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Dosage uniformity in hydrocortisone ointment B.P.

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The content uniformity of hydrocortisone in seven commercially available brands of hydrocortisone ointment B.P. 1% has been investigated. Fifty 5 mg samples were assayed by high pressure liquid chromatography and the results indicated that for 95% confidence levels only two of the ointments exhibited no positive skewness, one exhibited a significant degree of positive skewness and four exhibited a highly significant degree of positive skewness. The extent of the skewed distributions is discussed in relation to previously published particle/agglomerate distributions for these ointments. The content uniformity in terms of the coefficient of variation C_E calculated from the h.p.l.c. data is compared with the coefficient of variation C_P that can be predicted from mixing theory on the basis of the particle/agglomerate distribution of the hydrocortisone. The departure from normality in drug content uniformity in the ointment is attributed to the hydrocortisone particles not being individually available for randomization, a large number being in an agglomerated form. That is, the manufacturing process is failing to achieve the full potential of the formulation by dispersing all of the agglomerates. Theoretical and experimental models predict that percutaneous absorption of drug may be enhanced over areas, where agglomerates are located, possibly not only resulting in localized toxicity but increased systemic availability. Drug content variability in small samples (5 mg) of topical steroid formulations could also effect the degree of skin blanching response in Mackenzie-Stoughton type tests since 5 mg portions containing in excess of twice the labelled strength were found. Regulatory control of content uniformity should be considered for certain topical steroids if unintentional over-dosage on small discrete areas is to be avoided.

There is general recognition of the need to control the content uniformity of solid dosage forms containing potent drugs at high dilutions, but this has yet to be extended to topical products, which often present mixing problems of a similar magnitude. Hersey & Cook (1973) suggested that the content uniformity of ointments and similar topical products was worthy of further investigation and possibly should also be subjected to stringent control. They illustrated the point with a series of calculations indicating the range of particle sizes required to meet pre-set standards of mixing at different sample sizes and concentrations using Buslik's concept of homogeneity (Buslik 1973).

In general, the rationale for incorporating drugs as fine powders into topical formulations is to improve the rate of dissolution (Lees 1963), but it should also improve the homogeneity of the mix according to random mixing theory (Train 1960). However, fine particles tend to be cohesive and considerable energy is required to break down agglomerates. Skewed drug content distributions of tablets containing low concentrations of drug have been attributed to the presence of agglomerates composed of the fine particles initially incorporated into the formulations to improve the homogeneity of the final mix (Orr & Shotton 1973; Egermann 1974; Hess 1976; Orr & Sallam 1978). A microscopic study of hydrocortisone ointments (Orr et al 1980) demonstrating the presence of agglomerates of drug remaining undispersed in the base suggested that positively skewed drug content distributions would be obtained from single assays of small sample sizes. Highly skewed drug content distribution is considered pharmaceutically undesirable because of the increased chance of administering doses of drug substantially higher than the labelled strength either as a discrete dose, or over small, discrete areas as might occur with topical products.

Specification of a dose uniformity test for topical products is not possible in the absence of an appropriate unit dose. A unit dose would depend on the thickness of the layer applied on the skin and the area of skin that could be regarded as behaving independently of adjacent areas of skin whilst undergoing therapy. Some theoretical aspects of this problem have been discussed by Orr et al (1980) but it is worthwhile considering that 12–15 g of ointment is recommended for whole body coverage with a topical steroid when applied 'sparingly' (Rook et al 1972). Therefore, approximately 1 mg of ointment probably containing 10 μg or less of drug is con-

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sidered sufficient to treat 1 cm^2 of skin. The fact that pharmacological responses can be provoked in the skin from such small quantities of ointment and that their effects can be restricted to small areas, as demonstrated by Mackenzie-Stoughton vasoconstrictor assays (Mackenzie & Stoughton 1962; Barry & Woodford 1978) suggest that content uniformity should possibly be assessed at a scale of scrutiny of 1–10 mg.

