified. However, it is unfortunate that the chemical shifts are exclusively discussed in terms of delta ppm, without even a reference to the alternative (and now preferred) use of tau (10 – delta) units. Mass spectrometry and its potential development are merely mentioned in two valedictory paragraphs – but then it is difficult to see what could have been given in a book of these limited dimensions and scope.

The book should be judged as it is intended to be used, that is as a half-year introductory course for students who subsequently may proceed to a variety of life-science Honours courses. Given good tutorial support, orientated to the particular slant of the freshmen concerned, this text should provide a basic understanding of structural features and reaction driving forces, without overburdening memory with a welter of data in many cases unlikely ever to be retrieved. Honours chemistry schools would legitimately look elsewhere. G. F. PHILLIPS

## INFRARED SPECTRA OF ADSORBED SPECIES. L. H. Little. Pp. xii + 428 + Ill. (1966). *Academic Press, London/New York.* 100s. $16

Books on ir spectra are numerous but those devoted to adsorbed species are few and far between. A number of papers relating to this topic have been published and this book with its 700 odd references and supplementary chapters by A. V. Kiselev and V. I. Lygin is basically a review.

The primary appeal will be for scientists interested in catalysis and for metallurgists and geologists but the field covered in the book is very wide.

There is extensive discussion of (a) the adsorption of gases and hydrocarbons onto metals and metal oxides, the mechanism of catalysis and differentiation between chemisorption and physical adsorption, and (b) surface hydroxyl groups and the general adsorption of molecules within clays and zeolites.

There are other chapters covering early Russian work and studies of the pertur-

bation of physically adsorbed molecules. Relatively little space is devoted to experimental techniques. These are well described but there should perhaps have been more emphasis on the importance of ordinate expansion for weak absorbers and ATR for surface studies on non-metallic substrates. The techniques are a limiting factor particularly if the studies are to be extended to natural surfaces.

There is no direct reference to any application in the toiletry or cosmetic field but where the hydrogen bond is involved there must be a link, however oblique, with the cosmetic research chemists' interests.

The book is quite readable but somewhat heterogeneous in presentation. It is a useful review and provides background references for a topic which is likely to become important in the future. D. M. GABRIEL.

## INTERNATIONAL TABLES OF SELECTED CONSTANTS 15: SESQUITERPENOIDS. G. Ourisson, S. Munavalli and C. Ehret. Pp. 70 + xxx. (1966). *Pergamon Press, Oxford.* 90s.

At some time or another, most of us – particularly those working in perfumery – require information on materials of natural origin. High on the list are the sesquiterpenoids since they are relatively widespread and also rather complex materials. Days, weeks or even months may be spent searching for information on a specific material. Having found something one has to estimate its accuracy and value, or the relative value of conflicting pieces of information.

Here is a book which does most of it for us, saving countless hours of often fruitless searching. Information on nearly five hundred materials is recorded in tabular form. This information includes melting and boiling point, density, refractive index, specific rotation and wavelengths of the absorption peaks in the uv. Bibliographica references are given to all the data together with references to further data on synthesis, on uv, ir, Raman and nmr spectra, on rotatory dispersion or circular dichroism, and on mass spectrum, and X-ray structure. Facing each table are structural formulas for the materials included in the table, where these are known, and the number which are not known seems now to be remarkably small.

Basically the materials are arranged according to the number and type of their carbon ring structure although the authors found this to be not entirely satisfactory and varied it in some cases. Separately there is an alphabetical list of the substances with details of their source or origin.

Over 1750 bibliographical references are given and these are arranged in chronological order from 1840 to 1965. An index of authors is included. Where discrepancies arise in results from different sources the authors have critically evaluated the original papers and used the figures which in their opinion are the most reliable.

The volume is basically in French but English translations of the introductory sections and the symbols and abbreviations, combined with the fact that mostly it consists of tables of figures and structural formulas, makes this immaterial.

There are bound to be some errors in a book such as this but none were noticed (except for minor translational quirks) and the overall excellent presentation inspires confidence in the fact that these are very minimal.

This first-class volume is an essential reference work for everyone whose work nvolves the sesquiterpenoid compounds. R. P. REEVES

## INTERPRETED INFRARED SPECTRA. Vol. 2. H. A. Szymanski. Pp. ix + 304 + Ill. (1966). *Plenum Publishing, New York.* $12.50.

