VOL XVI NO 7 24 JUNE 1965

Journal of the Society of Cosmetic Chemists

Contents

Page

SUBJECT REVIEW ARTICLES

The preservation of ophthalmic preparations
M. R. W. Brown, Ph.D., and D. A. Norton, B.Sc., F.P.S. 369

Hygiene in manufacturing plant and its effect on the preservation of emulsions
Doreen L. Wedderburn, B.Sc. 395

The sebaceous glands
F. J. Ebling, M.Sc., Ph.D., M.I.Biol. 405

BOOK REVIEWS 413

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN

Annual Report 1964/65 421

Annual General Meeting 428

Obituary 430

4th I.F.S.C.C. CONGRESS viii

INDEX TO ADVERTISERS ii

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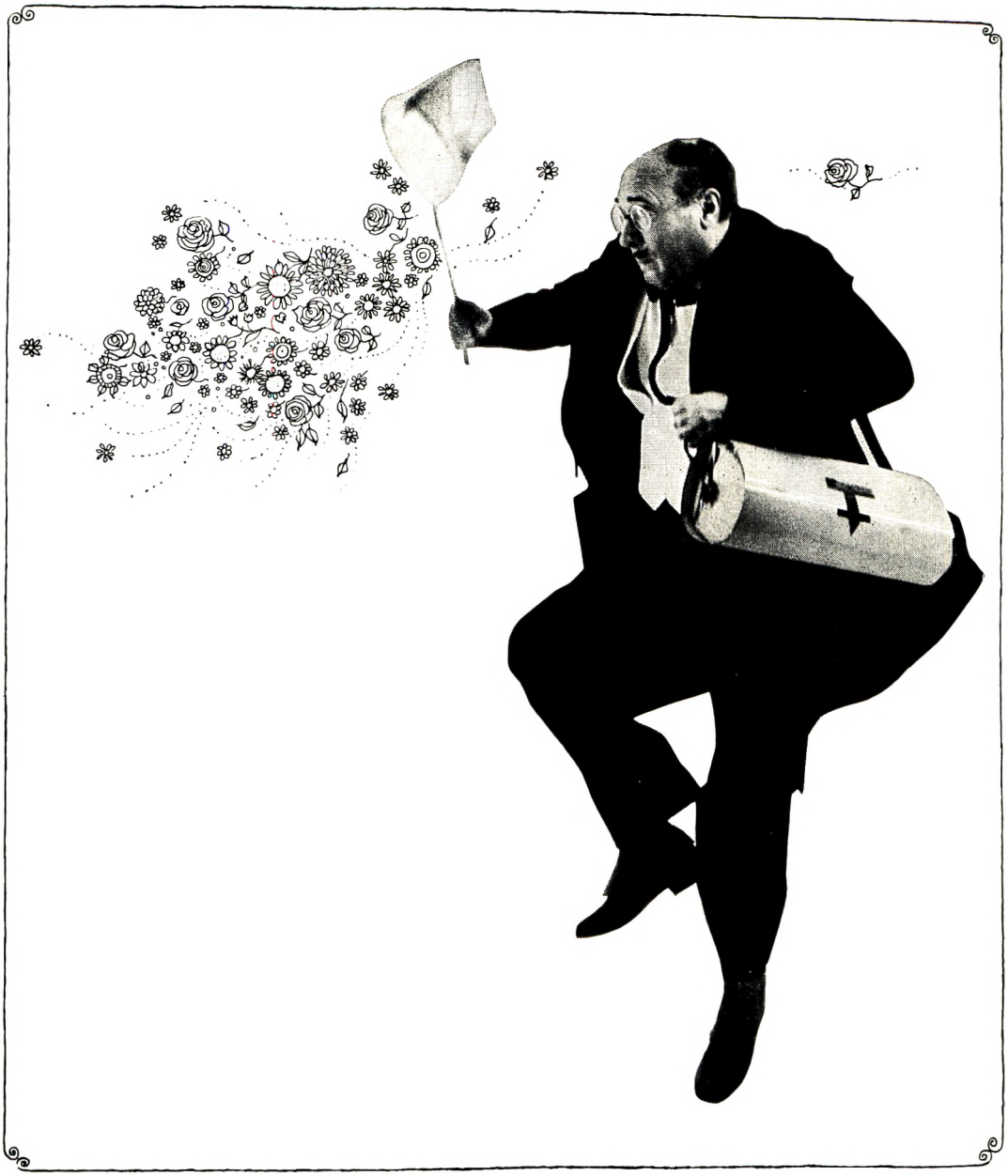

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INDEX TO ADVERTISERS

American Cholesterol Products, Inc. iii

D. F. Anstead Ltd. ix

A. Boake, Roberts & Co. Ltd. .. .. .. Inside Front Cover

W. J. Bush & Co. Ltd. xv

Antoine Chiris Ltd. vi

Croda Ltd. vii

Fritzsche Bros. Inc. xi

Givaudan & Co. Ltd. v

Glovers (Chemicals) Ltd. xvii

Haarmann & Reimer GmbH i

May & Baker Ltd. xiv

Pergamon Press Ltd. .. .. xvi, xviii

Proprietary Perfumes Ltd. xiii

P. Robertet & Cie. .. .. Outside Back Cover

Rhone-Poulenc iv

Schimmel & Co. Inc. x

Whittaker, Clark & Daniels, Inc. .. .. .. Inside Back Cover

Williams Ansbacher Ltd. xii

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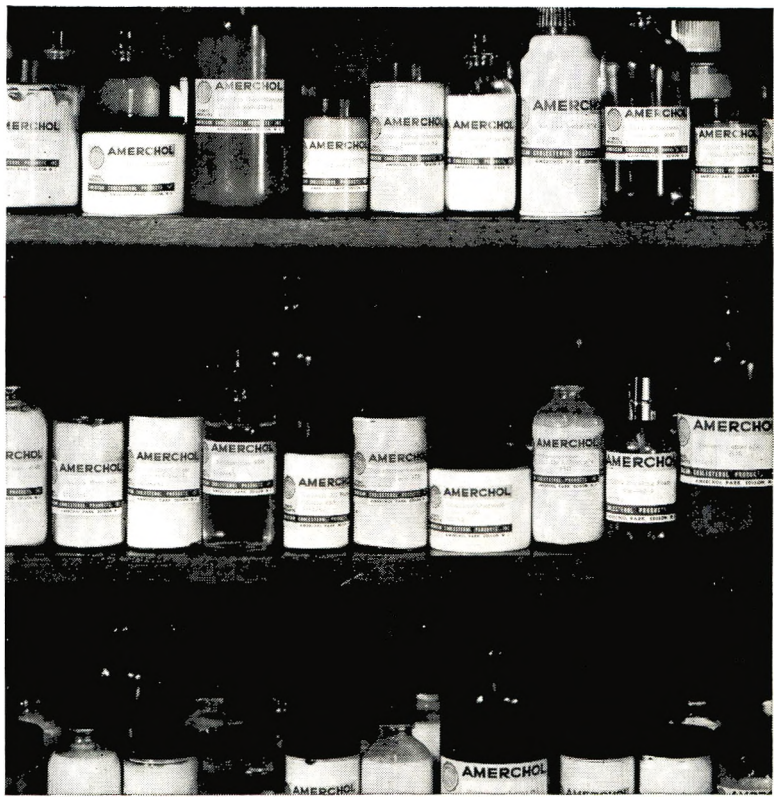

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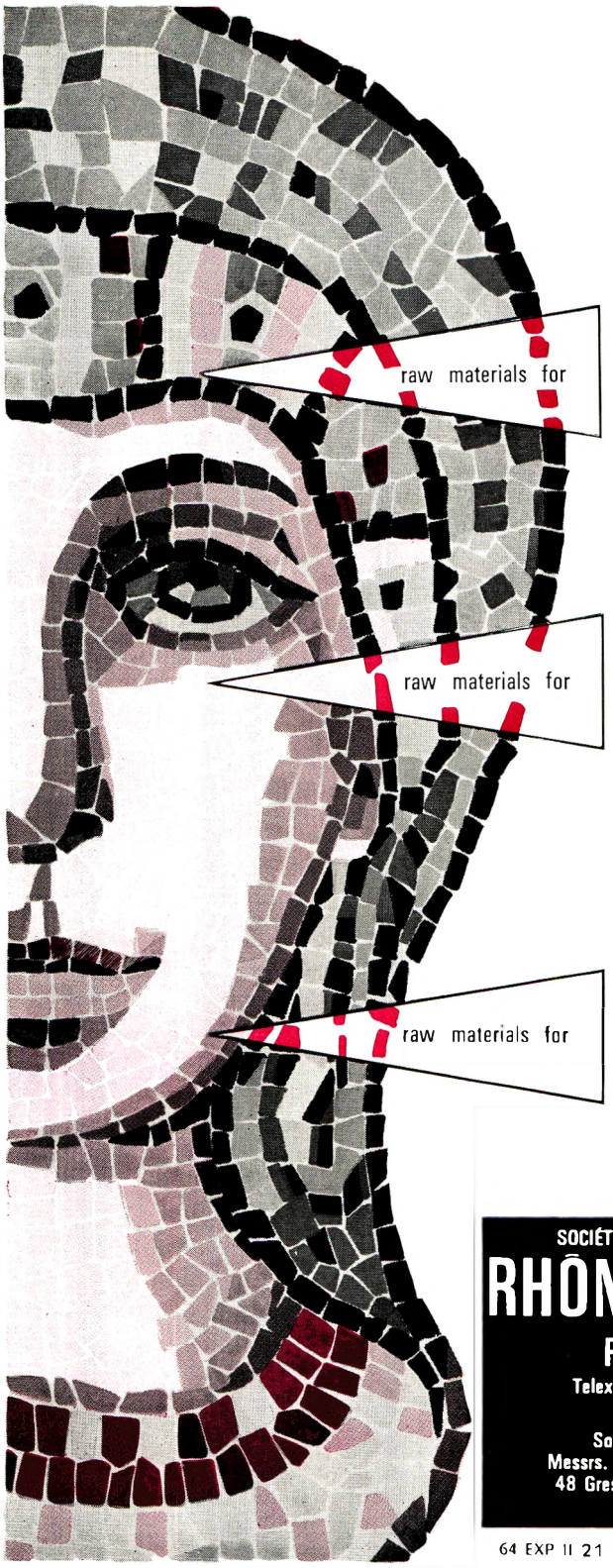

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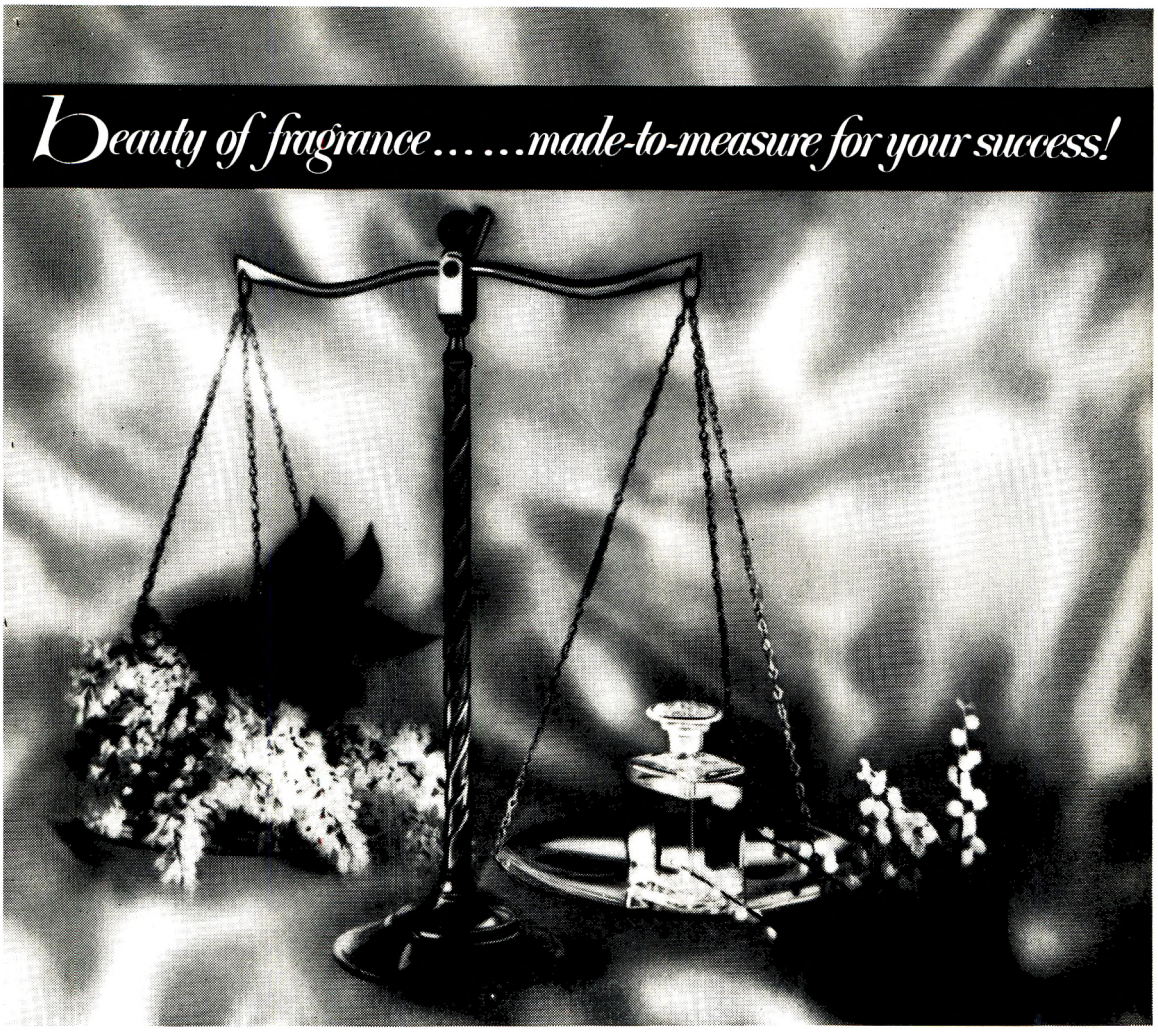

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Five issues for the Society of Cosmetic Chemists of Great Britain by Pergamon Press Ltd., Headington Hill Hall, Oxford, England.

Six issues by the Society of Cosmetic Chemists from 201 Tabor Road, Morris Plains, N.J., U.S.A.

Two issues by the Gesellschaft Deutscher Kosmetik-Chemiker, e.V. from Hamburg-Grossflottbek, Beselerstrasse 1, Germany.

Issue No.	*Publication Date*	*Country of Origin*
1	8th January	Great Britain
2	5th February	U.S.A.
3	4th March	U.S.A.
4	1st April	Great Britain
5	29th April	Germany
6	27th May	U.S.A.
7	24th June	Great Britain
8	22nd July	Great Britain
9	19th August	U.S.A.
10	16th September	U.S.A.
11	14th October	Great Britain
12	11th November	Germany
13	9th December	U.S.A.

Advertisements : All enquiries regarding advertisements in the British Editions of the Journal should be addressed to Mr. J. V. Robinson, Pergamon Press, Ltd., 4 & 5 Fitzroy Square, London, W.1.

Subscription : All members of the Society of Cosmetic Chemists of Great Britain receive one copy of each edition free. Further copies at non-member rates. Non-members: £10 per annum, post free, or £1 per issue, post free.

Missing Numbers : **Journals are despatched at Printed Paper rate. Claims for missing numbers can be entertained only from subscribers in the country of origin of the particular issue, and must be made within 30 days from date of issue.** Members and subscribers are urged to give notice of change of address to the Publication Offices.

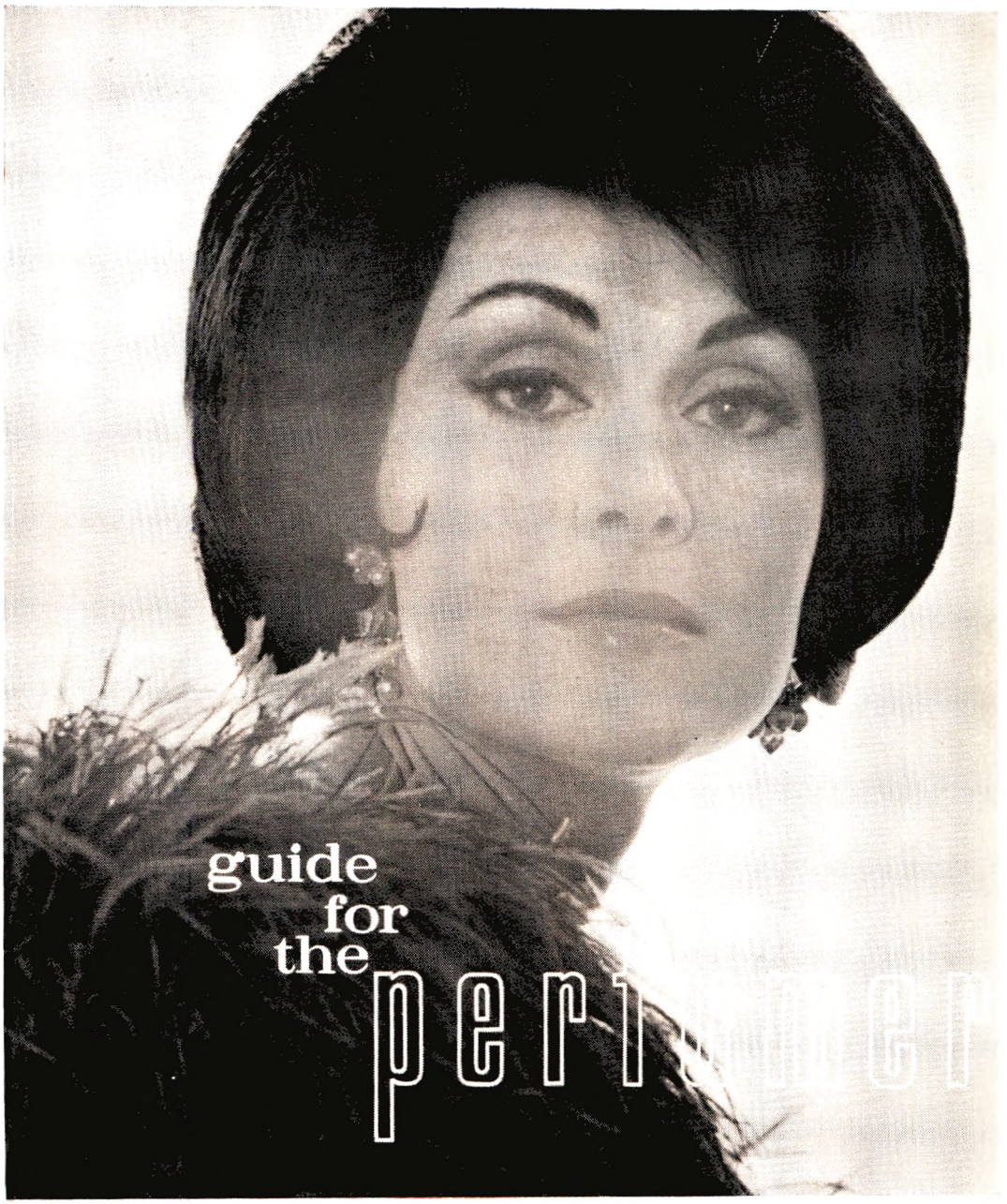
Responsibility for statements published : The Society of Cosmetic Chemists of Great Britain and its Hon. Editor assume no responsibility for statement or opinions advanced by contributors to this Journal.