The homogeneity of a drug in an ointment contained in a processing vessel may not necessarily be maintained during filling and packaging. The final homogeneity on the skin may be affected by extrusion, the method of use and subsequent application. In this investigation the uniformity of the midstream ointment after extrusion under standard conditions is examined.

The analytical assessment and quality control of topical dosage forms is complicated by the presence of excipients which may interfere with the assay at small scales of scrutiny. An assay technique of sufficient sensitivity and precision was essential to perform single unit dose assays at sample sizes in the range of 1–10 mg. Modification of the extraction technique used by Bailey & Brittain (1972) has enabled samples of 1–5 mg to be assayed quickly and accurately for content unformity.

MATERIALS AND METHODS

Materials

For extractability and reproducibility studies hydrocortisone ointment B.P. 1% was prepared by dispersing micronized hydrocortisone B.P. in white soft paraffin B.P. Content uniformity tests were performed on 7 commercially available hydrocortisone ointments B.P. 1% designated A–G, purchased from wholesalers.

Standard hydrocortisone solutions in the range of 200 to 40 $\mu g\ ml^{-1}$ were made from a freshly prepared concentrate of 0·1% w/v in methanol. A methanolic solution of approximately 100 $\mu g\ ml^{-1}$ of norethisterone was prepared for use as internal standard. The eluent was 75% v/v methanol–water degassed under vacuum at ambient temperature. The chemicals used were B.P. grade from local wholesalers except methanol (Koch-Light A.R. Puriss) and norethisterone (Sigma Chemicals Ltd).

Apparatus and conditions

A Perkin-Elmer LC2 liquid chromatograph was used in the isocratic mode with a 25 cm × 4·6 mm i.d. Spherisorb 5 ODS column (H.P.L.C. Technology Ltd). The detector system was a Pye-Unicam LC/UV variable wavelength u.v. detector set at 254 nm. Samples were introduced onto the column using a Rheodyne injection valve (model 7120) fitted with a 20 μl loop, both complete and partial loop filling methods were used.

The flow rate of the eluent was 1·4 ml min^{-1} giving a pressure of 2000 psi. The progress of the separation was followed on a Linseis LS2 chart recorder.

Sample preparation

To determine the average drug content of the ointment, accurately weighed samples of about 1 g were transferred to a 200 ml separating funnel and dispersed in 100 ml of 2,2,4-trimethylpentane (Koch-Light Puriss) by vigorous shaking. Approximately 20 ml of methanol was added and after shaking for several minutes the lower, methanolic layer was transferred to a 100 ml volumetric flask. This was repeated for a further three 20 ml portions of methanol each of which was transferred to the same volumetric flask, which was made up to the final volume with methanol. Five 1 ml samples were withdrawn and each added to 1 ml of the internal standard solution.

Mid stream sampling was achieved by extruding the entire contents of the 15 g tube to form a 60 cm long stream of ointment on a glass slab. Fifty 5 mg portions of ointment were removed using a mounted needle at 4 mm intervals over the central 20 cm. The samples were then transferred to preweighed glass coverslips and the weight determined to ± 0·005 mg on a microbalance (Stanton Model MC9). The coverslip and ointment was then placed in a 150 mm test tube, and the ointment dispersed in 1 ml of 2,2,4-trimethylpentane with vigorous shaking. One ml of internal standard solution was added to the tube, and shaken for a further 10–20 s to ensure that all of the hydrocortisone was in solution. The methanolic layer was then transferred using a Pasteur pipette, to a 2 ml sample tube, which was sealed and stored until required for assay.

Standards for the assay were prepared in a similar manner. Plain white soft paraffin B.P. was dispersed in 2,2,4-trimethylpentane and equal volumes of internal standard solutions and standard hydrocortisone solutions were pipetted into each tube and shaken. Blanks were also prepared by adding methanol alone to check that there was no interference from the ointment base in the assay. The standards were injected at the beginning and end of each assay run. When 50 or more assays were performed further injections were made after every 25 samples.