Dr. Szymanski continues this long-term course in the education of the tyro organic spectroscopist, who should ultimately be able with confidence to interpret his own ir spectra and make his own identifications. The pattern of volume 1 of the series [reviewed *J.***17** 434 (1966)] has been maintained: for successively complex functions, characteristic group frequencies are deduced and detailed correlation tables compiled for further prediction.

This second volume begins with the remaining hydrocarbon function not treated in volume 1: the alkynes. A full vibrational analysis is presented for eleven specific alkynes (including deuterium replacement) and then there is more general reference to acetylenic hydrogen and the CC triple bond in a large number of ethynyl and propargyl systems. The annotated spectra of 13 alkynes are reproduced. The only error observed (p. 7) is an assignment to aldehydic, rather than acetylenic, CH.

That section, however, only accounts for some 8% of the text: the major part is a remarkably full treatment of the aliphatic hydroxyl function: a family which – as the author recognises – seems to have attracted disproportionately less attention from other compilers. In particular little has been published on diol correlation studies. Dr. Szymanski supports his basic vibration analysis with a correlation table of group frequencies which can be used to identify primary, secondary and tertiary aliphatic alcohols, and proceeds to discuss in helpful detail four environmental factors (inductive effect; resonance phenomena; hydrogen bonding; conformation) which influence the group frequencies. There follows the annotated spectra of 247 alcohols, of which 51 are diols and 15 other substances containing more than two hydroxyl functions. In some cases, by virtue of the correlation deduced, Dr. Szymanski is able to extend the diagnosis of the originator of certain spectra to identify the specific isomer examined or detect components of mixed isomers or indicate the presence of congeneric impurities.

Another very useful facet of this volume is the provision of a cumulative index, wherein all compounds whose spectra have been interpreted in the two volumes are listed in ascending complexity of molecular (not "empirical" as wrongly cited in the text) formula. In each case the graphic formula is appended, which facilitates scanning the index for explicit structural features. This is similar to the provision made in *Formula index to nmr literature data* [reviewed *J.***18** 265 (1967)], although in that text the elements C and H are deliberately the last choice in the ascending complexity sequence.

Thus we now have two useful volumes comprehensively analysing the ir spectra of hydrocarbons and the aliphatic hydroxyl function; one looks forward to seeing a similarly authoritative coverage of the fascinating subject of the variation observed in the ir spectra for different carbonyl environments. G. F. PHILLIPS

## DISCUSSIONS OF THE FARADAY SOCIETY. No. 40 1965: INTERMOLECULAR FORCES. Pp. 291 + Ill. (1966). *Butterworth, London.* 80s.

The 40th of a series of 'General Discussion' meetings organised by the Faraday Society was held at Bristol University during three days in September 1965; the 276 participants included visitors from 19 other countries. The meeting was built around the manifold studies currently being conducted for the understanding of 'Intermolecular Forces'. The Society has now published under one cover the Spiers Memorial lecture with which Professor Longuet-Higgins opened the meeting, the 24 contributed papers, the text of the five general discussion sessions, and the synthesis of ideas and prospect in the summary by Professor C. A. Coulson.

Broadly speaking the papers fall into four groups. Those dealing with examination of interatomic forces in the gas phase are largely theoretical and require increasingly complex electronic computational assistance; a number of papers are concerned particularly with 3– (or even many–) body treatments and these concepts are extended to crystal structure. More complex discussion is needed to cater for the short-range repulsive interactions in the liquid and solid state; a few contributors have perhaps sought an interpretation that is too physical for some of their calculations. Useful papers quantify non-polar repulsion in a fluid or at a surface, but much more study is needed on absorption phenomena. The description of non-polar interactions in molecular crystals, and other polyatomic systems, are also largely of a repulsive character, depending little upon mutual orientation, but dipole-quadrupole interaction may lead to angular dependence. Of course where a molecule has a permanent dipole this may swamp the subtle defects described. Literally the most vital, and requiring the most indulgence in the way of assumptions, are interactions in biological systems. Crude estimations of non-bonding repulsion in peptide helixes lead to predictions of geometrical rigidity for a given amino-acid sequence.