Lectures : The Society shall have the right of first publication of any lecture or address delivered before it, but does not undertake to publish any given matter.

Manuscripts : These should be in accordance with the "Directions for Preparation of Manuscripts," copies of which are available from the Hon. Editor, Ashbourne House, Alberon Gardens, London, N.W.11.

4TH I.F.S.C.C. CONGRESS

The 4th I.F.S.C.C. Congress will take place in June 1966, in Paris. Anyone wishing to submit a paper should contact the Société Française de Cosmetologie, Maison de la Chimie, Rue Saint Dominique, Paris VIIe, France.



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SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 5″ × 3″ index cards for reference, without mutilating the pages of the Journal.

The preservation of ophthalmic preparations: M. R. W. BROWN and D. A. NORTON.
Journal of the Society of Cosmetic Chemists **16** 369-393 (1965)

Synopsis—The chemical and physical factors involved in ophthalmic formulations have been considered briefly. The problems of sterility for ophthalmic preparations have been discussed and the evidence relating to the activity of several preservatives examined. Chlorocresol, chlorbutol, benzalkonium chloride and chlorhexidine were shown to have scientific support for use as ophthalmic preservatives.

Hygiene in manufacturing plant and its effect on the preservation of emulsions: DOREEN L. WEDDERBURN.
Journal of the Society of Cosmetic Chemists **16** 395-403 (1965)

Synopsis—Emulsions, which under normal circumstances are adequately preserved against microbial decomposition, can and often do break down when unusually large numbers of micro-organisms are accidentally introduced. The origins of excessive contamination frequently lie in the resin beds of demineralized water units, in filters, and in poorly designed plant which cannot be cleaned and sterilized effectively.

Precautions can be taken to avoid circumstances in which bacteria and fungi can multiply in manufacturing plant, and the best ways of guarding against this are the elimination of ''blind ends'' and dead spaces in equipment, and meticulous attention to plant cleaning and sterilization. The washing of machinery with warm detergent solutions is often inadequate because residues of emulsion diluted with the wash-solution can provide a favourable environment for rapid microbial growth in stagnation areas.

Lessons in good plant design and hygienic practice can be learned from the food industry where good housekeeping is essential in the avoidance of spoilage. Modern pharmaceutical and cosmetic emulsions are more susceptible to microbial decomposition than they were in the past, because many new materials are biodegradable, and consequently more rigorous standards of cleanliness, and sterility are now needed in manufacture.

The sebaceous glands: F. J. EBLING.
Journal of the Society of Cosmetic Chemists **16** 405-411 (1965)

Synopsis—The structure, mode of secretion, distribution and development of the sebaceous glands are reviewed, the composition of sebum is described, and its function is debated. The actions of androgens, oestrogens, and progestogens on the sebaceous glands are discussed. It is concluded that while traditional views of the function of the sebaceous gland may be challengeable, the gland is far from vestigial in structure and, moreover, its sensitivity to male hormones is no less than that of the accessory male sexual organs. For these reasons a functional status for the gland ought to be assumed, though it is possible that this is not yet fully understood.

Journal of the Society of Cosmetic Chemists

This edition is published for
THE SOCIETY OF COSMETIC CHEMISTS
OF GREAT BRITAIN
by Pergamon Press, Ltd., Headington Hill Hall, Oxford
Editorial Office : Ashbourne House, Alberon Gardens, London, N.W.11.

VOL. XVI 24 JUNE 1965 No. 7

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J. Soc. Cosmetic Chemists **16** 369-393 (1965)

The Preservation of Ophthalmic Preparations

M. R. W. BROWN and D. A. NORTON*

Presented at the Symposium on "Preservatives and Antioxidants", organized by the Pharmaceutical Society of Great Britain and the Society of Cosmetic Chemists of Great Britain, in London on 17th November 1964.

Synopsis—The chemical and physical factors involved in ophthalmic formulations have been considered briefly. The problems of sterility for ophthalmic preparations have been discussed and the evidence relating to the activity of several preservatives examined. Chlorocresol, chlorbutol, benzalkonium chloride and chlorhexidine were shown to have scientific support for use as ophthalmic preservatives.

Ophthalmic preparations should be made so as to preserve them against physical, chemical and biological changes which may make them less effective therapeutically. The pharmacist must formulate such preparations with attention to many factors, most important of which are tonicity, pH, stability, viscosity and sterility. It is proposed briefly to consider the chemical and physical factors involved in ophthalmic formulations and to investigate more fully the problems of sterility and preservation against micro-organisms.

Physical and Chemical Factors

Tonicity

Lachrymal fluid is isotonic with blood and has a tonicity equivalent to that of 0.9% sodium chloride solution. Riegelman and Vaughan (1) have quoted results of their own, and of other workers, showing that an

*School of Pharmacy, Bristol College of Science and Technology, Bristol 7.

ophthalmic solution may safely range from the equivalent of 0.7% to 1.5% sodium chloride. Most ophthalmic drugs have high molecular weights, and may usually be added to an isotonic vehicle without altering the tonicity to painful levels. This applies particularly to eye drops used in small volumes ; large volumes of eye lotions should be approximately isotonic.

Hydrogen ion concentration

Normal tears have a pH of about 7.4. They have the capacity to bring the pH of an unbuffered solution at pH as low as 3.5, or as high as 10.5, to within tolerable limits instantaneously (1). Most ophthalmic drugs, e.g. alkaloidal salts, in distilled water or isotonic saline have a negligible buffer capacity although some drugs, notably salts of pilocarpine and epinephrine, may overtax the buffer capacity. The United States National Formulary XI recommends a 2% boric acid solution (about pH 4.7) as a general ophthalmic vehicle. This solution will tend to neutralize alkali leached from the glass container, it has a satisfactory tonicity, and is at a pH where most ophthalmic drugs are stable to autoclaving as well as being therapeutically active. The United States Pharmacopoeia XVI recommends an isotonic phosphate buffer as an ophthalmic vehicle in addition to boric acid solution.

Stability

The stability of ophthalmic preparations during prolonged storage, and the effects of heat sterilization processes, must be considered during formulation. The effects of temperature and pH are particularly important. Buffering certain drugs in the physiological pH range makes them unstable, particularly at high temperatures. Most of the common ophthalmic drugs, however, in 2% boric acid solution can be autoclaved without seriously affecting their therapeutic activity. The U.S.N.F. XI states that the oxidative discolouration of physostigmine, epinephrine and phenylephrine under these circumstances may be reduced by the addition of 0.2% sodium bisulphite to the vehicle. Alkaline drugs such as the sulphonamides and fluorescein sodium should be prepared in sterile distilled water.

Viscosity

Methylcellulose is sometimes added to ophthalmic solutions to increase contact time with the cornea. Calculated quantities of a concentrated, hydrated stock solution of methylcellulose may be added to normal

ophthalmic vehicles which may then be autoclaved, cooled, and the coagulated methylcellulose re-dispersed by agitation.

Preservation against Micro-Organisms

There has recently been much discussion about the difficulties of preserving ophthalmic solutions against micro-organisms. The main problem is the exceptionally high resistance of *Pseudomonas aeruginosa* to chemical antibacterial agents. This pathogen causes serious difficulties in the control of cross-infection in general (2,3) but especially in ophthalmology (4). Some species of *Aerobacter* are eye pathogens (5), and the problem is further complicated by the existence of pathogenic strains of the spore formers *Bacillus subtilis* (6) and *Clostridium welchii* (7).

Pseudomonads have been isolated from most naturally occurring waters (8), and *Ps.aeruginosa* in particular has exceptionally simple requirements and will grow in many ophthalmic solutions. This organism has been shown to use the hydroxybenzoates as a sole source of carbon (9). In addition, the temperature requirements for growth are wide. It is a virulent pathogen causing severe ocular infections (10). Relatively small inocula of about 50–100 cells have been shown to produce infection in rabbits' eyes (11,12). Fisher and Allen (13) have shown that *Ps.aeruginosa* produced an enzyme which destroys the cornea by degradation of corneal collagen.

Ps.aeruginosa has consequently been recommended as the main reference organism when selecting chemical preservatives for ophthalmic solutions. An adequate preservative should also be effective against a wide range of gram-positive and gram-negative organisms.

The case for sterility

An injured eye has less resistance to infection than the bloodstream, so that at least the same precautions should be taken in preparing ophthalmic solutions as in preparing solutions for intravenous use (14). The United States National Formulary XI states that all solutions for surgical use should be sterile, and prepared without a preservative because of danger of chemical damage to the inner structure of the eye. It has frequently been shown that a significant proportion of used, and unused, ophthalmic solutions were contaminated with *Ps.aeruginosa*, and that eye ointments are also liable to contamination (15,16,17). There is now widespread agreement that ideally ophthalmic medicaments should be dispensed in a sterile condition (11,15,18,4).

The United States Pharmacopoeia, the United States National Formulary and the International Pharmacopoeia all specify sterility for ophthalmic solutions. The British Pharmaceutical Codex 1963 stipulates sterility for eye drops only.

Autoclaving in the final container is the method of choice because of the danger from pathogenic spore formers. This is still so with chemically preserved solutions because of the possibility of germination on the cornea, which can apparently eliminate the carry-over activity of a wide range of antibacterial agents (11). It is, however, unlikely that pathogenic spores would germinate in the ophthalmic container in the presence of an antibacterial agent, and at room temperature. Gamma radiation has proved a useful sterilizing agent for some heat labile ophthalmic preparations and may prove to be of wide application (19).

Eye drop containers

Norton (20), and Richards *et al* (21) have shown that eye drop containers available then in Britain for extemporaneous preparations were not satisfactory, and that closure units must be modified to provide satisfactory sealing during autoclaving. British Standards Institution Committee P.188 is now engaged in producing a specification for a suitable multidose eye drop container.

It seems possible, and desirable, that in the field of ophthalmic preparations, as with other classes of medicaments, there will be progressive movement away from extemporaneously prepared products. The time is opportune for bulk-manufactured sterile products of standard formulae, packed in simple plastic single and multidose containers. Several manufacturers do this using methods which would normally be uneconomic for the individual pharmacist in a small business (22). Nevertheless, there is now available relatively inexpensive equipment for small scale extemporaneous sterile filtration employing a modified syringe with membrane filter (15).

Preservation of oils, creams and ointments

There is little published work on the preservation of oils, creams and ointments. Ridley (16) has isolated *Ps.aeruginosa* from eye ointments on several occasions. He proposed to eliminate the use of oils by replacing them with ointments dispensed in sterile tubes. The activity of bactericides in o/w dispersions has been shown to be influenced by many interacting factors and the *ad hoc* addition of preservatives shown to be

unsatisfactory (23). A practical ideal at present would appear to be the aseptic preparation of ophthalmic ointments, using sterile ingredients.

Preservation of solutions

The possibility of contamination from pathogenic organisms subsequent to sterilization makes it necessary for multidose containers to be preserved using a suitable antibacterial agent.

The capacity of small inocula of *Ps.aeruginosa* to cause infection, together with the neutralizing properties of the cornea towards antibacterial agents, make it preferable that these agents be capable of maintaining sterility.

Much of the earlier work assessing preservatives is of reduced value because of inadequate experimental procedures. Riegelman *et al* (11) quoted numerous papers which did not state the precautions taken to ensure the absence of bacteriostatic concentrations of preservative in subcultures testing for recovery. They discussed the inadequacy of dilution techniques designed to inactivate preservatives and introduced *in vivo* procedures as well as *in vitro* tests involving chemical antagonists. They consistently found that organisms would produce infections, and could be recovered from the cornea while *in vitro* cultures were apparently negative. Subsequent improvement of the antagonist/recovery broth reduced the discrepancy. Kohn *et al* (24,25) further developed this important principle of comparing *in vivo* with *in vitro* procedures and obtained exact correlation.

Several chemical agents have been proposed as suitable ophthalmic preservatives and it seemed appropriate to consider each separately.

Esters of p-hydroxybenzoic acid

A combination of methyl and propyl *p*hydroxybenzoates has been used in the form of "Solution for Eye Drops" until this preparation was replaced by chlorocresol in the British Pharmaceutical Codex 1963. Subsequently this Codex was amended (26), and Solution for Eye Drops reintroduced. Brown *et al* (4) have commented on these changes. Klein *et al* (27) tested two combinations of the esters at three concentrations up to 0.16% against 10^8/ml *Ps.aeruginosa* present in three eye-drop preparations—Sodium fluorescein 2%, atropine sulphate 0.5%, eserine salicylate 0.25%. Contact times were up to 24 hr after which subcultures were made testing for sterility. They found that both combinations 1 (*Nipasept*) and 2 (*Nipa 82121*) were ineffective at all concentrations in fluorescein drops, and growth occurred after subculture at each contact time up to 24 hr. 0.16%

Nipasept was necessary to produce sterility after 1 hr contact in atropine drops, while 0.106% sterilized after 2 hr. In eserine drops 0.16% *Nipasept* sterilized after 3 hr, and 0.106% after 5 hr. Combination 2 was more effective than *Nipasept* in the presence of atropine and eserine, and 0.08% sterilized after 1 hr. The esters were effective in some cases at 0.16%, but nevertheless it was found that this strength caused some burning sensation when introduced on the eye. The value of their results is reduced because they used a broth dilution experimental procedure, and inactivation of the agents was uncertain. Nonionic detergents are generally known to inactivate *p*hydroxybenzoic acid esters (28) and Kohn *et al* (24) have recommended *Tween 20* as an antagonist of these esters.

Lawrence (29) found that a mixture of 0.16% methylparaben and 0.02% propylparaben sterilized within 1 to 6 hr in every case with 26 strains of *Ps.aeruginosa*, and four species of Proteus in simple buffer at pH 6.2. He used undiluted 24 hr cultures as inocula. Activity was also tested against four strains of *Ps.aeruginosa* in the presence of non buffered aqueous solutions of several drugs. Sterility was achieved in less than 30 min in several cases and never after more than 6 hr. He did not use inactivating substances in the recovery broth for the parabens.

Ridley (16) stated that 0·1% methyl *p*hydroxybenzoate had been used successfully as a preservative in hospital practice for three years. He stated that 0.1% methyl *p*hydroxybenzoate was " . . . effective against a wide range of bacteria tested, including *Ps.pyocyanea*, *Streptococcus pyogenes*, and *Staphylococcus aureus* at room temperature, in conjunction with the drugs commonly used and in normal clinical concentrations, in three hours." No experimental evidence was provided to support this statement and the definition of "effective" was not given.

Kohn *et al* (24) found that a mixture of 0.2% methyl paraben and 0.04% propyl paraben sterilized *Ps.aeruginosa* suspensions (2×10^8/ml) in 3 hr. A mixture of 0.18% propyl paraben sterilized in 6 hr. These workers used 13 strains of *Ps.aeruginosa*, and the effectiveness of the inactivating agents in their recovery broth was established by a correlation between *in vivo* and *in vitro* results.

Anderson *et al* (30) tested 0.1% methyl hydroxybenzoate in six final eye drop preparations using *Staphylococcus pyogenes*, *Proteus vulgaris* and *Ps.aeruginosa* in concentrations of about 5×10^4/ml. They found that sterility was achieved within one day in every case except two. The viable count increased during 11 days with *Ps.aeruginosa* in gelatin eye drops, and sterility was achieved after two days but not after one day

with *Pr.vulgaris* in glycerine eye lotion. More useful information might have been obtained if tests had been made at intervals less than daily.

Hugo and Foster (9) have shown that the rate of growth of one strain of *Ps.aeruginosa* was little affected by the presence of the concentration of esters used in Solution for Eye Drops B.P.C. (0.034%), and that they could be used as a sole carbon source at the same concentration. These workers, [Foster (31), Hugo and Foster (32)], found that a mixture of two parts methyl and one part propyl *p*hydroxybenzoate sterilized aqueous suspensions containing 10 and 100/ml *Ps.aeruginosa* cells of one strain in 30 minutes at 18° using concentrations of 0.125% and 0.2% respectively. They did not use an inactivator in the recovery medium.

Chlorocresol

*p*Chloro-*m*-cresol was the first official ophthalmic antibacterial preservative used in Britain. Its present replacement by Solution for Eye Drops in the B.P.C. has been discussed (4).

Davis (33) found that 0.05% chlorocresol was as effective against *Staph.aureus* as was 0.5% phenol. In each case, sterility was achieved within one day. Details of reaction menstrum and recovery conditions were not given.

Klein *et al* (27) used a broth dilution procedure and found that 0.1% chlorocresol sterilized broth suspensions (10^8/ml) of three strains of *Ps.aeruginosa*. They also tested 0.1% and 0.03% chlorocresol in the presence of three eye drop preparations. In each case, sterility was achieved within 1 hr using 0.1%. 0.03% chlorocresol sterilized atropine drops within 4 hr, while fluorescein and eserine were sterilized between 6 and 24 hr. They found that 0.1% chlorocresol caused smarting. 0.05% chlorocresol sterilized inocula of 10/ml *Ps.aeruginosa* cells of one strain in aqueous suspensions at 18° within 30 min, but 0.07% was required to sterilize 100 cells/ml within the same time (32).