Accuracy and reproducibility

A plot of peak height ratios of hydrocortisone to norethisterone was a linear function of concentration of hydrocortisone over the range of concentrations used. Correlation coefficients of 0·9999 were obtained for the regression lines and standard solutions. The error of a prediction at 50 μg ml^{-1} was estimated from the regression data to be $\pm 1{\cdot}53$ μg ml^{-1} for 95% confidence limits.

An estimate of the extractability of hydrocortisone from the ointment base was made by adding an accurately weighed quantity of hydrocortisone (approximately 10 mg) to 1 g of white soft paraffin ointment base. Fifty 5 mg samples were assayed individually and the total amount of drug extracted was determined. The remaining ointment was assayed and the combined total of drug extracted was calculated as a percentage of the total amount of hydrocortisone added. Extractability was calculated to be 99·5 %, range 98·7–100% (n = 3).

The reproducibility of the assay was determined by preparing and assaying 50 individual replicate standard solutions. The standards were prepared by mixing equal volumes of internal standard solutions and standard hydrocortisone solutions. The error introduced by the double pipetting procedure was intended to compensate for the absence of weighing errors introduced during the normal assay procedure. The reproducibility of the assay was calculated to be equivalent to a coefficient of variation of 1·5%.

RESULTS

The results obtained from each of the fifty single 5 mg sample assays taken from the seven ointments studied are summarized in Table 1. The results for the minimum, maximum and mean values of drug content are expressed as percentage w/w of hydrocortisone in the ointment base and are independent of the variation in sample weight. The sample weights ranged from 4·31–5·94 mg with average weight 5·15 mg.

To determine if there was a significant difference between a normal distribution of unspecified mean and variance and the unknown distribution of the drug content of the samples, the non-parametric Lilliefors test was applied to the data (Lai et al 1974). The sample mean ($\bar{X}$) and standard deviation (s) were calculated for each set of 50 samples and the 'normalized' result (Z_1) for each sample value was calculated according to the equation:

$Z_1 = \bar{X}_1 - X/S$ where X_1 = observed sample value

A graphical method for determining the test statistic was used. The standard normal distribution function

Table 1. Summary of the results obtained on the assay of 50 5 mg samples of hydrocortisone ointment B.P. 1% from seven different manufacturers. The maximum, minimum and mean drug content are expressed as percentage w/w of drug in base. C_E% is the coefficient of variation of the experimental data, C_P% is the predicted coefficient variation for products A–F calculated from particle size data (Orr et al 1980) T_2 is the Lilliefors test statistic calculated to determine if the true distribution function (unknown) is significantly different from a normal distribution of unspecified mean and variance; the distribution is normal if $T \leqslant 0{\cdot}125$ (95% probability) $\sqrt{b_1}$ and $\sqrt{b_2}$ are the coefficients of skewness and Kurtosis respectively.

Ointment	Min result	Max result	Mean	T_2	$\sqrt{b_1}$	$\sqrt{b_2}$	C_E%	C_P%
A	0·94	1·05	0·99	0·19	0·35	2·54	2·55	0·49
B	0·88	1·20	1·01	0·24	0·51	6·47	5·84	8·00
C	0·82	1·19	0·97	0·10	1·21	5·79	6·80	2·40
D	0·85	1·51	1·00	0·28	3·56	15·95	11·47	0·67
E	0·93	1·89	1·03	0·28	5·92	39·71	13·00	7·40
F	0·96	1·11	1·02	0·18	0·60	3·18	3·52	5·90
G	0·91	2·02	1·04	0·29	4·77	28·52	15·63	30*

* C_P% for ointment G is estimated from the five largest particle/agglomerate size fractions present in the base.

F*(x) and the empirical distribution function of the normalized samples S(x) were plotted on the same co-ordinates and the maximum vertical distance between the two graphs were determined (Fig. 1). The value obtained represents the Lilliefors test statistic T_2 (Table 1).

The hypotheses tested were: H_0: the selected samples have a normal distribution with unspecified mean and variance.

vs H_1: the distribution function of X's are non-normal.