Perhaps the most striking feature of these papers is the difference in level of sophistication: some problems appear to require a profound study of forces between (say) gases; other (e.g. liquid-solid) interactions are examined from crude approximations and assumptions. The somewhat specialised interaction of the hydrogen-bond in organic systems was deliberately excluded from this General Discussion; no doubt a special meeting could be set aside – the Faraday Society last held one on this topic in 1940. This collection of papers is so broad ranging, and in many instances so specialised, that the book is unlikely to commend itself for general reading. Its value lies as a reference source to a broad spectrum of current thinking in the analytical treatment of the nature and magnitude of forces between molecules in many different physical states. This extension of physical chemistry was aptly summarised by Professor Coulson when he defined the discussion as a synthesis of theoretical physics and experimental chemistry. G. F. PHILLIPS

David E. Butterfield, M.A.
*President 1967-68*

*Facing page 499*

# Society of Cosmetic Chemists of Great Britain

## ANNUAL GENERAL MEETING

The Eighteenth Annual General Meeting of the Society took place on the 22nd May, at the Washington Hotel, Curzon Street, London, W.1.

The Annual Report was presented by the President, Dr. A. W. Middleton, who referred particularly to the acquisition of office accommodation in Central London, to the appointment of Mrs. S. Taylor as General Secretary, in succession to Mrs. D. Mott, and the retirement of Dr. M. Cantley as Hon. Education Secretary. The Treasurer's Report was presented by the Hon. Treasurer, Mr. G. A. C. Pitt.

In the voting for three new Members of Council the following were elected: Mr. R. L. Davis, Mr. K. M. Godfrey, Mr. K. Tomlinson.

The officers for 1967-68 are:—

| | |
|---|---|
| President: | Mr. D. E. Butterfield |
| Immediate Past President: | Dr. A. W. Middleton |
| Vice-President: | Mr. C. Pugh |
| Hon. Secretary: | Dr. J. J. Mausner |
| Hon. Treasurer: | Mr. G. A. C. Pitt |
| Hon. Editor: | Mr. A. Herzka |
| Hon. Education Secretary: | Dr. T. J. Elliott |

Mr. L. S. Smith was re-appointed Hon. Auditor, and Messrs. H. W. Fisher & Co. were re-appointed Auditors for the current year.

The business meeting was followed by a buffet supper.

## 1967-68 PROGRAMME

Lectures will be delivered on the following Thursdays:

*Venue:* The Royal Society of Arts, John Adam Street, London, W.C.2., *except 7th December.*

*Time:* 7.30 p.m.

5th October 1967
*Mouth odour*
Dr. A. Naylor (Dept. of Preventive Dentistry, Guy's Hospital)

7th December 1967, *at Havelock Arms, Gray's Inn Road, London W.C.1.*
*Lucent syrops tinct with cinnamon*
Dr. V. L. S. Charley (Beecham Products U.K.)

4th January 1968
*Synergism of antimicrobials*
Dr. E. E. Boehm (Nipa Laboratories Ltd.)

1st February 1968
Joint Meeting with British Society of Perfumers

7th March 1968
*The problems of Parliament*
D. Smith (Beecham Group Ltd.)

2nd May 1968
*Some rheological aspects of cosmetics*
B. Warburton and B. W. Barry (School of Pharmacy, University of London)

*MEDAL LECTURE:* Friday, 22nd March 1968
This will be given by
Dr. B. H. Crawford (Imperial College of Science and Technology, London, S.W.7.)

*1968 DINNER AND DANCE:* Friday, 9th February, at the Russell Hotel, Russell Square, London, W.C.2.

*ANNUAL GENERAL MEETING:* Monday, 20th May 1968, at the Washington Hotel, Curzon Street, London, W.1. at 7 p.m.

## SYMPOSIUM ON PROCESSING AND MANUFACTURING

A Symposium on PROCESSING AND MANUFACTURING will take place in Royal Leamington Spa, Warwicks., on 13th and 14th November 1967. *Programme Secretary:* Dr. J. J. Mausner, Helena Rubinstein Laboratories, Ltd., Central Avenue, West Molesey, Surrey.

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