Crompton and Anderson (34) have reported that injections of 0.05% chlorocresol in normal saline into the anterior chamber of rabbits' eyes caused the cornea to become opaque. This disadvantage would seem not to apply in ophthalmic solutions preserved and used with intact eyes.

Richards (35) reported that chlorocresol was more active in terms of reduction of viable count in final eye drop preparations at acid pH, than it was in simple solution.

Chlorbutol

Klein *et al* (27) used a broth dilution test, and found that three strains of *Ps.aeruginosa* (10^8/ml) were sterilized by 0.2% chlorbutol. They found that three eye drop preparations inoculated with 10^6/ml *Ps.aeruginosa* were sterilized after 1 hour's contact with 0.5% chlorbutol. Recovery media did not contain inactivators. These workers found that heat significantly reduced the antibacterial activity of this compound. Chlorbutol is slowly soluble in water, and heat is often used. This phenomenon may account for the contamination of 0.25% chlorbutol preserved eye drops reported by Crompton (6).

All of 35 strains of *Ps.aeruginosa* failed to grow on subculture after 24 hours' contact with 0.5% chlorbutol (36). These workers measured the percentage hydrolysis of buffered 0·5% chlorbutanol solutions after autoclaving at 121°. There was about 5% hydrolysis after 15 min at 121° at pH less than 5. At about pH 6, and higher, the percentage rose considerably. They found that autoclaving at acid pH (up to pH 6) did not inactivate the antibacterial effect of chlorbutanol. Murphy *et al* (36) reported an absence of endothelial damage or other ill effect upon the tissues within the eye, despite repeated use of 0.5% chlorbutol lotions during anterior chamber washes. This preservative had been in continuous hospital use for twenty-nine years.

Lawrence (29) found that 0.5% chlorbutol sterilized heavy inocula of 26 strains of *Ps.aeruginosa* and four species of *Proteus* in simple buffer after contact times of up to two days. The bactericidal action was more rapid in the presence of non-buffered solutions of several drugs, and sterility of four strains of *Ps.aeruginosa* took up to 6 hr. Recovery media did not contain inactivators.

Riegelman *et al* (11) found that 0.5% chlorbutol apparently sterilized broth suspensions of *Ps.aeruginosa* in times which varied with the inactivating recovery broth. Negative *in vitro* tests were obtained between 8 and 24 hr using optimum recovery broth, and these were correlated with *in vivo* tests. Apparent sterilization occurred after 45 min when recovery was made in nutrient broth alone. These workers found that concentrations in excess of 0.5% were irritating to the eye.

Anderson and Stock (37) found that chlorbutol 0.5% in water sterilized three strains of *Ps.aeruginosa* (about 10^5/ml) within 15 min, but was ineffective against one strain of *Staph.aureus* even after 1 hr. These workers recovered in nutrient broth without any inactivator.

Kohn *et al* (24) used 13 strains of *Ps.aeruginosa* and found that 0.5%

chlorbutol sterilized suspensions of about 2×10^6 cells/ml in 12 hr. These workers used inactivators in their recovery media and obtained good agreement between *in vivo* and *in vitro* experiments.

Organic mercurials

Phenylmercuric nitrate, phenylmercuric acetate and thiomersalate all sterilized three strains of *Ps.aeruginosa* (10^8/ml) at a dilution of 1 in 50,000 in broth (27). It was found that 0.005% thiomersalate sterilized this organism in 2 hr in fluorescein drops but a contact of 6 hr was necessary to sterilize atropine and eserine drops. These sterilizing times must be viewed critically because the efficiency of the recovery media was apparently not demonstrated.

Lawrence (29) found that organic mercurials were less active in the presence of common ophthalmic drugs than in their absence. 0.01% P.M.N. and 0.01% thimerosol sterilized four strains of *Ps.aeruginosa* in several ophthalmic solutions in contact times varying up to 48 hr. In the absence of any drug, 0.01% P.M.N. sterilized in times varying up to 3 hr. Recovery was in Brewer's fluid thioglycolate medium.

Riegelman *et al* (11) showed that 0.01% P.M.N. produced apparent sterility after 1 hour's contact with *Ps.aeruginosa* (10^8/ml) when subcultured into thioglycolate broth. However, corneal ulcers were produced by these apparently sterile suspensions. The use of lecithin-polysorbate 80-thioglycolate medium eliminated the discrepancy between *in vivo* and *in vitro* results, and the sterilizing time was in excess of a week.

0.01% P.M.N. failed to sterilize one strain of *Staph.aureus* and three strains of *Ps.aeruginosa* in contact times up to 1 hr using about 10^5/ml cells (37).

Ridley (16) stated that 0.004% P.M.N. had been used successfully in hospital practice and had been shown to be effective against several pathogenic species but omitted to give experimental procedures.

In vitro tests using inactivating recovery media together with *in vivo* tests showed that P.M.N. 0.01% and thimerosal 0.02% sterilized *Ps.aeruginosa* (2×10^6/ml) in aqueous suspensions in 6 hr. A two-fold dilution of these mercurials had little effect upon the sterilizing time (24).

Anderson *et al* (30) tested the activity of P.M.N. (0.001-0.004%) against *Staph.pyogenes*, *Proteus vulgaris* and *Ps.pyocyanea* (10^3-10^4/ml) using 18 eye drop formulations involving 12 drugs. Sterility was achieved in less than one day in all cases excluding fluorescein. P.M.N. 0.002% did not sterilize fluorescein drops after several days contact. In the one formu-

lation using 0.004% P.M.N. in fluorescein drops, sterility was achieved within a day. These workers did not use contact times of less than a day and the recovery media did not contain inactivators.

Phenylethyl alcohol

Lilley and Brewer (38) showed that 2-phenylethyl alcohol was particularly active against gram-negative bacteria including *Pseudomonads*.

Klein *et al* (27) tested phenylethyl alcohol against *Ps.aeruginosa* in the presence of atropine, eserine and fluorescein. In each case 0.6% sterilized within 1 hr while 0.5% required contact of up to 3 hr. Inactivators were not used during recovery.

This preservative was rejected by Murphy *et al* (36) on the grounds that two strains of *Ps.aeruginosa* could be serially transferred in the presence of 0.5%, and four strains of *Staph.aureus* grew in the presence of 0.6% in broth.

Lawrence (29) tested the activity of 0.5% phenylethyl alcohol against 26 strains of *Ps.aeruginosa* and four species of *Proteus* using inocula of undiluted broth culture. Sterilization occurred after contact times of up to 6 days in the presence of a series of ophthalmic drugs. In the absence of drugs, sterilization occurred after longer contact periods and in some instances had not occurred by the end of the experimental period (6 days).

Corneal ulcers were produced from intracorneal injections of a *Ps.aeruginosa* contaminated solution of 2% phenylethyl alcohol after 8 hours' exposure (longest experimental period) (11). These workers found that 0.75% solutions were irritating to the eye.

Phenylethyl alcohol 0·6% failed to sterilize three strains of *Ps.aeruginosa* in aqueous suspension during exposures up to 1 hr, but sterilized one strain of *Staph.aureus* within 1 hr using cell concentrations of about 10^5/ml (37).

Kohn *et al* (24) tested 0.5% phenylethyl alcohol against 13 strains of *Ps.aeruginosa* (2×10^6/ml) using *Tweens* as inactivating agents in the recovery media. Sterilization was not effected after 24 hours' contact, and *in vivo* experiments confirmed these *in vitro* results.

Phenylethanol 0.9% sterilized inocula of 100 cells/ml of one strain of *Ps.aeruginosa* in aqueous suspension in 30 min at 18°. Inactivators were not used in the recovery medium (31, 32).

Quaternary ammonium compounds

There is much published evidence about the use of these compounds as ophthalmic preservatives. Klein *et al* (27) found that a concentration

of 0.05% benzalkonium was required to sterilize broth suspensions (10^8/ml) of three strains of *Ps.aeruginosa*. Subcultures were taken after two days. These workers found that although 0.5% cetrimide sterilized, there was one instance when cetrimide was shown to have comparatively little effect against *Ps.aeruginosa*.

Benzalkonium chloride was found not to be a uniformly effective bactericide against *Ps.aeruginosa*. Five out of 30 strains were not sterilized by contact for 24 hours with 0.01% of the agent, at pH 7.4 (36).

Lawrence (39) in his review concluded that benzalkonium chloride, chlorbutanol and P.M.N. were the most suitable agents on the basis of published evidence. The same worker (29) tested benzalkonium chloride 0.02% and 0.01% against 26 strains of *Ps.aeruginosa*, and four species of Proteus using inocula of undiluted 24 hr broth cultures. Tests were made in (1) simple buffer, (2) in the presence of several ophthalmic drugs in buffer, and (3) in the presence of several drugs in aqueous solution. In every instance sterility was achieved in less than 30 min with 0.02% benzalkonium chloride. The majority of strains were sterilized by 0.01% in less than 30 min in all three systems but a few strains needed up to 6 hr. Chemical inactivators were used in the recovery broth.

These results conflicted with those of Riegelman *et al* (11) who found that benzalkonium chloride was only slowly bactericidal. They suggested that the discrepancy between their results, and those of earlier workers, was because of inadequate inactivation of benzalkonium chloride in the recovery broth used by other workers. They found that 0.01% benzalkonium chloride failed to sterilize 10^8/ml *Ps.aeruginosa* cells in saline in one week when tested by *in vivo* methods whereas the use of nutrient broth subculture indicated sterility after five minutes contact. The use of a lecithin-*Tween 80*-thioglycolate medium resulted in concordant *in vivo* and *in vitro* tests. Riegelman *et al* (11) reported incidents when resistance to benzalkonium chloride by *Ps.aeruginosa* was demonstrated. In one case, active growth occurred and pigment was produced in the presence of un-neutralized 0.02% benzalkonium chloride from an inoculum of 1 ml of a 24 hr culture.

Kohn *et al* (24) found that benzalkonium chloride 0.02% sterilized 13 strains of *Ps.aeruginosa* (2×10^6/ml) in aqueous suspension within 45 min, and that 0.01% required up to 9 hr. The effectiveness of the inactivators used in the *in vitro* recovery media was demonstrated by correlation with *in vivo* results. These workers found that of seven commonly used preservatives, only 0.02% benzalkonium chloride sterilized in less than 1 hr.

Inocula of 10 and 100/ml *Ps.aeruginosa* cells were sterilized in aqueous suspension at 18° in 30 min by benzalkonium chloride 0.05% and 0.08% respectively (32). Recovery was on media containing an inactivator.

Benzalkonium chloride has been accepted officially in the U.S.A. for many years but has not been widely used in Britain despite the large amount of favourable published work. There have been criticism against benzalkonium chloride on grounds other than bacteriological. Klein *et al* (27) stated "Quaternary ammonium compounds as a preservative for eye-drops should be used only in exceptional cases. Ginsburg and Robson found that detergents could prove harmful by causing 'solubilization' of the intercellular cement of the corneal epithelium." This reference to the work of Ginsburg and Robson (40) has been quoted by others (41). Recently, Anderson *et al* (30) have also quoted this work as evidence that benzalkonium chloride dissolves the intercellular cement substance of the cornea. Ginsburg and Robson (40) did not use a quaternary ammonium compound but worked with anionic and nonionic wetting agents. These workers found that the anionic detergent, dodecyl sodium sulphate (1%) damaged the cornea. They also found that although 1% nonionic *Lissapol N* caused slight temporary oedema, 0.5% caused no observable effect. These results are in accord with those of Buschke (42) who made direct measurements on intercellular cohesion in corneal epithelium. He found that six out of seven anionic detergents " . . . produced marked effects in concentrations of 1% or sometimes even in lower concentrations." Not one of a wide selection of nonionic or cationic detergents caused loss of intercellular cohesion. Benzalkonium chloride was used in concentrations up to 10%.

Recently, Anderson *et al* (43) have stated " . . . Swan has shown that wetting agents such as benzalkonium chloride are injurious to the corneal epithelium and especially to the endothelium. . . ." Swan (44) found that intraocular concentrations of benzalkonium chloride in excess of 0.01% caused serious damage. He also found that instillation of 0.1% benzalkonium chloride produced a severe reaction, and that 0.03% to 0.04% used over periods of up to 8 weeks produced a less severe reaction. In most cases the conjunctiva and cornea were normal within 12 hr. Nevertheless, Swan concluded, "The minimal concentrations of wetting agents producing irritation in the conjunctival sac are greater than those that are effective."

Hughson and Styron (45) replaced the aqueous humour of rabbits' eyes with benzalkonium chloride in saline, and found that concentrations of

1 in 3000 and 1 in 6000 produced endothelial oedema which disappeared in 6 to 8 weeks. The use of a concentration of 1 in 7500 gave no visible evidence of damage. Bell (46) used a 1 in 10,000 concentration of a quaternary ammonium agent as a routine irrigant in eye surgery for three years, and observed no hypersensitivity or allergic reaction, nor was there any evidence of irritation. Lawrence (39) has quoted numerous other workers with similar experiences using quaternary ammonium compounds.

Polymyxin

Wiggins (47) found that 85 strains of *Ps.aeruginosa* were sensitive to polymyxin B sulphate. He found that the minimum inhibitory concentration at 37° in broth lay between 0.08 to 2.5 μg/ml.

Antibiograms for 152 strains of *Ps.aeruginosa* showed that while there was marked resistance to several antibiotics, all strains were sensitive to polymyxin B (3).

Riegelman *et al* (11) found that 1000 units/ml polymyxin B sulphate sterilized *Ps.aeruginosa* (10^8/ml) in aqueous suspension within 30 min. Lecithin was found to be an effective inactivator for polymyxin. *In vivo* tests confirmed the *in vitro* results.

Polymyxin B sulphate was found to be only slowly bactericidal in aqueous solution against 13 strains of *Ps.aeruginosa* (2×10^6/ml). Sterility was achieved in 12 hr using 2000 units/ml, and in 18 hr using 1000 units/ml. The results of *in vivo* experiments were in agreement with results of recovery using lecithin broth (25).

Agents not widely used as preservatives

Anderson and Stock (37) showed that chlorhexidine acetate 0.01% in aqueous solution sterilized three strains of *Ps.aeruginosa* within 15 min, and one strain of *Staph.aureus* within 30 min using cell concentrations of about 10^5/ml. Egg yolk medium was used to inactivate the chlorhexidine on recovery. Much useful work on formulation of ophthalmic solutions with chlorhexidine has been done in Australia (48,49), and it has been included in the Australian Pharmaceutical Formulary. No instance of sensitivity had been detected after several years' hospital use of chlorhexidine preserved drops (6). Crompton (6) has stated that " . . . Chlorhexidine at present is the bacteriostatic of choice." He quoted evidence of the personal communications of two workers to support this belief but omitted to give sufficient detail to allow evaluation of experimental methods. Anderson *et al* (30) tested chlorhexidine against single strains of *Staph.*

pyogenes, *Proteus vulgaris* and *Ps.aeruginosa* using inocula of about 10^3–10^4 cells/ml. Tests were made at 28° in the presence of numerous ophthalmic drugs in 75 formulations. Counts were made of recovered cells, and the preservative was regarded as satisfactory if the count remained static or was reduced. In most instances sterility was achieved within the shortest test period of one day. Increased counts occurred with both serum and methylcellulose formulations in the presence of 0.005% chlorhexidine. Growth occurred in the presence of lignocaine in one out of two formulations. It was suggested that growth in the presence of pilocarpine was due to incompatibility with the 0.005% chlorhexidine. Chlorhexidine is not in fact used in the Australian Pharmaceutical Formulary for eye drops containing pilocarpine (50). The experimental procedures of these workers are open to question. They did not use conventional inactivators in their recovery medium but attempted to inactivate the agents tested, including chlorhexidine, using a washing procedure, the efficiency of which apparently was not demonstrated. They recovered chemically treated *Pr.vulgaris* cells in medium containing 0.1% phenol which might well reduce recovery as well as swarming. One other unusual aspect of their experimental procedure was an 18 hr recovery incubation period. Without evidence to the contrary, this would seem to be a short time for the recovery of chemically treated organisms. Hugo and Foster (32) found that inocula of *Ps.aeruginosa* (100/ml) were sterilized in aqueous suspension at 18° by 0.005% chlorhexidine, and at 30° by 0.002%.

Kohn *et al* (25) tested 51 chemicals not previously employed widely in ophthalmic solutions as preservatives. Thirteen strains of *Ps.aeruginosa* (2×10^6/ml) were used, and *in vitro* and *in vivo* methods employed. Six out of 37 quaternary ammonium compounds were found to possess equal or superior activity to benzalkonium chloride which they used as a standard. All of eight amphoteric surfactants were found to be inadequate while three iodophors were found to possess sterilizing times of less than 1 hr. Chlorhexidine and Colistin were each found to sterilize in less than 1 hr.

Richards (35) found that chlorhexidine was less active in final eye drop preparations at acid pH than it was in simple solution.

Wiseman (51) attempted to increase the resistance of several gram-positive and gram-negative species to chlorhexidine by training in its presence. He failed to increase the resistance of gram-positive organisms but found that gram-negative species showed a substantial increase in resistance. In particular the minimum inhibitory concentration against

Ps.aeruginosa increased progressively from an initial 4 μg/ml to 64 μg/ml after 8 subcultures in the presence of chlorhexidine diacetate.

Bronopol (2-bromo-2-nitropropane-1, 3-diol) is a new antimicrobial agent shown to be active against numerous strains of several gram-positive and gram-negative species including 22 strains of *Ps.aeruginosa* (52). Preliminary experiments showed that bactericidal concentrations are not irritant to the skin or mucous membranes. The authors failed to increase the resistance of *Ps.aeruginosa* and *Staph.aureus* to Bronopol by repeated subculture in its presence.

Summary and Conclusions

Consideration of the available published evidence reveals that at present there is no single ideal preservative for ophthalmic preparations. The capacity of strains of *Ps.aeruginosa* or closely related species to become resistant to quaternary ammonium compounds (11), chlorbutol (39), phenylethyl alcohol (36) chlorhexidine (51) and even to decompose phenol (53) and *p*hydroxy-benzoates (9) would suggest caution in accepting any new agent supposed not to have this defect against this enigmatic organism.