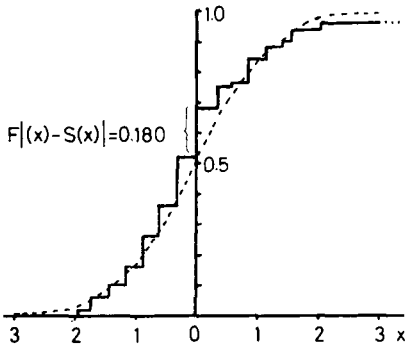

FIG. 1. Graphical determination of the test statistic T_2 for the Lilliefors test for normality. The value of the maximum vertical distance between the normal distribution function F* (x) (broken line) and the empirical distribution function of the data to be tested S(x) (solid line) is equivalent to the test statistic T_2. The critical level for fifty samples is $0{\cdot}125$ $P = 0{\cdot}05$ (Lai et al 1974). The example shown is that of the data obtained from ointment F. $T_2 = |F^*(x) - S(x)| = 0{\cdot}180$

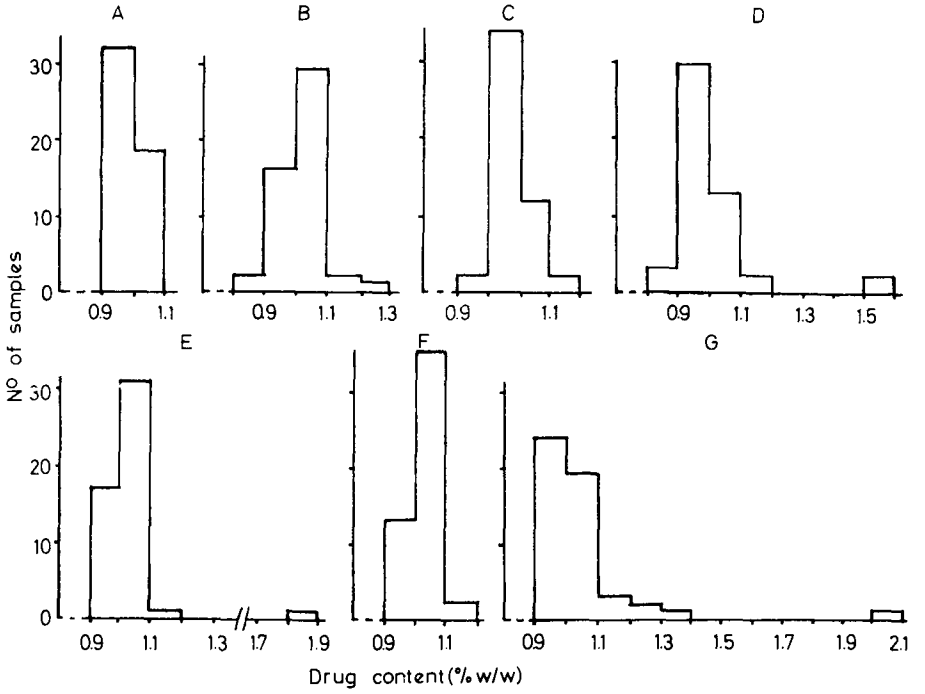

FIG. 2. Histograms based on the results of 50 individual assays of 5 mg samples of hydrocortisone ointments B.P. 1% designated A–G. The ordinate gives the number of samples and the abscissa the drug content expressed as percent w/w.

The test requires rejection of the null hypothesis at $P = 0{\cdot}05$ if the test statistic T_2 exceeds the 0·95 quantile. For 50 samples the 0·95 quantile = 0·125.

The limiting values of the coefficient of skewness, $\sqrt{b_1}$, for a normal distribution determined for 50 samples are $\pm 0{\cdot}583$ and $\pm 0{\cdot}787$ for probabilities of 0·05 and 0·01 respectively (Pearson & Hartley 1958). For a normal distribution, the coefficient of Kurtosis, $\sqrt{b_2} = 3$ but significant departure from normality can only be effectively determined for sample sizes > 200.

Histograms of the results are given in Fig. 2 expressed as the percentage hydrocortisone w/w in the base, to illustrate the shape of each distribution. They are plotted on the same scale so that the shape of each distribution can be compared.

DISCUSSION

The drug content of each of the ointments when determined on a 1 g sample complies with the limits of $\pm$ 7·5% of the labelled strength specified in the British Pharmacopoeia (1973). However, the range of the results at the 5 mg scale of scrutiny obtained for ointments B-G fall outside these limits, with excessively high results being more common than low ones, imparting positive skewness to the distributions. This is a pattern typical of mixtures containing low concentrations of drug when agglomerates of the active material are believed to remain undispersed in the final formulation (Orr & Shotton 1973; Egermann 1974; Orr & Sallam 1978; Orr et al 1980). Although an agglomerate may consist of many particles, this will have little net effect on the overall distribution of drug in the bulk of the mixture. The drug content of most of the samples withdrawn from the bulk will, consequently, deviate to a relatively small extent from the mean drug content if the particle size is sufficiently small. Therefore, the probability of selecting a dose low enough to compensate for a high dose due to the presence of an agglomerate is extremely low and the observed assymetry arises.

The Lilliefors test for normality demonstrated that the distribution of drug content in the ointments is not approximated by the normal distribution except

for ointment C and application of tests that assume normality should only be used with caution.

On a purely statistical basis it could be argued that the extreme results are outliers and may be regarded as 'unrepresentative' of most of the values obtained for drug content uniformity. The extent to which the high results effect the distribution become immediately apparent if they are discarded and the mean drug content, $C_E\%$, $\sqrt{b_1}$ and $\sqrt{b_2}$ are recalculated from the modified data (Table 2).

Table 2. Summary of results obtained on rejection of outliers. The extreme results were rejected if the value of the ratio

$$\frac{\text{Extreme value} - \text{Overall mean}}{\text{Overall standard deviation}}$$

exceeded the critical level of 3·13 for 50 samples ($P = 0{\cdot}05$). The number of results discarded using this procedure is shown for the seven ointments studied. The values of the mean, experimental coefficient of variation ($C_E\%$), predicted coefficient of variation ($C_P\%$) and coefficients of skewness ($\sqrt{b_1}$) and Kurtosis ($\sqrt{b_2}$) are calculated from the modified data.

Ointment	No. discarded results	Mean % drug w/w	C%	$\sqrt{b_1}$	$\sqrt{b_2}$
A	0	0·99	2·55%	0·35	2·54
B	1	1·00	5·00%	−0·11	6·25
C	2	0·96	5·12%	−0·01	3·03
D	2	0·98	4·93%	0·35	6·75
E	1	1·01	3·88%	0·40	3·13
F	0	1·02	3·52%	0·60	3·18
G	1	1·02	7·71%	2·33	9·49

The rejection procedure used was that suggested by Grubbs (1969) using the ratio

$$\frac{\text{Extreme value—Overall Mean}}{\text{Overall standard deviation}}$$

The outlier was rejected if the ratio exceeded a specified critical level. The critical level corresponding to $P = 0{\cdot}05$ for 50 samples is 3·13 and outliers were discarded if the ratio exceeded this value on the basis that the probability of observing such results were less than 0·05, assuming a normal distribution.

When agglomerates are known to be present in a mixture, to assume normality is probably inappropriate as indicated by the results of the Lilliefors test. The subjective nature of an observer's response to an 'outlier' (Collett & Lewis 1976) and its possible rejection is likely to lead to misinterpretation of the results if such procedures are followed blindly. (In the situation being discussed occasional high results are, in fact, to be expected.) If a test for skewness is applied to the data after rejecting 'outliers' the dose content distribution in six of the ointments could be accepted as approximately normal.