Many of the results in the literature are conflicting. Some of the discrepancies may be accounted for by reason of variation in such important factors as reaction temperature, composition of reaction mixture, and recovery medium. Importance must be attached to the results of those workers who have clearly defined their experimental conditions, and who have related the efficiency of the inactivators used in their recovery media to the results of *in vivo* tests. The capacity or not to cause disease would appear to be the ultimate, objective assessment, particularly when activity is measured in a final ophthalmic formulation.

An appraisal of the scientifically acceptable data on the *p*hydroxy-benzoates would suggest that nearly saturated solutions are capable of sterilizing heavy inocula (10^8) of *Ps.aeruginosa* in several hours. Such concentrations are too irritant for normal ophthalmic use. A concentration of about 0.1% mixture may be useful in some eye preparations excluding, in particular, fluorescein. The capacity of *Ps.aeruginosa* to utilise (9) these compounds as a source of carbon would seem to preclude their recommendation for widespread use.

The available evidence suggests that the concentration used in Solution for Eye Drops B.P.C. (0.0229% methyl, 0.0114% propyl) is unlikely to sterilize, and may possibly allow growth of *Ps.aeruginosa*.

The small amount of available evidence supports the use of chlorocresol 0.05% for use with intact, but not damaged eyes.

The considerable volume of work on chlorbutol has shown that almost all workers have found it to be consistently an effective, but relatively slow, sterilizing agent. It would seem that formulation aspects of this agent have been adequately investigated (36,54).

The mercurials have been shown to be effective sterilizing agents by nearly all workers. Riegelman *et al* (11) found 0.01% P.M.N. required longer than a week to sterilize a heavy inoculum of *Ps.aeruginosa*, but Kohn *et al* (24), using several strains and similar procedures to those of Riegelman and his co-workers, found that sterilization was effected in 6 hr.

It has been asserted that these mercurials may give rise to mercurial sensitization ("mercurialentis"), and that mercury may deposit on the lens capsule (6,26). There is little published evidence to support these assertions. Ridley (16) found very few cases of dermatitis medicamentosa with P.M.N. preservation. These few cases were with drops used for a long period of time, and in every case the reaction was slight. Work is in progress in our laboratory to elucidate this situation. Rabbits' eyes have been irrigated with mercurial solution, subsequently sectioned, and examined with an electron probe microanalyser. It is hoped that these procedures will provide useful information about local concentrations of mercury.

The available evidence offers little support for the use of phenylethyl alcohol as an ophthalmic preservative. The existence of resistant *Ps.aeruginosa* strains would not appear to be the result of inadvertent training due to faulty procedures.

The evidence about quaternary ammonium compounds is clouded by numerous quotations of opinions, assertions unsupported by data, and even errors in quoting the literature. A literature search has revealed very few instances where resistance to benzalkonium chloride has been demonstrated. Murphy *et al* (36) and Riegelman *et al* (11) have shown the existence of highly resistant strains of *Ps.aeruginosa*. On the other hand, it has been shown that faulty procedures may enable contaminants to acquire resistance, particularly to quaternary ammonium compounds (55).

The available evidence supports the conclusion of Riegelman and Vaughan (1) "with all its limitations, benzalkonium chloride is among the most effective and rapidly acting preservatives when the conditions of its use are properly controlled."

There is no evidence of resistance by *Ps.aeruginosa* to polymyxin B sulphate. This antibiotic is limited by its relatively low activity against gram-positive organisms, and some species of *Proteus* (47). The U.S.P. XI suggests the use of a combination of 1000 units/ml polymyxin B sulphate and 0.01% benzalkonium chloride.

The newer recommended ophthalmic preservatives include several promising compounds, nearly all of which require more work before their acceptance. Chlorhexidine is an exception in that it has been introduced into the Australian Pharmaceutical Formulary, and experience in practice has been gained. Nevertheless there are problems of compatibility with this compound, and it is not uniformly satisfactory with all ophthalmic solutions (50).

When suitably formulated, the following compounds have reasonable scientific support for their use as ophthalmic preservatives in some instances: 0.05% Chlorocresol, 0.5% chlorbutol, 0.01% benzalkonium chloride, 0.005% chlorhexidine. Experimental evidence is required about mercury deposition on the lens before the mercurials may be used with confidence.

Little attention has been paid to the use of combinations of agents. The recommendation of a combination of polymyxin B sulphate and benzalkonium chloride by U.S.N.F. XI would seem to be based on attaining broad spectrum activity rather than an expectation of synergism against any particular organism. Attention has been drawn to the dangers of indiscriminately combining antimicrobial agents (56,57). Preliminary work has shown synergism between phenylethyl alcohol and the organic mercurials (58).

It would appear that the future lies more in the understanding of the nature of the resistance of *Ps.aeruginosa* to chemical inactivation than in the discovering of other new agents. The literature possesses several monuments to the capacity of this beast, literally to eat the agents used against it. The acquired resistance to a quaternary ammonium compound has been eliminated in the presence of E.D.T.A. (59), and the U.S.N.F. XI has suggested its use for that purpose. It has been shown that cells grown in the presence of *Tween 80* are much less resistant to the action of several agents than are cells grown in plain broth (60,61). It has been suggested that the resistance of *Ps.aeruginosa* is connected with its slime production and its membrane permeability, both of which may be affected by *Tween 80*.

The problems of sterilization and preservation have for too long been

overshadowed in pharmacy curricula by consideration of tonicity, and the attendant calculations now shown to be largely unnecessary. The time would seem appropriate, and even overdue, to adjust long held ideas relating to ophthalmic formulations.

(Received : 17th September 1964)

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Introduction by Dr. M. R. W. Brown

I would like to draw attention very briefly to some of the more important elements. The ideal preservative should be effective against a wide range of organisms, must not be irritant, or in any way harmful to eye tissues, must be compatible with other medicaments and should withstand sterilization, preferably by a heat method, without production of harmful degradation products. Furthermore, careful distinction must be made between materials used for continuous application to intact eyes, and the solutions used on damaged eyes or during surgery. The latter solution should be sterile and should not contain preservatives.

Since this paper was submitted, we have been made aware of the recent work of Abrams (62) related to mercurialentis. He found that mercurialentis was slow to develop and no instance was found in glaucoma patients using myotics containing P.M.N. for less than three years. He furthermore stated, "as far as is known the appearance (mercurialentis) is not associated with any visual impairment and seems to be quite innocuous." There is scientific evidence to support the use of the following preservatives in appropriate instances: Benzalkonium chloride, phenylmercuric nitrate, thiomersal, chlorbutol, chlorocresol. At present there appears to be no scientifically based evidence to completely preclude their use under the controlled conditions of an ophthalmic formulation. Reports from Australian workers indicate decomposition of chlorhexidine on autoclaving, and the production of degradation products the freedom of which from toxicity has not been fully established and this would suggest caution regarding heat sterilization of preparations containing this compound.

The employment of these substances must be subject to normal considerations of formulation and compatibility and, of course, to correct clinical usage. There

(62) Abrams, J. D. *Trans. Ophthalm. Soc.* **83** 263 (1963).

is little evidence so far to confirm effectiveness of combinations of preservatives, and there are dangers in indiscriminately combining antimicrobial agents. There is no simple answer to the problem of preservation and each eye drop formulation must be considered individually.

In conclusion I would like to draw your attention to a reference which may possibly be misleading. In p. 003 reference (6) is mentioned and refers to the work of Roemer, cited by Crompton, regarding pathogenicity of *Bacillus subtilis*. It is in fact stated correctly as a citation in the reference. However, in p. 016, reference (6) was used in connection with mercurialentis and it was intended to refer directly to Crompton's paper. (Our attention to this was kindly drawn by Professor Neuwald.)

DISCUSSION

PROF. DR. F. NEUWALD: We have heard from Mrs. Wedderburn that every drug and every formulation needs special preservation. This applies especially to eye drops, and this is illustrated by *Table I* (63).

Table I

Eye-drop medicament	Buffer solution	Preservative
Ethylmorphine hydrochloride	P 5.3	Bz 0.02%
Silver diacetyltannin protein	B 6.8	Ph 0.002%
Silver protein	B 6.8	Ph 0.002%
Atropine sulphate	P 6.45	Bz 0.02%
2-Benzylimidazoline hydrochloride	P 6.85	HB 0.1%
Calcium chloride	B 6.8	Ph 0.002%
Carbamide	B 6.8	Ph 0.002%
Cinchocaine hydrochloride	P 6.05	Bz 0.02%
Cocaine hydrochloride	P 6.05	Bz 0.02%
Ephedrine hydrochloride	P 6.05	Bz 0.02%
Homatropine hydrobromide	P 6.45	Bz 0.02%
Mercuric oxycyanide	P 6.85	—
Potassium iodide	P 6.85	HB 0.1%
2-(Naphthyl-1-methyl)imidazoline nitrate	P 6.85	HB 0.1%
Sodium iodide	P 6.85	HB 0.1%
Sodium salicylate	B 6.8	Ph 0.002%
Physostigmine salicylate	P 6.05	HB 0.1%
Pilocarpine hydrochloride	P 6.85	Bz 0.02%
Procaine hydrochloride	P 6.05	Bz 0.02%
Resorcinol	P 6.05	Bz 0.02%
Scopolamine hydrobromide	P 6.45	Bz 0.02%
Adrenaline acid tartrate solution	B 5	Ph 0.002%
Tetracaine hydrochloride	P 5.3	Bz 0.02%
Zinc sulphate	B 6.3	Ph 0.002%

Abbreviations

B 5	Boric acid solution pH 5	P 6.85	Phosphate buffer solution pH 6.85
B 6.3	Borate buffer solution pH 6.3	Bz	Benzalkonium chloride
B 6.8	Borate buffer solution pH 6.8	Ph	Phenylmercuric acetate
P 5.3	Phosphate buffer solution pH 5.3	HB	Mixture of two parts of methyl hydroxybenzoate and one part of propyl hydroxybenzoate
P 6.05	Phosphate buffer solution pH 6.05		
P 6.45	Phosphate buffer solution pH 6.45		

DR. BROWN: Thank you. It is interesting to see that they are regarding each formulation as a separate entity rather than hoping that one preservative can be used for everything.

(63) *Österreichisches Arzneibuch 9* (1960).

DR. E. E. BOEHM : It has recently been pointed out by several authors (64–65) that a *p*hydroxybenzoate concentration of 0.0343%, as recommended in the B.P.C. for eye drops preservation is insufficient. It has already been pointed out by Williams *et al* (65), that the addition of at least 0.08% of *p*hydroxybenzoates is necessary for the successful inhibition of microbial growth in eye drops. They further pointed out that *p*hydroxybenzoate concentrations of the order as recommended by the B.P.C. were ineffective against less resistant micro-organisms and therefore it could hardly be expected that at these low concentrations of the *p*hydroxybenzoates they would be effective against a much more resistant organism such as *Pseudomonas aeruginosa*, which is also resistant against benzethonium chloride (67).

We have also shown that the addition of 0.06% *Nipasteril*, another *p*hydroxybenzoate combination, does not only inhibit the growth of an especially resistant strain of *Pseudomonas aeruginosa* in a buffered aqueous solution, but also killed the organism between 5 and 24 hr.

Recently Müller (68), on the basis of his own experience, recommended the addition of 0.07% methyl and 0.03% propyl *p*hydroxybenzoates to eye drops, and Montgomery *et al* (64) the addition of 0.063% methyl and 0.023% propyl *p*hydroxybenzoates.

DR. BROWN : I think you will find in the paper that we agree that the concentration recommended in the B.P.C. is not likely to produce sterility with such organisms as *Pseudomonas aeruginosa*. However, I personally think that this field has been cluttered up with all sorts of opinions and assertions. One can pick almost any chemical and it is possible to find three or four references to workers recommending it. If, however, one critically evaluates the experimental procedures the recommendation often lacks weight. We have to give particular emphasis to those workers who have precisely stated their experimental conditions, have stated how many cells they used, what the medium was, recovery conditions and have correlated the efficiency of their inactivating agents with *in vivo* tests. Few people have done this. Riegelman *et al* (1) in the U.S.A. have done this. Their work was followed by Kohn *et al* with two excellent papers (24,25). I am unable to comment on your reference to the experience of Müller because I have not had an opportunity to see this reference. I have seen the reference by Montgomery and Halsall (64), and although they recommend the hydroxybenzoates they give no experimental details about their findings. I am not criticising them for omitting details because their views were merely expressed in a short letter.

MR. J. E. JEFFRIES : In your summary you state that at present there is no single ideal preservative for ophthalmic preparations and in particular that strains of *Pseudomones aeruginosa* or closely related types become resistant to all known preservatives. Referring to the *Achromabacter* species, there would again appear to be no effective preservative and in any case differentiation between the two types is very difficult. Would you please indicate if, in fact, these organisms are very thermolabile, since I believe their optimum growth temperature is of the order of

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(64) Montgomery, M. F. and K. G. Halsall, *Pharmaceut. J.* **192** 407 (1964).
(65) Sabalitschka, Th., *Pharmaz. Ztg.* **108** 1723 (1963).
(66) Williams, R. and Boehm, E. E. *Lancet* **2** 790 (1963).
(67) Pivnick, H. *et al J. Pharmac.* Sc. **52** 883 (1963).
(68) Müller, F. *Pharmaz. Praxis* 71 (1964).

25°C whilst at 37° they can be killed off, certainly in the presence of preservatives such as the esters of *p*hydroxybenzoic acid at normal concentration after not more than two days' incubation. This might possibly be a method of getting out of difficulty if one had a batch of material that was subsequently shown to be contaminated heavily with *Achromobacter*, although it would of course suffer from the serious drawback that the products of metabolism would still be present in the finished product.

DR. BROWN: I would just like to make one slight correction. In our paper we did not state that strains of *Pseudomonas aeruginosa* become resistant to all known preservatives. People from several quarters have been saying that compound X is the thing. Exaggerated claims have been made for chlorhexidine. What we state above (p. 015) is rather different from claiming that *Pseudomonas* can become resistant to all known preservatives. The point we are making here is that we should be cautious in accepting any new chemical that is heralded as a new wonder preservative. I do not believe there are any. I am puzzled about your second point. In page 003 of our paper we mention *Pseudomonas*, we mention *Aerobacter subtilis* and *Clostridium*. We do not, in fact, mention *Achromobacter*. Do you in fact mean *Achromobacter*?

MR. J. E. JEFFRIES: Yes, I do mean *Achromobacter*. Surely this is a fairly common contaminant of water used in the production area and it is in fact extremely difficult to get rid of once you have it there. It appears particularly on columns from demineralized water.

DR. BROWN: I must confess I am not an expert in this field and as far as I know *Achromobacter* is not pathogenic, and is not particularly thermolabile. I looked it up in Bergley and the original type species is reported at 20° to 25° optimum growth. Unfortunately this was a very early work and certain important tests that now seem to be necessary were not carried out. There are many other species listed where the optimum growth temperature is more normal. As far as the heat resistance is concerned, I do not think it is particularly heat labile. You may have come across some odd strains that are, but I certainly have not.

DR. O. D. PRIDDLE: What, in your opinion, is a good preservative for fluorescein sodium solutions? Fluorescein, as a high molecular weight anion, should react with a cation such as benzalkonium and the phenylmercurate ion.

DR. BROWN: Mr. Norton and I agree that the ideal would be a sterile single dose unit for fluorescein sodium, which is mainly used as a diagnostic agent. I would also like to make the point that although I have in fact worked at one eye hospital I am informed that because fluorescein is used frequently in eye hospitals, it is quite common to have a bottle of fluorescein used for months and I suspect that because of this there have been so many reports of fluorescein contaminated by *Pseudomonas*. I suspect that if it were customary for cups of tea to be left around for months there would be numerous reports of tea being a marvellous medium for *Pseudomonas*. I think that the problem could be eliminated in this particular case where it has a specific use as a diagnostic agent by using sterile, single unit drops.

The Australian formulary which has just been published, uses 0.004% P.M.N. with fluorescein, and the B.P.C. of course used 0.002% P.M.N. It would appear

that they should react. I am not sure that anybody has shown whether in fact it does happen at this concentration.

DR. O. D. PRIDDLE : Can you make any comments on the physical stability of comparatively low molecular weight preservatives such as chlorobutanol or phenylethanol in solutions contained in polyethylene or other plastic bottles ?

DR. BROWN: It seems very likely that there is absorption of some sort between plastics and phenols and as far as we know, not that we know very much, very little is published on this. I think it is very important and we, at Bristol, are certainly coming to the conclusion that we should be progressing towards mass produced, small volume eye drop preparations, rather than extemporaneous preparations as the norm. Information about the effect of these low molecular weight preservatives in the presence of plastics would be useful.

MR. M. J. GROVES : We were interested to note that you refer to *Bronopol* under the heading of agents not widely used as preservatives, the inference being that it could possibly be considered as a preservative for eye drops. We are also interested in this but we would like to sound a note of warning since *Bronopol* is not stable at an alkaline pH. We have examined the eye drops officially formulated in the British Pharmaceutical Codex. A number of these are alkaloidal solutions and hence acidic in reaction. But of the 20 eye drops in the Codex, 10 would appear to be either directly incompatible with *Bronopol* or contraindicated because of the pH. The use of *Bronopol* as a preservative is therefore somewhat limited and in addition it would be necessary to establish beyond reasonable doubt, that this new antibacterial, and I emphasize the word new or untried, is completely safe to use in the eye. Some work in our own laboratories which has been undertaken on the effect of *Bronopol* in rabbit eyes had given encouraging results so far, but there is limited information at present on the effects on human eye. We can only reiterate Dr. Harold Davis' comment at the recent British Pharmaceutical Conference, to the effect that reliable information about safety must be obtained under the conditions in which the materials can be used.

DR. BROWN : I agree and I would like to point out that *Bronopol* is mentioned under the heading of agents not widely used as preservatives. In the summary and conclusions we have not made any reference to it at all. We have ignored it, because more work will clearly have to be done on it, as you have suggested.

MISS B. CROSHAW : There is a further word of caution I would like to add regarding *Bronopol*. Although we have shown that it is bacteriostatic against both gram-negative and gram-positive bacteria at similar concentrations, the bactericidal activity is much greater against gram-negative organisms than against gram-positive. For this reason we would not recommend the use of *Bronopol* alone as a satisfactory preservative for ophthalmic solutions. Although you do not like combinations, we would only consider it in combination with benzalkonium chloride or something similar. I do not think we should be arguing whether *Achromobacter* is or is not pathogenic to eyes. I think any organism in an eye drop must be regarded as a potential pathogen, and a preservative that does not deal with it can not really be considered as satisfactory.

Concerning the use of chlorhexidine and its breakdown on heating : Are the breakdown products bacteriologically active?

DR. BROWN : I agree completely with the first part of your remarks. I would just like to comment on your remark that we do not like combinations, as I do not think this is correct. We have stated that they should not be used indiscriminately. A great deal of work has been done on synergism, antagonism, and additive action. It was originally thought that if you had one antibiotic that killed cells in the body and another one that did the same, then obviously the two together would be twice as good. This, of course, was not true and we were merely referring to the fact that they should not be combined indiscriminately.

I agree that any organism which is happily surviving in the presence of the preservative, should be regarded as potentially dangerous. I only made the remark because I thought that Mr. Jeffries was perhaps referring to *Aerobacta*, and not *Achromobacter*.

We were rather timid about our reference to breakdown of chlorhexidine because the literature contains too many personal communications and statements of opinion. Nevertheless we have it on good authority that heating chlorhexidine in solution and autoclaving it, did in fact produce compounds which could well be toxic and that this would have to be examined before using this method. This would not preclude the use of chlorhexidine in a filtered solution, or sterilizing in some other way. I do not know whether these degradation products are harmful to the bacteria.

MR. A. G. HOPKINS : At the present time quaternary ammonium compounds are becoming suspect as causing indurated ulcers, especially in damaged tissue. Does this not rule out substances like benzalkonium chloride, cetrimide, etc.?

DR. BROWN : We have heard about many defects of the quaternary ammonium compounds, particularly benzalkonium chloride. It has been stated, for example, that it dissolves the intercellular cement of the cornea. I have never heard of this particular problem but I think perhaps the problem is eliminated if, when using the damaged eye, no preservative at all is used unless it is absolutely necessary. Certainly during an operation when you are irrigating, say, the anterior chamber, you should use sterile saline or sterile water, or whatever it is that is required, without any preservative. What real evidence there is about this type of ulcer I do not know. Is it more than a report ?

MR. A. G. HOPKINS : It is quite factual and there have been some reports in the last two or three weeks on this. In fact the use of Dequadin, Dequalinium chloride and even cetrimide is being curtailed because of these reports. I do not know whether anyone else has heard anything about this.

DR. BROWN: In America benzalkonium chloride has been used for a good number of years as a preservative of choice, and has been the official preservative. There are a good number of hospitals in America and I should have thought that benzalkonium chloride had been exposed to sufficient possibilities causing problems, to have really stood the test. We have only been able to find about two cases where there was proof that a resistant strain to benzalkonium chloride had arisen. I think it is easy, if you abuse the techniques, to produce resistant strains to benzolkonium chloride.

MR. J. A. MYERS : Are you now in a position to suggest a suitable preservative for any of the eye drops in the British Pharmaceutical Codex ?

DR. BROWN : We would like to suggest, and I am not sure, that the B.P.C.

Committee agrees, that each preparation be regarded in its own right and to eliminate the attitude of "which is the best preservative ?" Not to say, "we will use chlorhexidine, or we will use *p*hydroxybenzoates," but to look at each particular compound, and at each formulation, because each formulation could be different and could involve the necessity of using a different kind of preservative. It is therefore advisable to have in one's armoury as many preservatives as possible and not to eliminate any unless one has to. I think that this is the approach that will be taken in England from now on, i.e. that we will look at each preparation and consider it on its own merits. This, as Professor Neuwald has mentioned, occurs in Austria.

MR. W. TRILLWOOD : It seems to me that we are very little further forward than we were 20 years ago. We do not have a suitable container, we have one or two eye drops that are available as single dose containers, if we can call it that. I think we have surely reached the stage when, for surgical theatre work on damaged eyes, it must be a single dose container and yet we are limited to perhaps five drugs if we do this. We then turn to the things which are available commercially; none of these, as far as I know, are available in a single drop container. The point has been made about the improper use in hospital of injections by nurses. I wonder if it is generally realized what kind of a moving population we have to deal with in hospitals. The nurses are moving all the time they are in training. We have moving populations of medical staff, too. And whatever drill one lays down today is obsolete tomorrow unless people are continuously watched. With eye drops it is even worse. They are left in the hands of patients. They are liable to touch the margin which may be contaminated, and after the first drop is used, the dropper is put back again and the contents are contaminated. These are the practical problems we are up against in hospitals. For damaged eyes one must suggest single drop containers as far as possible, and the smallest possible container.

DR. BROWN : I believe there are single dose units on the market, and I believe that a B.S.S. for multidose containers is on its way. Mr. Norton has details of it.

MR. F. BROPHY : I confirm that there are already a small number of 2 ml single dose phials available on the British market, which are completely sterile. A balanced salt solution, which is completely sterile, is also shortly to be available here.

MR. D. A. NORTON : A committee of the British Standards Institution has been investigating pharmaceutical containers for the last two years or so, and one of the subcommittees has been dealing with the multidose eye drop container. We recognize, I think, that the need for extemporaneously prepared preparations will go on for some time and this standard recognizes it. The main aim of the specification is to produce a bottle which will withstand autoclaving when properly sealed and with the medicaments contained therein. This was the requirement for autoclaving laid down some time ago in earlier papers. I hope that this specification will be published quite shortly, and I understand that almost final agreement has been reached on this standard.

DR. HARRIS : The question concerning corneal ulceration following quaternary ammonium compounds is, I think, one of concentration. Some work has been published on this. Some work was done in Australia where I believe it was shown fairly clearly that the concentration normally used to preserve eye drops does not really give any trouble, not even over long periods.

J. Soc. Cosmetic Chemists **16** 395-403 (1965)

Hygiene in Manufacturing Plant and its Effect on the Preservation of Emulsions

DOREEN L. WEDDERBURN*

Presented at the Symposium on "Preservatives and Antioxidants", organized by the Pharmaceutical Society of Great Britain and the Society of Cosmetic Chemists of Great Britain, in London on 17th November 1964.

Synopsis—Emulsions, which under normal circumstances are adequately preserved against microbial decomposition, can and often do break down when unusually large numbers of micro-organisms are accidentally introduced. The origins of excessive contamination frequently lie in the resin beds of demineralized water units, in filters, and in poorly designed plant which cannot be cleaned and sterilized effectively.

Precautions can be taken to avoid circumstances in which bacteria and fungi can multiply in manufacturing plant, and the best ways of guarding against this are the elimination of "blind ends" and dead spaces in equipment, and meticulous attention to plant cleaning and sterilization. The washing of machinery with warm detergent solutions is often inadequate because residues of emulsion diluted with the wash-solution can provide a favourable environment for rapid microbial growth in stagnation areas.

Lessons in good plant design and hygienic practice can be learned from the food industry where good housekeeping is essential in the avoidance of spoilage. Modern pharmaceutical and cosmetic emulsions are more susceptible to microbial decomposition than they were in the past, because many new materials are biodegradable, and consequently more rigorous standards of cleanliness, and sterility are now needed in manufacture.

In most well run factories, gross contamination of products by undesirable chemicals and frank dirt is usually avoided because such contamination is readily detected during routine testing and quality control. The unseen contamination by unusually large numbers of bacteria or fungi, which can occur from time to time, however, is probably the biggest enemy in a factory producing emulsions. Firstly, because

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routine testing may not include microbial assay, and secondly even if it does, the examination of small batch samples may fail to reveal the potential dangers.

Emulsions designed for topical application to the skin such as cosmetic creams and lotions need not, of course, be completely free from non-pathogenic bacteria and fungi, but the few organisms present in any product at the time of manufacture must be prevented from multiplying during the product's shelf and user life by an effective preservative. In general, preservatives are included in these products at a low concentration which is only sufficient to hold moderate numbers of organisms in a quiescent state, and an increase in preservative concentration is often undesirable because of increased cost and, perhaps more important, the increased risk of toxicity to the skin and mucous membranes.

If, by any chance, unusually large numbers of organisms are introduced into a product during manufacture or packaging, a preservative which is normally perfectly adequate may be overcome with the result that the product is virtually unprotected against microbial spoilage. In these circumstances, the emulsion may break down because the emulsifier has been metabolized, off-odours may develop as a result of utilization of various constituents, gas may be produced, discolouration may occur if pigmenting bacteria are responsible, and perhaps worst of all because of its visibility to the customer, fungal growth may occur on the surface of the product and inside the pack.

There are no hard and fast rules about the "best" types of preservatives for use in emulsions nor about the concentrations at which they should be used since all emulsions differ in their physical characteristics, and in the nature and concentration of their constituents. Some constituents will restrict microbial activity, while others will provide nourishment for growth ; some may be grossly contaminated themselves. The pH of the product may favour or discourage growth of some organisms, and the ratio of water to oil will also influence the likelihood of the survival and multiplication of micro-organisms. For all these reasons, a preservative must be selected on the basis of its suitability for one particular system, and not because it has proved effective in other systems. Suggestions about the preservation of emulsions are given by Wedderburn (1). Once a compatible and effective preservative has been selected, however, steps must be taken to ensure that it does not fail in the occasional batch of product when manufacturing conditions are not all that they should be.

The Origins of Contamination

Water supplies, raw materials, sacks, bags, drums and vats in which ingredients are packed, air and dust, and the final product packing materials are all vehicles by which unusually large numbers of micro-organisms can enter even the best run factories. Unfortunately, spot-checks for microbial contamination do not provide a complete safeguard as pockets of organisms in otherwise satisfactory materials and equipment can often provide greater problems than moderate numbers evenly distributed throughout. These pockets can easily be missed by the usual microbiological test techniques, and the only solution to this insidious problem is awareness of possible danger points, constant vigilance, and high standards of factory control.

Demineralized water is contaminated more often than not, and the resin beds of ion exchangers are usually responsible. Baker (2) has cited a case where water entering an ion exchange unit contained four to five bacteria per ml, and left the apparatus carrying 500,000 per ml. Frequent sterilization of the resin beds in these units is desirable because the organic matter trapped in them provides excellent growth conditions for organisms. Eisman *et al* (3) have also studied this problem and suggest that if the deionizing units are operated daily, contamination is less likely to occur than if they are only used intermittently and water is stored in large quantities where rapid multiplication can occur in short periods. Cruickshank *et al* (4) found irrigation of ion exchange beds with 0.25 per cent formalin (0.1 per cent formaldehyde) satisfactory for sterilization.

In factories where filtered tap water is used instead of demineralized water, the same attention should be paid to the filters as to deionizing beds. Frequent renewal of filters is, of course, preferable to sterilization particularly if organic matter collects in the filter, as this is often present in sufficient quantity to render the sterilizing fluid inactive against the organisms present.

Stagnant water, and damp patches on walls, ceilings, and under stacks of raw materials are an eternal enemy to hygienic manufacture, as both bacteria and fungi will thrive in these conditions, become airborne, and eventually enter the plant. Equal, if less obvious, sources of aerial contamination are draughts which disturb dust and microbial spores.

Of the raw materials entering a factory those of natural origin, such as the gums tragacanth, acacia and karaya, and solids like chalk, talc, kaolin and rice starch, are much more liable to carry large numbers of micro-organisms than the refined oils, fats and waxes, or synthetic materials,

and should, where possible, be stored away from the plant and out of draughts which could disseminate their flora.

Plant Cleaning

Fully continuous production of a single formulation seldom occurs in any one piece of equipment, and many cosmetic and pharmaceutical factories make several formulations in the same plant. The intervals between use of the machinery may vary from hours to weeks, and the plant may be cleaned immediately after production of the last batch of one product or just before manufacture of the next. The time during which the equipment is idle is, however, important from the microbiological point of view regardless of whether it occurs before or after the cleaning process. Undisturbed residues in dark, damp and often warm areas of a plant are danger points, because these are the conditions best suited to rapid microbial multiplication. Favourable growth conditions also occur in a plant which has been sluiced through with warm detergent solution and left without further treatment. Many types of bacteria grow actively in dilute detergent solutions and a quick rinse through of any plant will remove some, but not all, of the previous product, leaving a nutritious mixture of detergent and product in which the preservative has been diluted beyond effectiveness.

Few manufacturing plants are entirely free from stagnation areas in pumps, joints, pipelines, pressure gauges and valves, and large masses of microbial growth can form in very small amounts of liquid left undisturbed for a few hours.

Sokolski *et al* (5) have reported an example of contamination of a product from an accumulation of organisms in a trap in a production pipeline. The product contained clay, which can often be heavily contaminated, and pectin which can be metabolized and give rise to rapid growth. It also contained methyl *p*hydroxybenzoate, and the product was usually perfectly satisfactorily protected against growth of the normal number of organisms present. However, when the trap in the pipeline became heavily contaminated with organisms, clumps of growth occasionally broke away and the numbers were sufficient to overcome the preservative and cause product spoilage. Another interesting observation in this case study was that the offending organism *Cladosporium resinae* was capable of utilizing the preservative, methyl *p*hydroxybenzoate when its concentration was reduced below 0.15 per cent.

Complete and thorough removal of product residues is essential if a

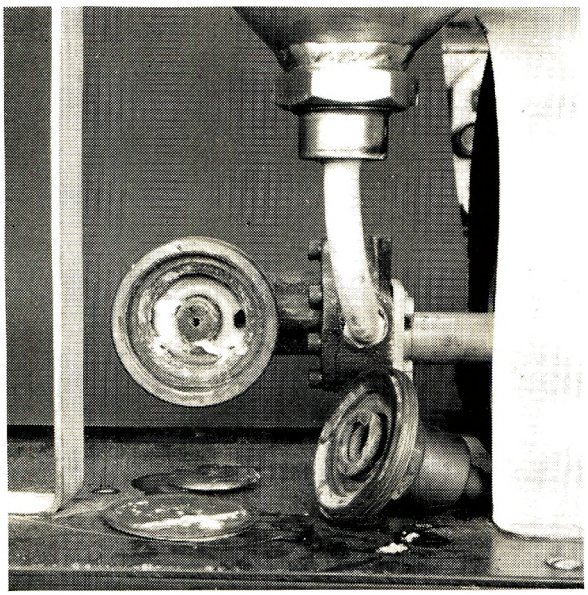

Figure 1

Gear pump with integral relief valve

An example of good engineering but poor plant design. The relief valve prevents damage by pumping against excessive pressure heads, but since it is awkward to clean, and it is not obvious when the valve has operated, it tends to be "missed" and so provides a potential microbial breeding ground.

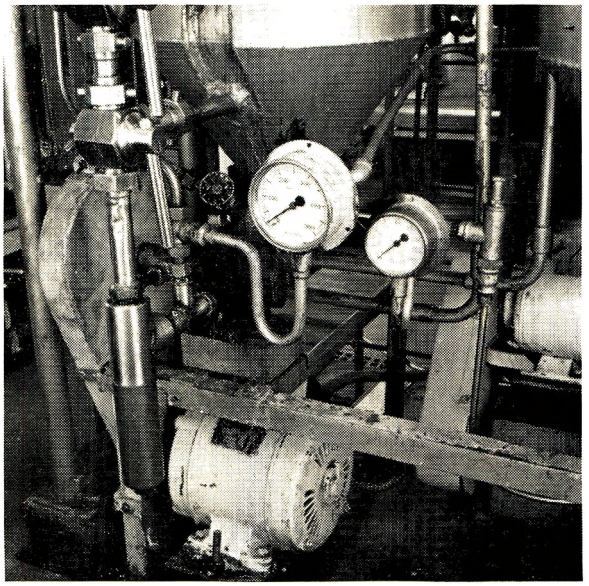

Figure 2

Good plant design spoilt by lack of attention to detail

There are a number of easily dismantled and easily cleaned cocks and pipeworks, but the plant is spoilt by the use of glycerine filled U-tubes to pressure gauges, and the presence of plug valves, and pressure glands on the gear pump. There is also a lack of drain cocks on fixed items, and too many right-angled joints exist, all making adequate cleaning impossible.

Facing page 398

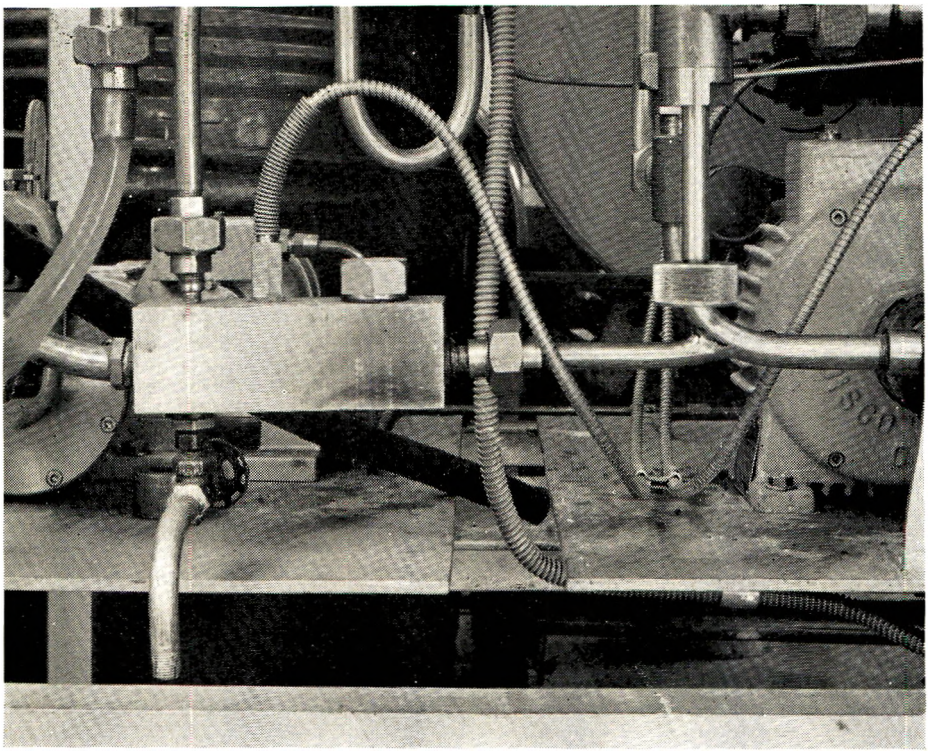

Figure 3

Manifold to reduce number of unions

The number of joints is reduced to a minimum by the use of a manifold. There are few right-angled internal corners ; dismantling, cleaning and sterilizing are therefore easy.