Mixing theory contains a number of assumptions about the particle size distribution of drug in the mixture to reduce the mathematical complexity of the true situation. For example, Stange (1954) assumes a normal distribution and Johnson (1972) assumes a Poissonian distribution which approximates to the normal distribution. The particle weight distribution of hydrocortisone suspended in ointment A & C-F were highly positively skewed. In ointment B the distribution was bimodal, a similar distribution is probably present in ointment G, since the agglomerates observed constitute a relatively greater proportion by weight than most particles, despite being relatively few in number. The probability of sampling and observing large agglomerates is small and errors in estimation of numbers of particles in these size classes may be considerable (Selden 1977). The predicted values for the coefficient of variation were of the right order but the differences in the predicted and measured variances were found to be significant ($P = 0{\cdot}05$) using the variance ratio test. This may be related largely to the error associated with particle sizing. It is necessary to consider the implications of storage of the ointment and the method of sampling used in this investigation.

It is unlikely that the storage conditions would influence the distribution of agglomerates. Sedimentation under the influence of gravity is unlikely to occur since the force required to instigate flow in the base, which is plastic in nature, is relatively large and particles would therefore remain in the same spatial relation to each other and the base. Similar considerations may also apply to sampling. Each section sampled undergoes shearing in a restricted area for a brief time and little or no effect may be expected on the drug distribution in neighbouring sections. Nevertheless this possibility was taken into account and fifty samples were taken at the maximum possible distance apart (4 mm) along a 20 cm length of ointment. Adopting a truly random sampling procedure may have required the selection of neighbouring portions possibly affecting the drug distribution and was, therefore, considered unsuitable.

The positively skewed drug distributions in 5 mg samples of ointment implies that doses greatly in excess of the labelled strength will be administered over small, discrete areas of skin. The subsequent potential for enhanced drug penetration over these areas could be such that toxic effects become a possibility.

Hersey & Cook (1974) suggested that lateral diffusion of drug in the ointment base would tend to decrease the homogeneity requirements of topical products. However as shown by Orr et al (1980) there is no evidence to show that substantial lateral diffusion occurs on application of a suspension ointment.

Normally, drug penetration of the stratum corneum in healthy skin is rate limiting and will reduce the effect of concentration variations over the skin. Disruption of the barrier function in experimental epidermal disease has been shown to increase the amount of percutaneous absorption in vitro (Solomon & Lowe 1979) and it is probable that drug absorption will also be enhanced by diseased states in vivo. Increasing drug concentration on the skin has been shown to increase the amount of drug absorbed in vivo from intact skin, using rhesus monkeys as a model (Wester et al 1979). It has also been shown earlier that a single high dose of hydrocortisone produced substantially more absorption than thrice daily application of smaller doses (Wester et al 1977).

Cases of toxic reactions in the skin arising from poor drug dispersion have previously been reported for dithranol (Seville 1966) and hexachlorophane (Baker & Lloyd 1967; Baker et al 1969). Topical steroid therapy may also be subject to the same problem. Toxic effects at localized sites will probably include atrophy of the epidermis and striae. In infants, systemic availability of topically absorbed drugs is of concern. The ratio of body surface area is approximately three times that of an adult, also infant skin shows higher drug penetrability, therefore the systemic availability of topically-applied drugs is greatly increased. Differences in systemic metabolism between adults and infants may exacerbate the effect. Similar considerations may also hold for patients with liver malfunction receiving concomitant topical therapy.

The content uniformity of topical steroid preparations should, ideally, be established before the comparison of relative activities using vasoconstrictor assays. The conclusions drawn from such trials using samples sizes of the order of 5 mg may be confounded by inter-sample drug content variation. Abnormally high scores could result if the drug content distribution was positively skewed.

Conclusion

Topical therapy should, in principle, allow drugs to be applied at the site of action with a high degree of safety. Skewed drug content distributions obtained on assaying small samples of ointment indicates that this is not necessarily the case over small areas of skin. The available evidence suggests that unless drug content uniformity is adequately controlled inconsistent rates of drug penetration may occur over discrete areas of skin. There is evidence to suggest that gross content disuniformity over discrete areas could be associated with toxic side effects for certain highly potent drugs in topical formulations.