Facing page 399

piece of machinery is to be left idle for even a few hours, as thin films of product may trap and protect organisms beneath them, and diluted pools of product will allow infected foci to develop. Hot detergent solutions are satisfactory for most emulsion plants, but, particularly in plants used to make w/o emulsions, it is usually necessary to couple circulation of the solution with mechanical scrubbing and the dismantling of all inaccessible joints. This inevitably calls attention to plant design, and the necessity for smooth surfaces to working parts, the absence of "blind ends," and the need for easily opened joints cannot be stressed too strongly. If these conditions cannot be met in older machinery, it is often better to leave equipment unwashed before idle time, assuming the last made product contained an effective preservative, than to wash through and risk dilution of the product and the ensuing dangers of growth in stagnation areas.

Figures 1 and *2* show examples of parts of plant which are difficult to dismantle and clean, while *Fig. 3* illustrates equipment of better design.

Plant Sterilization

Cleaning and the removal of product residues should always be followed by sterilization, especially if the plant is not to be used again immediately. The fact that a single bacterium can give rise in five hours to over 1,000,000 like itself shows the risks of delaying sterilization after washing, which in itself can never remove all micro-organisms.

Sterilization by heat is the most effective means of ensuring destruction of *all* micro-organisms. Vegetative bacteria and fungi are destroyed by boiling for 5 to 10 minutes, but the only certain methods of destroying bacterial and fungal spores are by autoclaving at 120°C for 20 min or by holding at dry heat of 170°C for 2 hours. These procedures are, of course, not practicable for large manufacturing plants, and pasteurization which involves holding at 65°C for half-an-hour is not recommended since this, although effective against some pathogenic bacteria, does not affect heat resistant bacteria or spores.

Chemical sterilization is an alternative to heat treatment, and is effective in most instances but, because spores are not always destroyed, must be preceded by thorough washing to remove mechanically as much contamination as possible. The use of strong concentrations of chemical disinfectants at very high temperatures is a safeguard if contamination from spores is suspected, but these measures are often difficult to carry out. Davis (6) has reviewed the application of chemical sterilization of

plant used in the processing of food, milk, beer and textiles, and gives the advantages and disadvantages of several procedures.

Chlorine or hypochlorite is the best sterilant for most purposes as it is active against almost all organisms, is cheap, and is easy to prepare for use. Its odour is, of course, a disadvantage when hot solutions are used, and it is also corrosive at pH lower than 9. Above this pH, however, corrosion is not a problem in stainless steel machinery, although it should not be used in aluminium vessels. Cold solutions of hypochlorite to give 200 to 250 ppm of available chlorine will sterilize metal and glass surfaces after 5 min provided all organic matter has been washed away previously.

Formaldehyde or formalin is also a convenient sterilizing material for use after plant washing. Like chlorine, it is substantially inactivated by organic matter, and although its action on micro-organisms is rapid, its use suffers from the disadvantage that many plant operators are sensitive to its vapours. Intense irritation in the mucous membranes of the eyes, nose and throat can develop in people who are hypersensitive, and cases of these symptoms have been known even if the cork is removed from a formalin bottle in the vicinity of one of these unfortunate victims. For this reason, a cold solution should be used at a concentration of about 0.5 per cent formalin (0.2 per cent formaldehyde) and then only when non-allergic operators are present. This concentration will sterilize surfaces, free from organic matter, in 10 minutes.

Quaternary ammonium compounds, although weight for weight less effective than chlorine and formaldehyde, have advantages such as being relatively odourless, much less toxic to man, and far less corrosive than chlorine. They do not, however, have such a wide spectrum of anti-microbial action and are more effective against gram positive bacteria and vegetative forms of fungi than against gram negative bacteria. As well as being rendered less effective by organic matter, they are also inactivated by anionic detergents. Being surface active themselves, they can in some cases be used to clean the plant as well as to sanitize, although their detergent action may be too weak where water-in-oil emulsions are manufactured. A concentration of 0.5 per cent of benzalkonium chloride at about 60°C will sterilize previously cleaned smooth surfaces in less than 10 minutes.

Iodophors are prepared by the action of iodine on nonionic detergents in acid solution, and have both mild detergent and sterilizing actions. It is claimed that iodine in this form is as effective as the available chlorine in hypochlorite, but as these compounds are relatively untried in treating

emulsion manufacturing plant, definite recommendations about usage cannot be made.

Treatment after Plant Sterilization

Residues of disinfectant solution must be removed from the plant after sterilization without recontaminating the machinery, and the best way of doing this is to irrigate with sterile water.

The risk of recontaminating sterile machinery is high if cold water from a distilled or demineralized water storage tank is used, and boiled or autoclaved water is preferable, particularly if the plant is to be left idle before the next batch of product is made.

Ideally, equipment should be washed, sterilized and rinsed immediately before use, but if it is necessary to stop operations at some point during this 3-stage process, the safest point to break off is after chemical sterilization, and before rinsing. Leaving the plant idle after detergent washing or after final rinsing is potentially dangerous from the microbiological point of view unless the machinery is thoroughly dried inside and out.

(Received : 11th September 1964)

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Introduction by the lecturer

In the cosmetic industry in particular many types of formulations are made in the same plant, and the intervals between making one formula and the next can vary from days to weeks, and the time during which the plant is idle can be very dangerous indeed from a microbiological point of view. Although most of the people concerned with the formulation of emulsions are fully aware of these dangers, plant operatives seldom are and a lot more ought to be done in their training. They are very seldom aware that an adequately preserved emulsion, under clean conditions, will behave very differently when there is massive and accidental contamination, either through faulty plant cleaning or through the use of a certain raw material that might, by chance, have been very heavily contaminated.

Factory hands also cannot possibly be expected to know how quickly bacteria can multiply and change raw materials completely. Nor can they be expected to know which of the many raw materials handled are likely to be very heavily

contaminated, e.g. the gums acacia, karaya and tragacanth, and chalk, kaolin, rice starch and various pigments, often carry a very rich flora themselves.

Dilute solutions of anionic and nonionic detergents, left in a plant between clean- and sterilizing can lead to very heavy contamination as micro-organisms, particularly the gram-negatives like *Pseudomonads* will grow quite readily in these solutions.

I did mention the importance of having plant that is easy to dismantle, and can therefore easily be cleaned. Dismantling is therefore important, as is cleaning. The plant should be washed thoroughly using mechanical means if necessary, particularly with w/o emulsions, which are not easily rinsed and removed from the plant. Follow this by sterilization, and then by rinsing.

These four procedures can very seldom be carried out in quick succession without a break.

In my opinion it is important not to have a break after the cleaning process, because of the danger of having product residues diluted with anionic detergents that can easily and very quickly be metabolized by micro-organisms. If a break is necessary, it should be either after dismantling but before cleaning, and preferably after sterilization. In that event the sterilizing fluid is left in the plant during the idle time. One should take care that rinsing is not carried out with water from contaminated demineralized storage units. Either boiled, or very hot water which is unlikely to carry rich flora, should be used for this purpose.

DISCUSSION

MR. N. J. VAN ABBÉ : I believe that the question of plant hygiene as discussed by you, is of great importance whether or not a finished product includes a preservative. For this reason, I should like to make one or two suggestions.

In the first place, mains, distilled or demineralized water is a principal constituent of many products, but few companies, I believe, observe the same quality control over it that they apply to other raw materials. Bearing in mind the labyrinth of pipework through which water usually passes on its way to the production plant, and which may well result in contamination, I would strongly advocate the setting-up of internal bacteriological standards for water, and routine checks on this.

Secondly, I feel that connections of plastic hose to metal pipework nozzles, as shown in *Fig. 3*, offer a distinct contamination risk. A film of stagnant product is liable to remain between the plastic and the metal and this could easily serve as a reservoir of contamination for the bulk passing through. If joints of this type must be used, it is always prudent to fit a Jubilee clip around the plastic as close to the nozzle outlet as possible.

THE LECTURER : I agree that daily bacteriological tests ought certainly to be carried out if one is suspicious of the water supply.

Concerning the plastic pipe, I would not really think that the addition of a Jubilee clip would solve the problem. I would hope that the whole assembly would be taken apart and cleaned thoroughly, and not cleaned *in situ*. It is one of the advantages of using plastic pipe that one can take it off and discard it quite frequently, because it is not very expensive, and is very easy to replace.

MR. G. SYKES : In terms of water supplies, I think that deionized water is the most dangerous product that has been introduced into the pharmaceutical industry in recent years, because if one runs a column continuously it appears to be all right. If, however, one stops the system overnight, and certainly over the weekend, an

enormous bacterial population builds up, which, I know from experience, never gets rid of itself. It is true that one can use formaldehyde in order to disinfect the columns, but then there is the problem of getting rid of the formaldehyde, which I understand is even more difficult than getting rid of the bacteria.

You say that the only certain method of destroying bacteria and fungal spores is by autoclaving. I would like to know if there are any fungal spores which will stand more than, say 100°C.

THE LECTURER : I believe that there are fungal spores amongst some of the Penicillia which will resist heating to 100°C or more.

MR. J. JEFFRIES : It is indeed very important to get rid of whatever one is using to clean up demineralized water, be it formaldehyde or chlorine, as all these compounds can play havoc, for example, with dyes in the finished product. We are in a difficult position in the problem of demineralized water versus distilled water because the former provides us with the only method of producing the volumes of water that we need in a comparatively short time, and there are many occasions when one can use thousands of gallons in a normal working day. One would never obtain this volume of water from a conventional still.

MRS. H. BUTLER : I have had the misfortune to produce a food emulsion which is sold through chemists and where it is impossible to use a preservative. Unfortunately we got a yeast infection. I think that the only way to make sure that water is sterile is to boil it well for a quarter of an hour just before use. Do you agree with that ?

I think that just rinsing is dangerous, because a water rinse which dissolves the water from your emulsion will leave a residue of oil on the surface, where spores can stay. So the rinsing must be combined with cleaning and we found that we had to pump chlorinated water round the system before making any product. Each batch had still to be tested for organisms.

THE LECTURER : I sympathize with you for having a yeast infection in your plant. This is a very nasty thing to get rid of. On the other hand, I do not really think that just boiling your water will be sufficient, because you may well have contaminants in the form of spore bearing organisms. This is one of the reasons why demineralized water is so good, and distilled water even better, provided your resin beds and the lines from your storage tanks to your actual manufacturing plant are kept scrupulously clean.

MRS. H. BUTLER : We actually boiled the water phase a quarter of an hour. We then added the ingredients of the water phase, we boiled again, and pumped without it being open to the atmosphere ; the same with the oil phase. For the last four or five years we have been manufacturing under those conditions without any micro-organisms at all. We found that yeast was resistant even to hypochlorite, to heat, and we still had contamination after cooking the oil phase for a quarter of an hour.

THE LECTURER : This is very interesting, but it is laborious, very expensive, and time-consuming.

J. Soc. Cosmetic Chemists **16** 405-411 (1965)

The Sebaceous Glands

F. J. EBLING*

Based on a lecture delivered before the Society on 25th February 1965.

Synopsis—The structure, mode of secretion, distribution and development of the sebaceous glands are reviewed, the composition of sebum is described, and its function is debated. The actions of androgens, oestrogens, and progestogens on the sebaceous glands are discussed. It is concluded that while traditional views of the function of the sebaceous gland may be challengeable, the gland is far from vestigial in structure and, moreover, its sensitivity to male hormones is no less than that of the accessory male sexual organs. For these reasons a functional status for the gland ought to be assumed, though it is possible that this is not yet fully understood.

Introduction

The human scalp and face may have as many as 900 sebaceous glands in each sq cm of skin (1). These glands produce a waxy secretion known as sebum, and in adults about 100 μg cm^2/hr of lipid material can be removed from the forehead by absorption on pads of cigarette paper (2–4). Thus a single forehead, say 100 cm^2 in area, produces nearly 2g of sebum in a week. What is sebum ? What is its function ? How it is produced and how is the activity of the sebaceous gland controlled ?

Structure, mode of secretion, distribution and development of Sebaceous Glands

The sebaceous gland is holocrine ; its secretion is formed by complete disintegration of the glandular cells. In man the gland consists of a series of lobes or acini, each connecting to the main sebaceous duct which itself normally opens into the pilary canal (1); in rodents the gland consists only of one or two simple sacs. Sebaceous cells are replaced by cell division at the periphery of the lobes, and the cells differentiate and disintegrate as

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they move towards the centre of the lobe and duct. In the human gland, the lobes are in different states of maturity; it seems that new acini constantly arise from walls of the duct, grow into sebaceous units, fuse with adjacent lobes, and ultimately degenerate (1).

Sebaceous glands occur over much of the body, though not normally on the palms or soles, and only sparsely on the dorsal surfaces of the hand and foot; they are largest and most numerous in the mid-line of the back, on the forehead and face, in the external auditory meatus and on the ano-genital surfaces (1).

In a number of sites sebaceous glands open directly to the surface of the skin and not by way of a hair follicle. Examples of such glands are the Meibomian glands of the eyelid and Tyson's glands of the prepuce (1). Free sebaceous glands are also found in the areolae of the nipples and along the red margin of the upper lip (5). In the last site such glands are often visible to the naked eye as pale yellow bodies which vary in size from minute specks to about 1.5 mm in diameter. These are known as Fordyce's spots, and appear to increase in number with age.

Large sebaceous glands not associated with hair follicles occur in many mammals (6). Such are the flask-shaped preputial glands – opening by single ducts alongside the urethra – of rodents, the inguinal glands of rabbits, the dorsal gland of kangaroo rats (7), the large supracaudal gland of guinea pigs (8), the abdominal glands of shrews, the intermandibular sebaceous glands of bats and pigs (6), the large "brachial glands", one on the ventral surface of each shoulder, of male lemurs (6), and glands in the cloacal region of marsupials (9).

In the human foetus the sebaceous gland can be seen, as a small knob on the mid-posterior wall of the developing hair follicles, by 17 weeks of age (10). It is interesting to note that each developing follicle carries an apocrine sweat gland as well as a sebaceous gland, but the apocrine gland subsequently disappears except in a few regions of the body.

The Composition and Function of Sebum

Sebum is a complex mixture of lipid substances, and its detailed chemical composition is still incompletely known. Information which is available is based not on pure sebum, but on the mixture of sebum and epidermal lipids which makes up the surface film.

As much as 30 per cent of the skin surface fat may consist of free fatty acids. Though more than half of these are saturated and unsaturated C_{16} and C_{18} acids, there is a wide range including branched and unbranched

C_{17}, C_{15}, C_{14} and some shorter carbon chains (11,12). The remaining material consists of esterified acids, wax alcohols, squalene, sterols, and a small quantity of paraffin hydrocarbons which some authors believe are of environmental and not of endogenous origin.

It seems probable that the long fatty acids, ranging from 5 to 22 carbons with an average length of 16, are synthesized within the sebaceous cell, and that most of these are made into triglycerides. However, the esters are subjected to lipolytic activity by enzymes present in the sebaceous ducts and on the skin surface, and are broken down to diglycerides, monoglycerides and free fatty acids. Indeed, hydrolysis of triglycerides of exogenous origin has been demonstrated on the skin surface ; C_{14} labelled tripalmitin was spread on the back, and 3 hr later labelled free fatty acids were isolated from the surface fat (11).

Several functions have been attributed to sebum but they are by no means undisputed. In hairy mammals the sebum may be important in waterproofing the hair and perhaps, as in cattle, it may seasonally reduce the thermal insulation by the coat (13). In man, it has been stated that the lipid film both controls moisture loss from the epidermis and protects the skin from fungal and bacterial infection.

Cornified epithelium such as a cutting from a plantar callus becomes hard and brittle if it is allowed to dry out, but remains pliable as long as it contains 10 per cent by weight of water (14). The stratum corneum receives moisture from below and loses it by evaporation at the skin surface, but the major barrier against water loss through the skin is not at the surface but either near the base of the stratum corneum or throughout the entire cornified layer. At relative humidities of 60 per cent or more the moisture content remains high enough to maintain the pliability of the keratin. Under the lower relative humidity of winter weather or in rapidly flowing air the stratum corneum can, however, dry out with consequent chapping of the skin surface.

If the cornified epithelium is treated with organic solvents and then extracted with water, the water-holding capacity of the epithelium is greatly decreased (15). This suggests that the water-holding power of the cornified epithelium depends on the presence of lipids, but an alternative explanation might simply be that lipid solvents damage natural barriers. It may be unnecessary to doubt the view dear to cosmetic chemists that under adverse environmental conditions the application of lipid materials helps to keep the stratum corneum pliable and to prevent the chapping of skin, but it is debatable how far naturally-occurring lipids play such a

role and, if they do, whether sebum contributes to any protective surface film, or whether the lipid is mainly supplied by the epidermal cells themselves. To complicate the issue further, it is perhaps worth commenting that the hydrolysis of triglycerides on the skin surface must produce a small quantity of glycerol (11). Though this substance has never been demonstrated in sebum, it would have a moisturizing effect by virtue of its hygroscopic properties.

It is widely believed that free fatty acids on the skin surface hinder the growth of pathogenic organisms [for review see (16)]. Circumstantial evidence supports the view that sebum, or at least the product of its hydrolysis, is fungistatic. Fungi causing athlete's foot preferentially colonize areas which are not supplied by sebaceous glands ; ringworm of the scalp becomes rare after puberty when sebum production increases. It is also evident that free fatty acids have a limited action against certain bacteria, for example *Streptococcus pyogenes* (16). Both these properties of sebum have been challenged by Kligman (17). Admitting that fatty acids by themselves are antifungal *in vitro*, he claims that this potential is inactivated in the presence of horny material. He also states that the addition of sebum has no effect on streptococci or staphylococci growing on discs of isolated stratum corneum in agar culture. He therefore suggests that the sterilizing power of the skin is due largely to desiccation, a process supported equally by a wide range of inanimate surfaces. A critic of these studies has suggested that they only demonstrate the self-evident proposition that sebum does not prevent the growth of organisms which normally occur on the skin surface. They tell us nothing about the possible role of sebum in prevention of infection by other organisms which might prove pathogenic (18).

Control of Sebaceous Glands

Sebaceous glands are primarily under the control of androgens and this fact has been established by many studies in animals and man. For example, androgens enlarge sebaceous glands in rats (19), rabbits (20), hamsters (21), and mice (22), and also affect homologues such as the preputial gland of the rat or the supracaudal gland of the guinea pig (8). The sebaceous glands of man are minute during the prepuberal period but undergo vast enlargement at puberty, when the sebum output of males increases more than fivefold (23). Administration of testosterone increases the sebum output of prepuberal boys (23), but not of adult males where the glands would appear to be already under maximal stimulation by endogenous androgens (23). In eunuchs, who secrete only about half as much

sebum as normal males, the glands can, however, be stimulated by administered testosterone (24).

The stimulating action of androgens cannot be produced in the absence of the pituitary body, as shown by experiments in rats (25,26). It has been claimed that the necessary pituitary hormone is separable from other pituitary fractions, and it has been given the name of "Sebotropin" (27).

The production of sebum by eunuchs appears to be correlated with the urinary excretion of 17-hydroxycorticoids and 17-oxosteroids (24) ; thus the activity of the sebaceous glands of eunuchs, who lack gonadal testosterone, appears to be dependent on adrenal androgens.

The secretion of sebum by adult women is only a little less than that by normal men. Up to the age of 50 it is greater than in castrate men, but after that age it falls (23). This pattern suggests that the sebaceous glands in premenopausal women may be responding to androgens secreted not only by the adrenals but also from other sources. The ovary may be implicated, though it has not been possible to demonstrate any change in sebum secretion after bilateral ovariectomy (28).

Oestrogens decrease the size of the sebaceous glands of animals (19–30), and reduce sebum production in man (3,4,31). When large amounts of oestrogen are given, however, the glands will still respond to small amounts of androgens given at the same time and thus oestrogens do not seem to antagonize androgens at their site of action. As they were unable to demonstrate a local, as distinct from a systemic, effect by local application of oestrogen (23), Strauss *et al* suggested the possibility that oestrogens could act systemically by suppressing endogenous androgen secretion. There are, however, several pieces of evidence against this view. Though it was possible to suppress sebum secretion in castrates by administration of oestrogen, the treatment appeared to have no parallel effect on the excretion of 17-hydroxycorticoids or 17-oxosteroids (24). Moreover, from animal experiments it appears that while the effect of androgens is produced by a stimulation of cell division and by an increase in the anabolic capacity of each sebaceous cell, oestrogens have different points of action. In rats which have been treated with oestrogen and androgen over the same period, the rate of cell division in the sebaceous glands is very little less than that in animals treated with androgen alone ; nevertheless, the sebaceous glands are smaller (32). Thus it seems that oestrogens decrease the size of the glands by increasing the rate of cell breakdown so that the synthetic potential is reduced, rather than by any interference with cell division. In addition, this action of oestrogens, unlike that of androgens, does not

depend on the presence of pituitary (33). Finally, the effect of oestrogens can be demonstrated in adrenalectomized or in spayed rats (32,33). In animal experiments oestrogens can thus be shown to act independently of androgens at the peripheral site and independently of the presence of androgen-secreting glands ; but this does not, perhaps, exclude the possibility that they could have some systemic action as well.

The possible effect of progesterone on the sebaceous glands has been a matter of controversy. Some authors have stated that sebum production is increased during the luteal phase of the menstrual cycle (34), others that it has not (35), and the claim of Smith (36) that progesterone increases sebum production in elderly women is not consonant with other negative findings (35,37). Rothman *et al* published evidence that progesterone stimulated the sebaceous glands of spayed adult rats, claiming that its effect was comparable to that of testosterone (38). Although administration of progesterone has been tried in a number of different circumstances, an effect of physiological doses has not been confirmed (39). However, some slight effect of progesterone may be detected in rats given the extremely large dose of 10 mg/day (40).

Conclusions

The evidence in support of the traditional view that the secretion of the sebaceous glands plays a part in maintaining the hydration and pliability of the stratum corneum and in inhibiting the growth of pathogenic fungi and bacteria is by no means conclusive. It is difficult nevertheless to accept the view that in man sebum is functionless and the sebaceous gland is vestigial. Over much of man's body the hair follicle is reduced to a vestige producing a wisp of vellus hair, and the arrector muscle is similarly degenerate. Yet it is in these very regions that the sebaceous gland shows its greatest development. If the sebaceous gland were truly vestigial and the sebum had no function, why has it not suffered the same fate as the apocrine gland, which becomes visible during development but subsequently disappears in most regions of the body ? Moreover, the sebaceous gland in both men and women has retained a response to male hormones no less sensitive than that of the accessory male sexual structures themselves.

Zoological hypothesis would thus appear to be at one with the vested interest of the cosmetic chemist in maintaining a functional status for the gland. But why is sebum secretion so clearly linked with the sex hormones? Is the true function of the sebaceous glands and their product yet fully understood ?

(*Received : 23rd March 1965*)

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Book reviews

THE HALOGENATED HYDROCARBONS OF INDUSTRIAL AND TOXICOLOGICAL IMPORTANCE. W. F. Von Oettingen. Pp. x + 300 + Ill. (1964.) *Elsevier Publishing Company/Amsterdam/London/New York.* 60s.

Those who have had occasions to consult the literature on the toxicology of the halogenated hydrocarbons will certainly be familiar with the name of the author, Dr. Von Oettingen, who has compiled this volume. To cosmetic chemists, the subject is primarily of interest in connection with the use of aerosol propellants and they will find here a comprehensive review of relevant material, even though it does not deal specifically with all the propellants in current use.

The book is quite small and easy to follow, provided that the reader has some knowledge of physiology. The compounds with which it deals are divided into methane, ethane, ethylene and Butadiene derivatives, each chapter being subdivided for individual compounds such as methylene chloride and vinyl chloride, for example. Each of these selected compounds has a concise monograph, outlining its chemistry, manufacture, uses, detection and determination, absorption rate and excretion, determination in biological materials, toxicity for animals and man, prognosis, treatment and prevention of toxic effects. The paragraphs dealing with toxic effects are written mainly as reviews of the literature and are well referenced. There is a subject index, simply and adequately arranged for ease of consulting the main items in the book.

The cosmetic chemist is quite likely to encounter situations demanding a decision on whether a particular compound is safe for a special application and he cannot expect to find a ready-made answer in this or any other work of reference. He will still have to estimate or determine the acute and chronic exposure in the actual conditions of use and then utilize information such as Dr. Von Oettingen provides, in order to evaluate the hazards. For such purposes, this is an excellent contribution and it will be useful as a guide to the toxic hazards that might arise in manufacturing and packaging areas, as well as in relation to the final consumer. N. J. VAN ABBE.

L'AEROSOL EN PARFUMERIE. Tran Anh Tuan. Pp. 28 + Ill. (1964.) *Editions Varia, Paris.* 20s.

This booklet deals very briefly with general principles, with propellants, active ingredients, containers, valves, filling methods and laboratory techniques.

It is always difficult to deal with a large subject in a relatively brief manner, and this publication suffers from the faults which one would expect in such circumstances. It is generally conceded that it does take quite a while to publish a textbook and parts of it could therefore become out-of-date between writing and actual publication. These difficulties are not, however, inherent in a small booklet and it is therefore incomprehensible why this particular publication should be so out-of-date, e.g. in the note on propellants reference is made to a German manufacturer who has not supplied propellants for some time, and what is remarkable is that the trade names of a group of French propellants is given wrongly. The obsolete commercial numbering of Arctons and Algofrene is given; in fact these propellants were renumbered to the generally accepted form in 1960. Numerous spelling mistakes also occur.

EVALUATION OF DRUG ACTIVITIES : PHARMACOMETRICS. Editors : D. R. Laurence and A. L. Bacharach. Vol. 1. Pp. xvii + 456 + Ill. 95s. Vol. 2. Pp. vii + 457–900 + Ill. 90s. (1964.) *Academic Press, London and New York.*

The coining of new hybrid words for subdivisions of sciences which have expanded sufficiently to have exclusive books written on them is always an interesting intellectual exercise. Whether in fact it necessarily contributes to the science of communication is another matter. When confronted with such a term one tends to work through a mental list – pharmacography, pharmacodynamics, etc., and arrive at the meaning rather slowly by a process of elimination.

Pharmacometrics is defined as the identification and the comparative evaluation, qualitative and quantitative, of drug activities. It comprises the screening tests used in development of new drugs, and is distinguished from bioassay in which the object is to measure quantities of active materials.

The objective of the book is to provide a review and a critical discussion of general and special pharmacological techniques used in the search for new drugs. It is aimed at the pharmacologist who needs a

quick guide to the methods available for a particular screening job, at other scientists working in pharmacological laboratories but trained in other disciplines, such as biology, physiology, and biochemistry, and at the clinical workers who receive the end products of pharmacometrics for final evaluation. It is not a handbook, although a variable amount of experimental detail is given in the different chapters.

Vol. 1 is divided into two parts, the first comprising chapters on general principles underlying screening and clinical trials, and discussing limitations of animal tests. The second part of *Vol. 1*, and the whole of *Vol. 2*, contains chapters on particular applications to the different types of pharmacological activity.

Statistics quoted by J. R. Vane, in his chapter entitled "A Plan for Evaluating Potential Drugs," show that in a survey of over one hundred pharmaceutical companies, dermatological preparations form the largest single group of products. It would be satisfying to record that such effort in the marketing field receives suitable pharmacometric backing. A. Jarrett, in discussing dermatological aspects, however, feels that studies of alterations in the skin of man and laboratory animals have provided little information about the pharmacological activities of substances used in dermatology ; for the cosmetic chemist, this is a disappointingly short chapter.

Other chapters on different types of drug activity vary considerably in length and the extent to which they fulfil the editors' objectives. There is perhaps a tendency to fall between the two stools of being on the one hand insufficiently informative for the pharmacologist, and on the other hand too detailed for the more casual reader. Nevertheless, both will find much of use and interest in the book. An index is not included, but chapter headings are self-explanatory, and the two volumes are generally well produced. B. G. OVERELL.

DISINFECTION AND STERILIZATION. G. Sykes. 2nd edn. Pp. xx + 486 + Ill. (1965.) *E. & F. N. Spon Ltd., London.* (84s. U.K. only).

It is no mean achievement to have a publication recognized as one of the foremost reference books on a subject and to gain this standing as quickly as Mr. Sykes has done. Eloquent testimony to this effect is given by the speed with which this second edition has come along.

The second edition has naturally incorporated new facts and new ideas but the author planned the original well enough not to have found it

necessary to introduce any radical changes. It is interesting to note that practically every chapter has been expanded to some extent, with the exception of the one on dyes; presumably this is one group of antiseptics that is tending to wane in popularity. Literature citations have been extended to 1963 and apparently the early part of 1964. More attention is now devoted to the background theory of sterilization, in the light of the current state of knowledge on the effects of heat, cold, radiation and desiccation. In the section dealing with sterilization by gases and vapours, it is interesting to observe that βpropiolactone is now elevated to the status of having its own monograph.

Methods of testing disinfectants and antiseptics have long been the happy hunting ground for microbiologists with a taste for dialectics; Mr. Sykes has brought the subject up to date in his text, though still leaving the reader with the impression that current methodology is as debatable as ever it has been since the turn of the century.

Chapter 17 dealing with Preservation has been re-written and extended considerably, though the amount of detail it is possible to give on individual aspects is still rather limited. The author devotes only two paragraphs to the inactivation of preservatives by the nonionics. It is of interest to wonder whether he feels that this topic has been unduly magnified by others, or whether he was just a bit weary by the time he came to write these very last paragraphs of the book.

Mr. Sykes is well known as a powerful exponent of the need for terminological exactitude in the field of -cides and -stats. But his writing style is neither pedantic nor unduly academic and it has an easy flow; coupled with the care taken in adequately quoting the published literature and the comprehensiveness of the volume as a whole, this helps to produce a readable text book as well as a serviceable treatise for general reference purposes.

THE CHEMISTRY OF NATURAL PRODUCTS 3. I.U.P.A.C.

Pp. vii + 191 + Ill. (1964.) *Butterworths, London.* 60s.

The International Union of Pure and Applied Chemistry republish lectures by senior contributors to its symposia on the chemistry of natural products. At Prague (1962) the eleven papers included a number of specialist topics, e.g. chemotaxonomy, conformational analysis and photochemical transformations, but at the third symposium, held in Kyoto, Japan, in April 1964, the emphasis shifted more towards physical methods. In addition to three speakers specifically surveying physical techniques,

many of the structural studies reported rely heavily on instrumentation.

In a useful historical review, *S. Sugasawa*, traces the development of organic chemistry in Japan up to 1940, from early training in the famous *fin de siecle* European schools of Willstatter, Hofmann and Emil Fischer. Investigations in agricultural chemistry include various vital growth factors derived from rice, and naturally occurring pesticides. The pharmaceutical field embraces classical work on ephedrine and its congeners, and more recent attention to the amaryllidaceae alkaloids. Reference is made to the impressive contribution made to lichen chemistry by Prof. Asahina. Other groups of plant products studied include components of Japan lacquer, phenolic pigments, heartwood tropolones, triterpenes, flavones and biflavones ; fish toxins and hormone and bile steroids are cited as examples of studies on factors of animal origin.

Prof. *Buchi* (M.I.T.) concerns himself with the chemistry of certain dimeric indole alkaloids. The special interest in bisindoles derives from calabash curare, but at least three types of linkage are now distinguished ; simple dimers such as dihydrotoxiferin-C, an aminoacetal, e.g. geissospermine, and C-C linked moieties, as occur in a number of Voacanga and Vinca rosea bases ; intensive interest in the latter bisindoles has been prompted by their reputed anti-tumour activity. Particular emphasis is laid on the support that mass-spectroscopic and NMR studies have given to the elucidation of these exceedingly diverse series of indole alkaloids.

Prof. *D. H. R. Barton* (Imperial College, London) traces his active interest in biogenesis from his revision of the structure of Pummerer's ketone (the crystalline oxidation product of *p*cresol) and an elegant synthesis of the lichen product usnic acid. He proceeds from a generalisation of phenolate radical coupling to a lucid exposition of mechanisms of alkaloid biogenesis, giving special attention to a derivation of the morphine group from a benzylisoquinoline tricyclic intermediate, to demonstration of the natural occurrence of dienone *iso*quinoline intermediates in biogenesis of the apophine series and to speculation regarding the interrelationships of the Stephanie alkaloids.

Prof. *R. B. Woodward* (Harvard) follows with a fascinating account of the elucidation of the structure of one of the most virulent poisons known : Tetrodotoxin – found in some organs of Japanese puffer fish. The investigation is notable for many features, including the considerable initial difficulty of obtaining crystalline substances to determine even the molecular formula, the diagnostic use of NMR spectroscopy at every stage, the caution in interpreting facile rearrangements in a polyfunctional

product and the presence of a hemilactal system – entirely unique in natural and synthetic products.

Shemyakin and *Antonov* (Moscow Natural Products Institute) present a most useful review of intramolecular enzymic transpeptidation. They cite examples of formation of *N*-acyl amides important in metabolism and biosynthesis and of the determination of structures resulting from incorporation of hydroxyl or aminoacyl groups, using UV, IR, NMR and mass spectroscopy. They discuss the criticality of ring size in the expansion of *N*-acyl cyclic lactams to form peptide lactones and outline a number of biogenetic pathways for peptide antibiotics ; such considerations may also be applied to proteins and hormones.

K. Biemann (M.I.T.) describes in considerable detail the utility of high resolution mass spectroscopy in the examination of natural products, particularly for the very accurate determination of molecular weights and the study of fragmentation mechanisms leading to structural assignments. There are two distinct systems : Both employ preliminary electrostatic focussing in addition to the conventional electromagnetic stage. Beynon employs a single focus low resolution scan followed by double focussing to examine peaks of special interest. Biemann considers this time-consuming for general use ; he employs a computer to analyse a photographic record of a complete HR mass spectrum, which assigns mass with a precision of one part in 100,000 by comparison with an internal standard, and is then able to deduce the correct elemental composition of fragments that are superposed in conventional low resolution instruments. Examples from steroid and alkaloid fields, using both techniques, are discussed in detail ; one at present totally unknown structure is included to demonstrate the potential of his version.

Prof. *V. Prelog* (ETH, Zurich) refers to his earlier examination of microbiological reduction of alicyclic ketones (especially decalin-1,4-diones), frequently with the production of a preferred enantiomer, which has led naturally into an investigation of product stereospecificity in enzyme reactions. Whilst the enzyme infra-structure plays an important role, such interaction cannot be the sole factor. The development of Prelog's diamond lattice theory for the spatial relation of the reactive groups is therefore particularly significant.

Prof. *Stork* (Columbia University, New York) gives a detailed account of annelation reactions that he has found especially useful in the total synthesis of natural alicyclic systems. Having shown his versatility in the construction of steroid rings B and A, from a basic decalenone starting

point, he proceeds to a stimulating description of the solution of structural and stereochemical problems in the manufacture of a tricyclic quinolone which should yield members of the aspidospermine alkaloid family.

Prof. *Th. Wieland* (University of Frankfurt/Main) contributes a condensed but most readable account of the elucidation of the structure of a group of bicyclic peptide toxins extracted from a lethal species of mushroom found in Central Europe. He first reviews his earlier work on the more rapidly toxic phalloidine heptapeptide congeners and then reports his most recent studies establishing the structure of the four related amanitine toxins ; the members of latter group differ in being octapeptides with a 6-hydroxyl in the interesting 2(S)-cysteinyl-tryptophan moiety.

The progress of mass spectroscopy is survey by *Carl Djerassi* (Stanford University, California). He briefly comments on electronic control in fragmentation mechanisms and then develops his main theme – the importance of isotopic labelling in mass spectroscopic studies. Instances are cited revealing the composite nature of cleavage products having the same empirical composition, i.e. which would not be distinguished even by high resolution mass spectroscopy. Details are given of his extensive experience with deuterium labelling whereby hydrogen transfer rearrangements may be detected.