The treatment of skin disease has been advanced by a better understanding of the factors affecting drug delivery and subsequent improvements in formulation design. Formulations containing hydrocortisone dissolved in propylene glycol are generally more active (in terms of percutaneous absorption) than the simpler suspension ointments and are more suitable for the treatment of persistent dermatoses. However, while a requirement for a weak topical steroid for less severe skin conditions remains, particulate suspensions will remain in use. In addition other topical products prepared extemporaneously contain particulate material e.g. dithranol ointment B.P. for which the same problem of producing a good dispersion must arise. Control of drug distribution on the skin may be among the factors that will lead to optimum therapeutic effect which has yet to be investigated. If this proves to be the case regulatory control of drug content uniformity in some formulations should be considered.

Improved understanding of mixing theory will undoubtedly help the development of suitable formulations. However, a number of shortcomings in random mixing theory have become apparent, particularly with respect to the estimation of variation in dose content uniformity from particle size data. The methods used and the errors associated with the determination of the particle size distribution are likely to influence the final result. The currently accepted theory may need to be expanded to cover this aspect of particle sizing to give improved estimates of probable dosage variations in mixtures containing agglomerates.

REFERENCES

Bailey, F., Brittain, P. N. (1972) J. Pharm. Pharmacol. 24: 425–428

Baker, H., Lloyd, M. J. (1967) Br. J. Derm. 79: 723–728

Baker, H., Ive, F. A., Lloyd, M. J. (1969) Arch. Derm. 99: 693–696

Barry, B. W., Woodford, R. (1978) J. Clin. Pharm. 3: 43–65

British Pharmacopoeia (1973) London H.M.S.O.

Buslik, D. (1973) Powder Technol. 7: 111–116

Collett, D., Lewis, T. (1976) Appl. Stat. 25: 228–237

Egermann, H. (1974) Scientia Pharm. 42: 1–19

Grubbs, F. E. (1969) Technometrics 11: 1–21

Hersey, J. A., Cook, P. C. (1973) J. Pharm. Pharmacol. 25: Suppl. 121P

Hersey, J. A., Cook, P. C. (1974) Ibid. 26: 126–133

Hess, H. (1976) Acta Pharm. Technol. 22: Suppl. 2, 49–64

Johnson, M. C. R. (1972) Pharm. Acta Helv. 47: 546–559

Lai, F. S., Wang, R. H., Fan, L. T. (1974) Powder Technol. 10: 13–21

Lees, K. A., J. Pharm. Pharmacol. (1963) 15: 43T–55T

Mackenzie, A. W., Stoughton, R. B. (1962) Arch. Derm. 86: 608–610

Orr, N. A., Shotton, E. (1973) Chem. Eng. 269: 12–19

Orr, N. A., Sallam, E. A. (1978) J. Pharm. Pharmacol. 30: 741–747

Orr, N. A., Hill, E. A., Smith, J. F. (1980) International Journal of Pharmaceutical Technology & Product Manufacture, in the press

Pearson, E. S., Hartley, H. O. (1958) Biometrika Tables for Statisticians, Vol. 1 Cambridge, University Press

Rook, A., Wilkinson, D. S., Ebling, F. J. G. (1972) Textbook of Dermatology Vol. 2, 2nd Ed. Blackwell, Oxford

Selden, M. G. (1977) Microscope 26: 127–129

Seville, R. H. (1966) Br. J. Derm. 78: 269–272

Solomon, A. E., Lowe, N. J. (1979) Ibid. 100: 717–722

Stange, K. (1954) Chem. Ing. Tech. 26: 331–337

Train, D. (1960) Pharm. J. 131: 129–134

Wester, R. C., Noonan, P. K., Maibach, H. I. (1977) Arch. Dermatol. 113: 620–622

Wester, R. C., Noonan, P. K., Maibach, H. I. (1979) J. Soc. Cosmet. Chem. 30: 297–307