Finally, Prof. *J. Monteath Robinson* (Glasgow University) presents a concise, polished review of the present status of X-ray crystallographic solution of natural product structures. To illustrate the use of his heavy atom labelling technique, he cites derivatives of natural products with both favourable and unfavourable phase situations and then gives examples employing isomorphous replacement or solvated crystals. He mentions a number of terpenoid bitter principles elucidated during a most fruitful collaboration with the natural product schools in Glasgow of Barton and, currently, Raphael.

Altogether, this book contains a fascinating collection of lectures, admirably balanced despite the contemporary preoccupation with physical techniques. It is a worthy successor to the two previous volumes ; all three are essential reading for any chemist having an interest in natural products and are profitable study for those in less specialised fields.

G. F. PHILLIPS.

Society of Cosmetic Chemists of Great Britain

ANNUAL REPORT 1964-65

The work of the Society has again been maintained at a high level and the most noteworthy developments have been the institution of the Medal Lecture, the adoption of a new format for the *Journal*, and the Society's election to membership of the Parliamentary and Scientific Committee.

1. Membership

The membership of the Society on 31st March 1965 is shown below in comparison with the figures at the same time during the two preceding years.

	1963	1964	1965
Honorary Members	6	6	6
Members	329	351	359
Associates	57	49	48
TOTAL	392	406	413

Although the total number of members have not increased to any great extent during the year, it is a source of gratification that amongst the new members elected have been persons who are counted as leaders in their fields of activity.

1.1. *Obituary:* The Council records with regret the passing of Mr. W. P. Pepper, a Founder, who had served on the Council. The death is also recorded of Mr. E. Polan, another Founder who was the Society's first Hon. Treasurer, and who subsequently also served on Council. [Obituary notices : *J.* **16** 430 (1965)]

2. Scientific Meetings

2.1. *Lectures :* When planning the 1964/1965 lecture programme it was decided to broaden the scope of the lectures and this was reflected

in the choice of subjects. This trend will be continued during 1965/66. The scientific meetings were devoted to the following :—

Date	Subject	Lecturers
13th October 1964 :	The application of digital computers in the chemical industry.	R. E. Giles.
16th December 1964 :	The dermal testing of cosmetics in experimental animals and in man.	A. Rook and K. H. Harper.
26th January 1965 :	Storage testing in the cosmetic industry. A discussion with contributions by	D. E. Butterfield, E. K. Clarke, and J. J. Mausner.
25th February 1965 :	The sebaceous gland.	F. J. Ebling.

The Council wishes to record its thanks to Mr. S. J. Bush for organizing the lecture programme and the film evening.

2.2. *Medal Lecture:* The Council agreed to institute an Annual Medal Lecture, and it was decided that "the recipient of the Annual Medal should be a leading personality who has made an outstanding contribution to science, public life or the arts. The lecture shall be on cosmetics or allied subjects."

The first Medal Lecture was delivered by Sir Edward Charles Dodds, M.V.O., F.R.S., Courtauld Professor of Biochemistry, University of London, on "The Hormonal Background of the Skin," in the presence of Members of Parliament and other distinguished guests. The lecturer was presented with the Society's Silver Medal, specially struck for this occasion. Subsequently, the lecturer and representatives of kindred societies were entertained to dinner by the President, and three of the Past-Presidents.

2.3. *Film Evening:* A film evening was held on 13th May 1965 and members, together with their ladies, spent an enjoyable evening.

3. Symposia

The symposium on "Hair," briefly referred to in the last Annual Report, attracted an audience of 155. During November 1964, a one-day symposium on "Preservatives and Antioxidants", organized jointly with The Pharmaceutical Society of Great Britain, was held at the Connaught Rooms, London, and 258 delegates attended. From 30th March to 1st

April 1965 a symposium on "Emulsions" took place at Harrogate, Yorks., and attracted 132 participants.

The Council records its appreciation to Messrs. R. E. Eckton, N. J. Van Abbé and R. F. L. Thomas for concerning themselves with the organization of these three functions, respectively.

4. Journal

The *Journal*, as every member will appreciate, is unique by being published from three different locations, in two different languages, and yet with consecutive pagination. This has become even more arduous as the gap between each issue is only four weeks.

For some time the need has been felt for modernizing the appearance of the *Journal* and protracted negotiations, spanning almost two years and involving numerous meetings between the three editors, resulted in the adoption of the format used since the January 1965 issue. Minor differences in style are bound to continue but there can be little doubt that the new format considerably improves the appearance of the *Journal*.

A proposal on the part of our Society to change the name of the *Journal* failed to find the necessary support.

The Annual Subscription for non-members is £10 (£1 per single issue) while members continue to receive free copies as part of their membership subscription.

5. Education

The one-year, part-time day release course for the Society's Diploma is progressing satisfactorily. Although Brunel College will be granted University status shortly, it is hoped that the course can continue at the college.

Seventeen candidates sat for the examination as a result of attending the new one-year course, and seven students after completing the second year of the old course. Eleven and five students respectively passed the examinations and the £5 prizes were awarded to Mr. W. W. F. Scotland for being the best student in the new course, and to Miss V. J. Lavin in respect of the old course.

Having regard to the importance which the Society attaches to education, a Special Meeting was convened at which the post of Hon. Education Secretary was created, the office holder being ex-officio a member of Council with full voting rights. Dr. M. Cantley is the first holder of that office.

Additional changes were made in the Constitution and Rules to indicate the higher standard of the Society's Diploma.

6. Scientific Committee

This met several times under the chairmanship of the Vice-President, Mr. R. Clark, in order to discuss broad lines of investigation.

6.1. *The Toxicology Subcommittee* (Chairman : Mr. N. J. Van Abbé) has taken an interest in the following matters :—

6.1.1. Cosmetic Hazards and *The Times*.

Following the publication of a rather alarmist article in *The Times*, detailed correspondence was entered into which dealt with issues such as the inhalation toxicity of hair sprays and the supposed need for legislative control of toiletries. It is not possible yet to foresee whether that paper will in the future consult with the Society in advance of going to print.

6.1.2. Hair sprays

The inhalation toxicity of hair sprays is still the subject of various investigations and, whilst "thesaurosis" now seems to be almost universally discounted, the notion of possible hypersensitivity is still around, and this is under discussion.

6.1.3. Use of methanol in industrial spirits

The sub-committee has reached the view that there is no evidence of any marked toxic hazard in this case, though accidental acute poisoning could possibly arise. It was felt that members should bear in mind the desirability of eventually persuading the excise authorities to employ a less toxic tracer material.

6.1.4. Warning labels on aerosol packs

A query was raised on the desirability of standardizing label warnings on the hazards and misuse of aerosols. This was considered to be in the province of organizations more closely concerned with aerosol packaging.

6.1.5. Collaborative patch testing

The sub-committee is currently studying detailed proposals for a collaborative patch-testing technique, with a view to initiating work in several laboratories to aid in standardizing tests of primary irritation.

6.1.6. Cream shampoos

The sub-committee had followed up the paper by I. Gaunt and K. H. Harper [*J.* **15** 209 (1964)], by proposing to publish a recommendation that cream shampoos should normally be tested at 10%

concentration in rabbit eye studies. Publication has been deferred pending the results of new studies on a range of leading U.K. cream shampoos.

6.1.7. Toxicity of aerosol propellants
The sub-committee initiated consideration of the desirability of evolving a new toxicity rating system for aerosol propellants. This is being progressed initially by means of a review of data on propellant 11.

6.2. *The Methods of Analysis Sub-Committee* (Chairman : Mr. S. J. Bush) is investigating tests for the analysis of hexachlorophene contained in toilet preparations.

7. Relationship with other Bodies

7.1. As indicated in the last Annual Report, the Society applied for, and was granted, membership of the Parliamentary and Scientific Committee, an informal body of members of both Houses of Parliament and representatives of scientific bodies. Mr. A. Herzka has been appointed one of the Society's representatives for a minimum period of three years, and the second representative alternates between members of Council and of the Society. The Parliamentary and Scientific Committee plans to visit a cosmetic research laboratory situated in the vicinity of London, in the course of the next 18 months, and there is also a possibility that the General Committee might be addressed on cosmetic legislation.

7.2. A symposium sponsored jointly with The Pharmaceutical Society of Great Britain was held during the year, as already referred to above, and it is hoped to arrange similar joint functions from time to time. A department of The Pharmaceutical Society of Great Britain continues to send a representative to the meetings of the Scientific Committee Analytical Sub-committee.

7.3. Since 1953 the Society has been represented by Dr. H. W. Hibbott on a B.S.I. Committee concerned with Test Methods for Powder Properties ; Mr. A. Herzka has represented the Society since January 1965 on the B.S.I. Technical Committee P/189 – Aerosol Dispensers.

7.4. Two meetings have taken place with representatives of the Toilet Preparations Federation to maintain harmonious relations. Each body now sends one representative to the meetings of each other's

scientific committee, Mr. R. Clark being the Society's representative. A further result has been the preparation of a joint Press Release relating to the Labelling of Food Bill 1965.

8. Cosmetic Industry Exhibition

The Society has sponsored two technical exhibitions of suppliers to the cosmetic and allied industries. The first was held in Brighton, Sussex, during April 1964 and the second in Harrogate, Yorks., from 30th March to 1st April 1965. The Society originally specified that the first two exhibitions be held in the provinces, with the third one in London in 1967. Support for the second exhibition was less than for the first one, and although this could be due to the fact that provincial exhibitions do not command the customary support, the whole project is currently under review.

9. International Federation of Societies of Cosmetic Chemists

9.1. The Third Congress of the International Federation took place in New York at the end of June 1964, and a large contingent of members, with their families, travelled to the U.S.A. for this occasion by means of a special charter flight. The congress, and the attendant social functions, will always be remembered by those privileged to be present. The next (4th) Congress is due to be held in Paris in June 1966.

9.2. The Council of the I.F.S.C.C. met twice in New York, and unanimously adopted the modified Policy Document, which was subsequently ratified by the Council of this Society. The Society's delegates to the Council were Mr. A. Herzka, Dr. H. W. Hibbott, and Mr. J. B. Wilkinson.

9.3. Mr. J. B. Wilkinson, a former Council member, is serving as I.F.S.C.C. President until September 1965, being the second member of the British Society to hold this high office since the formation of the Federation in 1959.

10. Social Activities

10.1. *Soirée*

A Soirée was held at the School of Pharmacy, Brunswick Square, London, on 16th November 1964, on the eve of one of the symposia. It is hoped that this function will become an annual event and will enable all members to attend a social function at a reasonable cost. The Guest of Honour was Mr. R. W. Murphy (accompanied by Mrs.

Murphy), Immediate Past-Chairman of the Toilet Preparations Federation, who stood in at very short notice for his chairman, Mr. A. S. May, whom business commitments prevented from attending. During the evening, Diplomas and the two prizes were presented to those successful in the 1964 examinations.

10.2. *Dinner*

The Annual Dinner and Dance was held at the Connaught Rooms, London on 13th February 1965, and was attended by 232 members and guests. Sir Owen Wansbrough-Jones, Executive Vice-Chairman of Albright & Wilson Ltd., and Chairman of the Council of B.I.B.R.A., was the Guest of Honour. Other guests included the President of the I.F.S.C.C., the President of The Pharmaceutical Society of Great Britain, the Chairman of the T.P.F., and Mr. W. Howie, M.P.

11. Tie

A tie, with the Society's crest in red and yellow woven onto a blue background, is now available.

12. Administration

A new Register of Members, as at 1st June 1964, was published which incorporated a number of new features, such as lists of past and present Officers, details of past service to the Society, a list of Diploma holders, etc.

At the same time a further booklet entitled "Objects" was issued, as a means of publicising the Society amongst the general public and prospective members. The call for this has proved so extensive that a further reprint is being undertaken.

Mrs. D. Mott, having completed her trial year to the Council's satisfaction, has been asked, and has agreed, to continue in the position of General Secretary.

13. The Council

The following changes in Council took effect at the 15th Annual General Meeting, held on 28th May 1964.

Mr. A. Herzka succeeded Mr. S. J. Bush as President.
Mr. R. Clark succeeded Mr. A. Herzka as Vice-President.
Mr. D. E. Butterfield, Acting Hon. Secretary became Hon. Secretary.
Mr. R. E. Spate succeeded Mr. D. F. Anstead as Hon. Treasurer.

Seven candidates had been nominated for the three vacancies on

Council, and the following were elected :—Mr. E. K. Clarke, Dr. H. W. Hibbott, and Dr. J. J. Mausner.

A complete list of Hon. Officers and Committees for 1964/1965 was published in January 1965 (*J.* **16** 1).

Meetings of the Council were held in each month, except August, October and January, and all the committees and sub-committees met at frequent intervals. It is now clear that only persons who are regularly able to devote time to the Society should offer themselves for election.

Mr. S. J. Bush, Immediate Past-President, retires from Council at the forthcoming Annual General Meeting, after five years' continuous and devoted service. The Society is fortunate that Mr. Bush will continue to serve on some of the committees, and that he has agreed to arrange the scientific programme for one of the 1966 symposia.

The following also retire from Council after two years' service : Mr. R. E. Eckton, Mr. R. F. L. Thomas, Mr. N. J. Van Abbé. Mr. Van Abbé has agreed to look after the scientific programme of the other 1966 symposium. Council is appreciative of the service given by these gentlemen during their term of office, particularly Mr. Thomas who resides in Yorkshire but has attended most Council meetings.

14. The Future

Council is of the opinion that the range of activities outlined above is indicative of the progress made by the Society during the current year. Council further believes that progress will continue to be made, particularly if some of the projects now under consideration bear fruit, e.g. Council feels that more attention should be paid to public relations. It is also felt that steps should be taken to devise ways and means of encouraging the submission of original work for possible publication in the *Journal*, and of improving the standard of the papers given at symposia and lectures.

ANNUAL GENERAL MEETING

The Sixteenth Annual General Meeting of the Society took place on the 24th May, at the usual venue–55 Park Lane, London, W.

The Annual Report was presented by the President, Mr. A. Herzka (*J.*421–428). The Treasurer's Report was presented by the Hon. Treasurer, Mr. R. E. Spate.

In the voting for three new Members of Council the following were elected: Mrs. H. Butler, Mr. C. Pugh, and Professor A. N. Worden.

Ronald Clark, A.R.I.C.

President 1965-66

Facing page 429

The officers for 1965-66 are:—

President:	Mr. R. Clark
Vice-President:	Dr. A. W. Middleton
Immediate Past President:	Mr. A. Herzka
Hon. Secretary:	Mr. D. E. Butterfield
Hon. Treasurer:	Mr. R. E. Spate

The Chairman then explained that since the Special General Meeting on the 25th February 1965, a member drew the attention of the Council to the fact that Higher National Certificate was equated wrongly with the General Certificate of Education (Advanced Level). After due consideration, the Council proposed that this anomaly be rectified, and that corresponding alterations be made in various parts of Rule 3. The Council's Resolution to effect these changes was put to the vote and carried unanimously.

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

As usual, an informal supper followed the formal proceedings.

SYMPOSIUM ON EMULSIONS

A Symposium on Emulsions, organised by the Society, took place from the 30th March to 1st April 1965, at the Royal Hall, Harrogate. It was attended by 132 participants, including visitors from France, Germany, Holland, Norway, Poland, Switzerland and U.S.A. A Civic Reception to participants and their ladies, was given by his Worship the Mayor of Harrogate, Councillor H. S. Hitchen, M.A., J.P. at the Lounge Hall, and the Mayor also addressed a few words of welcome to delegates before the symposium was formally opened by the President of the Society, A. Herzka, Esq., B.Sc., F.R.I.C.

SECOND COSMETIC INDUSTRY EXHIBITION

The Second Cosmetic Industry Exhibition, sponsored by the Society and organised by Events Promotions, Ltd, was held at the Exhibition Hall, Harrogate from 30th March to 1st April 1965. The following companies exhibited:

Consolidated Vacuum Corporation, Croda Ltd., Flexile Metal Co. Ltd., Flexitainers Ltd., Lautier Fils Ltd., J. H. Little & Co. Ltd., Lustroid Ltd., Pergamon Press Ltd., Specialities, Thomson & Joseph Ltd.

By invitation of the Society, a stand was taken by the British Industrial Biological Research Association.

OBITUARY

W. P. Pepper

It is with regret that we have to record the death of Mr. William P. Pepper who died in March after a road accident. Mr. Pepper, a chemistry graduate of Liverpool University, had served the cosmetic industry for many years with Unilever, Port Sunlight, then with Pond's Extract Co. and later Chesebrough-Pond's.

He was a Founder Member of the Society and had served on the Council. A qualified teacher, he helped greatly in the establishing of the Society's Educational Course at Acton Technical College and later at Brunel College. He was a member also of the Standards Committee of the Toilet Preparations Federation. He had served on this committee (which is responsible for Raw Material Specifications published by the T.P.F.) since its inception some ten years ago. In all these activities his sound chemical sense had been invaluable and was much appreciated by those who worked with him.

Of his other interests one remembers his affection for cricket and his abiding passion for chess. His love of chess dated from his undergraduate days when he represented the University. He maintained this interest in local chess circles in Ealing where he lived. Many friends will miss him and mourn his death at a comparatively early age.

E. Polan

Members may have heard with regret of the death in October 1964 of Mr. Edmond Polan, a Founder Member of the Society. Second son of the Rev. Mark Polan, he spent a lifetime in the soap-making industry. For many years he was chief chemist with J. C. & J. Field Ltd. in Lambeth until the factory suffered air-raid destruction in 1941. After serving in Royal Ordnance factories in Scotland, he returned to the soap-making industry with Mangers, then Bristows Ltd., and finally John Green of Barking.

He was an active participant in the formation of the British Society in 1948, was its first Treasurer and subsequently also served as a Member of Council. His quiet, thoughtful ideas were appreciated by his fellow officers in the early development of the Society. It was his suggestion which led to the installation by the Society of cosmetic treatment salons in two mental hospitals.

His social interests were wide and he gave much of his time over many years to the work of the St. John Ambulance organization. He also maintained his interest in the Society up to his retirement, and many members will remember him with affection.

Published on behalf of the Society of Cosmetic Chemists of Great Britain by Pergamon Press, Ltd., Headington Hill Hall, Oxford. Printed and Bound by Cheltenham Press Ltd., Swindon Road, Cheltenham and London, Great Britain.